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).

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.

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.

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*

≥65 years) showed increases in reported adverse events and laboratory abnormalities in older patients, but these differences did not present a barrier to treatment as no premature discontinuation of study treatment occurred in any patient. Analysis of safety data according to the presence or absence of cirrhosis did not indicate clinically important differences in safety or tolerability of the 12-week sofosbuvir plus ribavirin regimen.

Consistent with previous reports, the results of this study confirm the high barrier to resistance afforded by the sofosbuvir plus RBV treatment regimen. Rapid viral suppression was observed with all patients achieving HCV RNA undetectable status by week 4, with no virologic breakthrough observed during treatment in any of the 153 patients. The percentage of patients who relapsed after treatment was low (3%), and none of the subjects who relapsed had S282T or other nucleoside inhibitor resistance-associated variants. No change in susceptibility to sofosbuvir or ribavirin compared with the corresponding baseline or wild-type reference was observed at the relapse time point.

The main limitation of this study was the lack of a control arm to allow direct comparison with interferon-based regimens. Several considerations guided our choice of an uncontrolled study design. Adding an interferon-based control arm would have required exclusion of patients who were ineligible to receive or intolerant of interferon – an important and substantial proportion of patients – as well as previously treated patients, for whom further interferon treatment is not an option. Moreover, given that Peg-IFNα is administered by subcutaneous injection, blinding of treatment arms would not have been possible.

In conclusion, treatment with the all-oral, interferon-free combination of sofosbuvir and RBV resulted in high rates of sustained virologic response in both treatment-naïve and previously treated Japanese patients with chronic genotype 2 HCV infection. The degree of antiviral efficacy coupled with a favourable safety and tolerability profile, including patients with cirrhosis and those aged 65 and older, suggest that this combination may fill an important unmet medical need in Japan.

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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 Tanaka J, Kumgai J, Katayama K *et al.* Sex- and age-specific carriers of hepatitis B and C viruses in Japan estimated by the prevalence in the 3.485.648 first-time blood donors during 1995–2000. *Intervirology* 2004; 47: 32–40.
3 Mizokami M, Tanka Y, Miyakawa Y. Spread times of hepatitis C virus estimated by the molecular clock differ among Japan, the United States and Egypt in reflection of their distinct socioeconomic backgrounds. *Intervirology* 2006; 49: 28–36.
4 Thein HH, Yi Q, Dore GJ, Krahn MD. Estimation of stage-specific fibrosis progression rates in chronic hepatitis C virus infection: a meta-analysis and metaregression. *Hepatology* 2008; 48: 418–431.
5 Toyoda H, Kumada T, Takaguchi K, Shimada N, Tanaka J. Changes in hepatitis C virus genotype distribution in Japan. *Epidemiol Infect* 2014.
6 Kumada H, Okanoue T, Onji M *et al.* Guidelines for the treatment of chronic hepatitis and cirrhosis due to hepatitis C virus infection for the fiscal year 2008 in Japan. *Hepatol Res* 2010; 40: 8–13.
7 Sovaldi (sofosbuvir) Tablets: US Prescribing Information. Foster City, CA: Gilead Sciences, December 2013. Available at: http://www.gilead.com/~/media/Files/pdfs/medicines/liver-disease/sovaldi/sovaldi_pi.pdf.
8 Lawitz E, Mangia S, Wyles D *et al.* Sofosbuvir for previously untreated chronic hepatitis C infection. *N Engl J Med* 2013; 368: 1878–1887.
9 Jacobson IM, Gordon SC, Kowdley KV *et al.* Sofosbuvir for hepatitis C genotype 2 or 3 in patients without treatment options. *N Engl J Med* 2013; 368: 1867–1877.
10 Zeuzem S, Dusheiko GM, Salupere R *et al.* Sofosbuvir and ribavirin in HCV genotypes 2 and 3. *N Engl J Med* 2014; 370: 1993–2001.
11 Kirby B, Gordi T, Symonds WT, Kearney BP, Mathias A. Population pharmacokinetics of sofosbuvir and its major metabolite (GS-331007) in healthy and HCV-infected adult subjects. AASLD Annual Meeting 2013.
12 Kanda T, Imazeki F, Azemoto R *et al.* Response to peginterferon-alfa 2b and ribavirin in Japanese patients with chronic hepatitis C genotype 2. *Dig Dis Sci* 2011; 56: 3335–3342.
13 Inoue Y, Hiramatsu N, Oze T *et al.* Factors affecting efficacy in patients with genotype 2 chronic hepatitis C treated by pegylated interferon alpha-2b and ribavirin: reducing drug doses has no impact on rapid and sustained virological responses. *J Viral Hepat* 2010; 17: 336–344.
14 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: 518–527.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Patient disposition.

Table S1. Reasons for screen failure.

Table S2. Calculation of the adjusted historical control rate.

Table S3. SVR12 by subgroup.

Table S4. Characteristics of patients who relapsed.

Table S5. Common adverse events by age group.

Table S6. Common adverse events by cirrhosis status.

Table S7. Gilead sciences grading scale for severity of adverse events and laboratory abnormalities.