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Efficacy of Sofosbuvir Plus Ribavirin With or Without Peginterferon-Alfa in Patients With Hepatitis C Virus Genotype 3 Infection and Treatment-Experienced Patients With Cirrhosis and Hepatitis C Virus Genotype 2 Infection

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CLINICAL LIVER

See editorial on page 1326.

BACKGROUND & AIMS: We conducted an open-label, randomized, phase 3 trial to determine the efficacy and safety of sofosbuvir and ribavirin, with and without peginterferon-alfa, in treatment-experienced patients with cirrhosis and hepatitis C virus (HCV) genotype 2 infection and treatment-naïve or treatment-experienced patients with HCV genotype 3 infection. **METHODS:** The study was conducted at 80 sites in Europe, North America, Australia, and New Zealand Patients were randomly assigned (1:1:1) to groups given sofosbuvir and ribavirin for 16 weeks (n = 196); sofosbuvir and ribavirin for 24 weeks (n = 199); or sofosbuvir, peginterferon-alfa, and ribavirin for 12 weeks (n = 197). The primary end point was the percentage of patients with HCV RNA <15 IU/mL 12 weeks after stopping therapy (sustained virologic response [SVR12]). From October 2013 until April 2014, we enrolled and treated 592 patients—48 with genotype 2 HCV and compensated cirrhosis who had not achieved SVR with previous treatments and 544 with genotype 3 HCV (279 treatment-naïve and 265 previously treated). Overall, 219 patients (37%) had compensated cirrhosis. The last post-treatment week 12 patient visit was in January 2015. **RESULTS:** Rates of SVR12 among patients with genotype 2 HCV were 87% and 100%, for those receiving 16 and 24 weeks of sofosbuvir and ribavirin, respectively, and 94% for those receiving sofosbuvir, peginterferon, and ribavirin for 12 weeks. Rates of SVR12 among patients with genotype 3 HCV were 71% and 84% in those receiving 16 and 24 weeks of sofosbuvir and ribavirin, respectively, and 93% in those receiving sofosbuvir, peginterferon, and ribavirin. On-treatment virologic failure occurred in 3 patients with HCV genotype 3a receiving sofosbuvir and ribavirin for 24 weeks. The most common adverse events were fatigue, headache, insomnia, and nausea. Overall, 1% of patients discontinued treatment due to adverse events. **CONCLUSIONS:** Among patients with genotype 3 HCV infection, including a large proportion of treatment-experienced patients with cirrhosis, the combination of sofosbuvir, peginterferon, and ribavirin for 12 weeks produces high rates of SVR. Treatment-experienced patients with cirrhosis and genotype 2 HCV infection had high rates of SVR in all groups. EudraCT ID 2013-002641-11.

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With the approval of second-wave direct-acting antiviral agents, highly effective interferon-free treatment regimens are now available for the majority of patients chronically infected with the hepatitis C virus (HCV).[1,2] However, a number of questions remain concerning the optimal treatment for certain subgroups of patients infected with genotypes 2 and 3 HCV. Historically, these 2 genotypes, which account for approximately 40% of all HCV infections globally,[3] have been grouped together in treatment guidelines. However, it is now recognized that patients with genotype 3 HCV have more rapid disease progression, are less responsive to treatment than patients with genotype 2 HCV, and show variable susceptibility to different direct-acting antiviral agents.[4–7] Although most patients with genotype 2 HCV respond well to the interferon-free combination of sofosbuvir and ribavirin for 12 weeks, treatment-experienced patients with genotype 2 HCV, especially those with cirrhosis, have lower rates of response than treatment-naïve patients.[8] Current guidelines recommend the following options for patients with genotypes

Abbreviations used in this paper: **HCV, hepatitis C virus; LLOQ, lower limit of quantification; RAV, resistance-associated variants; SVR, sustained virologic response.**

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2 and 3 HCV: sofosbuvir and ribavirin for 12−24 weeks; 12 weeks of sofosbuvir plus peginterferon and ribavirin; or, more recently, 12 weeks of sofosbuvir plus daclatasvir.[1,2] Response rates for all-oral regimens in patients with genotype 3 HCV and cirrhosis have been well below the 90% rates seen in patients infected with other HCV genotypes.[8–10] Alternative options need to be considered. Phase 2 data suggest that sofosbuvir plus peginterferon and ribavirin may have value in patients with genotype 3 HCV,[11] but no adequately powered study to inform clinicians about the comparative safety and efficacy of these regimens has been performed to date.

We therefore conducted a large, phase 3 trial to compare the efficacy and safety of sofosbuvir plus ribavirin with and without peginterferon-alfa in treatment-experienced patients with genotype 2 HCV and cirrhosis and in treatment-naïve and treatment-experienced patients with genotype 3 HCV.

Methods

Study Design and Patients

We conducted this randomized, phase 3, open-label trial at 80 sites in the United Kingdom, Australia, the United States, Canada, and New Zealand. Patients were enrolled between October 18, 2013 and April 1, 2014; the last post-treatment week-12 visit was on January 7, 2015. Eligible patients were at least 18 years old and were chronically infected with HCV, with plasma HCV RNA $\geq 10^4$ IU/mL. There were no upper limits on age or body mass index. Patients were required to have a platelet count of $\geq$60,000/mm^3 and albumin level $\geq$3.5 g/dL. We enrolled patients with genotype 2 HCV and compensated cirrhosis who had not achieved sustained virologic response (SVR) after prior HCV treatment for at least 12 weeks with an interferon-based regimen, and patients with genotype 3 HCV with and without compensated cirrhosis who had either never previously received treatment for HCV or who had not achieved SVR after previous HCV treatment. A maximum of 50% of patients with genotype 3 HCV infection with cirrhosis were eligible for inclusion. Previously treated patients who had stopped prior treatment prematurely due to an adverse event were not eligible. Presence of cirrhosis was determined by liver biopsy or by a Fibroscan result of >12.5 kPa. Liver biopsy was required for all patients with genotype 3 HCV except those with contraindications (eg, hemophilia). The study was conducted in collaboration with STOP-HCV, a consortium sponsored by the Medical Research Council, United Kingdom, which assisted in the design of the study.

All patients provided written informed consent before undertaking any study-related procedures. The study protocol was approved by each institution's review board or ethics committee before study initiation. The study was conducted in accordance with the International Conference on Harmonization Good Clinical Practice Guidelines and the Declaration of Helsinki.

Procedures

Randomization was conducted by interactive web and voice-response system (Bracket Global, Wayne, PA). The random allocation sequence was list-based, controlled by a seed, and the list had 4800 slots and a block size of 6. Patients were randomized in a 1:1:1 ratio to 1 of 3 treatment regimens: sofosbuvir and ribavirin for 16 weeks, sofosbuvir and ribavirin for 24 weeks, or sofosbuvir plus peginterferon-alfa and ribavirin for 12 weeks. Enrollment of approximately 600 patients was planned, of which 516 would have genotype 3 HCV infection. Approximately equal numbers of treatment-naïve and treatment-experienced HCV genotype 3 patients were to be enrolled. Stratification into treatment groups was based on HCV genotype and, for patients with genotype 3 HCV, absence or presence of cirrhosis and history of treatment. No specific target was set for the enrollment of patients with genotype 2 HCV.

Sofosbuvir was administered as an oral tablet of 400 mg once daily. Ribavirin was administered orally twice daily, 1000 or 1200 mg/d in a divided dose based on body weight of <75 kg or $\geq$75 kg, respectively. Peginterferon-alfa 2a 180 μg was administered once weekly via subcutaneous injection. This was an open-label study, treatment assignments were not masked to patients or study administrators or investigators at any point.

The use of erythropoietin-stimulating agents, granulocyte colony-stimulating factors, or platelet boosters was prohibited from 28 days before baseline through the end of treatment.

Assessments

Samples for determining plasma HCV RNA levels were drawn at screening, on-treatment time points, including day 1, weeks 1, 2, 4, 8, 10, 12, and, where applicable, 16, 20, and 24, and at post-treatment weeks 4, 12, and 24. Plasma HCV RNA was analyzed using the Roche Cobas TaqMan HCV RNA CAP/CTM Test, v2.0 for use with the High Pure System (Roche Molecular Systems, Inc., Branchburg, NJ), which has a lower limit of quantification (LLOQ) of 15 IU/mL.

For analysis of viral resistance, we collected blood samples before dosing (baseline) and at each subsequent visit for all patients. For patients with virologic failure, we compared samples taken at baseline and time of failure to detect any amino acid changes in the nonstructural 5B polymerase region that might confer resistance to sofosbuvir. We report resistance-associated variants (RAVs) that were present in at least 1% of deep sequence reads.

Safety data collected during treatment included reported adverse events, physical examinations, clinical laboratory tests, vital signs, and electrocardiography recordings. Concomitant medication intake was also recorded. Treatment-emergent clinical and laboratory adverse events were summarized using the Medical Dictionary for Regulatory Activities (version 17.1).

End Points and Statistical Analyses

The primary efficacy end point was the percentage of patients in each study group with SVR12, defined as HCV RNA less than LLOQ (15 IU/mL) 12 weeks after stopping the study drug. For the primary efficacy analysis, the SVR12 rate was calculated with a 2-sided 95% exact confidence interval using the Clopper-Pearson method. Patients without HCV RNA result for post-treatment week 12 for any reason were counted as treatment failures.

The primary statistical hypothesis testing was performed in all patients with genotype 3 HCV who were enrolled and received at least one dose of study treatment. A sequence of noninferiority/superiority hypotheses comparing the treatment groups was conducted using a gatekeeping approach to control the familywise error rate. The comparison of the SVR12 rates between any 2 treatment groups was performed using a

Cochran-Mantel-Haenszel test stratified by prior treatment experience and presence/absence of cirrhosis. In stage 1 of the analysis, we assessed the equivalence of the SVR12 rates for patients receiving 16 or 24 weeks of sofosbuvir and ribavirin. Equivalence was evaluated using a two 1-sided testing approach. The hypotheses at later stages were defined separately, depending on the outcomes of stage 1. See Supplementary Material for the sequence of hypotheses. The SVR12 rate of one group was considered superior to that of another group if the *P* value of the superiority test (Cochran-Mantel-Haenszel test) was less than the α levels prespecified ($\alpha = .025$ one-sided for 24 weeks compared with 16 weeks and 12 weeks compared with 16 weeks, and $\alpha = .0125$ one-sided for 12 weeks compared with 24 weeks) (Supplementary Material).

Secondary efficacy end points included the percentage of patients in each group with HCV RNA less than LLOQ during treatment and rates of relapse and breakthrough.

We calculated that a sample size of 172 subjects per arm would provide >83% power to detect a difference of 15% in SVR12 rates between 16 and 24 weeks of sofosbuvir plus ribavirin at a .0125 significance level using a 1-sided continuity-corrected χ^2 test. Assuming an SVR12 rate of 85% for the groups receiving 24 weeks of sofosbuvir and ribavirin and 12 weeks of sofosbuvir plus peginterferon and ribavirin, this sample size would provide >80% power to establish noninferiority of 24 weeks of sofosbuvir plus ribavirin to 12 weeks of sofosbuvir plus peginterferon and ribavirin (with a noninferiority margin of 12%) at a signicance level of .0125 using a 1-sided test.

Role of the Funding Source

The sponsor of the study designed and undertook the study, and collected and analyzed the data, in collaboration with external investigators. The sponsor and Graham R. Foster interpreted data. The manuscript was drafted by Jennifer King of August Editorial, who was paid by the sponsor. All authors had full access to study data and approved the final manuscript.

Results

Baseline Characteristics

We screened 776 patients, of which 601 were randomized, and 592 received at least one dose of study treatment (Supplementary Material). The baseline demographic and disease characteristics of the patients in the 3 treatment groups are shown in Table 1. A majority of patients were white (84%) and 67% were male. Forty-eight (8%) patients had genotype 2 HCV and 544 (92%) had genotype 3 HCV (of the 522 patients with genotype 3 HCV who could be subtyped, 512 [98%] had genotype 3a HCV). Overall, 37% of patients (100% of those with genotype 2 HCV and 31% of those with genotype 3 HCV) had compensated cirrhosis. Mean baseline HCV RNA levels were similar among the treatment groups.

Patients With Genotype 2 Hepatitis C Virus

Among treatment-experienced patients with genotype 2 HCV and cirrhosis, reduction in viral load to less than

Table 1. Patient Demographics and Baseline Characteristics

	SOF-RBV for 16 wks (n = 196)	SOF-RBV for 24 wks (n = 199)	SOF-RBV+PEG for 12 wks (n = 197)
Age, *y*, mean (SD)	51 (9.7)	49 (9.8)	50 (10.2)
Male, n (%)	134 (68)	129 (65)	132 (67)
Race, n (%)			
White	162 (83)	168 (84)	165 (84)
Asian	29 (15)	26 (13)	25 (13)
Black or African American	2 (1)	2 (1)	2 (1)
Hawaiian or Pacific Islander	0	1 (1)	2 (1)
American Indian or Alaska Native	2 (1)	0	0
Other	0	1 (1)	1 (1)
Not disclosed	1 (1)	1 (1)	2 (1)
BMI <30 kg/m^2, n (%)	145 (74)	140 (70)	139 (71)
Genotype, n (%)			
2	15 (8)	17 (9)	16 (8)
3	181 (92)	182 (92)	181 (92)
HCV RNA, *log$_{10}$ IU/mL*, mean (SD)	6.3 (0.68)	6.2 (0.71)	6.3 (0.69)
Prior HCV treatment, n (%)			
Treatment naive	91 (46)	94 (47)	94 (48)
Relapse/breakthrough	79 (75)	79 (75)	83 (81)
Nonresponse	26 (25)	26 (25)	20 (19)
IL-28B, n (%)			
CC	75 (38)	73 (37)	78 (40)
CT	94 (48)	95 (48)	98 (50)
TT	27 (14)	31 (16)	21 (11)
Cirrhosis present, n (%)	72 (37)	73 (37)	74 (38)
ALT, *U/L*, median (range)	83 (17–490)	93 (12–485)	76 (14–410)

ALT, alanine aminotransferase; PEG, peginterferon-alfa; RBV, ribavirin; SOF, sofosbuvir.

LLOQ was markedly more rapid in those receiving peginterferon than in those receiving sofosbuvir and ribavirin: 88% of patients (14 of 16) receiving sofosbuvir plus peginterferon and ribavirin had HCV RNA less than LLOQ by week 2, as compared with 40% of patients (6 of 15) in the 16-week group and 41% (7 of 17) in the 24-week group. By week 8 of treatment, all 48 patients had HCV RNA less than LLOQ.

Rates of SVR12 were similar across treatment groups: 87% of patients (13 of 15) who received 16 weeks of sofosbuvir and ribavirin, 100% of patients (17 of 17) who received 24 weeks of sofosbuvir and ribavirin, and 94% of patients (15 of 16) who received 12 weeks of sofosbuvir plus peginterferon and ribavirin. Three patients with genotype 2 HCV did not achieve SVR12, two patients receiving 16 weeks of sofosbuvir and ribavirin relapsed by post-treatment week 4, and 1 patient receiving sofosbuvir plus peginterferon and ribavirin was lost to follow-up after having HCV RNA less than LLOQ at post-treatment week 4. Deep sequencing in the 2 relapsers did not reveal the sofosbuvir RAVs S282T, L159F, or V321A, at baseline or time of virologic failure.

Patients With Genotype 3 Hepatitis C Virus

Among patients with genotype 3 HCV, on-treatment response was also more rapid in those receiving peginterferon than in those receiving sofosbuvir and ribavirin. By week 2 of treatment, 65% of patients with genotype 3 HCV receiving sofosbuvir plus peginterferon and ribavirin had HCV RNA less than LLOQ, as compared with 54% and 55%, respectively, for genotype 3 patients receiving sofosbuvir and ribavirin for 16 and 24 weeks ($P = .007$ for both comparisons). By week 8 of treatment, virtually all evaluable patients (99%) had HCV RNA less than LLOQ.

Among patients with genotype 3 HCV, overall rates of SVR12 were 71% in those who received 16 weeks of sofosbuvir and ribavirin, 84% in those who received 24 weeks of sofosbuvir and ribavirin, and 93% in those who received 12 weeks of sofosbuvir plus peginterferon and ribavirin (Table 2). The SVR12 rate among patients who received 12 weeks of sofosbuvir plus peginterferon and ribavirin was statistically superior to that among patients who received 16 weeks of sofosbuvir and ribavirin (by 22 percentage points; 95% confidence interval: 15%−30%; $P < .001$) and to that of patients who received 24 weeks of

Table 2. Treatment Response

	SOF-RBV for 16 wks (n = 196)	SOF-RBV for 24 wks (n = 199)	SOF-RBV+PEG-IFN for 12 wks (n =197)
SVR			
Overall, n (%)			
Wk 4	143 (73)	171 (86)	189 (96)
Wk 12 (SVR12)	141 (72)	170 (85)	183 (93)
95% CI	65−78	80−90	88−96
By subgroups, n/N (%)			
Genotype 2	13/15 (87)	17/17 (100)	15/16 (94)
Genotype 3			
Overall	128/181 (71)	153/182 (84)	168/181 (93)
By treatment history, n/N (%)			
Naïve	70/91 (77)	83/94 (88)	89/94 (95)
Experienced	58/90 (64)	70/88 (80)	79/87 (91)
By cirrhosis status, n/N (%)			
No	99/124 (80)	109/126 (87)	117/123 (95)
Yes	29/57 (51)	44/56 (79)	51/58 (88)
By cirrhosis status and treatment history, n/N (%)			
Naïve non-cirrhotic	58/70 (83)	65/72 (90)	68/71 (96)
Naïve cirrhotic	12/21 (57)	18/22 (82)	21/23 (91)
Experienced non-cirrhotic	41/54 (76)	44/54 (81)	49/52 (94)
Experienced cirrhotic	17/36 (47)	26/34 (76)	30/35 (86)
Virologic failure,[a] n/N (%)			
Overall	52 (27)	27 (14)	9 (5)
Breakthrough	0	2 (1)[b]	0
Nonresponse	0	1 (<1)[c]	0
Relapse	52/195 (27)	24/195 (12)	9/195 (5)
Genotype 2	2/15 (13)	0/17	0/16
Genotype 3	50/180 (28)	24/178 (14)	9/179 (5)

ALT, alanine aminotransferase; CI, confidence interval; PEG-IFN, peginterferon; RBV, ribavirin; SOF, sofosbuvir.
[a]Ten patients with missing data were imputed to have treatment failure: 3 receiving 16 weeks of sofosbuvir and ribavirin, 2 receiving 24 weeks of sofosbuvir and ribavirin, and 5 receiving 12 weeks of sofosbuvir plus peginterferon and ribavirin.
[b]Two patients with genotype 3 HCV.
[c]One patient with genotype 3 HCV.

sofosbuvir and ribavirin (by 9 percentage points; 95% confidence interval: 2%−16%; $P = .008$). In addition, the SVR12 rate among patients who received 24 weeks of sofosbuvir and ribavirin was statistically superior to that among those who received 16 weeks of sofosbuvir and ribavirin (by 13 percentage points; 95% confidence interval: 5%−22%; $P = .002$).

The same pattern of response—the lowest rates of SVR12 among patients receiving 16 weeks of sofosbuvir and ribavirin and the highest among patients receiving 12 weeks of sofosbuvir plus peginterferon and ribavirin—was seen in every major subgroup of patients with genotype 3 HCV, including treatment-naïve and treatment-experienced patients, those with and without cirrhosis, and in subgroups by combined treatment history and cirrhosis status (Table 2, Figure 1, and Supplementary Material).

Among patients with genotype 3 HCV, 83 had virologic relapse after the end of treatment—50 (28%) of those receiving 16 weeks of sofosbuvir and ribavirin, 24 (13%) of those receiving 24 weeks of sofosbuvir and ribavirin, and 9 (5%) of those receiving 12 weeks of sofosbuvir plus peginterferon and ribavirin. We performed an exploratory multivariable logistic regression analysis using the forward selection method to discover baseline factors associated with relapse among patients with genotype 3 HCV. Our results showed that male sex and non-CC allele of the IL28B gene were independently associated with relapse among patients receiving 16 weeks and 24 weeks of sofosbuvir and ribavirin (Table 3). The presence of cirrhosis was independently associated with relapse after treatment with 16 weeks of sofosbuvir and ribavirin and 12 weeks of sofosbuvir plus peginterferon and ribavirin, but not 24 weeks of sofosbuvir and ribavirin.

Among patients receiving sofosbuvir and ribavirin alone, on-treatment response was somewhat slower in those who relapsed than in those who achieved SVR12. Among those who relapsed, 38% of patients receiving 16 weeks of sofosbuvir and ribavirin and 33% of those receiving 24 weeks of sofosbuvir and ribavirin had HCV RNA less than LLOQ at week 2 of treatment, as compared with 61% ($P = .007$) and 58% ($P = .028$) of those who achieved SVR12. By week 4 of treatment, this difference was not as marked (Supplementary Table 10). Among patients receiving sofosbuvir plus peginterferon and ribavirin, on-treatment response among those who relapsed and those who achieved SVR12 was similar.

Deep sequencing of the NS5B polymerase region of the HCV RNA was attempted at baseline and at time of virologic

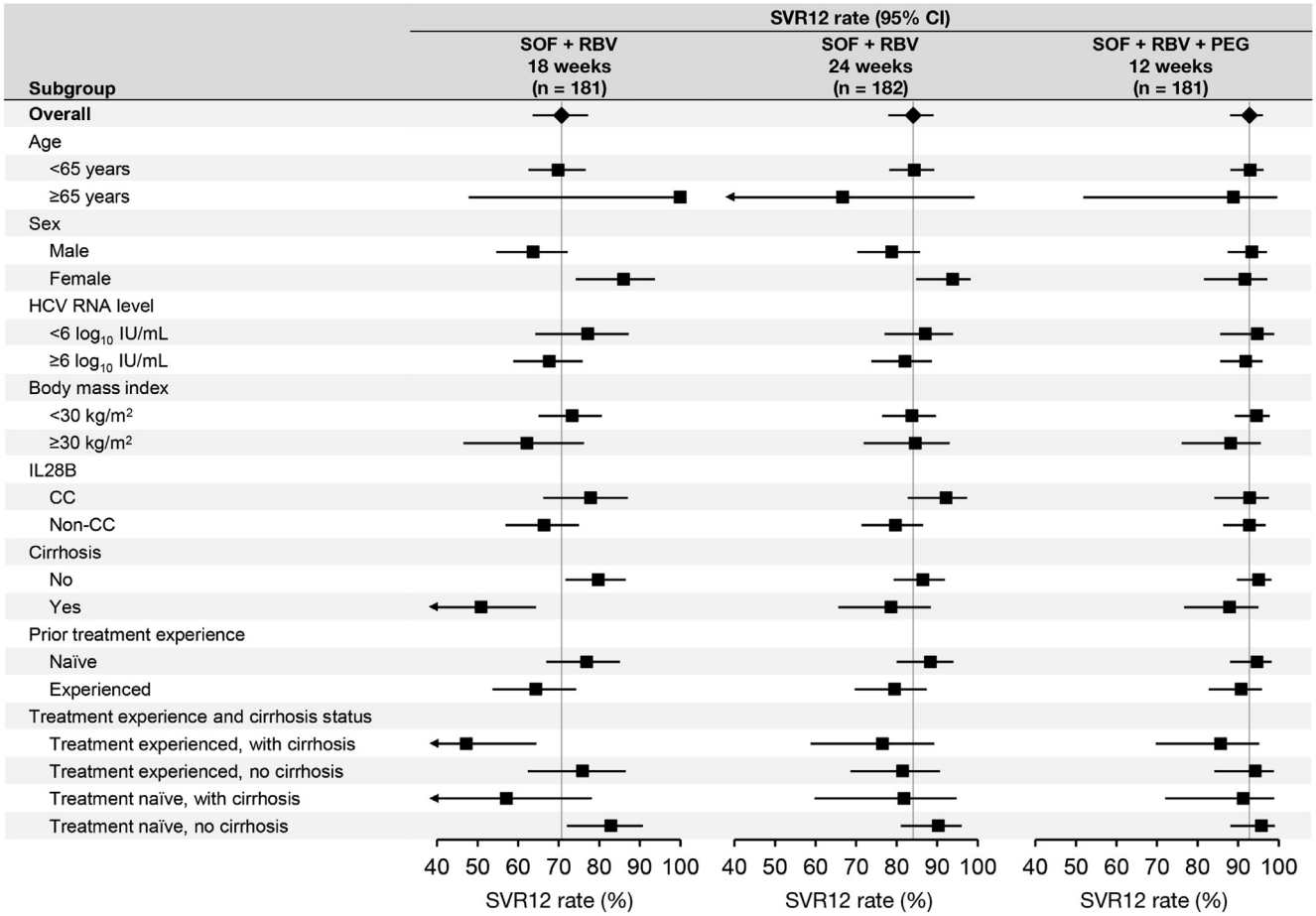

Figure 1. Rates of SVR12 by subgroup in patients with genotype 3 HCV. The position of the *squares* indicates the rate of virologic response 12 weeks after the end of treatment in the subgroup. *Horizontal lines* indicate 95% confidence intervals. *Vertical lines* represent the overall rates of SVR.

Table 3. Multivariate Analysis of Factors Associated With Relapse in Patients With Genotype 3 HCV

Variable	Odds ratio	95% CI	2-Sided *P* values
Model 1: SOF+RBV 16 wks (n = 177)			
Male vs female	4.13	1.57−10.84	.0040
Cirrhosis vs non-cirrhosis	4.81	2.24−10.35	<.0001
IL28B CT vs CC	2.84	1.23−6.58	.0145
IL28B TT vs CC	2.21	0.67−7.34	.1941
Model 2: SOF+RBV 24 wks (n = 180)			
Male vs female	6.06	1.71−21.45	.0052
IL28B CT vs CC	3.58	1.11−11.60	.0333
IL28B TT vs CC	7.10	1.85−27.18	.0042
Model 3: SOF+RBV+PEG 12 wks (n = 177)			
Cirrhosis vs non-cirrhosis	4.59	1.10−19.07	.0361
Model 4: 3 arms combined (n = 534)			
16 wks SOF+RBV vs 12 wks PEG+SOF+RBV	9.32	4.22−20.59	<.0001
24 wks SOF+RBV vs 12 wks PEG+SOF+RBV	3.70	1.62−8.43	.0019
Cirrhosis vs non-cirrhosis	2.77	1.61−4.75	.0002
Male vs female	3.03	1.54−5.98	.0014
BL HCV RNA ≥ vs <6 log	2.01	1.10−3.68	.0229
BL ALT > vs ≤1.5 × ULN	2.05	1.09−3.87	.0261
IL28B CT vs CC	3.40	1.81−6.40	.0001
IL28B TT vs CC	5.63	2.41−13.15	<.0001

NOTE. For Arm 2, logistic regression was also performed excluding "other" and on-treatment failures (n = 3). The model remained the same (ie, final model includes sex and IL28B status) with only small numerical changes to odds ratio, 95% confidence interval, and *P* values (data available upon request).
ALT, alanine aminotransferase; BL, baseline; CI, confidence interval; PEG, peginterferon-alfa; RBV, ribavirin; SOF, sofosbuvir.

failure in 83 patients with genotype 3 HCV who relapsed. Full-length NS5B sequencing was successful for 81 of the 83 patients at baseline, and from 76 of 83 at time of relapse. The S282T RAV was not present in any patient at any time point. The L159F RAV was identified at levels ranging from 1.2% to >99% of viral sequence reads in 8 patients at the time of relapse, including 1 who had the variant at baseline. The V321A RAV was identified in 1 patient (in 35.1% of viral sequence reads) at the time of virologic failure. One patient who had a low level of V321A (3.8%) at baseline relapsed after treatment, but amplification of NS5B at the time of relapse was not successful. All patients with L159F and V321A at time of relapse were in the sofosbuvir and ribavirin arms. None of the patients receiving sofosbuvir plus peginterferon and ribavirin who were successfully sequenced after virologic relapse had S282T, L159F, or V321A RAVs.

Patients With On-Treatment Virologic Failure

Three patients, all with genotype 3a HCV and all in the group receiving 24 weeks of sofosbuvir and ribavirin, experienced virologic failure during treatment. Two of these patients, both 41-year-old white males, experienced virologic breakthrough. One had HCV RNA less than LLOQ from week 2 through week 8 of treatment, but had quantifiable HCV RNA at the treatment week 12 visit; no additional data are available because the patient was subsequently lost to follow-up after week 12. The other patient with virologic breakthrough had HCV RNA less than LLOQ for the first time at the week 8 visit, but had quantifiable HCV RNA at the following visit (week 12) and at all other visits through post-treatment week 12. The third patient with on-treatment virologic failure, a 50-year-old Asian male, failed to achieve HCV RNA less than LLOQ through week 8 and was therefore discontinued from treatment, after which the patient withdrew consent. Pharmacokinetic analyses of all 3 patients showed detectable plasma levels of sofosbuvir and its metabolites GS-331007 and GS-566500, consistent with drug intake at the scheduled visits. Although drug levels in these 3 patients' results do not indicate lack of adherence, pill counts suggest that 1 patient with virologic breakthrough might not have been adherent. The S282T, L159F, or V321A RAVs were not detected at baseline in any of the 3 patients with on-treatment virologic failure. Amplification was possible for samples taken at the time of virologic failure from 2 of these 3 patients. One was a partial responder who had no RAVs at the time of virologic failure at week 2. In the other patient, who had breakthrough at week 12, the V321A variant was detected at a low level (2.5%).

Safety

The majority of patients experienced at least one adverse event during treatment (Table 4), but most adverse events were mild to moderate in severity. The most common adverse events in all 3 treatment groups were fatigue, headache, and insomnia. Numerically greater proportions of patients in the peginterferon-containing group than in the sofosbuvir and ribavirin−only groups experienced fatigue, nausea, rash, decreased appetite, myalgia, cough, influenza-like illness, pyrexia, and chills. Serious adverse events were experienced at similar frequencies across treatment arms. The proportion of patients with moderate (grade 3) and severe (grade 4) adverse events was not substantially higher in the peginterferon-containing arm (8%) than in the interferon-free arms (6% and 4%, respectively, in patients receiving 16 and 24 weeks of sofosbuvir and ribavirin).

Six (1%) patients, 5 receiving sofosbuvir and ribavirin and 1 receiving sofosbuvir plus peginterferon and ribavirin, discontinued all study treatment because of an adverse event. Three of these discontinuations were not thought to be related to study drugs. Of the 3 discontinuations thought to be related to study treatment, 2 were in patients receiving 16 weeks of sofosbuvir and ribavirin (sleep disturbances in 1 patient and worsening depression/suicidal thoughts in the other) and 1 in a patient receiving sofosbuvir plus peginterferon and ribavirin (depression). Two of these 6 patients achieved SVR12, 2 relapsed, and 2 discontinued the study early.

A numerically greater proportion of patients receiving sofosbuvir plus peginterferon and ribavirin for 12 weeks

Table 4. Treatment-Emergent Adverse Events and Laboratory Abnormalities

	SOF-RBV for 16 wk (n = 196)	SOF-RBV for 24 wk (n = 199)	SOF-RBV+PEG-IFN for 12 wk (n = 197)
Patients with any AE	185 (94)	188 (95)	195 (99)
Patients with moderate or severe AE	11 (6)	7 (4)	15 (8)
Patients with a serious AE	8 (4)	10 (5)	12 (6)
Patients with AEs leading to discontinuation of any study drug	3 (2)	3 (2)	2 (1)
Patients with AEs leading to discontinuation of sofosbuvir	3 (2)	2 (1)	1 (<1)
Deaths, n	0	0	0
Common AEs[a]			
Fatigue	74 (38)	83 (42)	92 (47)
Headache	61 (31)	72 (36)	70 (36)
Insomnia	47 (24)	56 (28)	50 (25)
Nausea	32 (16)	34 (17)	50 (25)
Rash	24 (12)	27 (14)	39 (20)
Pruritus	21 (11)	24 (12)	22 (11)
Dyspnea exertional	22 (11)	22 (11)	30 (15)
Diarrhea	21 (11)	18 (9)	27 (14)
Decreased appetite	13 (7)	16 (8)	35 (18)
Irritability	17 (9)	25 (13)	21 (11)
Dry skin	15 (8)	22 (11)	25 (13)
Myalgia	12 (6)	19 (10)	33 (17)
Cough	10 (5)	19 (10)	28 (14)
Arthralgia	10 (5)	16 (8)	25 (13)
Influenza-like illness	7 (4)	8 (4)	39 (20)
Dizziness	14 (7)	16 (8)	21 (11)
Vomiting	10 (5)	21 (11)	19 (10)
Pyrexia	5 (3)	7 (4)	29 (15)
Chills	3 (2)	4 (2)	21 (11)
Serious adverse events in >1 patient			
Atrial fibrillation	0	1 (<1)	1 (<1)
Chest pain	1 (<1)	0	0
Depression	1 (<1)	0	1 (<1)
Gallbladder pancreatitis	1 (<1)	1 (<1)	0
Syncope	0	1 (<1)	1 (<1)
Laboratory abnormalities			
Hemoglobin			
<8.5 g/dL	0	0	2 (1)
<10 g/dL	7 (4)	12 (6)	24 (12)
Neutrophils, <750/mm^3	0	0	31 (16)
Lymphocytes, <500/mm^3	2 (1)	3 (2)	18 (9)
WBC, <1000/mm^3	0	0	14 (7)
Platelets, <50,000/mm^3	1 (<1)	0	9 (5)

NOTE. Values are n (%).
AE, adverse events; PEG-IFN-alfa, peginterferon; RBV, ribavirin; SOF, sofosbuvir; WBC, white blood cells.
[a]In ≥10% of patients in any treatment group.

had grade 3 and 4 laboratory abnormalities than patients receiving sofosbuvir and ribavirin for 16 or 24 weeks. The most common grade 3 and 4 laboratory abnormalities were hematologic. The median reduction in hemoglobin level at the end of treatment was 2.6 g/dL among patients receiving sofosbuvir plus peginterferon and ribavirin, as compared with 2.0 g/dL and 1.8 g/dL, respectively, among patients receiving sofosbuvir and ribavirin for 16 and 24 weeks. Two patients, both in the peginterferon-containing group, experienced declines in hemoglobin to <8.5 g/dL. Four patients, 3 receiving sofosbuvir plus peginterferon and ribavirin and 1 sofosbuvir-ribavirin had blood transfusions (2 in the post-treatment period due to gynecologic bleeding in 1 patient randomized to sofosbuvir plus peginterferon and ribavirin, and the other in a diabetic patient randomized to sofosbuvir and ribavirin who developed pneumonia associated with urinary tract infection). Other grade 3 and 4 cytopenias were more common among patients in the peginterferon-containing arm than among patients receiving sofosbuvir and ribavirin (Supplementary Material).

Discussion

The results of this phase 3 trial have shown that patients with genotype 3 HCV achieve superior rates of SVR12 with 12 weeks of sofosbuvir plus peginterferon and ribavirin than they do with 16 or 24 weeks of sofosbuvir and ribavirin. Numerically superior SVR 12 rates were observed

across all major subgroups of genotype 3 patients who received triple therapy as compared with those receiving IFN-free treatment. Additionally, genotype 2 treatment-experienced HCV-infected patients with cirrhosis had high SVR rates in all treatment groups.

For patients with genotype 3 HCV, current treatment guidelines suggest the following options: 24 weeks of sofosbuvir and ribavirin, 12 weeks of sofosbuvir plus peginterferon and ribavirin, or, in Europe, sofosbuvir and daclatasvir for 12 weeks in treatment-naïve or peginterferon-experienced patients, and sofosbuvir and daclatasvir plus ribavirin for 12 or 24 weeks in treatment-experienced patients.[1,2] The first option was based on results of the VALENCE trial, in which 85% (213 of 250) of treatment-naïve and previously treated patients achieved SVR12 after 24 weeks of sofosbuvir and ribavirin.[8] Among treatment-experienced patients with cirrhosis, the SVR12 rate was only 62%. The second treatment option is based on results from a small cohort in the phase 2 LONESTAR-2 trial.[11] Of the 24 patients with genotype 3 who received sofosbuvir plus peginterferon and ribavirin, 20 (83%) achieved SVR12. In this trial, there was no difference in response between patients with and without cirrhosis. The third and fourth option is based on results from the ALLY-3 trial, in which 90% (91 of 101) of treatment-naïve patients and 86% (44 of 51) of treatment-experienced patients achieved SVR12.[9] The SVR12 rate in this trial was substantially lower among patients with cirrhosis receiving 12 weeks of treatment without ribavirin.

Most patients with genotype 2 HCV in the VALENCE trial[8] were able to achieve SVR12 with 12 weeks of sofosbuvir and ribavirin. However, a small subgroup (n = 9) of treatment-experienced patients with compensated cirrhosis had an SVR12 rate of 78%, which was considerably lower than rates seen in treatment-naïve and treatment-experienced non-cirrhotic patients. In the LONESTAR-2 trial,[11] a small subgroup (n = 14) of treatment-experienced genotype 2 patients with cirrhosis had an SVR12 rate of 93% after treatment with 12 weeks of sofosbuvir plus peginterferon and ribavirin. On the basis of these data, current treatment guidelines for patients with genotype 2 HCV who failed previous treatment with peginterferon and ribavirin include, among other options, 16−20 weeks of sofosbuvir and ribavirin or 12 weeks of sofosbuvir plus peginterferon and ribavirin. Our results, albeit in a small group of patients, provide further support for these recommendations, but raise a new question about the optimal duration of sofosbuvir and ribavirin for these patients. Two patients in the 16-week arm relapsed after the end of treatment as compared with no patients in the other 2 treatment groups. Although this suggests that 24 weeks of sofosbuvir and ribavirin is optimal, our study was not powered for formal comparisons among patients with genotype 2 HCV.

On-treatment virologic failures have been reported rarely in clinical trials of sofosbuvir. Most cases on record have been attributable to nonadherence to study drug dosing by patients. In our study, there was no evidence for nonadherence, as the study drug and its metabolites were detectable in the plasma in samples taken during study visits.

In this study, the S282T variant of the NS5B protein—the first identified sofosbuvir RAV—was not observed in any patient at any time point. A recent analysis of 282 patients who did not achieve SVR in phase 2 and 3 studies with sofosbuvir identified 2 additional variants, L159F and V321A, that confer a slightly reduced susceptibility to sofosbuvir.[12] In our study, L159F and V321A were observed in a subset of patients with virologic failure, confirming their association with sofosbuvir treatment. All patients with L159F and V321A at the time of relapse were in the sofosbuvir and ribavirin arms, suggesting that the addition of peginterferon to sofosbuvir and ribavirin reduces the emergence of these variants.

The well-known side effects of interferon have made its elimination from the treatment of HCV a primary clinical and research objective.[13–15] One of the main reasons that interferon was so difficult for patients to tolerate was the length of treatment—most interferon-based regimens had durations of 6 months to 1 year, at the end of which patients were faced with a relatively high likelihood of relapse. With a shorter duration of 12 weeks and greater efficacy, the side effects of interferon have proven to be less formidable.[5] In our study, the safety profile of the 12-week regimen of sofosbuvir plus peginterferon and ribavirin was unexpectedly not markedly different from that of 16−24 weeks of sofosbuvir and ribavirin. Some interferon-associated side effects were more common in patients receiving this drug (fatigue, nausea, decreased appetite, myalgia, flu-like illness, and rash), but these were typically mild and the rates of adverse events leading to discontinuation of the study drug were similar in all arms. Safety and tolerability did not differ by treatment experience; similar rates of adverse events, serious adverse events, and treatment discontinuation for adverse events were observed in treatment-naïve and previously treated patients (Supplementary Material).

The results of this study are limited by the small number of patients with genotype 2 HCV, which tempers any conclusions about differences between SVR12 rates by regimen in patients with genotype 2 HCV. The study was also not powered for comparisons among subgroups of patients with genotype 3 HCV. However, our results were generally in keeping with those previously reported in phase 2 and 3 trials. Other features of the study population—its covering multiple sites in different continents, and the inclusion of a large proportion of cirrhotic patients with minimum platelets cell counts lower than that usually recommended for interferon-based treatment—support the generalizability of the overall efficacy and safety results to a broad population of patients. The open-label design of the study is unlikely to have biased the reporting of efficacy results because HCV RNA is an objective laboratory assessment, but bias in the reporting of safety events cannot be ruled out. Although the field of HCV treatment is moving rapidly away from interferon-based regimens, our results suggest that a short course of highly effective interferon-containing treatment may still have a role in subpopulations without better options. In particular, SVR rates in treatment-experienced patients with genotype 3 HCV and cirrhosis receiving all-oral combinations—sofosbuvir plus daclatasvir,

sofosbuvir plus ledipasvir, and sofosbuvir plus ribavirin—range from 62% to 73%.[8–10]

In conclusion, our results provide clear evidence that sofosbuvir plus peginterferon and ribavirin for 12 weeks should continue to be considered a treatment option for eligible patients with genotype 3 HCV. Our results support the use of sofosbuvir and ribavirin for 24 weeks as an option for patients who cannot take interferon or are unwilling to do so.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2015.07.043.

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