ORIGINAL ARTICLE

Nucleotide Polymerase Inhibitor Sofosbuvir plus Ribavirin for Hepatitis C

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ABSTRACT

BACKGROUND

The standard treatment for hepatitis C virus (HCV) infection is interferon, which is administered subcutaneously and can have troublesome side effects. We evaluated sofosbuvir, an oral nucleotide inhibitor of HCV polymerase, in interferon-sparing and interferon-free regimens for the treatment of HCV infection.

METHODS

We provided open-label treatment to eight groups of patients. A total of 40 previously untreated patients with HCV genotype 2 or 3 infection were randomly assigned to four groups; all four groups received sofosbuvir (at a dose of 400 mg once daily) plus ribavirin for 12 weeks. Three of these groups also received peginterferon alfa-2a for 4, 8, or 12 weeks. Two additional groups of previously untreated patients with HCV genotype 2 or 3 infection received sofosbuvir monotherapy for 12 weeks or sofosbuvir plus peginterferon alfa-2a and ribavirin for 8 weeks. Two groups of patients with HCV genotype 1 infection received sofosbuvir and ribavirin for 12 weeks: 10 patients with no response to prior treatment and 25 with no previous treatment. We report the rate of sustained virologic response 24 weeks after therapy.

RESULTS

Of the 40 patients who underwent randomization, all 10 (100%) who received sofosbuvir plus ribavirin without interferon and all 30 (100%) who received sofosbuvir plus ribavirin for 12 weeks and interferon for 4, 8, or 12 weeks had a sustained virologic response at 24 weeks. For the other patients with HCV genotype 2 or 3 infection, all 10 (100%) who received sofosbuvir plus peginterferon alfa-2a and ribavirin for 8 weeks had a sustained virologic response at 24 weeks, as did 6 of 10 (60%) who received sofosbuvir monotherapy. Among patients with HCV genotype 1 infection, 21 of 25 previously untreated patients (84%) and 1 of 10 with no response to previous therapy (10%) had a sustained virologic response at 24 weeks. The most common adverse events were headache, fatigue, insomnia, nausea, rash, and anemia.

CONCLUSIONS

Sofosbuvir plus ribavirin for 12 weeks may be effective in previously untreated patients with HCV genotype 1, 2, or 3 infection. (Funded by Pharmasset and Gilead Sciences; ClinicalTrials.gov number, NCT01260350.)

Recent progress in the treatment of chronic hepatitis C virus (HCV) infection — the approval of the first-generation protease inhibitors telaprevir and boceprevir — has benefited many but not all patients with HCV infection. One quarter of patients with HCV genotype 1 infection who have not received previous therapy and 71% of those with no response to previous therapy do not have a sustained virologic response with protease-inhibitor–based regimens.[1] No direct-acting antiviral agents have yet been approved for patients with HCV genotype 2 or 3 infection.

The standard of care for all patients with HCV infection continues to include 24 to 48 weeks of treatment with peginterferon-alfa 2a.[2] Treatment with interferon is associated with troublesome side effects, including influenza-like symptoms, depression, fatigue, and cytopenias,[3] and requires weekly subcutaneous injections. A substantial proportion of patients with HCV infection are either unable or unwilling to receive an interferon-based regimen.[4,5] Therefore, the development of an interferon-free, all-oral treatment regimen would represent an important advance.

Sofosbuvir (formerly known as GS-7977) is a direct-acting nucleotide polymerase inhibitor that is being developed as an oral drug for the treatment of chronic HCV infection.[6] Nucleotide analogues are phosphorylated within the host hepatocyte to the active nucleoside triphosphate, which competes with the natural nucleotides, thereby causing termination of RNA replication in the nascent viral genome. The active triphosphate of nucleotide analogues such as sofosbuvir targets the highly conserved active site of the HCV-specific NS5B polymerase, acting as a nonobligate chain terminator, an effect that is independent of the viral genotype.[7,8] Monotherapy with sofosbuvir at a dose of 400 mg for 7 days resulted in a profound reduction in the level of HCV RNA in patients with HCV genotype 1 infection.[9] Twelve weeks of treatment with sofosbuvir in combination with peginterferon and ribavirin resulted in substantial decreases in the level of HCV RNA during therapy, leading to a sustained virologic response at 24 weeks after treatment in 92% of patients with HCV genotype 2 or 3 infection (23 of 25 patients).[10] The same regimen followed by 12 weeks of treatment with peginterferon and ribavirin resulted in a sustained virologic response 24 weeks after treatment in 89% of patients with HCV genotype 1 infection (42 of 47 patients).[11] To date, no virologic breakthrough has been observed during sofosbuvir therapy.

We report results from the Electron trial, a multipart, phase 2a study designed to test the safety and efficacy of sofosbuvir and ribavirin in various interferon-sparing and interferon-free regimens for the treatment of patients with HCV genotype 1, 2, or 3 infection.

METHODS

PATIENTS

We enrolled patients at two centers in New Zealand from December 2010 through December 2011. Eligible patients were men and women, 19 years of age or older, who had chronic HCV infection (serum HCV RNA level, >50,000 IU per milliliter) without cirrhosis. Prescribed maintenance therapy with methadone was allowed. The absence of cirrhosis was determined either by means of liver biopsy within the previous 3 years or by means of transient elastography within the previous 12 months. Patients with positive tests for hepatitis B surface antigen, hepatitis B core IgM antibodies, or antibodies against the human immunodeficiency virus were excluded. All patients provided written informed consent.

STUDY DESIGN

In the first part of this open-label study, patients with HCV genotype 2 or 3 infection were randomly assigned in a 1:1:1:1 ratio to one of four treatment groups. Patients in all four groups received sofosbuvir (Gilead Sciences) and ribavirin (Copegus, Roche) for 12 weeks. Patients in group 1 received only sofosbuvir and ribavirin, whereas patients in groups 2, 3, and 4 received 4, 8, and 12 weeks of concomitant peginterferon alfa-2a (Pegasys, Roche), respectively. Sofosbuvir was administered orally at a dose of 400 mg once daily. Ribavirin was administered orally twice daily, with doses determined according to body weight (1000 mg daily in patients with a body weight of <75 kg, and 1200 mg daily in patients with a body weight of ≥75 kg). Peginterferon alfa-2a was administered once weekly at a dose of 180 μg subcutaneously. Randomization was stratified according to HCV genotype (genotype 2 vs. genotype 3) and host IL28B genotype, as assayed for the rs12979860 single-nucleotide polymorphism (CC vs. CT or TT).

After completion of dosing in the original four treatment groups, the protocol was amended to include two additional groups of previously untreated patients with HCV genotype 2 or 3 infection: 10 patients treated for 12 weeks with sofosbuvir, at a dose of 400 mg once daily, as monotherapy, and 10 patients treated with a triple-drug regimen — sofosbuvir plus peginterferon and ribavirin — for a total duration of 8 weeks.

A total of 35 patients with HCV genotype 1 infection were also enrolled: 10 patients who had not had a response to prior treatment with at least 12 weeks of treatment with peginterferon and ribavirin (decline in the HCV RNA level, <2 $\log_{10}$ IU per milliliter) and 25 who had not received prior treatment for HCV infection.

STUDY OVERSIGHT

The study was approved by the institutional review board at both participating sites and was conducted in compliance with the Declaration of Helsinki, Good Clinical Practice guidelines, and local regulatory requirements. The study was designed and conducted by the sponsors (Pharmasset and Gilead Sciences) in collaboration with the principal investigators. The sponsor collected the data and monitored the study conduct. PharStat (Research Triangle Park, NC) performed the statistical analyses. The investigators, participating institutions, and sponsor agreed to maintain confidentiality of the data. The first draft of the manuscript was prepared by the first author. The final drafts and revisions were prepared by a medical writer employed by Gilead Sciences, with input from all the authors. All the authors had access to the data and assume responsibility for the integrity and completeness of the reported data. All the authors made the decision to submit the manuscript for publication. The study was conducted according to the protocol, available with the full text of this article at NEJM.org.

EFFICACY ASSESSMENTS

Serum HCV RNA levels were measured with the use of the COBAS AmpliPrep/COBAS TaqMan HCV Test, version 2.0 (Roche Molecular Systems), with a lower limit of quantification of 43 IU per milliliter and a lower limit of detection of 15 IU per milliliter. Serum HCV RNA levels were measured at the following points during the study: at screening; at baseline; at 6, 12, 24, 48, and 72 hours after the first dose; weekly throughout the 8-week or 12-week treatment period; and at 2, 4, 8, 12, and 24 weeks after treatment.

Resistance Monitoring

Serum samples obtained at each time point were stored for drug-resistance monitoring. Samples for NS5B genotypic and phenotypic monitoring were collected before the dose was administered on dosing days. Population sequencing of the HCV NS5B–encoding region of the viral polymerase was performed with the use of standard population-sequencing technology on all baseline (pretreatment) viral samples and on any sample with an HCV RNA level of 1000 IU per milliliter or higher. In addition, resistance monitoring was planned for all patients who did not have a response or who had a rebound, breakthrough, or plateau in the serum HCV RNA level between day 1 and the end of week 12.

Safety Assessments

Vital signs were assessed, electrocardiography and symptom-directed physical examinations were conducted, and laboratory assessments were performed for biochemical analysis, hematologic testing, and urinalysis. All adverse events were recorded and graded according to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events.[12]

STATISTICAL ANALYSIS

This study was not designed to evaluate formal statistical hypotheses, and no sample-size calculations were performed. Adverse events, vital signs, laboratory tests, magnitude of decline in the HCV RNA level, and rates of virologic response during and after treatment were descriptively compared across the treatment groups. For the virologic response, two-sided 95% confidence intervals were calculated with the use of the exact binomial distribution.

RESULTS

CHARACTERISTICS OF THE PATIENTS

A total of 95 patients were enrolled and received at least one dose of the study drug. All patients completed treatment with sofosbuvir and ribavirin. One patient in group 4 discontinued peginterferon alfa-2a. The baseline characteristics of the patients are presented in Table 1.

Table 1. Baseline Demographic and Clinical Characteristics.*

Characteristic	HCV Genotype 2 or 3 Infection						HCV Genotype 1 Infection	
	12 Wk of Sofosbuvir–Ribavirin				12 Wk of Sofosbuvir (N=10)	8 Wk of Sofosbuvir–Ribavirin–Peginterferon (N=10)	12 Wk of Sofosbuvir–Ribavirin	
	no peginterferon (N=10)	4 wk of peginterferon (N=9)	8 wk of peginterferon (N=10)	12 wk of peginterferon (N=11)			no response to prior therapy (N=10)	no prior therapy (N=25)
Male sex — no. (%)	8 (80)	5 (56)	5 (50)	9 (82)	4 (40)	5 (50)	7 (70)	15 (60)
White race — no. (%)†	7 (70)	4 (44)	8 (80)	9 (82)	10 (100)	7 (70)	9 (90)	20 (80)
Age — yr								
Mean	47	48	49	46	43	39	48	49
Range	36–53	29–66	30–62	37–57	22–58	19–54	30–58	22–69
Body-mass index‡								
Mean	28	26	25	24	26	25	28	26
Range	24–36	21–32	18–33	21–28	18–39	21–35	20–36	19–38
HCV RNA $\log_{10}$ — IU/ml								
Median	6.7	6.6	6.5	6.5	5.9	6.0	7.0	6.2
Range	5.7–7.1	5.6–7.4	5.5–7.2	5.1–7.3	4.6–7.4	4.3–7.3	5.6–7.5	4.4–7.2
HCV genotype — no. (%)								
1a	—	—	—	—	—	—	9 (90)	22 (88)
1b	—	—	—	—	—	—	1 (10)	3 (12)
2	4 (40)	3 (33)	4 (40)	4 (36)	3 (30)	0	—	—
3	6 (60)	6 (67)	6 (60)	7 (64)	7 (70)	10 (100)	—	—
IL28B genotype — no. (%)								
CC	5 (50)	4 (44)	4 (40)	4 (36)	2 (20)	3 (30)	2 (20)	11 (44)
CT	4 (40)	4 (44)	4 (40)	5 (45)	6 (60)	6 (60)	5 (50)	12 (48)
TT	1 (10)	1 (11)	2 (20)	2 (18)	2 (20)	1 (10)	3 (30)	2 (8)

* HCV denotes hepatitis C virus.
† Race was self-reported.
‡ The body-mass index is the weight in kilograms divided by the square of the height in meters.

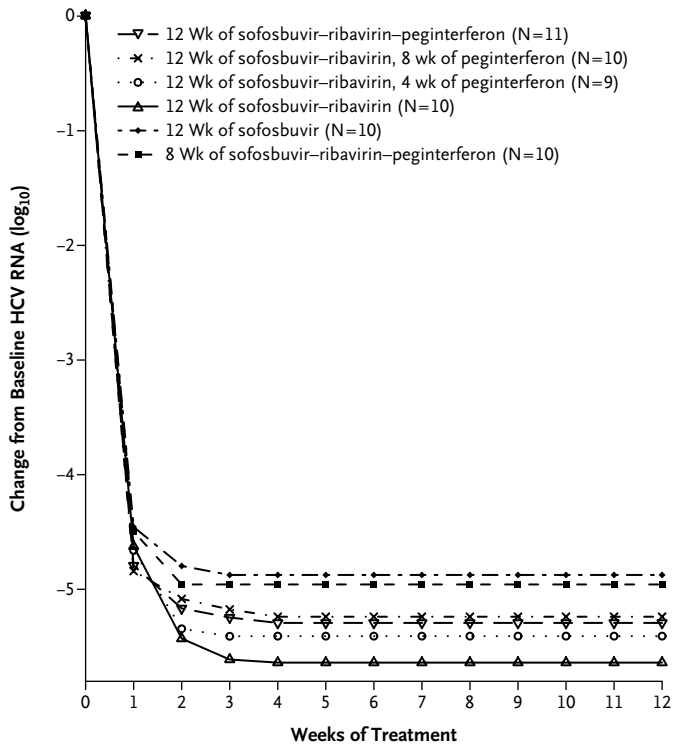

Figure 1. Mean Change from Baseline in Hepatitis C Virus (HCV) RNA Level during Treatment.

All patients with HCV genotype 2 or 3 infection completed the 12 weeks of treatment. All patients had an HCV RNA level below the limit of detection (i.e., <15 IU per milliliter) from week 4 until the end of treatment. See Figure S1 in the Supplementary Appendix for data from patients with HCV genotype 1 infection.

VIROLOGIC RESPONSE

Virologic Response during Treatment

The viral kinetics during treatment were nearly identical in all treatment groups. Viral suppression was rapid in all patients, regardless of genotype, status with respect to previous treatment, baseline viral load, race or ethnic group, IL28B status, and presence or absence of interferon in the regimen (Fig. 1; and Fig. S1 in the Supplementary Appendix, available at NEJM.org). All 95 patients had an undetectable level of HCV RNA by week 4, with viral suppression sustained through the end of treatment (Table 2). No virologic breakthrough was observed in any patient during the dosing period. All 41 patients with elevated levels of alanine aminotransferase at baseline had normal levels at the end of treatment.

Sustained Virologic Response

All 40 patients with HCV genotype 2 or 3 infection who received sofosbuvir and ribavirin for 12 weeks had an undetectable level of serum HCV RNA at 2, 4, 8, 12, 24, and 48 weeks after treatment. All 10 patients who received sofosbuvir plus peginterferon and ribavirin for 8 weeks had a sustained virologic response at 12 weeks after treatment (and all 9 patients for whom data were available had a sustained virologic response at 24 weeks after treatment). The presence or absence of peginterferon alfa-2a appeared to have no effect on viral kinetics or rate of sustained virologic response.

Six of the 10 patients in the sofosbuvir-monotherapy group had a sustained virologic response at 12 and 24 weeks after treatment. Of those who had a relapse, 2 patients, both with HCV genotype 3 infection, had a detectable level of HCV RNA at 2 weeks after treatment; the other 2, both with HCV genotype 2 infection, had a detectable level of HCV RNA at 4 weeks after treatment. Standard population sequencing in these 4 patients who had a relapse showed development of the S282T mutation in 1 patient with HCV genotype 2b infection. Deep sequencing confirmed the presence of S282T in this patient in 99% of the sequenced viral genomes. Deep sequencing showed no NS5B mutations at position 282 in the other 3 patients.

Of the 25 previously untreated patients with HCV genotype 1 infection, 21 (84%) had a sustained virologic response at 24 weeks after treatment. The other 4 patients had a relapse during follow-up. Three of the 4 patients had a relapse within 4 weeks after the end of treatment. The S282T mutation was not detected in any of these 3 patients by means of either population sequencing or deep sequencing. The fourth patient had a relapse between 12 and 24 weeks after the end of treatment. Another patient had a detectable level of HCV RNA at 12 weeks after treatment. This patient's viral load was too low (HCV RNA level, <200 IU per milliliter) for sequencing analysis. At 24 weeks after treatment, this patient's viral load was undetectable. Of the 10 patients with HCV genotype 1 infection who did not have a response to prior treatment, 1 (10%) had a sustained virologic response at 24 weeks after treatment. Population sequencing and deep sequencing showed no S282T-containing mutations in any of the 9 patients with HCV genotype 1 infection

Table 2. Patients with an HCV RNA Level below the Limit of Detection.*

Timing of Response	HCV Genotype 2 or 3 Infection						HCV Genotype 1 Infection	
	12 Wk of Sofosbuvir–Ribavirin				12 Wk of Sofosbuvir (N=10)	8 Wk of Sofosbuvir–Ribavirin–Peginterferon (N=10)	12 Wk of Sofosbuvir–Ribavirin	
	no peginterferon (N=10)	4 wk of peginterferon (N=9)	8 wk of peginterferon (N=10)	12 wk of peginterferon (N=11)			no response to prior therapy (N=10)	no prior therapy (N=25)
During the 12-wk treatment period — no. (% [95% CI])								
Wk 2	8 (80 [44–98])	7 (78 [40–97])	8 (80 [44–98])	9 (82 [48–98])	8 (80 [44–98])	10 (100 [69–100])	7 (70 [34–93])	17 (68 [47–85])
Wk 4	10 (100 [69–100])	9 (100 [66–100])	10 (100 [69–100])	11 (100 [72–100])	10 (100 [69–100])	10 (100 [69–100])	10 (100 [69–100])	25 (100 [86–100])
Wk 8	10 (100 [69–100])	9 (100 [66–100])	10 (100 [69–100])	11 (100 [72–100])	10 (100 [69–100])	10 (100 [69–100])	10 (100 [69–100])	25 (100 [86–100])
Wk 12	10 (100 [69–100])	9 (100 [66–100])	10 (100 [69–100])	11 (100 [72–100])	10 (100 [69–100])	10 (100 [69–100])	10 (100 [69–100])	25 (100 [86–100])
During the post-treatment period								
Wk 4 — no. (% [95% CI])	10 (100 [69–100])	9 (100 [66–100])	10 (100 [69–100])	11 (100 [72–100])	6 (60 [26–88])	10 (100 [69–100])	1 (10 [0–45])	22 (88 [69–98])
Wk 8 — no. (% [95% CI])	10 (100 [69–100])	9 (100 [66–100])	10 (100 [69–100])	11 (100 [72–100])	6 (60 [26–88])	10 (100 [69–100])	1 (10 [0–45])	21 (84 [64–96])
Wk 12 — no. (% [95% CI])	10 (100 [69–100])	9 (100 [66–100])	10 (100 [69–100])	11 (100 [72–100])	6 (60 [26–88])	10 (100 [69–100])	1 (10 [0–45])	21 (84 [64–96])
Wk 24 — no./total no. (% [95% CI])	10/10 (100 [69–100])	9/9 (100 [66–100])	10/10 (100 [69–100])	11/11 (100 [72–100])	6/10 (60 [26–88])	9/9 (100 [66–100])	1/10 (10 [0–45])	21/25 (84 [64–96])

* The limit of detection was 15 IU per milliliter.

Table 3. Adverse Events and Hematologic Abnormalities during the Treatment Period.*

Event	HCV Genotype 2 or 3 Infection						HCV Genotype 1 Infection	
	12 Wk of Sofosbuvir–Ribavirin				12 Wk of Sofosbuvir (N=10)	8 Wk of Sofosbuvir–Ribavirin–Peginterferon (N=10)	12 Wk of Sofosbuvir–Ribavirin	
	no peginterferon (N=10)	4 wk of peginterferon (N=9)	8 wk of peginterferon (N=10)	12 wk of peginterferon (N=11)			no response to prior therapy (N=10)	no prior therapy (N=25)
	number of patients with event (percent)							
Adverse event†								
Headache	4 (40)	7 (78)	9 (90)	8 (73)	8 (80)	6 (60)	4 (40)	8 (32)
Fatigue	1 (10)	3 (33)	3 (30)	5 (45)	3 (30)	7 (70)	4 (40)	12 (48)
Insomnia	3 (30)	6 (67)	5 (50)	5 (45)	6 (60)	1 (10)	4 (40)	5 (20)
Nausea	0	3 (33)	4 (40)	2 (18)	3 (30)	2 (20)	2 (20)	11 (44)
Rash	3 (30)	3 (33)	3 (30)	3 (27)	1 (10)	6 (60)	3 (30)	4 (16)
Anemia‡	1 (10)	4 (44)	2 (20)	3 (27)	0	3 (30)	3 (30)	5 (20)
Dizziness	4 (40)	4 (44)	2 (20)	3 (27)	2 (20)	1 (10)	1 (10)	1 (4)
Myalgia	2 (20)	3 (33)	3 (30)	3 (27)	1 (10)	4 (40)	0	1 (4)
Diarrhea	2 (20)	0	2 (20)	2 (18)	0	3 (30)	1 (10)	6 (24)
Irritability	2 (20)	0	1 (10)	4 (36)	1 (10)	2 (20)	1 (10)	5 (20)
Pruritus	1 (10)	3 (33)	3 (30)	2 (18)	0	2 (20)	2 (20)	0
Decreased appetite	0	2 (22)	0	3 (27)	0	5 (50)	0	2 (8)
Upper respiratory tract infection	0	1 (11)	1 (10)	1 (9)	2 (20)	1 (10)	1 (10)	4 (16)
Arthralgia	0	1 (11)	3 (30)	1 (9)	0	1 (10)	1 (10)	3 (12)
Back pain	1 (10)	2 (22)	1 (10)	1 (9)	1 (10)	2 (20)	0	2 (8)
Pyrexia	1 (10)	1 (11)	1 (10)	2 (18)	0	5 (50)	0	0

Hematologic abnormality								
Anemia§								
Grade 1	2 (20)	5 (56)	4 (40)	1 (9)	1 (10)	4 (40)	2 (20)	12 (48)
Grade 2	2 (20)	2 (22)	4 (40)	4 (36)	0	3 (30)	3 (30)	7 (28)
Grade 3	1 (10)	2 (22)	2 (20)	5 (45)	0	1 (10)	3 (30)	3 (12)
INR of grade 3¶	0	0	0	0	0	0	1 (10)	0
Lymphopenia‖								
Grade 3	0	1 (11)	2 (20)	0	0	0	0	0
Grade 4	0	0	0	0	1 (10)	0	0	0
Neutropenia**								
Grade 3	0	0	2 (20)	3 (27)	0	2 (20)	0	0
Grade 4	0	2 (22)	1 (10)	2 (18)	0	0	0	0
Leukopenia of grade 3††	0	2 (22)	0	3 (27)	0	0	0	0

* All adverse events were graded according to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events.[12]
† The following adverse events are those that were reported in at least 10 patients.
‡ Anemia was defined as a diagnosis of anemia or hemolytic anemia but not iron-deficient anemia.
§ Grade 1 anemia was defined as a hemoglobin level of 10.0 to 10.9 g per deciliter, grade 2 as 9.0 to 9.9 g per deciliter, and grade 3 as 7.0 to 8.9 g per deciliter.
¶ Grade 3 international normalized ratio (INR) was defined as a value from 2.1 to 3.0 times the upper limit of the normal range (normal range, 0.9–1.1).
‖ Grade 3 lymphopenia was defined as an absolute lymphocyte count of 350 to 499 per cubic millimeter, and grade 4 as less than 350 per cubic millimeter.
** Grade 3 neutropenia was defined as an absolute neutrophil count of 500 to 749 per cubic millimeter, and grade 4 as less than 500 per cubic millimeter.
†† Grade 3 leukopenia was defined as an absolute leukocyte count of 1000 to 1499 per cubic millimeter.

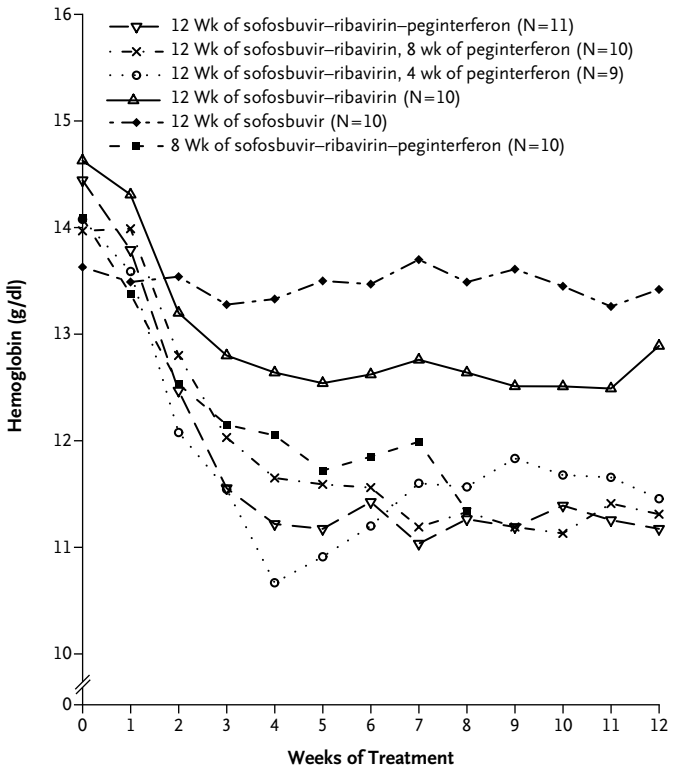

Figure 2. Mean Hemoglobin Level over Time during Treatment.

Data are shown for all patients with HCV genotype 2 or 3 infection for whom assessments were available. See Figure S2 in the Supplementary Appendix for data from patients with HCV genotype 1 infection.

and no response to prior therapy who had a relapse with the study treatment. No other mutations at conserved sites were consistently observed among the patients who had a relapse during this study.

ADVERSE EVENTS

All 95 patients completed treatment. The most common adverse events were headache, fatigue, insomnia, nausea, rash, and anemia (Table 3). One serious adverse event occurred during treatment. The event, urethral injury, occurred in a previously untreated patient with HCV genotype 1 infection who was receiving 12 weeks of treatment with sofosbuvir and ribavirin. Two other serious events occurred during follow-up: a previously untreated patient with HCV genotype 2 infection who received 12 weeks of treatment with sofosbuvir and ribavirin had an episode of furunculosis 13 days after the last day of treatment, and a previously untreated patient with HCV genotype 3 infection who received 8 weeks of treatment with sofosbuvir, peginterferon alfa-2a, and ribavirin had an episode of angina pectoris 24 days after the end of treatment.

Reduced levels of hemoglobin were common (Table 3 and Fig. 2, and Fig. S2 in the Supplementary Appendix). Hematologic abnormalities were more common among patients who received interferon than among those who did not (Table 3, and Fig. S2 and S3 in the Supplementary Appendix). Patients in the interferon-free groups generally had less severe reductions in hemoglobin level during treatment than did those in the groups that received peginterferon alfa-2a.

Likewise, neutropenia and thrombocytopenia were not observed in the groups that did not receive interferon. However, sofosbuvir monotherapy was associated with a modest decrease in the hemoglobin level (mean decrease, 0.54 g per deciliter), and 2 of 10 patients treated with sofosbuvir and ribavirin had grade 2 anemia. No patient had a grade 3 or 4 elevation of the total bilirubin level; 1 patient (in group 8) who had a relapse within 2 weeks after the end of treatment had a grade 3 elevation of the alanine aminotransferase level during week 4 after treatment.

DISCUSSION

In this study involving previously untreated and previously treated patients with HCV genotype 1, 2, or 3 infection, HCV RNA levels in all patients declined rapidly after the initiation of treatment. By week 4 of treatment, all 95 patients in the study had an undetectable level of HCV RNA, which was maintained until the end of treatment. The uniform response during treatment is notable because the eight treatment groups represented diverse patient populations — patients who had an infection with HCV genotype 1, 2, or 3; those who had not received prior treatment for HCV infection; and those who had not had a response to prior treatment with interferon and ribavirin. A total of 63% of patients had a non-CC IL28B genotype (CT or TT), which correlates with a poor response to treatment.[13] Of those patients with HCV genotype 1 infection, 89% were infected with HCV genotype 1a, which, when compared with HCV genotype 1b infection, is associated with lower rates of response to current telaprevir-based or boceprevir-based triple therapy.

All 50 previously untreated patients with HCV genotype 2 or 3 infection who received 8 or 12 weeks of treatment with sofosbuvir and ribavirin, with or without peginterferon-alfa 2a, had a sustained virologic response at 24 weeks after treatment. Results from the 10 patients with HCV genotype 2 or 3 infection who received sofosbuvir alone suggest a role for ribavirin in the maintenance of an antiviral response. Although all 10 patients had a rapid response and had an undetectable level of HCV RNA by week 4 of treatment, which was maintained for the duration of treatment, 4 patients had a relapse after the end of treatment. Sofosbuvir monotherapy led to a sustained virologic response in the other 6 patients. The exact mechanism by which ribavirin prevents relapse when added to interferon or direct-acting antiviral agents remains uncertain.[14,15]

The post-treatment response that was observed in patients with HCV genotype 1 infection differed according to their status with respect to prior treatment, with much higher rates of sustained virologic response among patients who had never received treatment for HCV infection, as compared with those who had not had a response to previous treatment with pegylated interferon plus ribavirin.

The absence of viral breakthrough in any patient during treatment in the study confirms that sofosbuvir has a high barrier to resistance. S282T, the only mutation that has been identified in vitro as having a reduced susceptibility to nucleotide NS5B inhibitors, including sofosbuvir,[16] has very poor replicative fitness, as indicated by its absence in untreated patients with HCV infection.[17,18] Although in vitro resistance is observed in the replicon model after only 3 to 5 days of monotherapy with nonnucleoside NS5B, NS3-4A, and NS5A inhibitors, no in vitro resistance has been observed after 7 to 14 days of monotherapy with various nucleoside or nucleotide analogues.[19-22] Finally, although mutations in the HCV NS3 and NS5B regions associated with resistance to protease and nonnucleoside inhibitors are present in more than 5% of previously untreated patients with HCV infection, the signature NS5B mutation associated with resistance to nucleoside polymerase inhibitors (S282T) is not detectable in such patients.[18]

The adverse events that occurred in the three groups of patients who received various durations of treatment with peginterferon in addition to sofosbuvir and ribavirin were generally those associated with peginterferon and ribavirin. The patients in the groups that did not receive interferon had fewer hematologic abnormalities than did those in the groups receiving peginterferon. No patients discontinued sofosbuvir or ribavirin in any group.

Previous studies have shown that interferon-free regimens with direct-acting antiviral agents can suppress HCV RNA. In the Interferon-free Regimen for the Management of HCV genotype 1 (INFORM-1) study, which involved patients with HCV genotype 1 infection who had not received previous therapy and those who had not had a response to prior therapy, treatment with the investigational nucleotide polymerase inhibitor mericitabine and the NS3-4A protease inhibitor danoprevir resulted in substantial, dose-dependent reductions in the HCV RNA level.[23]

In a phase 2a, open-label study conducted in Japan, 10 patients with HCV genotype 1b infection who had not had a response to prior treatment (<2 $\log_{10}$ reduction in HCV RNA level by week 12) received the NS5A replication complex inhibitor daclatasvir in combination with the NS3 protease inhibitor asunaprevir for 24 weeks.[24] All 9 patients who completed treatment had a sustained virologic response.

In another phase 2a study, patients with predominantly HCV genotype 1a infection who had not had a response to previous treatment received daclatasvir and asunaprevir with or without peginterferon alfa-2a and ribavirin for 24 weeks.[25] Although all 11 patients receiving daclatasvir plus asunaprevir without peginterferon and ribavirin had a rapid and dramatic decrease in the HCV RNA level during treatment, only 4 (36%) had a sustained virologic response; all 10 patients in the quadruple-therapy group had a sustained virologic response.

Preliminary results have also been reported from a phase 2a study evaluating sofosbuvir and daclatasvir for 24 weeks in previously untreated patients with HCV genotype 1, 2, or 3 infection.[26] Patients received sofosbuvir at a dose of 400 mg daily plus daclatasvir at a dose of 60 mg daily with or without ribavirin. All 44 patients with HCV genotype 1 infection had a sustained virologic response at 4 weeks after treatment. Among patients with HCV genotype 2 or 3 infection, rates of sustained virologic response at 4 weeks ranged from 88 to 100%.

In conclusion, sofosbuvir combined with ribavirin was associated with a sustained virologic

response in all previously untreated patients with HCV genotype 2 or 3 infection and in the majority of previously untreated patients with HCV genotype 1 infection. These early results potentially pave the way to address several unmet medical needs for such patients, who may benefit from a short-duration, all-oral regimen for HCV infection that does not include interferon. The pangenotypic antiviral efficacy of sofosbuvir supports the continued investigation of sofosbuvir with ribavirin alone or in combination with other direct-acting antiviral agents in various populations of patients with HCV infection.

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