Research Article

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

Simeprevir with peginterferon/ribavirin for treatment-naïve hepatitis C genotype 1 patients in Japan: CONCERTO-1, a phase III trial

Norio Hayashi[1], Namiki Izumi[2], Hiromitsu Kumada[3], Takeshi Okanoue[4], Hirohito Tsubouchi[5], Hiroshi Yatsuhashi[6], Mai Kato[7], Rito Ki[7], Yuji Komada[7], Chiharu Seto[7], Shoichiro Goto[7,*]

[1]*Kansai Rosai Hospital, Hyogo, Japan;* [2]*Musashino Red-Cross Hospital, Tokyo, Japan;* [3]*Toranomon Hospital, Tokyo, Japan;* [4]*Saiseikai Suita Hospital, Osaka, Japan;* [5]*Kagoshima University Medical and Dental Hospital, Kagoshima, Japan;* [6]*National Hospital Organization Nagasaki Medical Center, Nagasaki, Japan;* [7]*Research & Development, Janssen Pharmaceutical K.K., Tokyo, Japan*

Background & Aims: In a Japanese Phase II study, the hepatitis C virus NS3/4A protease inhibitor simeprevir demonstrated potent antiviral activity and significantly improved sustained virologic response rates when added to peginterferon α-2a/ribavirin in treatment-naïve patients infected with hepatitis C virus genotype 1.

Methods: CONCERTO-1 was a Phase III, randomized, double-blind, placebo-controlled trial. Treatment-naïve adults (⩽70 years) with chronic hepatitis C virus genotype 1 infection (hepatitis C virus RNA ⩾5 $\log_{10}$ IU/ml) were randomized (2:1) to simeprevir 100 mg once-daily with peginterferon α-2a/ribavirin for 12 weeks then response-guided therapy with peginterferon α-2a/ribavirin for 12 or 36 weeks, or to placebo with peginterferon α-2a/ribavirin for 12 weeks then peginterferon α-2a/ribavirin for 36 weeks.

Results: Overall, 183 patients were treated. Sustained virologic response 12 weeks after treatment end (primary efficacy endpoint) was achieved in 88.6% of simeprevir- and 61.7% of placebo-treated patients (p <0.0001 for stratum-adjusted between-group difference). Overall, 91.9% of simeprevir-treated patients met response-guided therapy criteria and completed treatment at week 24; sustained virologic response rate at 12 weeks in these patients was 92.0%. One simeprevir- (0.8%) and two placebo-treated patients (3.3%) experienced viral breakthrough; respective viral relapse rates were 7.6% and 30.6%. Overall adverse event profile in simeprevir-treated patients was comparable to that in patients who received peginterferon α-2a/ribavirin alone.

Conclusions: Simeprevir once daily with peginterferon α-2a/ribavirin significantly improved sustained virologic response rate 12 weeks after treatment end in treatment-naïve patients with chronic hepatitis C virus genotype 1 infection, with a shorter 24-week treatment duration in most patients.

Keywords: Genotype 1; Hepatitis C virus; Once-daily; Peginterferon; Protease inhibitor; Ribavirin; Simeprevir; TMC435; Treatment-naïve.

Received 12 December 2013; received in revised form 26 March 2014; accepted 1 April 2014; available online 12 April 2014

* Corresponding author. Address: Research & Development, Clinical Science Division, Janssen Pharmaceutical K.K., Nishi-kanda 5-2, 3-chome, Chiyoda-ku, Tokyo, Japan. Tel.: +81 3 4411 5639; fax: +81 3 4411 5086.

E-mail address: SGOTO2@its.jnj.com (S. Goto).

Abbreviations: HCV, hepatitis C virus; PegIFN, peginterferon; RBV, ribavirin; SVR, sustained virologic response; PI, protease inhibitor; AE, adverse event; QD, once-daily; CONCERTO, Clinical Optimization of New treatment strategy with TMC435 in Combination with peginterferon plus Ribavirin for Treatment-naïve and treatment-experienced patients infected with HCV genotype 1 (One); ALT, alanine aminotransferase; AST, aspartate aminotransferase; RGT, response-guided therapy; SVR12, sustained virologic response 12 weeks after the end of treatment; SVR24, sustained virologic response 24 weeks after the end of treatment.

Introduction

Japan has one of the highest rates of hepatitis C virus (HCV) infection worldwide, with around 2 million people estimated to be infected [1]. Of these, about 70% are infected with HCV genotype 1b, 20% with genotype 2a, and the remainder with genotype 2b or other genotypes. Hepatocellular carcinoma is a leading cause of cancer mortality in Japan, with approximately 70% of cases related to HCV infection [2].

The goal of chronic HCV infection treatment is virus eradication, to prevent progression to cirrhosis and hepatocellular carcinoma. Until recently, standard of care was combination therapy with peginterferon (PegIFN) and ribavirin (RBV) for 24–48 weeks [3,4], resulting in sustained virologic response (SVR) in approximately 50% of patients [5,6]. However, HCV genotype 1 appears less responsive to PegIFN-based therapy than other genotypes [7].

The development of direct-acting antiviral agents represents a major breakthrough in the treatment of chronic HCV infection. These have been shown to improve SVR rates when combined with PegIFN/RBV in treatment-naïve and treatment-experienced patients [8,9]. Current Japanese guidelines recommend triple therapy with the HCV NS3/4A protease inhibitor (PI) telaprevir plus PegIFN/RBV for chronic genotype 1 HCV infection [10]. However, first-generation HCV PIs, such as telaprevir and boceprevir, are associated with two- or three-times daily dosing, the potential for adverse events (AEs) including anemia, rash, and renal dysfunction; and relatively rapid emergence of resistance in patients who do not achieve SVR [8,9,11–13].

Simeprevir (TMC435) is a potent, oral, once-daily (QD), HCV NS3/4A PI which has recently been approved in Japan [14]. In a Phase I study, simeprevir plasma exposure was found to be higher in healthy Japanese adult male volunteers than in healthy

Research Article

Caucasian volunteers [15]. Based on this finding, simeprevir doses of 50 and 100 mg QD were selected for use in further studies in Japan. A Phase II study in Japanese treatment-naïve patients with HCV genotype 1 infection and high viral load reported simeprevir (50 or 100 mg QD, for 12 or 24 weeks) in combination with PegIFNα-2a/RBV to be generally well tolerated and associated with improved SVR rates and shorter 24-week treatment duration in most patients [16]. The Clinical Optimization of New treatment strategy with TMC435 in Combination with peginterferon plus Ribavirin for Treatment-naïve and treatment-experienced patients infected with HCV genotype 1 (One; CONCERTO) studies were initiated to further explore efficacy and safety of simeprevir combined with PegIFN/RBV in patients with HCV genotype 1 infection in Japan. We present results of the CONCERTO-1 study (ClinicalTrials.gov: NCT01292239) in treatment-naïve patients.

Patients and methods

Patients

Treatment-naïve male and female patients aged 20–70 years with documented chronic genotype 1 HCV infection and plasma HCV RNA $\geqslant 5.0$ $\log_{10}$ IU/ml at screening were eligible. Key exclusion criteria included liver cirrhosis, hepatic failure, any other liver disease of non-HCV etiology and co-infection with HIV-1, HIV-2, hepatitis B, or non-genotype 1 HCV. Additional exclusion criteria are summarized in the Supplementary data. All patients provided written informed consent before study entry.

Study design

This Phase III, multicenter, randomized, double-blind, placebo-controlled trial assessed efficacy and safety of simeprevir combined with PegIFNα-2a/RBV in treatment-naïve patients with chronic genotype 1 HCV infection. The study was conducted at 37 sites in Japan from January 17, 2011 to October 22, 2012. The study protocol conformed to Good Clinical Practice Guidelines and to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institutions' human research committee. (ClinicalTrials.gov: NCT01292239).

Eligible patients, stratified by age (<65 or $\geqslant$65 years old) and *IL28B* genotype (rs8099917; TT, TG, and GG), were randomized centrally 2:1 to simeprevir 100 mg QD plus PegIFNα-2a/RBV for 12 weeks followed by response-guided therapy (RGT) with PegIFNα-2a/RBV alone for 12 or 36 weeks, or placebo with PegIFNα-2a/RBV for 12 weeks followed by PegIFNα-2a/RBV for 36 weeks (see Supplementary data for details of treatment administration). Randomization was balanced by using randomly permuted blocks. Patients, study personnel, and the sponsor were blinded to treatment groups. According to RGT criteria, PegIFNα-2a/RBV therapy could be stopped at week 24 in simeprevir-treated patients who achieved HCV RNA <1.2 $\log_{10}$ IU/ml detectable or undetectable at week 4 and had undetectable HCV RNA at week 12. RGT was not permitted in the placebo group. All patients were followed for 72 weeks after treatment initiation.

Virologic stopping criteria were implemented to ensure that patients with a suboptimal response discontinued treatment in a timely manner in order to limit the risk of drug resistance and reduce unnecessary exposure to PegIFNα-2a/RBV. Patients with HCV RNA >3.0 $\log_{10}$ IU/ml at week 4 discontinued simeprevir or placebo but continued PegIFNα-2a/RBV. Patients with HCV RNA $\geqslant 1.2$ $\log_{10}$ IU/ml at week 36 stopped PegIFNα-2a/RBV.

The primary efficacy endpoint was the proportion of patients with undetectable plasma HCV RNA at end of treatment and 12 weeks after end of treatment (SVR12). Other efficacy endpoints included: the proportion of patients with undetectable plasma HCV RNA at end of treatment and 24 weeks after end of treatment (SVR24); the proportion of patients with undetectable plasma HCV RNA at week 4 (rapid virologic response) or week 12 (complete early virologic response); incidence of viral breakthrough (>1 $\log_{10}$ IU/ml increase in plasma HCV RNA level from the lowest level reached, or plasma HCV RNA level >2.0 $\log_{10}$ IU/ml in patients whose plasma HCV RNA level had previously been <1.2 $\log_{10}$ IU/ml detectable or undetectable); viral relapse (detectable or quantifiable plasma HCV RNA at last available measurement during post-treatment follow-up in patients who had undetectable plasma HCV RNA at end of treatment), and the proportion of patients who had achieved normalization of ALT levels.

Study assessments

To assess virologic response, plasma HCV RNA was quantified at screening, baseline, day 3, weeks 1, 2, 3, 4, 8, 12, 16, 20, 24, 28, 36, 48, 60, and 72 using the COBAS® TaqMan® HCV Auto Assay System (Roche, USA; lower limit of quantification, 1.2 $\log_{10}$ IU/ml). Plasma HCV RNA levels were also measured at weeks 42 and 52 in patients receiving PegIFNα-2a/RBV until week 48. ALT levels were measured at regular intervals throughout the study. Normal ALT levels, i.e., $\leqslant$40 IU/ml, were defined according to laboratory reference ranges.

Sequence analysis of the HCV NS3 protease domain was performed at baseline and in patients with simeprevir treatment failure (see Supplementary data for further details).

Safety was evaluated throughout the study. AEs were assessed during the treatment period and 28 days after the last dose of study medication. AE severity was graded by investigators using the World Health Organization grading scale. Certain AEs were identified as being of special or clinical interest (see Supplementary data for further details). Vital sign monitoring, electrocardiogram, and clinical laboratory tests were performed at regular intervals during treatment and the post-treatment follow-up period. Severity of laboratory abnormalities was classified according to the World Health Organization grading scale.

Statistical analysis

The sample size of the study (183 subjects) was calculated based on published data [16], and was considered to be sufficient to show superiority of simeprevir *vs.* placebo for the proportion of subjects achieving SVR12 with 90% power (see Supplementary data for further details). Efficacy analyses were performed on the full analysis set, which comprised all randomized patients who had received $\geqslant 1$ dose of study medication for whom post-baseline efficacy assessment data were available. SVR rates between treatment groups were compared using the Cochran-Mantel-Haenszel test adjusted for the stratification factors (age and *IL28B* genotype). The 95% confidence interval of the stratum-adjusted difference in the proportions between the treatment groups was calculated.

The safety population comprised all patients who received $\geqslant 1$ dose of study medication. Incidence of AEs and other safety endpoints was summarized for each treatment group.

Statistical analyses were performed using SAS® version 9.2 (SAS Institute Inc., Cary, NC, USA).

Results

Study population

Overall, 223 patients were screened for study participation and 188 were randomized, and 183 received $\geqslant 1$ dose of study medication (123 received simeprevir and 60 received placebo) (Fig. 1). Eleven patients (6.0%) discontinued the study during follow-up (3.3% and 11.7% in the simeprevir and placebo groups, respectively). The most common reason for study discontinuation was withdrawal of consent. In the simeprevir group, 92.7% completed the treatment period, as did 75.0% in the placebo group. Reasons for non-completion of all study medication are summarized in the Supplementary data.

Baseline demographic and disease characteristics were generally similar between treatment groups (Table 1). All patients were Japanese, 34.4% were male, and median age was 55 years (range 23–69 years). Most patients had the major alleles TT for *IL28B* 8099917 and CC for *IL28B* 12979860 associated with higher likelihood of response to pegIFN/RBV (66.7% and 64.2% in the simeprevir group, respectively, and 70.0% for both in the placebo group). Almost all patients had HCV genotype 1b (98.4%), except for three patients with HCV genotype 1a (two in the simeprevir

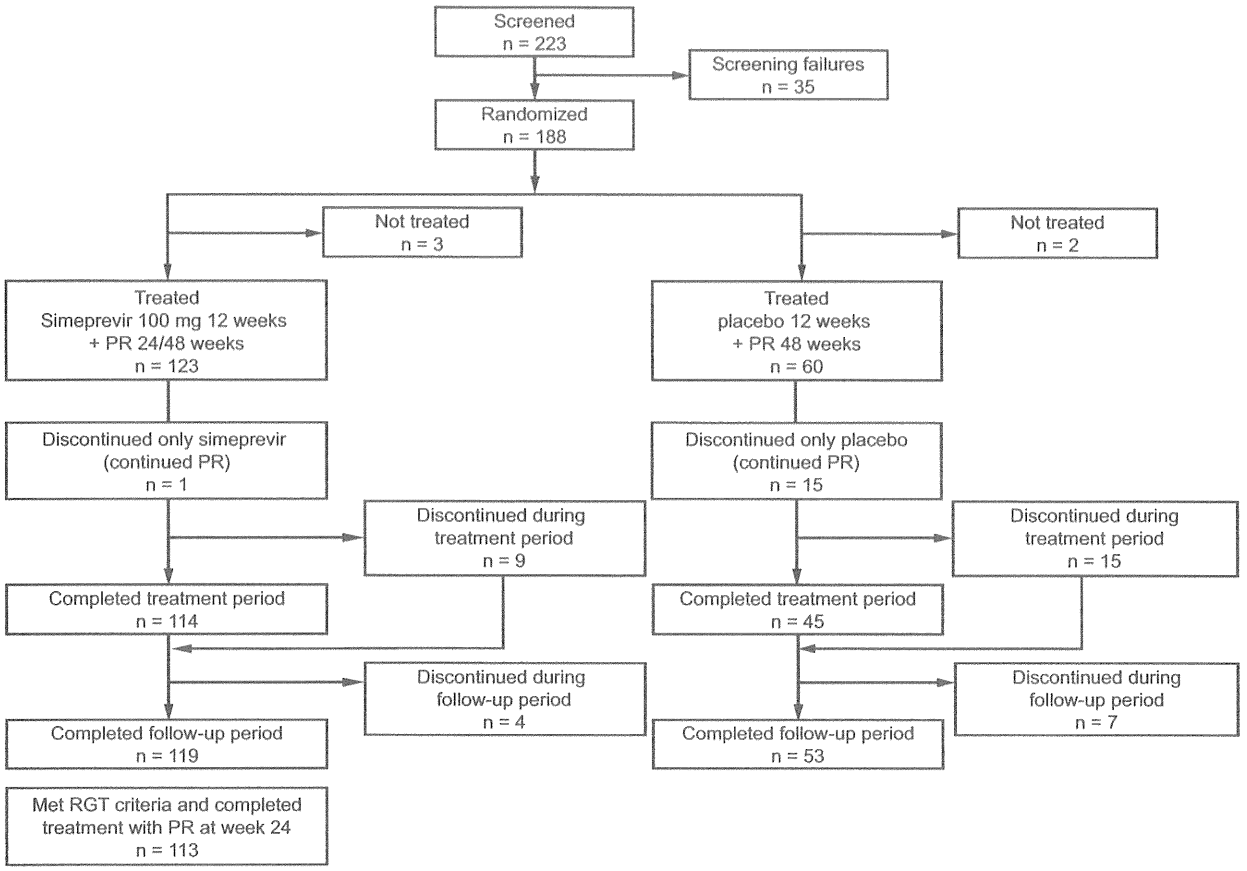

Fig. 1. Subject disposition. PR, peginterferon/ribavirin; RGT, response-guided therapy.

group and one in the placebo group). Median baseline HCV RNA was 6.3 $\log_{10}$ IU/ml (range 3.3–7.4 $\log_{10}$ IU/ml), with most patients in both groups having baseline HCV RNA levels $\geq$6 $\log_{10}$ IU/ml (74.0% for simeprevir and 76.7% for placebo).

Sustained virologic response

SVR12 was achieved in 88.6% of patients receiving simeprevir *vs.* 61.7% of patients receiving placebo (Table 2). The stratum-adjusted difference in SVR12 rate between the two groups was statistically significant (27.5% [95% confidence interval, 14.38–40.56%]; p <0.0001). Most (113/123; 91.9%) simeprevir-treated patients met RGT criteria and completed treatment at week 24. The SVR12 rate in these patients was 92.0%. Only one patient in the simeprevir group did not meet RGT criteria; this patient discontinued treatment with simeprevir only and completed treatment with PegIFNα-2a/RBV. The remaining nine patients discontinued the study before assessment of RGT criteria at week 24.

The proportion of patients achieving SVR24 was 88.6% in the simeprevir group and 56.7% in the placebo group (Table 2). Efficacy of simeprevir combined with PegIFNα-2a/RBV was statistically significantly superior to placebo combined with PegIFNα-2a/RBV (p <0.0001).

On-treatment virologic response

Onset of antiviral activity was more rapid with simeprevir than with placebo (Fig. 2). In the simeprevir group, 84.4% of patients had undetectable HCV RNA at week 4 *vs.* 13.3% in the placebo group (Table 2). The proportion of patients with undetectable HCV RNA at week 12 was also higher with simeprevir than with placebo (99.2% and 68.5%, respectively) (Table 2).

A notable difference in the proportion of patients achieving undetectable HCV RNA at weeks 4, 12, and 24 according to *IL28B* genotype at rs8099917 was seen between the simeprevir and the placebo groups (Fig. 3). With simeprevir, undetectable HCV RNA levels at week 4 were achieved by 87.8% of patients with the *IL28B* major allele (TT) and 75.6% of those with *IL28B* minor alleles (TG and GG). The proportion of simeprevir-treated patients with undetectable HCV RNA levels was increased at week 12 (96.3% with TT and 97.6% with TG and GG) and remained high at week 24 (92.7% with TT and 90.2% with TG and GG), irrespective of *IL28B* genotype. Undetectable HCV RNA levels at week 4 were achieved in 19.0% of placebo-treated patients with the *IL28B* major allele. No placebo-treated patients with *IL28B* minor alleles achieved undetectable HCV RNA at this time. Undetectable HCV RNA levels were achieved in 85.7% of placebo-treated patients with the *IL28B* major allele at weeks 12 and 24, and in

Research Article

Table 1. Baseline demographics and clinical characteristics.

Characteristic	Simeprevir group (n = 123)	Placebo group (n = 60)
Male, n (%)	39 (31.7)	24 (40.0)
Age (yr), median (range)	56.0 (23-69)	54.5 (30-69)
Age ≥65 years, n (%)	22 (17.9)	10 (16.7)
BMI (kg/m^2), median (range)	22.0 (16.9-32.9)	22.5 (17.3-33.2)
IL28B genotype (rs8099917), n (%)		
TT	82 (66.7)	42 (70.0)
TG/GG	41 (33.3)	18 (30.0)
IL28B genotype (rs12979860), n (%)		
CC	79 (64.2)	42 (70.0)
CT/TT	44 (35.8)	18 (30.0)
HCV genotype, n (%)		
1a	2 (1.6)	1 (1.7)
1b	121 (98.4)	59 (98.3)
Baseline HCV RNA (Log_{10} IU/ml), median (range)	6.3 (4.5-7.2)	6.4 (3.3-7.4)
Baseline HCV RNA category (Log_{10} IU/ml), n (%)		
<6.0	32 (26.0)	14 (23.3)
≥6.0 to <7.0	85 (69.1)	45 (75.0)
≥7.0	6 (4.9)	1 (1.7)
Metavir fibrosis stage,* n/N (%)		
0: No fibrosis	3/41 (7.3)	0
1: Periportal fibrosis expansion	28/41 (68.3)	18/24 (75.0)
2: P-P septae (>1 septum)	8/41 (19.5)	5/24 (20.8)
3: P-C septae	2/41 (4.9)	1/24 (4.2)
4: Cirrhosis	0	0
Platelets (×10^9/L), n (%)		
<150	30 (24.4)	10 (16.7)
≥150	93 (75.6)	50 (83.3)
ALT (IU/ml)		
<50	74 (60.2)	39 (65.0)
≥50	49 (39.8)	21 (35.0)
Total bilirubin (mg/dl), median (range)	0.7 (0.3-1.7)	0.7 (0.2-1.5)
Hemoglobin (g/dl), median (range)	13.9 (11.1-17.2)	14.1 (12.3-17.0)
Neutrophils (×10^2/μl), median (range)	24.2 (11.4-54.7)	22.5 (11.6-45.8)
Platelets (×10^4/μl), median (range)	18.3 (9.5-29.0)	20.2 (10.6-31.3)

*Available for patients who had a liver biopsy within 2 years before informed consent or during the screening period.
BMI, body mass index; HCV, hepatitis C virus; P-P, portal-portal; P-C, portal-central; ALT, alanine aminotransferase.

11.1% and 44.4% of placebo-treated patients with *IL28B* minor alleles at week 12 and week 24, respectively.

Viral breakthrough, viral relapse, and treatment failure

Viral breakthrough was observed in one patient (0.8%) in the simeprevir group, occurring at week 17 during treatment with Peg-IFNα-2a/RBV alone. Viral breakthrough was reported in two patients in the placebo group (3.3%) at weeks 2 and 21.

Viral relapse rates were lower in the simeprevir group than in the placebo group (7.6% *vs.* 30.6%, respectively). Viral relapse mostly occurred at follow-up week 12 (9/9 patients in the simeprevir group; 12/15 patients in the placebo group).

The proportion of patients meeting virologic stopping criteria was lower in the simeprevir group compared with the placebo group (0.8% *vs.* 40.0%, respectively). All of these patients met virologic stopping criteria at week 4 (plasma HCV RNA levels >3.0 log_{10} IU/ml).

Sustained virologic response according to baseline characteristics

SVR12 rates were higher in the simeprevir group than in the placebo group for all baseline demographic and disease characteristic subgroups. SVR12 rate by baseline demographic and disease characteristics is summarized in Supplementary Table 1.

NS3 sequencing analysis

Most patients (87.4% in the simeprevir group; 84.5% in the placebo group) had no baseline polymorphisms at any NS3 positions associated with reduced antiviral activity to simeprevir *in vitro*. Further information is provided in the Supplementary data section.

Alanine aminotransferase

In most patients with abnormal ALT levels at baseline, ALT levels were normalized by end of treatment (73.5% in the simeprevir group; 76.2% in the placebo group).

Table 2. Virologic response rates.

Endpoint, n/N (%)*	Simeprevir group (n = 123)	Placebo group (n = 60)
SVR12		
All patients	109/123 (88.6)	37/60 (61.7)
Patients meeting RGT criteria	104/113 (92.0)	n.a.
SVR24	109/123 (88.6)	34/60 (56.7)
Undetectable HCV RNA at Wk 4 of treatment*	103/122 (84.4)	8/60 (13.3)
Undetectable HCV RNA at Wk 12 of treatment*	117/118 (99.2)	37/54 (68.5)
Viral breakthrough	1/123 (0.8)	2/60 (3.3)
Viral relapse**	9/118 (7.6)	15/49 (30.6)

SVR12, sustained virologic response 12 weeks after the end of treatment; RGT, response guided therapy; SVR24, sustained virologic response 24 weeks after the end of treatment; n.a., not available.

*Subjects who discontinued all study medication prior to assessment of undetectable HCV RNA at week 4 and week 12 of treatment were not included in the denominator for calculation of these on-treatment response rates.

**Viral relapse was only assessed in patients with undetectable HCV RNA at end of treatment and with at least one follow-up HCV RNA measurement.

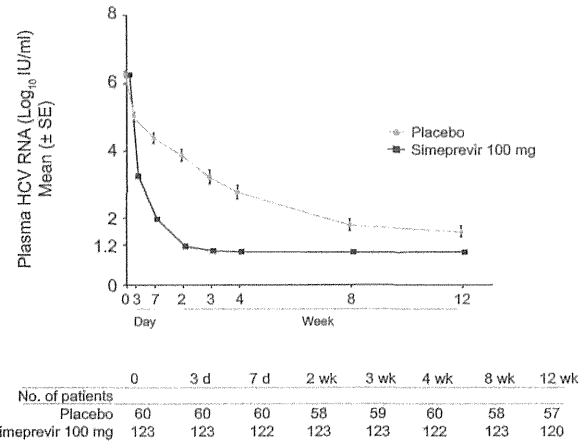

Fig. 2. Mean change from baseline in plasma HCV RNA levels up to week 12. HCV, hepatitis C virus; SE, standard error.

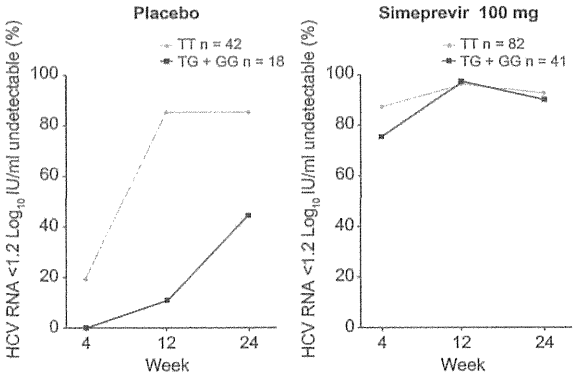

Fig. 3. Proportion of patients with plasma HCV RNA <1.2 log_{10} IU/ml undetectable according to *IL28B* genotype at rs8099917. HCV, hepatitis C virus.

Safety

For the entire treatment period, no clinically significant differences in the type and incidence of AEs were observed between groups (Table 3; Supplementary Table 2). Most AEs were grade 1/2 (72.4% in the simeprevir group; 66.7% in the placebo group). Rates of treatment discontinuation and PegIFNα-2a/RBV dose modification due to AEs were similar between groups, with no AEs leading to permanent discontinuation of simeprevir only. Serious AEs were observed in four patients (3.3%) in the simeprevir group and six patients (10.0%) in the placebo group. There were no deaths reported.

For AEs of special/clinical interest (Table 3), incidence was higher in the simeprevir group than in the placebo group for increased bilirubin-related AEs (22.8% *vs.* 10.0%) and photosensitivity conditions (1.6% *vs.* 0%). Incidence was lower in the simeprevir group than in the placebo group for rash (any type) and selected gastrointestinal events. For anemia, neutropenia, and pruritus-related AEs, incidence rates were similar in both groups. In the simeprevir group, all AEs of special/clinical interest were grade 1/2, except for grade 3 anemia-related AEs in one patient (0.8%) and grade 3/4 neutropenia-related AEs in 28 patients (22.8%). None of the neutropenia-related AEs were serious and none led to discontinuation of any study medications.

Fig. 4 shows changes in laboratory parameters over the entire duration of treatment and follow-up. Notably, only one patient in the simeprevir group continued treatment with PegIFNα-2a/RBV from week 24 onwards. Except for total bilirubin, no differences between groups were observed in median change from baseline for any of these laboratory parameters. Median total bilirubin levels increased in the simeprevir group from baseline to week 2 and then decreased to near baseline levels from week 4 to week 12. During this period, median levels remained within normal limits. From week 12, median values returned to baseline. Increases in total bilirubin levels were not associated with increased ALT or AST levels.

No consistent clinically significant differences in any other laboratory parameters, electrocardiogram parameters, or vital signs were observed between groups.

Discussion

This study was undertaken to assess efficacy and safety of simeprevir in combination with PegIFNα-2a/RBV in treatment-naïve

Research Article

Table 3. Summary of AEs reported in the entire study treatment period and during treatment with simeprevir plus PegIFNα-2a and ribavirin (safety population).

	Entire treatment period		Simeprevir/Placebo + PegIFNα-2a/RBV	
No. of patients, n (%)	Simeprevir group (n = 123)	Placebo group (n = 60)	Simeprevir group (n = 123)	Placebo group (n = 60)
Treatment discontinuation due to AE				
Simeprevir/placebo only†	0	1 (1.7)	0	1 (1.7)
All study medication‡	6 (4.9)	5 (8.3)	4 (3.3)	3 (5.0)
Dose modification due to AE				
PegIFNα-2a	47 (38.2)	24 (40.0)	n.a.	n.a.
RBV	67 (54.5)	27 (45.0)	n.a.	n.a.
SAE	4 (3.3)	6 (10.0)	3 (2.4)	3 (5.0)
Death	0	0	0	0
AEs of special/clinical interest*				
Increased bilirubin-related AEs	28 (22.8)	6 (10.0)	28 (22.8)	6 (10.0)
Blood bilirubin increased	20 (16.3)	4 (6.7)	20 (16.3)	4 (6.7)
Hyperbilirubinemia	8 (6.5)	2 (3.3)	8 (6.5)	2 (3.3)
Rash (any type)	71 (57.7)	42 (70.0)	64 (52.0)	27 (45.0)
Rash	57 (46.3)	37 (61.7)	54 (43.9)	24 (40.0)
Erythema	17 (13.8)	4 (6.7)	13 (10.6)	2 (3.3)
Other events	6 (4.9)	3 (5.0)	4 (3.3)	2 (3.3)
Pruritus-related AEs	37 (30.1)	19 (31.7)	29 (23.6)	5 (8.3)
Pruritus	35 (28.5)	18 (30.0)	28 (22.8)	5 (8.3)
Pruritus generalized	2 (1.6)	1 (1.7)	1 (0.8)	0 (0)
Photosensitivity conditions	2 (1.6)	0	1 (0.8)	0 (0)
Photosensitivity reaction	2 (1.6)	0	1 (0.8)	0 (0)
Neutropenia-related AEs	97 (78.9)	49 (81.7)	90 (73.2)	42 (70.0)
White blood cell count decreased	78 (63.4)	41 (68.3)	74 (60.2)	35 (58.3)
Neutrophil count decreased	69 (56.1)	37 (61.7)	59 (48.0)	30 (50.0)
Neutropenia	8 (6.5)	1 (1.7)	8 (6.5)	1 (1.7)
Anemia-related AEs	97 (78.9)	45 (75.0)	74 (60.2)	30 (50.0)
Anemia	70 (56.9)	36 (60.0)	55 (44.7)	24 (40.0)
Hemoglobin decreased	27 (22.0)	9 (15.0)	19 (15.4)	6 (10.0)
Selected gastrointestinal events	53 (43.1)	38 (63.3)	46 (37.4)	28 (46.7)
Decreased appetite	28 (22.8)	20 (33.3)	28 (22.8)	14 (23.3)
Diarrhea	20 (16.3)	17 (28.3)	14 (11.4)	12 (20.0)
Nausea	16 (13.0)	12 (20.0)	15 (12.2)	9 (15.0)
Constipation	9 (7.3)	5 (8.3)	7 (5.7)	2 (3.3)
Vomiting	6 (4.9)	5 (8.3)	4 (3.3)	1 (1.7)

†Permanent discontinuation of simeprevir or placebo alone, PegIFNα-2a/RBV continued.
‡Discontinuation of simeprevir/placebo and PegIFNα-2a/RBV at the same time, or discontinuation of PegIFNα-2a/RBV after completion or discontinuation of simeprevir/placebo).
*See Supplementary data for further details.
AEs, adverse events; PegIFNα-2a, peginterferon α-2a; RBV, ribavirin; SAE, serious adverse event; n.a., not available.

patients with chronic HCV genotype 1 infection and high viral load in Japan. A simeprevir dose of 100 mg with a 12 week duration for triple therapy was selected for use in this study based on results of a previous Phase II trial [16]. Oral once-daily treatment with simeprevir 100 mg for 12 weeks was associated with a significant improvement in SVR12 rate in this patient population. An RGT strategy was employed to allow individualized shortening of PegIFNα-2a/RBV treatment duration to 24 weeks, based on early virologic response. Almost all simeprevir-treated patients (91.9%) met RGT criteria and were eligible to stop PegIFNα-2a/RBV at week 24. The SVR12 rate in these patients was 92.0%, supporting this approach. A shorter overall treatment duration is highly desirable in patients with chronic HCV infection as it reduces PegIFNα-2a/RBV exposure and the potential for treatment-related AEs [17].

Even in patients with unfavorable demographic and baseline disease characteristics for a response to PegIFNα-2a/RBV (e.g., older age, high baseline HCV RNA, and low baseline platelet counts), treatment with simeprevir was associated with significantly improved SVR rates (Supplementary Table 1). As expected, *IL28B* single nucleotide polymorphism was predictive of therapeutic effect for PegIFNα-2a/RBV. It is well documented that

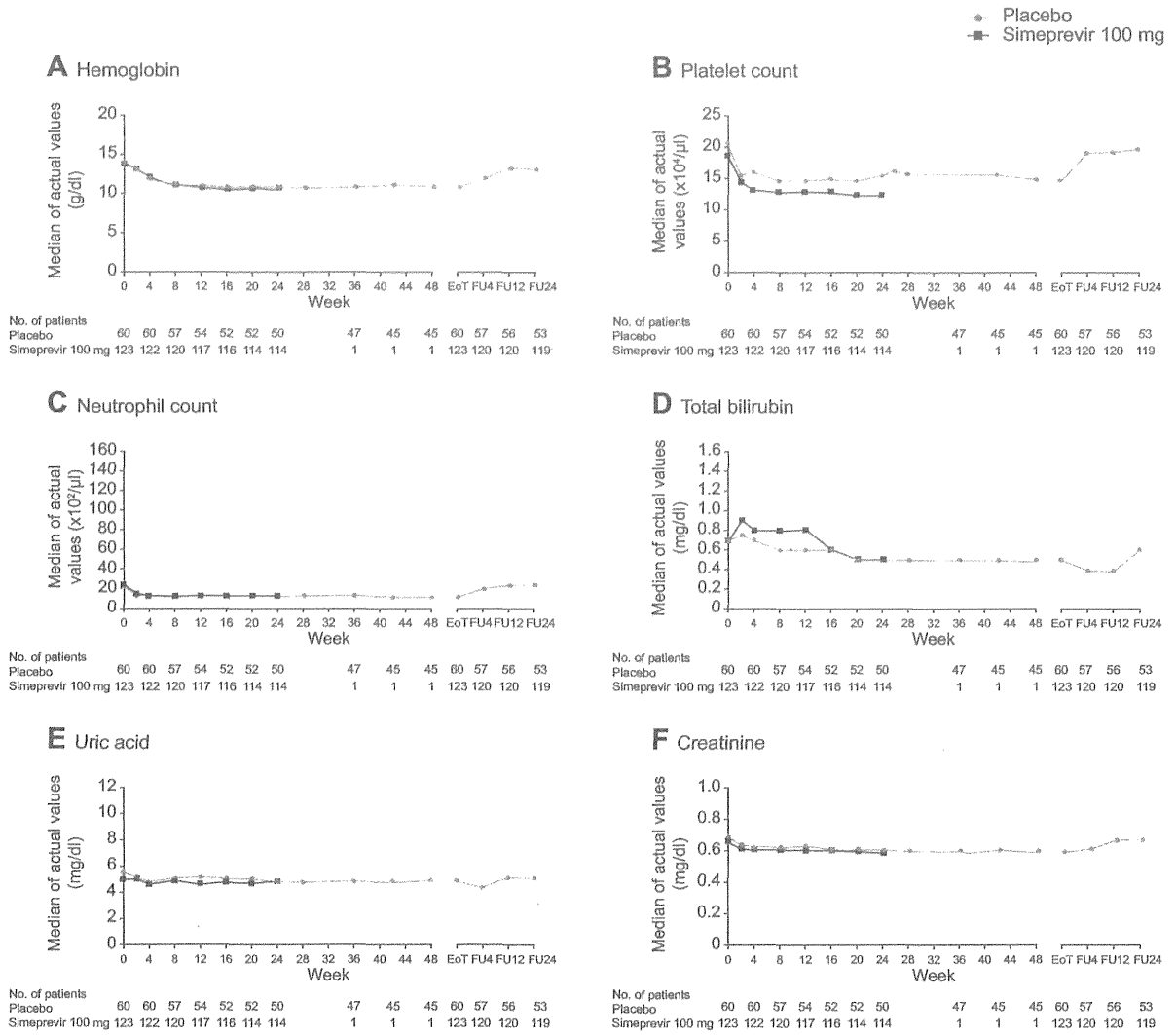

Fig. 4. Median changes in serum levels. (A) hemoglobin, (B) platelet count, (C) neutrophil count, (D) total bilirubin, (E) uric acid, and (F) creatinine over the entire duration of treatment and follow-up.*Only one patient in the simeprevir group continued treatment with peginterferon α-2a/ribavirin from week 24 onwards. EoT, end of treatment; FU, follow-up.

patients carrying *IL28B* minor alleles show greater resistance to PegIFN/RBV than those carrying major alleles [18–21]. Approximately two-thirds of patients in this study had favorable *IL28B* genotypes associated with higher likelihood of response to peg-IFN/RBV therapy. Nevertheless, our results suggest that addition of simeprevir to PegIFNα-2a/RBV reduces the impact of unfavorable *IL28B* minor alleles at rs8099917 and rs12979860. Consistent with the epidemiology of HCV infection in Japan [1], genotype 1b was dominant among patients enrolled in this study (98.4%). It has been suggested that HCV genotype 1a infection has a lower genetic barrier to resistance relative to genotype 1b infection [22].

The SVR rate in patients treated with PegIFNα-2a/RBV for 48 weeks is consistent with the original assumption for sample size estimation and is similar to that reported in a previous Phase III study of PegIFNα-2a/RBV in a comparable Japanese population [5]. The SVR rate observed in simeprevir-treated patients in this study is within the range reported in a previous dose-ranging Phase II study of simeprevir in treatment-naïve patients with chronic HCV genotype 1 infection in Japan [16].

Viral breakthrough was observed in one patient (0.8%) in the simeprevir group and two patients (3.3%) in the placebo group. Viral relapse occurred infrequently in simeprevir-treated patients compared with the placebo group, being observed in 7.6% of simeprevir-treated patients with undetectable plasma HCV RNA at end of treatment compared with 30.6% of patients who received placebo.

Sequence analysis of the HCV NS3 protease domain focused on detecting previously characterized HCV genotype 1 amino acid substitutions in the NS3 region that have been associated

with reduced susceptibility to simeprevir and other HCV NS3 PIs *in vitro* (43, 80, 155, 156, and 168) (see Supplementary data) [23,24]. Overall, 86.4% of patients did not have baseline polymorphisms at these positions. Emerging mutations were detected in most simeprevir-treated patients who experienced treatment failure, with D168V the most frequent emerging mutation. Emerging mutations identified at the time of treatment failure were generally not detectable at later time points, suggesting that these mutations do not persist and may resolve over time.

Simeprevir had a clinically favorable safety and tolerability profile in this patient population, with no notable differences in the type and incidence of AEs or discontinuations due to AEs observed between the simeprevir and placebo groups. In contrast to first-generation PIs, such as telaprevir [8,9,12,13], incidence of anemia- and rash-related AEs observed in simeprevir-treated patients was no higher than in placebo-treated patients, and no cases of severe anemia or severe rash were seen in simeprevir-treated patients in this study. Renal dysfunction has also been identified as a potential safety concern with currently marketed first-generation HCV PIs, including telaprevir; no clinically relevant changes in renal function parameters were reported in this study. Cases of sepsis and pneumonia have recently been reported in telaprevir-treated patients during post-marketing surveillance in Japan (Mitsubishi Tanabe, personal communication). However, no severe infectious diseases were reported in this study. Mild reversible transient increases in bilirubin levels were observed in simeprevir-treated patients, as reported previously [14,16]. *In vitro* data suggest that this results from inhibition of OATP1B1 and MRP2 transporters by simeprevir, as both play a role in bilirubin clearance [25].

In summary, results of the CONCERTO-1 study in treatment-naïve patients with HCV genotype 1 and high viral load show that oral once-daily simeprevir in combination with PegIFNα-2a/RBV significantly improves SVR rates and shortens treatment duration in most patients. Simeprevir has now been approved in Japan for the treatment of chronic HCV infection. Ongoing studies are investigating simeprevir in IFN-free combinations, including all oral regimens [26].

Financial support

This study was funded by Janssen Pharmaceutical K.K. Writing and editorial support were provided by Complete Medical Communications and funded by Janssen Research & Development.

Conflict of interest

Drs Okanoue, Tsubouchi, Kumada, and Hayashi consult and advise Janssen Pharmaceuticals. Drs Ki, Komada, Seto, and Goto are employed by Janssen Pharmaceuticals.

Acknowledgments

The authors would like to thank all patients, their families, investigators, and their staff at the 37 study sites. The study sites are listed in the Supplementary data.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhep.2014.04.004.

References

[1] Chung H, Ueda T, Kudo M. Changing trends in hepatitis C infection over the past 50 years in Japan. Intervirology 2010;53:39–43.
[2] Umemura T, Ichijo T, Yoshizawa K, Tanaka E, Kiyosawa K. Epidemiology of hepatocellular carcinoma in Japan. J Gastroenterol 2009;44:102–107.
[3] European Association for the Study of the Liver. EASL Clinical Practice Guidelines: management of hepatitis C virus infection. J Hepatol 2011;55:245–264.
[4] Izumi N. Diagnostic and treatment algorithm of the Japanese society of hepatology: a consensus-based practice guideline. Oncology 2010;78:78–86.
[5] Kuboki M, Iino S, Okuno T, Omata M, Kiyosawa K, Kumada H, et al. Peginterferon alpha-2a (40 KD) plus ribavirin for the treatment of chronic hepatitis C in Japanese patients. J Gastroenterol Hepatol 2007;22:645–652.
[6] Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001;358:958–965.
[7] McHutchison JG, Lawitz EJ, Shiffman ML, Muir AJ, Galler GW, McCone J, et al. Peginterferon alfa-2b or alfa-2a with ribavirin for treatment of hepatitis C infection. N Engl J Med 2009;361:580–593.
[8] Bacon BR, Gordon SC, Lawitz E, Marcellin P, Vierling JM, Zeuzem S, et al. Boceprevir for previously treated chronic HCV genotype 1 infection. N Engl J Med 2011;364:1207–1217.
[9] Jacobson IM, McHutchison JG, Dusheiko G, Di Bisceglie AM, Reddy KR, Bzowej NH, et al. Telaprevir for previously untreated chronic hepatitis C virus infection. N Engl J Med 2011;364:2405–2416.
[10] Chayama K, Hayes CN, Ohishi W, Kawakami Y. Treatment of chronic hepatitis C virus infection in Japan: update on therapy and guidelines. J Gastroenterol 2013;48:1–12.
[11] Buti M, Agarwal K, Horsmans Y, Sievert W, Janczewska E, Zeuzem S, et al. Telaprevir twice daily is noninferior to telaprevir every 8 h for patients with chronic hepatitis C. Gastroenterology 2014;146:744–753.
[12] Hezode C. Boceprevir and telaprevir for the treatment of chronic hepatitis C: safety management in clinical practice. Liver Int 2012;32:32–38.
[13] Thompson AJ, Locarnini SA, Beard MR. Resistance to anti-HCV protease inhibitors. Curr Opin Virol 2011;1:599–606.
[14] Tanwar S, Trembling PM, Dusheiko GM. TMC435 for the treatment of chronic hepatitis C. Expert Opin Investig Drugs 2012;21:1193–1209.
[15] Verloes R, Shishido A. Phase I safety and PK of TMC435 in healthy volunteers and safety, PK and short-term efficacy in chronic hepatitis C infected individuals (abstract O-32). In: Japanese Hepatology Congress, Kobe, Japan, June 4–5, 2009.
[16] Hayashi N, Seto C, Kato M, Komada Y, Goto S. Once-daily simeprevir (TMC435) with peginterferon/ribavirin for treatment-naïve hepatitis C genotype 1-infected patients in Japan: the DRAGON study. J Gastroenterol 2014;49:138–147.
[17] Reddy KR, Lin F, Zoulim F. Response-guided and -unguided treatment of chronic hepatitis C. Liver Int 2012;32:64–73.
[18] Estrabaud E, Vidaud M, Marcellin P, Asselah T. Genomics and HCV infection: progression of fibrosis and treatment response. J Hepatol 2012;57:1110–1125.
[19] Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 2009;461:399–401.
[20] Hayes CN, Kobayashi M, Akuta N, Suzuki F, Kumada H, Abe H, et al. HCV substitutions and IL28B polymorphisms on outcome of peg-interferon plus ribavirin combination therapy. Gut 2011;60:261–267.
[21] Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, et al. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet 2009;41:1105–1109.
[22] Cento V, Mirabelli C, Salpini R, Dimonte S, Artese A, Costa G, et al. HCV genotypes are differently prone to the development of resistance to linear and macrocyclic protease inhibitors. PLoS One 2012;7:e39652.

[23] Kieffer TL, Kwong AD, Picchio GR. Viral resistance to specifically targeted antiviral therapies for hepatitis C (STAT-Cs). J Antimicrob Chemother 2010;65:202–212.
[24] Lenz O, Verbinnen T, Lin T-I, Vijgen L, Cummings MD, Lindberg J, et al. *In vitro* resistance profile of the hepatitis C virus NS3/4A protease inhibitor TMC435. Antimicrob Agents Chemother 2010;54:1878–1887.
[25] Huisman MT, Snoeys J, Monbaliu J, Martens M, Sekar V, Raoof A. In vitro studies investigating the mechanism of interaction between TMC435 and hepatic transporters. In: Poster 278 presented at the 61st Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), Boston, USA, 29 October–2 November, 2010.
[26] Schinazi R, Halfon P, Marcellin P, Asselah T. HCV direct-acting antiviral agents: the best interferon-free combinations. Liver Int 2014;34:69–78.

Hepatology Research

Hepatology Research 2013 doi: 10.1111/hepr.12237

Case Report

Sequential occurrence of acute hepatitis B among members of a high school Sumo wrestling club

Sung Kwan Bae, Hiroshi Yatsuhashi, Ikuko Takahara, Yoko Tamada, Satoru Hashimoto, Yasuhide Motoyoshi, Eisuke Ozawa, Shinya Nagaoka, Kenji Yanagi, Seigo Abiru, Atsumasa Komori and Hiromi Ishibashi

Clinical Research Center, National Hospital Organization (NHO), Nagasaki Medical Center, Kubara Omura, Nagasaki, Japan

A 17-year-old male was admitted to our hospital and diagnosed with acute hepatitis B. Six weeks later, a 15-year-old male was admitted with acute hepatitis B as well. They were Sumo wrestling players in the same club. A detailed survey in the club revealed that a 28-year-old male coach was a hepatitis B surface antigen carrier with high-level viremia. The consistency of hepatitis B virus (HBV) DNA in the infected players was revealed by analyzing the complete HBV genome sequences. Sumo players are more likely to get injured, including cuts and bleeding, compared with players of other sports because of the characteristic wrestling style. Several past reports have suggested that highly viremic HBV carriers have high HBV DNA titers in both their blood and other body fluids such as sweat. In our cases, percutaneous HBV transmission through the bleeding wounds was the most probable infection route. We conclude that a universal HBV immunization program should be introduced urgently in Japan, similar to those implemented in other countries worldwide.

Key words: hepatitis B virus, horizontal transmission, Sumo, universal vaccination

INTRODUCTION

THE HORIZONTAL TRANSMISSION of hepatitis B virus (HBV) occurs in limited situations such as sexual intercourse with HBV positive partners, the transfusion of HBV-contaminated blood, and the re-use of needles and syringes used for the i.v. administration of drugs.[1-3] In addition, there are several reports of horizontal HBV transmission in elementary schools and day-care centers due to bites and scratches or exposure to blood or blood-contaminated fluids among children.[4-7] Although it is rare, HBV horizontal transmission has been reported in various sports as well, including Sumo wrestling and American football, because of contact with open wounds during training.[8,9]

In this paper, we report a sequential occurrence of acute hepatitis B in members of a high school Sumo wrestling club. After a detailed field survey, a 28-year-old male coach was determined to be a hepatitis B surface antigen (HBsAg) carrier with a high-level of viremia. This individual was identified as the source of transmission by analyzing the complete HBV genome sequences.

CASE REPORT

A 17-YEAR-OLD MALE (case 1) was admitted to our hospital with a 1-week history of jaundice and itching. He had no past medical history, except pediatric asthma, and was not taking any medications currently. There were no HBV carriers in his family. He reported no alcohol consumption, recent travel or sexual contact. He was a member of a high school Sumo wrestling club. On examination, the patient was slightly icteric with stable vital signs. Blood test results (Table 1) revealed the following: total bilirubin (T-Bil), 3.9 mg/dL; aspartate aminotransferase (AST), 1152 IU/L; alanine aminotransferase (ALT), 2856 IU/L; HBsAg, 12 229.87 IU/mL; hepatitis B e-antigen (HBeAg), 473.29 S/CO; anti-hepatitis B core (anti-HBc), 4.0 S/CO; immunoglobulin

Correspondence: Dr Hiroshi Yatsuhashi, Clinical Research Center, National Hospital Organization (NHO), Nagasaki Medical Center, 2-1001-1 Kubara Omura, Nagasaki 856-8562, Japan.
Email: yatsuhashi@nmc.hosp.go.jp
Conflict of interest: None.
Financial disclosure: None to declare.
Received 5 August 2013; revision 2 September 2013; accepted 3 September 2013.

Table 1 Laboratory findings at the initial visit

	Case 1	Case 2	Case 3	Reference range
White blood cells (10^3/μL)	5.8	4.0	4.2	3.9–9.8
Red blood cells (10^6/μL)	5.38	5.18	5.11	4.10–5.30
Hemoglobin (g/dL)	15.8	14.7	16.4	13.5–17.6
Platelet (10^3/μL)	293	232	198	131–362
Prothrombin time (%)	100.6	89.7	106.0	72.0–130.0
APTT (s)	38.7	36.6	38.7	24.8–40.4
Total bilirubin (mg/dL)	3.9	3.2	0.9	0.3–1.2
Direct bilirubin (mg/dL)	2.5	2.0	0.1	0.0–0.2
AST (IU/L)	1152	1567	42	13–33
ALT (IU/L)	2856	2526	76	8–42
ALP (IU/L)	729	1167	237	115–359
γ-GT (IU/L)	176	170	46	10–47
Albumin (g/dL)	4.9	4.1	4.9	4.0–5.0
Immunoglobulin G (mg/dL)	1600	1730	1080	870–1700
Immunoglobulin A (mg/dL)	220	297	281	110–410
Immunoglobulin M (mg/dL)	120	107	114	33–190
HBsAg (IU/mL)	12 229.87	12 381.98	62 276.59	<0.05
HBeAg (S/CO)	473.29	553.41	1394.20	<1.00
Anti-HBe (INH%)	0.0	0.0	0.0	<50
Anti-HBc (S/CO)	4.00	7.88	7.34	<1.00
IgM anti-HBc (S/CO)	24.10	19.30	0.78	<1.00
HBV DNA (log copies/mL)	6.1	6.1	>9.1	Non-detectable
HBV genotype	C	C	C	–
Precore mutations	Wild	Wild	Wild	–
Core promoter mutations	Wild	Wild	Wild	–
HCV RNA (log IU/mL)	Non-detectable	Non-detectable	Not tested	Non-detectable
IgM anti-HAV (S/CO)	<0.40	<0.40	Not tested	<0.40
EBV-VCA IgG	0.8	3.3	Not tested	<0.5
EBV-VCA IgM	0.0	0.0	Not tested	<0.5

ALP, alkaline phosphatase; ALT, alanine aminotransferase; anti-HBc, anti-hepatitis B core; anti-HBeAg, hepatitis B e-antibody; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; EBV-VCA, Epstein–Barr virus viral capsid antigen; HBeAg, hepatitis B e-antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; IgM anti-HAV, immunoglobulin M anti-hepatitis A virus; IgM anti-HBc, immunoglobulin M anti-hepatitis B core; γ-GT, γ-glutamyl transferase.

M anti-hepatitis B core (IgM anti-HBc), 24.1 S/CO; HBV DNA, 6.1 log copies/mL; and HBV genotype, C. On the basis of these results, a diagnosis of acute hepatitis B was confirmed, although the exact route and source of infection could not be identified. The patient recovered naturally and was discharged 12 days after admission. The clinical course was uneventful, and HBsAg clearance was achieved 157 days after admission.

Six weeks after discharge of case 1, a 15-year-old male (case 2) from the same high school Sumo wrestling club was admitted to our hospital with elevated transaminases. He had no past medical history, except atopic dermatitis, and was not taking any medications currently. There were no HBV carriers in his family, except that his father was an inactive HBsAg carrier. He reported no alcohol consumption, recent travel or sexual contact. On examination, the patient was slightly icteric with stable vital signs. Blood test results (Table 1) revealed the following: T-Bil, 3.2 mg/dL; AST, 1567 IU/L; ALT, 2526 IU/L; HBsAg, 12 381.98 IU/mL; HBeAg, 553.41 S/CO; anti-HBc, 7.88 S/CO; IgM anti-HBc, 19.30 S/CO; HBV DNA, 6.1 log copies/mL; and HBV genotype, C. On the basis of these results, this individual was diagnosed with acute hepatitis B as well. However, as in the first case, we could not identify the precise route or source of infection. The patient recovered naturally and was discharged 30 days after admission. The clinical course was uneventful, and HBsAg clearance was achieved 96 days after admission.

Because acute hepatitis B was observed to occur successively in the same high school Sumo wrestling club in a relatively short time period, we suspected the presence

of an infection route and source within the club. After obtaining informed consent from all the club members and coaches, they were tested for HBsAg and hepatitis B surface antibody (anti-HBs) by the school health service. Consequently, a 28-year-old male coach (case 3) was observed to be HBsAg and HBeAg positive with a high level of viremia. There were no HBV carriers in his family. His blood test results (Table 1) revealed the following: T-Bil, 0.9 mg/dL; AST, 42 IU/L; ALT, 76 IU/L; HBsAg, 62 276.59 IU/mL; HBeAg, 1394.20 S/CO; anti-HBc, 7.34 S/CO; IgM anti-HBc, 0.78 S/CO; HBV DNA, more than 9.1 log copies/mL; and HBV genotype, C. To identify the infection source, we performed an analysis of the complete HBV genome sequences in the two patients with acute hepatitis B as well as in the coach suspected to be the source of HBV transmission. Three isolates obtained from the two patients (cases 1 and 2) and the coach (case 3) had the same genomic length of 3215. Between case 1 and case 3, one base (nt1272) had mutated from T to G, with 99.97% (3214/3215) HBV DNA being consistent. Further, between case 2 and case 3, the HBV DNA sequence was 100% (3215/3215) consistent. Using Basic Local Alignment Search Tool (BLAST) analysis, which is a sequence similarity search program to compare a query to a database of sequences, we found that the sample from case 1 was most genetically similar to samples from cases 2 and 3 among other pooled samples. A phylogenetic tree of full-length HBV, obtained using a neighbor-joining method, revealed that the three isolates in this study (cases 1, 2 and 3) were most closely related to each other, and classified into subgenotype C2 (Fig. 1). On the basis of these results, the coach was determined to be the infection source for the successive occurrence of acute hepatitis B in this Sumo wrestling club.

DISCUSSION

ALTHOUGH SEXUAL INTERCOURSE is known as the major route for the horizontal transmission of HBV, several other routes have been reported in the past as well. Iatrogenic routes, including dental or oral surgery,[10] sharing of needles,[11] fingerstick blood-sampling devices,[12] surgical procedures[13] and acupuncture,[14] have been revealed as possible routes of horizontal HBV transmission. On the other hand, non-iatrogenic routes for horizontal HBV transmission include bites and scratches in children's day-care centers or institutions for the mentally retarded,[4–7] household contact,[15–19] tattooing,[20] sharing knives among butchers,[21] and needle pricks or scissor cuts in barbers.[22]

With regard to the field of sports, Kashiwagi *et al.* reported an acute hepatitis B outbreak in a high school Sumo wrestling club in 1982.[8] They confirmed five cases of acute hepatitis B among 10 club members within a 1-year period. Investigations identified an asymptomatic carrier who was judged to be the source of transmission for the hepatitis infection that occurred during the training sessions. Thereafter, in 2000, Tobe *et al.* reported an outbreak of acute hepatitis B in an American university football team.[9] During a period of 19 months, they confirmed five cases of acute hepatitis B among 65 team members and detected a single HBeAg carrier in the same training group. Consequently, they concluded that the carrier was the source of the hepatitis infection that occurred during the training sessions. They performed HBsAg analysis (subtype adr) and suggested that horizontal HBV transmission can occur in sports, probably because of contact with open wounds during training.

We also experienced successive occurrence of acute hepatitis B in a high school Sumo wrestling club similar to that reported by Kashiwagi *et al.*[8] We initiated an investigation in the club after confirming the diagnosis in the second patient. Sumo players wrestle on hard soil in an almost naked style, except for the Sumo belt, which is referred to as "Mawashi" in Japanese. Therefore, they are more likely to be injured and incur cuts and bleeding compared with athletes in other sports. Several recent reports have suggested that HBV carriers may exhibit high HBV DNA titers in other body fluids such as sweat, saliva, tears, nasopharyngeal fluid and urine.[23–27] In our cases, we could not determine whether the intermediate for HBV was blood or other body fluids. However, during their daily training, the players take turns wrestling with one another and continue even when injured or while bleeding from wounds. The nature of this training and our test results suggested that HBV was transmitted through cuts and bleeding wounds sustained during training. We eventually identified the carrier as the source of transmission by analyzing the complete HBV genome sequences in the infected patients. Several cases of horizontal HBV transmission have been reported previously; however, in the field of sports, this is the first report that confirmed the consistency of HBV DNA in the infected patients. Meanwhile, identification of the exact route of HBV transmission was difficult in the three patients in this outbreak. The mean incubation period for acute hepatitis B is 2–3 months after exposure but can range 1–6 months after exposure.[28] This implies that it is possible for one of the two patients with acute hepatitis B to have

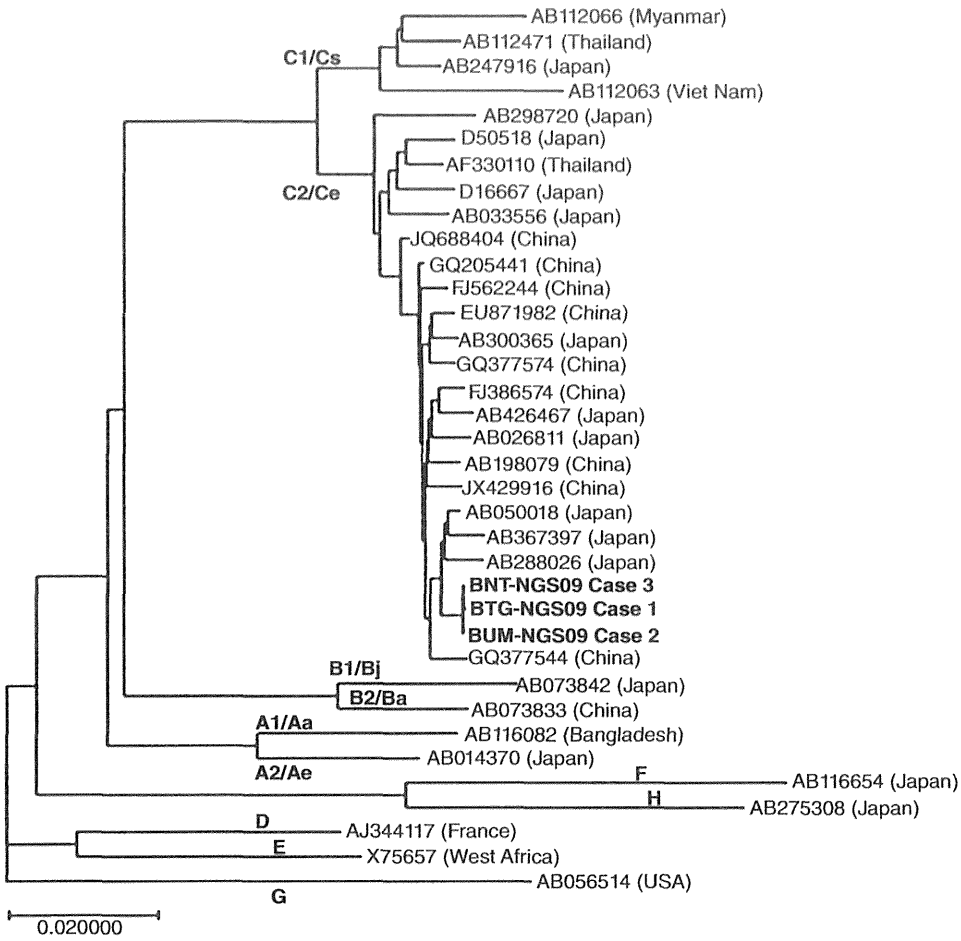

Figure 1 Phylogenetic tree of full-length hepatitis B virus by the neighbor-joining method. Isolates obtained in this study (cases 1, 2 and 3) are shown in bold.

infected the other during the incubation period. In our cases, the coach could have been the origin of transmission and could have infected at least one player, although we could not determine whether or not he infected the other player.

It is remarkable that all three reports of horizontal HBV transmission in the field of sports were from Japan. In 1992, the World Health Organization recommended that all countries should integrate hepatitis B vaccination into their national immunization programs by 1997. By the end of 2009, 177 countries had implemented a universal HBV immunization program for newborns, infants and/or adolescents. However, at the time of drafting of this manuscript (2013), Japan had not introduced this universal HBV immunization program yet. In 1986, Japan initiated a national prevention program comprising selective vaccination for newborns delivered by HBV carrier females. However, this does not aim at preventing horizontal HBV transmission but prevents vertical transmission alone.

Although the number of professional Sumo wrestling players in Japan is very few, the Japanese Ministry of Education revised the guidelines for junior high school education to include compulsory "Budo" (Japanese martial arts) education in 2008. Nowadays, all the students in Japanese junior high schools are taking martial arts classes such as Sumo and Judo. This means that they have a certain risk of exposure to HBV through body fluids or blood during the classes, even though most of them are negative for anti-HBs. In addition, recently

8 million foreign tourists visit Japan and 18 million Japanese nationals travel abroad each year. This has resulted in the rapid development of Japan's internationalization. Consequently, HBV genotype A infections as a sexually-transmitted disease have increased in urban areas of Japan, and then spread to other areas.[29] Thus, this might have increased the risk of horizontal HBV transmission in Japan, particularly in young individuals without HBs antibodies. Therefore, there is an urgent need to prevent horizontal HBV transmission in Japan, and thus the introduction of a universal HBV immunization program is both needed and desirable.

ACKNOWLEDGMENTS

THE AUTHORS ARE grateful to S. Bekki, R. Hamada, M. Fukuda, R. Nakao, T. Hirano and K. Yano for their valuable support in the preparation of this manuscript.

REFERENCES

1 Szmuness W, Much I, Prince AM *et al.* On the role of sexual behavior in the spread of hepatitis B infection. *Ann Intern Med* 1975; **83**: 489–95.
2 Seeff LB, Wright EC, Zimmerman HJ *et al.* Type B hepatitis after needle-stick exposure: prevention with hepatitis B immune globulin. Final report of the Veterans Administration Cooperative Study. *Ann Intern Med* 1978; **88**: 285–93.
3 van Duynhoven YT, van de Laar MJ, Schop WA *et al.* Prevalence and risk factors for hepatitis B virus infections among visitors to an STD clinic. *Genitourin Med* 1997; **73**: 488–92.
4 Cancio-Bello TP, de Medina M, Shorey J, Valledor MD, Schiff ER. An institutional outbreak of hepatitis B related to a human biting carrier. *J Infect Dis* 1982; **146**: 652–6.
5 Livengood JR, Miller GE, Coulter D, Foster LR. Hepatitis B and workers in institutions for the mentally retarded: risk of infection for staff in patient care. *Am J Prev Med* 1989; **5**: 170–4.
6 Shapiro CN, McCaig LF, Gensheimer KF *et al.* Hepatitis B virus transmission between children in day care. *Pediatr Infect Dis J* 1989; **8**: 870–5.
7 McIntosh ED, Bek MD, Cardona M *et al.* Horizontal transmission of hepatitis B in a children's day-care centre: a preventable event. *Aust N Z J Public Health* 1997; **21**: 791–2.
8 Kashiwagi S, Hayashi J, Ikematsu H, Nishigori S, Ishihara K, Kaji M. An outbreak of hepatitis B in members of a high school sumo wrestling club. *JAMA* 1982; **248**: 213–4.
9 Tobe K, Matsuura K, Ogura T *et al.* Horizontal transmission of hepatitis B virus among players of an American football team. *Arch Intern Med* 2000; **160**: 2541–5.
10 Shaw FE, Jr, Barrett CL, Hamm R *et al.* Lethal outbreak of hepatitis B in a dental practice. *JAMA* 1986; **255**: 3260–4.
11 Webster GJ, Hallett R, Whalley SA *et al.* Molecular epidemiology of a large outbreak of hepatitis B linked to autohaemotherapy. *Lancet* 2000; **356**: 379–84.
12 Nosocomial hepatitis B virus infection associated with reusable fingerstick blood sampling devices - Ohio and New York City, 1996. *MMWR Morb Mortal Wkly Rep* 1997; **46**: 217–21.
13 Harpaz R, Von Seidlein L, Averhoff FM *et al.* Transmission of hepatitis B virus to multiple patients from a surgeon without evidence of inadequate infection control. *N Engl J Med* 1996; **334**: 549–54.
14 Kent GP, Brondum J, Keenlyside RA, LaFazia LM, Scott HD. A large outbreak of acupuncture-associated hepatitis B. *Am J Epidemiol* 1988; **127**: 591–8.
15 Bernier RH, Sampliner R, Gerety R, Tabor E, Hamilton F, Nathanson N. Hepatitis B infection in households of chronic carriers of hepatitis B surface antigen: factors associated with prevalence of infection. *Am J Epidemiol* 1982; **116**: 199–211.
16 Toukan AU, Sharaiha ZK, Abu-el-Rub OA *et al.* The epidemiology of hepatitis B virus among family members in the Middle East. *Am J Epidemiol* 1990; **132**: 220–32.
17 Alter MJ. Epidemiology of hepatitis B in Europe and worldwide. *J Hepatol* 2003; **39** (Suppl 1): S64–9.
18 Shimizu T, Horiuchi T, Kamitsuji H, Murakami T, Tamai H. Horizontal transmission of hepatitis B from a father to two brothers. *Eur J Pediatr* 2003; **162**: 804–5.
19 Sciveres M, Maggiore G. Hepatitis B "by proxy": an emerging presentation of chronic hepatitis B in children. *J Pediatr Gastroenterol Nutr* 2007; **44**: 268–9.
20 Limentani AE, Elliott LM, Noah ND, Lamborn JK. An outbreak of hepatitis B from tattooing. *Lancet* 1979; **2**: 86–8.
21 Mevorach D, Eliakim R, Brezis M. Hepatitis B–an occupational risk for butchers? *Ann Intern Med* 1992; **116**: 428.
22 Candan F, Alagozlu H, Poyraz O, Sumer H. Prevalence of hepatitis B and C virus infection in barbers in the Sivas region of Turkey. *Occup Med (Lond)* 2002; **52**: 31–4.
23 van der Eijk AA, Niesters HG, Hansen BE *et al.* Paired, quantitative measurements of hepatitis B virus DNA in saliva, urine and serum of chronic hepatitis B patients. *Eur J Gastroenterol Hepatol* 2005; **17**: 1173–9.
24 Kidd-Ljunggren K, Holmberg A, Blackberg J, Lindqvist B. High levels of hepatitis B virus DNA in body fluids from chronic carriers. *J Hosp Infect* 2006; **64**: 352–7.
25 Bereket-Yucel S. Risk of hepatitis B infections in Olympic wrestling. *Br J Sports Med* 2007; **41**: 306–10; discussion 10.
26 Heiberg IL, Hoegh M, Ladelund S, Niesters HG, Hogh B. Hepatitis B virus DNA in saliva from children with chronic hepatitis B infection: implications for saliva as a potential mode of horizontal transmission. *Pediatr Infect Dis J* 2010; **29**: 465–7.

27 Komatsu H, Inui A, Sogo T, Tateno A, Shimokawa R, Fujisawa T. Tears from children with chronic hepatitis B virus (HBV) infection are infectious vehicles of HBV transmission: experimental transmission of HBV by tears, using mice with chimeric human livers. *J Infect Dis* 2012; **206**: 478–85.
28 Liang TJ. Hepatitis B: the virus and disease. *Hepatology* 2009; **49**: S13–21.
29 Tamada Y, Yatsuhashi H, Masaki N *et al.* Hepatitis B virus strains of subgenotype A2 with an identical sequence spreading rapidly from the capital region to all over Japan in patients with acute hepatitis B. *Gut* 2012; **61**: 765–73.

JOURNAL OF VIRAL HEPATITIS
JVH

Journal of Viral Hepatitis, 2014, 21, 762–768 doi:10.1111/jvh.12312

Sofosbuvir plus ribavirin in Japanese patients with chronic genotype 2 HCV infection: an open-label, phase 3 trial

Masao Omata,[1] Shuhei Nishiguchi,[2] Yoshiyuki Ueno,[3] Hitoshi Mochizuki,[1] Namiki Izumi,[4] Fusao Ikeda,[5] Hidenori Toyoda,[6] Osamu Yokosuka,[7] Kazushige Nirei,[8] Takuya Genda,[9] Takeji Umemura,[10] Tetsuo Takehara,[11] Naoya Sakamoto,[12] Yoichi Nishigaki,[13] Kunio Nakane,[14] Nobuo Toda,[15] Tatsuya Ide,[16] Mikio Yanase,[17] Keisuke Hino,[18] Bing Gao,[19] Kimberly L. Garrison,[19] Hadas Dvory-Sobol,[19] Akinobu Ishizaki,[19] Masa Omote,[19] Diana Brainard,[19] Steven Knox,[19] William T. Symonds,[19] John G. McHutchison,[19] Hiroshi Yatsuhashi[20] and Masashi Mizokami[17]

[1]*Yamanashi Prefectural Hospital Organization, Yamanashi, Japan;* [2]*Hyogo College of Medicine, Hyogo, Japan;* [3]*Yamagata University, Yamagata, Japan;* [4]*Musashino Red Cross Hospital, Tokyo, Japan;* [5]*Okayama University, Okayama, Japan;* [6]*Ogaki Municipal Hospital, Gifu, Japan;* [7]*Chiba University, Chiba, Japan;* [8]*Nihon University, Tokyo, Japan;* [9]*Juntendo University, Tokyo, Japan;* [10]*Shinshu University, Nagano, Japan;* [11]*Osaka University, Osaka, Japan;* [12]*Hokkaido University, Hokkaido, Japan;* [13]*Gifu Municipal Hospital, Gifu, Japan;* [14]*Akita City Hospital, Akita, Japan;* [15]*Mitsui Memorial Hospital, Tokyo, Japan;* [16]*Kurume University, Kurume, Japan;* [17]*National Center for Global Health and Medicine, Tokyo, Japan;* [18]*Kawasaki Medical School, Okayama, Japan;* [19]*Gilead Sciences, Inc., Foster City, CA, USA; and* [20]*National Hospital Organization Nagasaki Medical Center, Nagasaki, Japan*

Received June 2014; accepted for publication August 2014

SUMMARY. Genotype 2 hepatitis C virus (HCV) accounts for up to 30% of chronic HCV infections in Japan. The standard of care for patients with genotype 2 HCV – peginterferon and ribavirin for 24 weeks – is poorly tolerated, especially among older patients and those with advanced liver disease. We conducted a phase 3, open-label study to assess the efficacy and safety of an all-oral combination of the NS5B polymerase inhibitor sofosbuvir and ribavirin in patients with chronic genotype 2 HCV infection in Japan. We enrolled 90 treatment-naïve and 63 previously treated patients at 20 sites in Japan. All patients received sofosbuvir 400 mg plus ribavirin (weight-based dosing) for 12 weeks. The primary endpoint was sustained virologic response at 12 weeks after therapy (SVR12). Of the 153 patients enrolled and treated, 60% had HCV genotype 2a, 11% had cirrhosis, and 22% were over the aged 65 or older. Overall, 148 patients (97%) achieved SVR12. Of the 90 treatment-naïve patients, 88 (98%) achieved SVR12, and of the 63 previously treated patients, 60 (95%) achieved SVR12. The rate of SVR12 was 94% in patients with cirrhosis and in those aged 65 and older. No patients discontinued study treatment due to adverse events. The most common adverse events were nasopharyngitis, anaemia and headache. Twelve weeks of sofosbuvir and ribavirin resulted in high rates of SVR12 in treatment-naïve and previously treated patients with chronic genotype 2 HCV infection. The treatment was safe and well tolerated by patients, including the elderly and those with cirrhosis.

Keywords: Hepatitis C virus, HCV genotype 2, direct-acting antiviral agents, nucleotide polymerase inhibitor.

INTRODUCTION

Approximately two million people in Japan – nearly 2% of the population – are chronically infected with the hepatitis C virus (HCV) [1]. The population of patients with chronic HCV infection in Japan differs from that of other countries; patients are generally older, have more advanced liver disease and are more likely to have received previous treatment for HCV infection [2,3]. It is estimated that 15–30% of Japanese patients with HCV will develop serious complications, including liver cirrhosis, end-stage liver disease and hepatocellular carcinoma [4]. Although genotype 1 HCV is currently the most prevalent strain of the virus in Japan, genotype 2 HCV, which now accounts for up to 30% of infections, is rising in prevalence [5]. The current standard of care regimen for the treatment of chronic genotype 2 HCV infection in Japan is 24 weeks of pegylated interferon alpha (Peg-IFNα) and ribavirin (RBV) [6]. Although relatively high rates of SVR

Abbreviations: CI, confidence interval; GCP, Good Clinical Practice; HCV, hepatitis C virus; ICH, International Conference on Harmonization; Peg-IFNα, pegylated interferon alpha; PK, pharmacokinetics; RBV, ribavirin; SVR12, 12 weeks after therapy.

Correspondence: Masao Omata, Yamanashi Prefectural Hospital Organization, 1-1-1 Fujimi, Kofu City, Yamanashi 400-0027, Japan. E-mail: momata-tky@umin.ac.jp

Trial registration details: ClinicalTrials.gov number NCT01910636.

have been reported in clinical trials with this regimen (71–86%), the use of Peg-IFNα+RBV in an ageing population with progressive liver disease is limited by safety and tolerability issues. Moreover, a substantial number of patients have absolute or relative contraindications to interferon. As a result, many Japanese patients with chronic genotype 2 HCV infection have no available treatment options and are thus at risk for worsening of liver disease and complications of cirrhosis, including hepatocellular carcinoma.

Sofosbuvir (Gilead Sciences) is an oral nucleotide analogue inhibitor of the HCV-specific NS5B polymerase that has recently been approved in the United States and Europe for the treatment of chronic HCV infection [7]. The labelled use for patients with chronic genotype 2 HCV infection is sofosbuvir and RBV for 12 weeks. In phase 3 studies, 12 weeks of treatment with sofosbuvir plus RBV in patients infected with genotype 2 HCV resulted in rates of SVR12 of 97% in treatment-naïve patients, 93% in patients ineligible to receive interferon and 86–90% in previously treated patients [8–10].

We conducted a phase 3 trial to determine the efficacy and safety of 12 weeks of sofosbuvir and RBV in treatment-naïve and previously treated Japanese patients with chronic genotype 2 HCV infection with and without compensated cirrhosis.

METHODS

Patients

Patients were enrolled between 16 July 2013 and 30 September 2013 at 20 sites in Japan. Eligible patients were aged 20 years or older with a body weight of at least 40 kg. Patients were required to be chronically infected with genotype 2 HCV and with HCV RNA levels $\geq 10^4$ IU/mL at screening. Planned enrolment was for approximately 84 treatment-naïve and 50 previously treated patients. See Supplement for definitions of types of response to prior treatment.

Up to 40% of enrolled subjects in each group (i.e. treatment naive or treatment experienced) could have evidence of compensated cirrhosis at screening (Child-Pugh A). Cirrhosis was defined as liver biopsy showing a Metavir score of 4 or Ishak score ≥5 or a FibroScan score of >12.5 kPa. Patients were required to have ALT and AST ≤10 × upper limit of the normal range, platelet count ≥50 000 per μL, haemoglobin ≥11 g/dL for women and ≥12 g/dL for men and albumin ≥3 g/dL. There were no upper limits on age or body mass index. Similarly, no restriction was applied to white blood cell or absolute neutrophil count at screening.

Study design

In this multicenter, open-label trial, all patients received 12 weeks of treatment with 400 mg of sofosbuvir, administered orally once daily, and ribavirin (Copegus®, Chugai Pharmaceutical Co., Ltd, Tokyo, Japan), administered orally twice daily, with doses determined according to body weight (600 mg daily in patients with a body weight of ≤60 kg, 800 mg daily in patients weighing >60 and ≤80 kg, and 1000 mg daily in patients with a body weight of >80 kg).

In addition to the main study of efficacy and safety, sparse PK samples were collected from all patients over the course of the study for population PK analyses and all patients were eligible to participate in an optional substudy to determine the steady-state pharmacokinetics (PK) of sofosbuvir (and its predominant circulating metabolite GS-331007). The target enrolment per treatment group was approximately 15 patients. For the PK substudy, intensive serial pharmacokinetic samples were collected (samples obtained over 24 h postdose) at either the week 2 or week 4 treatment visits.

Study assessments

Screening assessments included serum HCV RNA levels and IL28B (rs12979860) genotyping, as well as standard laboratory and clinical tests. Serum HCV RNA was measured with the COBAS® TaqMan® HCV Test, version 2.0 for Use with the High Pure System (Roche Molecular Systems, West Sussex, UK), which has a lower limit of quantification (LLOQ) of 25 IU/mL. HCV genotype and subtype were determined at screening using the Siemens VERSANT HCV Genotype INNO-LiPA 2.0 assay.

On-treatment assessments included standard laboratory testing, serum HCV RNA, vital signs, electrocardiography and symptom-directed physical examinations. All adverse events were recorded and graded according to a standardized scale (see Supplementary Table S7).

NS5B amplification and deep sequencing was performed at DDL Diagnostics Laboratory (Rijswijk, The Netherlands) for all subjects who did not achieve SVR12. Deep sequencing of HCV NS5B was performed at the first virologic failure time point if a plasma/serum sample was available and HCV RNA was >1000 IU/mL, along with the respective baseline samples. Amino acid substitutions in NS5B in the samples collected at virologic failure were compared with the genotype 2 reference and the respective baseline sequence for each patient.

The population pharmacokinetic parameters for sofosbuvir and GS-331007 were computed for all subjects from concentration data from intensive and/or sparse samples using the previously established sofosbuvir and GS-331007 population PK models [11].

Statistical analysis

For treatment-naïve patients without cirrhosis, the SVR12 rate was compared to an adjusted historical SVR rate of 69%, using a two-sided exact one-sample binomial test. The historical control rate was calculated from the weighted average of historical SVR rates for noncirrhotic,

treatment-naïve Japanese patients with genotype 2 HCV infection receiving 24 weeks of Peg-IFNα+RBV (79% with a 10% discount applied due to the expected improvement in safety profile and shorter treatment duration – see Supplementary Table S2 for further details). We calculated that a sample size of 50 patients would provide 80% power to detect an 18% improvement in the SVR12 rate over the adjusted historical rate at a significance level of 0.05. For SVR12 rates for the overall population, for treatment-naïve patients with cirrhosis, and for previously treated patients, statistical hypothesis testing was not performed. For these outcomes, we calculated point estimates of SVR12 rates with two-sided 95% exact confidence interval using the binomial distribution (Clopper–Pearson method).

Study oversight

This trial was approved by the institutional review board or independent ethics committees at all participating sites and was conducted in accordance with local regulations and with recognized international scientific and ethical standards, including the International Conference on Harmonization (ICH) guideline for Good Clinical Practice (GCP) and the original principles embodied in the Declaration of Helsinki. The study was designed and conducted according to protocol by the sponsor (Gilead Sciences) in collaboration with the principal investigators. The sponsor collected the data, monitored study conduct and performed the statistical analyses. The manuscript was prepared by Gilead Sciences with input from all authors.

RESULTS

Baseline characteristics

Of the 188 patients who were initially screened, 153 (90 treatment-naïve and 63 previously treated patients) were enrolled and began treatment (Table S1 and Figure S1). The demographic and baseline clinical characteristics of the patients are provided in Table 1. Overall, the majority of patients were female (54%), and all were Japanese. The mean age was 57 years (ranging from 25 to 74 years) and 22% were aged 65 or older.

Previously treated patients were slightly older than the treatment-naïve patients, with a higher percentage of males, higher baseline viral load, with a higher prevalence of cirrho-

Table 1 Baseline Demographic Characteristics

Characteristic	Overall (N = 153)	Treatment naïve (n = 90)	Previously treated (n = 63)
Mean age, years (range)	57 (25, 74)	55 (25, 73)	60 (34, 74)
Mean BMI, kg/m^2 (range)	24 (16.5, 34)	24 (17, 34)	24 (16.5, 34)
Male, *n* (%)	70 (46)	33 (37)	37 (59)
Mean HCV RNA, $\log_{10}$ IU/mL ± SD	6.3 (0.84)	6.2 (0.92)	6.5 (0.66)
HCV RNA ≥5 $\log_{10}$ IU/mL, *n* (%)	140 (92)	78 (87)	62 (98)
HCV genotype, *n* (%)			
2a	92 (60)	52 (58%)	40 (63%)
2b	61 (40)	38 (42%)	23 (37%)
Cirrhosis, *n* (%)			
No	136 (89)	82 (91)	54 (86)
Yes	17 (11)	8 (9)	9 (14)
IL28B genotype, *n* (%)			
CC	121 (79)	73 (81)	48 (76)
CT	28 (18)	17 (19)	11 (17)
TT	4 (3)	0	4 (6)
Median baseline ALT, U/L (range)	34 (12, 412)	32 (12, 412)	36 (12, 232)
Baseline ALT >1.5 × ULN, *n* (%)	43 (28)	28 (31)	15 (24)
Interferon eligibility, *n* (%)*			
Interferon eligible	72 (80)	72 (80)	Not applicable
Interferon ineligible	5 (6)	5 (6)	Not applicable
Interferon unwilling	13 (14)	13 (14)	Not applicable
Response to prior HCV treatment, *n* (%)			
Nonresponse	15 (24)	Not applicable	15 (24)
Relapse/breakthrough	45 (71)	Not applicable	45 (71)
Interferon intolerant	3 (5)	Not applicable	3 (5)
Median eGFR, mL/min (range)	85 (51, 209)	86 (52, 175)	84 (51, 209)

*Interferon eligibility was determined by the site investigator based on whether or not, in their judgment, the patient had contraindications to interferon therapy.

sis and non-CC IL28B genotype. Overall, 11% of participating subjects had cirrhosis. The proportions of patients infected with genotype 2a and 2b HCV were 60% and 40%, respectively, which is similar to previous reports of HCV subtype distribution in the Japanese population [4]. Most (80%) of the treatment-naïve patients were considered eligible for interferon therapy, with 6% having contraindications to interferon therapy and 14% unwilling to receive this treatment. Most (71%) of the previously treated patients had experienced virologic breakthrough or relapse after previous treatment, with 24% reporting nonresponse to prior therapy.

Efficacy

Overall, 148 of the 153 patients (97%, 95% confidence interval [CI] 93–99%) achieved SVR12 (Table 2). By prior treatment history, 88 of the 90 treatment-naïve patients (98%, 95% CI, 92–100%) and 60 of the 63 previously treated patients (95%, 95% CI, 87–99%) achieved SVR12. Of the 82 treatment-naïve patients without cirrhosis, 80 (97%, 95% CI 91–100%) achieved SVR12, thus meeting the primary efficacy endpoint for this group of superiority to the adjusted historical control rate of 69% ($P < 0.001$). Of note, all eight treatment-naïve patients (100%) with cirrhosis and eight of the nine previously treated patients with cirrhosis (89%) achieved SVR12. Overall, 16 of the 17 patients with cirrhosis (94%, 95% CI 71–100%) achieved SVR12.

Patient responses according to baseline characteristics are shown in Supplementary Table S3. Rates of SVR12 were high in all subgroups of patients. Patients with characteristics historically associated with poor response to interferon-based treatment – non-CC IL28B genotype, high baseline viral load, elderly patients, cirrhosis – had rates of SVR12 similar to those in patients without these characteristics.

Relapse accounted for all cases of virologic failure; there were no patients with virologic breakthrough or nonresponse during treatment. Among all patients treated, 97% had HCV RNA <LLOQ by treatment week 2, and 100% achieved HCV RNA <LLOQ by treatment week 4. Overall, five patients experienced virologic relapse after the end of therapy: two (2%) treatment-naïve patients and three (5%) treatment-experienced patients. Four patients relapsed by post-treatment week 4, and one patient relapsed between post-treatment weeks 4 and 12. Characteristics of patients who relapsed are provided in Table S4. There were no consistent host or viral characteristics in the five subjects who relapsed; however, the number of virologic failures is too small for any conclusions to be drawn concerning predictors of virologic failure. No patient relapsed after post-treatment week 12. All 148 SVR12 patients (100%) also achieved SVR24.

Viral resistance testing

The NS5B region was deep sequenced in samples collected from the five relapsers at baseline and at the time of relapse. No S282T variant – known to be associated with reduced susceptibility to sofosbuvir – or any other nucleotide inhibitor resistance-associated variants were detected in any patient at relapse. Phenotypic analysis of the NS5B gene showed no change in susceptibility to either sofosbuvir or ribavirin.

Pharmacokinetics

Population pharmacokinetic analysis was performed to estimate the pharmacokinetics of sofosbuvir and its major circulating nucleoside metabolite, GS-331007. The mean (CV%) of steady-state AUC_{0-24} and C_{max} were 973 (31.2) ng*h/mL and 544 (33.6) ng/mL for sofosbuvir ($N = 45$), respectively, and 10 400 (27.2) ng h/mL and 818 (27.9) ng/mL for GS-331007 ($N = 153$), respectively. Within the Japanese study population, there were no clinically relevant differences in the pharmacokinetics of GS-331007 and sofosbuvir, based on age, sex, BMI, cirrhosis status, prior treatment experience or SVR12 outcome.

Safety

Overall, 73% of patients experienced at least one adverse event; however, the majority of patients experiencing

Table 2 Response during and after Treatment

Response	Overall ($N = 153$)	Treatment naïve ($n = 90$)	Previously treated ($n = 63$)
HCV RNA <LLOQ during treatment, *n* (%)*			
At week 2	148 (97%)	88 (98%)	60 (95%)
At week 4	153 (100%)	90 (100%)	63 (100%)
HCV RNA <LLOQ after end of treatment, *n* (%)			
SVR4	149 (97%)	89 (99%)	60 (95%)
SVR12	148 (97%)	88 (98%)	60 (95%)
95% confidence interval	92.5–99%	92–>99%	87–99%
On-treatment failure	0	0	0
Relapse, *n*/*n* (%)	5 (3%)	2 (2%)	3 (5%)

*LLOQ denotes lower limit of quantification, which is 25 IU/mL. SVR denotes sustained virologic response.

adverse events (84%) had only mild (grade 1) events. The most common treatment-emergent adverse events were nasopharyngitis (upper respiratory viral illness), anaemia, headache, malaise and pruritus (Table 3). No patient in the study discontinued treatment prematurely due to adverse events (or for any other reason). Twenty-two patients (14%) had adverse events that led to modification or interruption of a study drug; 20 patients had ribavirin dose reductions to manage anaemia, and one patient interrupted sofosbuvir and RBV for 1 day because of an event of nasopharyngitis. All but one of the 22 patients with modification or interruption of study drugs achieved SVR12. Two patients experienced treatment-emergent serious adverse events: one treatment-experienced 63-year-old woman had a worsening of anaemia for which she was hospitalized, and one treatment-naïve 36-year-old woman had a severe anaphylactic reaction to a bee sting. No patient experienced a life-threatening (grade 4) adverse event, and only three patients experienced severe (grade 3) events, two of which were deemed to be related to study treatment, the above-mentioned case of anaemia and one case of transient, ribavirin-associated hyperbilirubinaemia in a treatment-experienced 65-year-old man, which resolved during follow-up.

The overall rates of adverse events in younger (<65 years) and older (≥65 years) patients did not differ substantially (72% *vs* 76%, respectively), although there was a higher incidence of anaemia and pruritus in older patients (Table S5). The incidence and severity of adverse events in patients with and without cirrhosis at baseline were similar (Table S6).

Overall, the mean change in haemoglobin from baseline to week 12 of treatment was −1.2 g/dL. For patients aged 65 and older, the mean change in haemoglobin was −1.7 g/dL, as compared with 1.0 g/dL in patients under the age of 65. Of all 153 patients enrolled and treated, 19 (12%) had at least one postbaseline haemoglobin value of <10.0 g/dL, and one (1%) had a postbaseline haemoglobin value of <8.5 g/dL. Two patients (1%) had grade 3 hyperbilirubinaemia; no grade 4 hyperbilirubinaemia occurred. One patient, who had grade 2 neutropenia at baseline, had transitory grade 3 neutropenia.

Table 3 Discontinuations, Adverse Events and Laboratory Abnormalities by Age

Parameter	Overall (N = 153)
Discontinuation of any study drug due to adverse event	0
Serious adverse events	2 (1%)
Anaemia	1 (1%)
Anaphylactic reaction	1 (1%)
Any adverse event	112 (73%)
Common adverse events*	
Nasopharyngitis	45 (29%)
Anaemia	18 (12%)
Headache	15 (10%)
Malaise	11 (7%)
Pruritus	9 (6%)
Laboratory abnormalities, n (%)	
Decreased haemoglobin concentration	
<10 g/dL	19 (12%)
<8 g/dL	1 (1%)
Neutropenia (500–<750 per mm^3)	1 (1%)
Hyperglycaemia (>250–500 mg/dL)	3 (2%)
Hyperbilirubinaemia (>2.5–5.0 × ULN)	2 (1%)

ULN, upper limit of normal.
*Adverse events occurring in at least 5% of patients.

DISCUSSION

In this phase 3 trial, twelve weeks of treatment with sofosbuvir and RBV resulted in high rates of sustained virologic response (>95%) in treatment-naïve and previously treated Japanese patients with chronic genotype 2 HCV infection. Patients with host and viral characteristics that have historically been predictive of lower rates of SVR – older age, presence of cirrhosis, high viral load, non-CC IL28B alleles – had rates of SVR12 similar to patients without these characteristics. In patients who had been previously treated for HCV infection, the nature of the prior response was not associated with significant differences in rates of SVR following treatment with sofosbuvir and ribavirin; patients who had nonresponse to prior treatment had similar response rates as patients who had previously experienced relapse or viral breakthrough. No clear or consistent baseline predictors of treatment failure were evident among the five patients who relapsed after treatment.

The current standard-of-care treatment for Japanese patients with chronic genotype 2 HCV infection is 24 weeks of Peg-IFNα+RBV. Although patients who received this regimen in clinical trials achieved SVR12 rates ranging from 72% to 86%, these studies were restricted to patients <65 years of age [12,13]. However, the Japanese population chronically infected with genotype 2 HCV includes many patients with characteristics that make the use of interferon-based therapy problematic – older age, progressive liver disease, prior treatment experience and comorbid conditions such as diabetes and cardiovascular disease [14]. Moreover, many patients cannot receive interferon therapy due to relative or absolute contraindications. The interferon-free combination of sofosbuvir and ribavirin may represent a promising treatment option for these patients.

Given the characteristics of the patient population in Japan with HCV infection – generally older, and more likely to have advanced liver disease – safety and tolerability of therapeutic regimens is an important issue. In the present study, 22% of patients were aged 65 or older and 11% had cirrhosis. Analyses of safety data by age (<65 *vs*