Gastroenterology 2015;148:108–117

Sofosbuvir and Ribavirin for Treatment of Compensated Recurrent Hepatitis C Virus Infection After Liver Transplantation

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CLINICAL LIVER

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BACKGROUND & AIMS: Interferon alfa–based regimens used to treat recurrent hepatitis C virus (HCV) infection after liver transplantation are poorly tolerated, associated with generally modest efficacy, and can interact with immunosuppressive agents. We evaluated the efficacy and safety of an interferon-free regimen of the nucleotide polymerase inhibitor sofosbuvir combined with ribavirin for 24 weeks in treating post-transplantation HCV infection. **METHODS:** In a prospective, multicenter, open-label pilot study, we enrolled patients with compensated recurrent HCV infection of any genotype after a primary or secondary liver transplantation. All patients received 24 weeks of sofosbuvir 400 mg daily and ribavirin starting at 400 mg daily, which was adjusted according to creatinine clearance and hemoglobin values. The primary end point was sustained virologic response 12 weeks after treatment. **RESULTS:** Of the 40 patients enrolled and treated, 78% were male, 85% were white, 83% had HCV genotype 1, 40% had cirrhosis (based on biopsy), and 88% had been previously treated with interferon. Sustained virologic response 12 weeks after treatment was achieved by 28 of 40 patients (70%; 90% confidence interval: 56%−82%). Relapse accounted for all cases of virologic failure. No patients had detectable viral resistance during or after treatment. The most common adverse events were fatigue (30%), diarrhea (28%), and headache (25%). In addition, 20% of the subjects experienced anemia. Two patients discontinued study treatment because of adverse events, which were considered unrelated to study treatment. No deaths, graft losses, or episodes of rejection occurred. No interactions with any concomitant immunosuppressive agents were reported. **CONCLUSIONS:** Sofosbuvir and ribavirin combination therapy for 24 weeks is an effective and well-tolerated interferon-free treatment for post-transplantation HCV infection. EudraCT, Number: 2012-002417-19; ClinicalTrials.gov, Number: NCT01687270.

Keywords: Antiviral Agent; DAA; Clinical Trial; NS5B Polymerase Inhibitor.

Patients with detectable hepatitis C virus (HCV) RNA at the time of liver transplantation universally develop recurrent allograft infection post transplantation.[1] Recurrent HCV infection is the most common cause of mortality and graft loss after transplantation, and up to 30% of patients with recurrent infection develop cirrhosis within 5 years.[2] Post-transplantation patient and graft survival have been shown to be greatly improved by viral eradication,[3,4] but treatment options for recurrent HCV infection after liver transplantation are limited. Therapy with interferon-alfa and ribavirin results in low virologic response rates (especially for genotype 1 HCV infection), is poorly tolerated, and is associated with high rates of treatment discontinuation.[5–9] Adding the HCV protease inhibitors telaprevir or boceprevir to peginterferon and ribavirin increases the efficacy of treatment, but also the incidence and severity of adverse events, including mortality.[10,11] In a multicenter study, triple therapy with a protease inhibitor after liver transplantation was associated with anemia in 92% of patients, with one third requiring red blood cell transfusions, and frequent cyclosporine and tacrolimus dose reductions.[10] In a retrospective cohort study of 81 patients infected with HCV genotype 1 who received triple therapy after liver transplantation, 61% of patients achieved SVR, but 57% required blood transfusions, 27% were hospitalized, 15% discontinued because of adverse events, and 9% died.[11] Many of the severe

Abbreviations used in this paper: **AUC, area under the curve; CI, confidence interval; HCV, hepatitis C virus; LLOQ, lower limit of quantification; SVR12, sustained virologic response 12 weeks after treatment; ULN, upper limit of normal.**

0016-5085/$36.00
http://dx.doi.org/10.1053/j.gastro.2014.10.001

adverse events associated with these compounds post transplantation are a consequence of their potent inhibition of cytochrome P450 3A4 activity, which results in toxicity from the concurrent immunosuppressive agents.[12] A well-tolerated and effective treatment protocol for recurrence of HCV infection after liver transplantation is an important unmet clinical need.

Sofosbuvir is a potent inhibitor of the HCV NS5B polymerase. In combination with ribavirin, with or without peginterferon, sofosbuvir is indicated for the treatment of chronic HCV infection, based on results from phase 3 studies in patients infected with HCV genotypes 1–6.[13,14] Sofosbuvir has pangenotypic activity, a high genetic barrier to resistance, and a favorable safety profile. Most adverse reactions reported in clinical studies with sofosbuvir have been attributable to concurrent use of peginterferon or ribavirin,[15] but the safety of sofosbuvir in the post-transplantation setting has not yet been established. Sofosbuvir is administered orally once daily, has no clinically significant food effect,[16] and does not alter tacrolimus or cyclosporine concentrations in a clinically significant manner.[17] Coadministration of sofosbuvir and tacrolimus or cyclosporine does not affect GS-331007 (the primary analyte of interest) or sofosbuvir plasma concentrations in a clinically significant manner and does not necessitate sofosbuvir dose modification.[17]

We therefore evaluated the efficacy and safety of 24 weeks of sofosbuvir plus ribavirin in patients with recurrent hepatitis C of all genotypes after liver transplantation.

Methods

Patients

Eligible patients had chronic HCV (all genotypes) and were at least 18 years old, had HCV RNA plasma concentration of $\geq 10^4$ IU/mL, and had received a primary or secondary liver, or a combined liver and kidney transplant from a deceased or living donor. Liver transplantation was required to have taken place from 6 to 150 months before screening. Stage of fibrosis (METAVIR) and the absence of organ rejection were documented by a post-transplantation liver biopsy taken within 12 months of the first on-study treatment dose. Eligibility was restricted to patients with Child-Turcotte-Pugh scores ≤ 7 and Model for End-Stage Liver Disease scores ≤ 17, as this was the first study undertaken in this population with an anti-HCV nucleoside inhibitor. Patients with any of the following conditions or characteristics were excluded from participation: decompensated liver disease; heart or lung transplant; concurrent use of corticosteroids at any dose equivalent to >5 mg prednisone/d; HIV co-infection; hepatitis B virus co-infection; serum creatinine $>2.5 \times$ upper limit of normal (ULN); white blood cells $>20 \times 10^9$/L; absolute neutrophil count <1000 cells/mm^3; hemoglobin <10 g/dL; platelets $<25{,}000$/mm^3; bilirubin $\geq 4 \times$ ULN; alanine aminotransferase, aspartate aminotransferase, or alkaline phosphatase $\geq 10 \times$ ULN; or use of T-cell depleting or masking antibodies, systemic antineoplastic agents, cyclosporine >300 mg/d, sirolimus, or everolimus. All patients provided written informed consent before undertaking any study-related procedures.

Study Design

This was a multi-center, open-label study. Patients received sofosbuvir 400 mg and ribavirin 200–1200 mg orally every day for 24 weeks. After treatment, patients underwent follow-up for up to 48 weeks. The initial dose of ribavirin was 400 mg/d in 2 divided doses. If the hemoglobin level was ≥ 12 g/dL, ribavirin was increased as tolerated by 200 mg/d at weeks 2, 4, and up to every 4 weeks until the appropriate dose was reached. Target ribavirin dose was determined per label according to the patient's 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). If the hemoglobin level was 10–12 g/dL, the current dosing was continued. And if the hemoglobin level was 8–10 g/dL, the ribavirin dose was reduced by 200 mg/d. Erythropoiesis-stimulating agents were allowed during the study for patients whose hemoglobin levels fell to <10 g/dL. Erythropoiesis-stimulating agents use was to target a hemoglobin level sufficient to avoid transfusion. Ribavirin dosing was stopped if hemoglobin was <8 g/dL despite growth factor use, if bilirubin level was rising and had a direct fraction >10 mg/dL, or serum creatinine was >2.5 mg/dL.

Treatment was discontinued for patients with the following virologic criteria: confirmed HCV RNA plasma concentrations at or above the lower limit of quantification (LLOQ) after 2 consecutive HCV RNA plasma concentrations below the LLOQ, confirmed HCV RNA plasma concentrations >1 $\log_{10}$ increase from nadir, or HCV RNA plasma concentrations at or above the LLOQ through 8 weeks of treatment. In addition, treatment was stopped for patients with any of the following safety reasons: alanine aminotransferase or aspartate aminotransferase $>5 \times$ baseline or $5 \times$ nadir; alanine aminotransferase $>15 \times$ ULN; total bilirubin $>10 \times$ ULN; total bilirubin $>3 \times$ baseline or $3 \times$ nadir; any grade 2 or higher rash associated with constitutional symptoms; any nonlaboratory grade 4 event assessed as related to study treatment; progressing hepatic decompensation; or steroid-resistant acute allograft rejection.

The study protocol was approved by each institution's review board before study initiation. The study was conducted in accordance with the Declaration of Helsinki or the International Conference on Harmonization guidelines. The sponsor collected the data, monitored study conduct, and performed the statistical analyses. All authors had access to the data and assumed responsibility for the integrity and completeness of the reported data. The manuscript was prepared by Gilead Sciences with input from all authors. All authors approved the final manuscript.

Efficacy Assessments

Plasma samples for determining HCV RNA plasma concentration levels were drawn at screening; on days 1 and 3 of treatment; at weeks 1, 2, 3, 4, 8, 12, 16, 20, and 24 of treatment; and at follow-up weeks 2, 4, 8, 12, 24, and 48. Plasma HCV RNA concentration was analyzed by using the Roche COBAS TaqMan HCV Test, v2.0 for use with the High Pure System (Roche Molecular Systems, Inc., Branchburg, NJ), which has an LLOQ of 25 IU/mL.

Pharmacokinetic Assessments

A single blood sample was collected from patients at all on-treatment visits for pharmacokinetic analyses of sofosbuvir and ribavirin. Plasma concentrations of sofosbuvir, GS-331007

(ie, renally eliminated, predominant circulating metabolite of sofosbuvir, and the primary analyte of interest in clinical pharmacology studies), and ribavirin were determined by validated liquid chromatography tandem mass spectrometry assays. Steady-state pharmacokinetic parameters of sofosbuvir, GS-331007, and ribavirin were estimated by population pharmacokinetic modeling.[13,14] Mean daily ribavirin area under the curve (AUC_{24}) was calculated for each patient based on ribavirin dose modifications. Steady-state exposure (AUC_{tau}: AUC over the dosing interval) of GS-331007 and sofosbuvir were estimated for each patient.

Resistance Monitoring

Plasma samples were collected at baseline/day 1 and at each visit for viral sequence analysis and possible phenotypic testing. For all patients who experienced virologic failure, deep sequencing of the NS5A and NS5B regions was conducted at both baseline and at the time of failure.

Safety Assessments

Safety data were collected during treatment and for up to 30 days after the last dose of study drug. The data included reported adverse events, physical examinations, clinical laboratory tests, vital signs, and electrocardiograph recordings. Concomitant medication intake was also recorded. Treatment-emergent clinical and laboratory adverse events were summarized using a standardized scale (see Supplementary Table 4).

End Points and Statistical Analyses

The primary efficacy end point was the percentage of subjects with SVR12, defined as HCV RNA below the LLOQ (25 IU/mL) 12 weeks after stopping study drug. No inferential statistics or statistical comparisons were planned for efficacy end points. Along with the percentage of patients with SVR12, a 2-sided exact 90% confidence interval (CI) was constructed by using the Clopper-Pearson method. With a sample size of 40 patients, if the SVR12 rate was 50%, the lower bound of a 1-sided 95% CI (ie, same as lower bound of 2-sided 90% CI) would be 36.1%.

Results

Study Population

Of the 49 patients screened, 40 patients were enrolled (see Supplementary Table 1 for reasons for screen failure) and treated between October 2012 through February 2014 at 12 international study sites (8 in the United States, 1 in Germany, 1 in France, 1 in New Zealand, and 1 in Spain). Baseline characteristics of the patient population are given in Table 1. The majority of patients were white (85%), male (78%), and had undergone prior HCV treatment (88%). Of the 35 patients who had received prior treatment, 11 were treated before transplantation, 18 were treated after transplantation, 5 were treated before and after transplantation, and 1 was likely treated before and after transplantation, but this could not be confirmed. Nine of the 35 previously treated patients (26%) had received triple therapy with a protease inhibitor. Mean age was 59 years. Eighty-three percent of patients were infected with HCV genotype 1 and 80% had HCV RNA plasma concentration levels that were $\geq$6 $\log_{10}$ IU/mL. At baseline, 40% of patients had cirrhosis (METAVIR equivalent score of F4). Median time since prior liver transplantation was 4.3 years (range, 1.0–10.6 years). The majority of patients were receiving tacrolimus (70%) for maintenance immunosuppression.

Efficacy

Antiviral Response. HCV RNA plasma concentration levels declined rapidly upon initiation of treatment, from a mean of 6.55 $\log_{10}$ IU/mL at baseline to 2.43 $\log_{10}$ IU/mL after 1 week of treatment. All patients receiving sofosbuvir and ribavirin had HCV RNA plasma concentration below the LLOQ by week 4 of therapy. Of the 40 patients who were treated with sofosbuvir and ribavirin, 28 (70%, 90% CI: 56%−82%) achieved SVR12 (Table 2). Twelve patients (30%) experienced virologic relapse after the end of treatment: seven relapsed by follow-up week 2, four at follow-up week 4, and one at follow-up week 12. All 28 patients who achieved SVR12 also had HCV RNA plasma concentration below the LLOQ at 24 weeks after stopping therapy (SVR24).

Patients' virologic response by other baseline characteristics is shown in Table 3 and Supplementary Table 2. Although the study was not powered for subgroup comparisons, patients with a number of characteristics that have been found to be associated with reduced response to interferon-based therapy appear to have had numerically lower rates of response to sofosbuvir and ribavirin in the post-transplantation setting: male sex, presence of cirrhosis, and non-CC IL28B genotypes. In contrast to outcomes with interferon-based treatment, patients infected with HCV genotype 1a had numerically higher rates of SVR than patients with HCV genotype 1b (73% vs 55%). The small number of black patients (n = 3) and those with HCV genotype 3 (n = 6) all achieved SVR. High or low baseline viral load did not appear to be associated with any differences in response. Supplementary Table 3 shows characteristics of the 12 patients who relapsed. All but one were white men, and all but one had HCV genotype 1 infection.

Resistance Monitoring. Baseline NS5B deep sequencing was successfully obtained for 39 of the 40 patients enrolled in the study. The NS5B S282T variant, which is associated with reduced susceptibility to sofosbuvir, was not detected in any patient at baseline or any other time point. At baseline, only one patient had a variant associated with resistance to nucleotide inhibitor treatment. This patient, who had L159F (>99%) that was observed with C316N (a variant associated with resistance to non-nucleoside inhibitors) >99%, achieved HCV RNA <25 IU/mL at early termination visit and then was lost to follow-up. A total of 12 patients experienced viral relapse. Low levels of V321A (3.95%), a sofosbuvir treatment-emergent variant with no phenotypic resistance to sofosbuvir, were detected in one patient with HCV genotype 1a who relapsed. Phenotypic analysis of the NS5B gene, which was successfully performed for 10 of the 12 patients who relapsed, did not show any evidence of reduced susceptibility to sofosbuvir or ribavirin.

Table 1. Baseline Characteristics of the Study Population

Baseline characteristics	Sofosbuvir + ribavirin for 24 weeks (n = 40)
Age, *y*, median (range)	59 (49–75)
Male, n (%)	31 (78)
Race, n (%)	
White	34 (85)
Black	3 (8)
Asian	2 (5)
Other[a]	1 (3)
BMI <30, kg/m^2, n (%)	30 (75)
Genotype, n (%)	
1a	22 (55)
1b	11 (28)
2	0
3	6 (15)
4	1 (3)
HCV RNA, log_{10} *IU/mL*, median (range)	6.74 (4.49–7.59)
Hemoglobin, *g/dL*, median (range)	13.2 (10.4–17.5)
Platelet count, $\times 10^3/\mu L$, median (range)	124 (46–348)
Albumin, *g/dL*, median (range)	3.8 (2.8–4.8)
Total bilirubin, *mg/dL*, median (range)	0.8 (0.4–2.5)
INR, median (range)	1.0 (0.9–2.5)
ALT, *U/L*, median (range)	68 (16–391)
AST, *U/L*, median (range)	79 (30–319)
Creatinine clearance, *mL/min*, median (range)[b]	79.8 (39.1–171.5)
Prior HCV treatment, n (%)	35 (88)
Peginterferon or interferon only, n (%)	3/35 (9)
Peginterferon and ribavirin, n (%)	22/35 (63)
Protease inhibitor plus peginterferon and ribavirin, n (%)	9/35 (26)
Other plus peginterferon and ribavirin, n (%)	1/35 (3)
Response to last HCV regimen, n (%)	
Breakthrough	4/35 (11)
Relapse	9/35 (26)
Partial (≥2 log drop at week 12)	7/35 (20)
Nonresponse (<2 log drop at week 12)	11/35 (35)
Unknown	4/35 (11)
IL28B, n (%)	
CC	13 (33)
CT	16 (40)
TT	11 (28)
METAVIR-equivalent fibrosis stage, n (%)	
None or minimal (F0)	1 (3)
Portal fibrosis (F1−F2)	14 (35)
Bridging fibrosis (F3)	9 (23)
Cirrhosis (F4)	16 (40)
Years since liver transplantation, median (range)	4.3 (1.0–10.6)
Child-Pugh score	
5	24 (60)
6	12 (30)
7	4 (10)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; INR, international normalized ratio.
[a]Patient was of mixed white/Asian race.
[b]Estimated by Cockcroft-Gault.

Pharmacokinetic Assessments

GS-331007, the major circulating metabolite of sofosbuvir, and sofosbuvir AUC_{tau} were increased (96% and 26%, respectively) in the post-transplantation setting relative to a phase 2/3 historical control population, irrespective of concomitant cyclosporine-based therapy. Additionally, mean GS-331007 and sofosbuvir AUC_{tau} were comparable between patients achieving SVR12 or those experiencing relapse (Figure 1). Similarly, the mean daily ribavirin dose and AUC_{24} did not differ in those with SVR vs relapse (Figure 2).

Safety

There were no deaths, graft losses, or episodes of rejection. Six patients experienced grade 3 adverse events;

Table 2. Hepatitis C Virus RNA <25 IU/mL During and After Treatment

	Sofosbuvir + ribavirin (n = 40)
During treatment, n (%)	
Day 3	1/40 (3)
Week 2	23/40 (58)
Week 4	40/40 (100)
Week 12	40/40 (100)
Week 24	38/38 (100)[a]
After treatment, n (%)	
Week 2	33/40 (83)
Week 4	29/40 (73)
Week 8	29/40 (73)
Week 12	28/40 (70)
90% CI, %[b]	56–82
Week 24, n (%)	28/40 (70)
90% CI, %[b]	56–82

[a]Two patients discontinued treatment because of adverse events (hepatocellular carcinoma and pneumonia) and did not have HCV RNA plasma concentration collected at week 24. These patients, who discontinued treatment at weeks 16 of treatment and week 2 of follow-up, respectively, had HCV RNA <25 IU/mL at the time of discontinuation.
[b]The 2-sided exact 90% confidence interval is based on the Clopper-Pearson method.

one of the events, fatigue, was reported as being treatment related. Six patients had 10 serious adverse events (Table 4); all of these events were considered unrelated to study treatment. Two patients had adverse events leading to treatment discontinuation; the events—pneumonia (on study day 106) and hepatocellular carcinoma (on study day 113—were considered unrelated to study treatment.

Ribavirin dose increases as outlined were generally well tolerated. At week 4, the median ribavirin dose was 600 mg/d, and at weeks 8 through 24, the median dose was 800 mg/d (except at week 16, when it was 600 mg/d).

Most subjects had at least one laboratory abnormality during the study. Maximum post-baseline grade 3 and grade 4 laboratory abnormalities were reported in 11 patients (27.5%) each. Consistent with an immunosuppressed post-transplantation population, lymphopenia was the most common hematologic abnormality, with 35% of patients (14 of 40) experiencing a grade 3 (350 to $<500/mm^3$) or a grade 4 ($<350/mm^3$) abnormalities. Creatinine levels were stable during treatment and follow-up: median serum creatinine levels at baseline and week 24 were 1.13 and 1.16 mg/dL, respectively. Consistent with the expected safety profile of ribavirin, decreases in hemoglobin during treatment were common. The median hemoglobin level declined from 13.2 g/dL (range, 10.4–17.5 g/dL) at baseline to 11.1 g/dL (range, 9.4–14.3 g/dL) at week 20, but rose to 12.6 g/dL (range, 9.3–15.6 g/dL) by week 4 of follow-up. One third of patients had at least one post-baseline hemoglobin value of <10 g/dL. Although only one patient had a hemoglobin nadir of <8.5 g/dL, 11 patients (28%) required a ribavirin dose reduction. In addition, 8 patients (20%) received epoetin (n = 5) and/or blood products (n = 6) based on investigator discretion (Figure 3).

Sofosbuvir had no reported interactions with any of the concomitant immunosuppressive agents, which included tacrolimus (28 patients [70%]), mycophenolate (14 patients

Table 3. Characteristics of Patients by Response

Characteristics	Relapse patients (n = 12)	Patients who achieved SVR12 (n = 28)
Age, *y*, median (range)	60 (49–75)	59 (49–71)
Male, n (%)	11 (92)	20 (71)
Race, n (%)		
Black	0	3 (11)
White	11 (92)	23 (82)
Asian	0	2 (7)
Other	1 (8)	0
BMI, kg/m^2, median (range)	26.8 (22.6–36.4)	26.1 (20.7–42.5)
HCV RNA, log_{10} *IU/mL*, median (range)	6.7 (5.5–7.4)	6.8 (4.5–7.6)
Creatinine clearance, *mL/min*, median (range)	85.1 (51.4–112.5)	75.1 (39.1–171.5)
HCV genotype, n (%)		
1a	6 (50)	16 (57)
1b	5 (42)	6 (21)
3	0	6 (21)
4	1 (8)	0
IL28B, n (%)		
CC	3 (25)	10 (36)
Non-CC	9 (75)	18 (64)
Baseline METAVIR score, n (%)		
F0	0	1 (4)
F1–2	4 (33)	10 (36)
F3	2 (17)	7 (25)
F4	6 (50)	10 (36)
Years from transplantation, median (range)	3.6 (1.3–9.4)	4.5 (1.0–10.6)

BMI, body mass index.

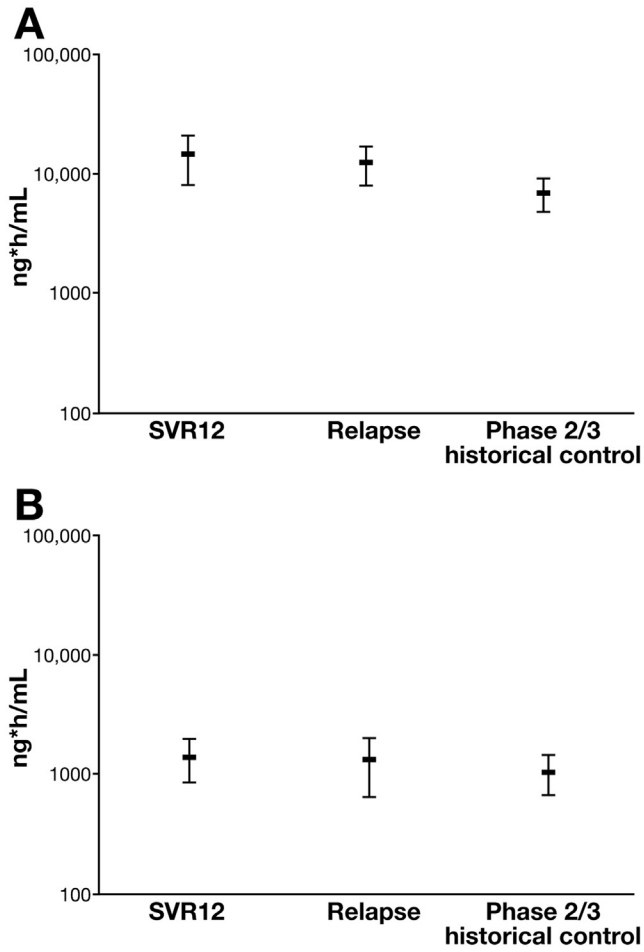

Figure 1. GS-331007 and sofosbuvir AUC_{tau} in patients with SVR12 and relapse. (*A*) GS-331007 AUC_{tau} in patients with SVR12 and relapse vs historical control, mean (SD). (*B*) Sofosbuvir AUC_{tau} in patients with SVR12 and relapse vs historical control, mean (SD).

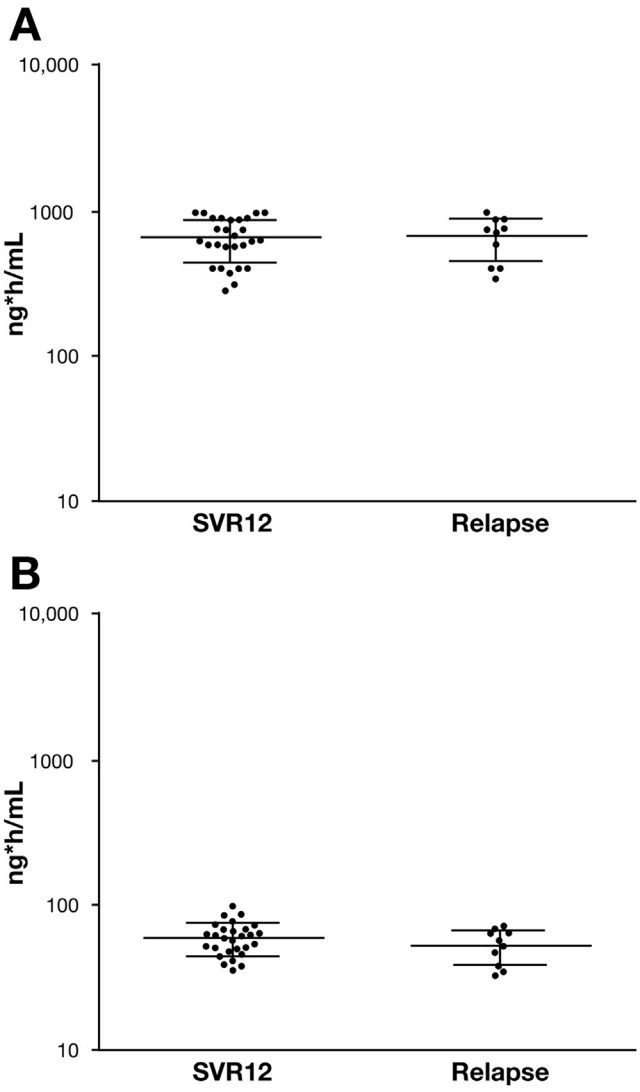

Figure 2. Ribavirin (RBV) mean daily dose and AUC_{tau} in patients achieving SVR12 vs relapse. (*A*) RBV mean daily dose in patients achieving SVR12 vs relapse, mean (SD). (*B*) RBV AUC_{tau} in patients achieving SVR12 vs relapse, mean (SD).

[35%]), prednisone (11 patients [28%]), cyclosporine (10 patients [25%]), and azathioprine (2 patients [5%]). Eight patients required increased tacrolimus dosing during sofosbuvir and ribavirin therapy. Four patients had tacrolimus dose increases of 40%−50% (1.5–3 mg twice a day) in the first 90 days of treatment. Five patients required reductions in tacrolimus dosing while on study treatment, all per institutional protocol, with the exception of one patient whose dose was reduced due to tacrolimus toxicity. Four patients required reductions in cyclosporine while on study treatment, all per institutional protocol, with the exception of one patient with renal impairment.

Discussion

Liver failure and hepatocellular carcinoma related to HCV infection have been the most common indications for liver transplantation in the 2 decades since the discovery of the hepatitis C virus.[18] Unfortunately, the benefits of liver transplantation can be short-lived, because patients with detectable HCV RNA at the time of transplantation universally experience recurrence of HCV infection, which is the most common cause of graft failure.[19] With increasing pressure on a diminishing supply of donor organs, there is a growing need to attenuate the impact of HCV recurrence on graft survival and other important post-transplantation outcomes. An ideal therapy for post-transplantation HCV infection would have 4 core attributes: a high degree of efficacy, good tolerability, lack of interaction with commonly administered immunosuppressive agents, and absence of potentiating allograft rejection. Pegylated interferon-alfa, ribavirin, telaprevir, and boceprevir have been of limited efficacy and tolerability in studies of liver transplant recipients with recurrent HCV.[10,11,20] In a recent retrospective study of post-transplantation treatment of recurrent HCV infection, treatment with telaprevir or boceprevir with peginterferon and ribavirin resulted in an overall SVR rate of 63%, with 9% mortality and many nonlethal adverse events.[11] A safer and more tolerable treatment strategy has been eagerly awaited.

Table 4. Treatment-Emergent Adverse Events and Laboratory Abnormalities

Adverse events and laboratory abnormalities	Sofosbuvir + ribavirin (n = 40)
Patients with any adverse event, n (%)	39 (98)
Patients with any serious adverse event, n (%)	6 (15)
Adverse event leading to discontinuation, n (%)	2 (5)
Deaths, n	0
Adverse events occurring in at least 10% of patients, n (%)	
Fatigue	12 (30)
Diarrhea	11 (28)
Headache	10 (25)
Arthralgia	9 (23)
Nausea	8 (20)
Anemia	8 (20)
Cough	7 (18)
Insomnia	5 (13)
Anxiety	5 (13)
Asthenia	4 (10)
Dyspnea	4 (10)
Irritability	4 (10)
Vomiting	4 (10)
Muscle spasms	4 (10)
Serious adverse events, n (%)[a]	
Pyrexia	2 (5)
Ascites	1 (3)
Jaundice	1 (3)
Pneumonia	1 (3)
Urinary tract infection	1 (3)
Hemarthrosis	1 (3)
Osteoporotic fracture	1 (3)
Confusional state	1 (3)
Hallucination	1 (3)
Laboratory abnormalities, n (%)	
Hemoglobin $<$10 g/dL	13 (33)
Hemoglobin $<$8.5 g/dL	1 (3)
Lymphocytes 0.35 to $<0.5 \times 10^3/\mu$L	5 (13)
Lymphocytes $<0.35 \times 10^3/\mu$L	9 (23)
Serum glucose 250 to 500 mg/dL	3 (8)
Serum glucose $\geq$500 mg/dL	1 (3)
White blood cells 1 to $<1.5 \times 10^3/\mu$L	3 (8)
Total bilirubin $>$6 mg/dL	1 (3)

[a]One patient was diagnosed with hepatocellular carcinoma, which did not meet the criteria for serious adverse event.

The most important result of our current study is that, on an intention-to-treat basis, 24 weeks of sofosbuvir and ribavirin without interferon led to SVR12 (and SVR24) in 70% of patients (28 of 40). The SVR12 rate of 70% is similar to that achieved in clinical trials using the same dose and duration of sofosbuvir and ribavirin in the non-transplantation setting.[13,21–23] The most directly comparable data, however, were generated by Osinusi et al[22] in a study in which the combination of sofosbuvir plus ribavirin without peginterferon was evaluated in 60 treatment-naïve patients with HCV genotype 1 with unfavorable treatment characteristics (eg, advanced fibrosis). In that study, 50 participants with any stage of liver fibrosis were randomized to 400 mg sofosbuvir with weight-based or low-dose ribavirin (600 mg daily, similar to that used in our post-transplantation study) for 24 weeks. The SVR24 rate was 68% (17 of 25) in the weight-based group and 48% (12 of 25) in the low-dose group. Among participants with advanced fibrosis, 7 of the 13 (54%), including all 4 with cirrhosis, experienced relapse, suggesting optimal ribavirin dosing may be important. In light of these results reported in the nontransplantation setting, the 70% SVR12 observed in our immunosuppressed cohort of liver transplant recipients with a high prevalence of advanced fibrosis/cirrhosis and treatment experience and low ribavirin dosing is encouraging. As ribavirin is almost exclusively excreted renally, our study population of liver transplant recipients, which included patients with impaired renal function and ubiquitous use of calcineurin inhibitors that decrease glomerular filtration, is likely to have had relatively greater ribavirin exposure than would have been predicted based on ribavirin dosing alone. The mean daily ribavirin exposure (AUC_{0-24}; 57.2 h*μg/mL) observed in this study is comparable with mean exposure observed in HCV-infected subjects with normal renal function after administration of 1200 mg/d ribavirin (2 times steady-state ribavirin AUC_{0-12}: 25.4 h*μg/mL).[24]

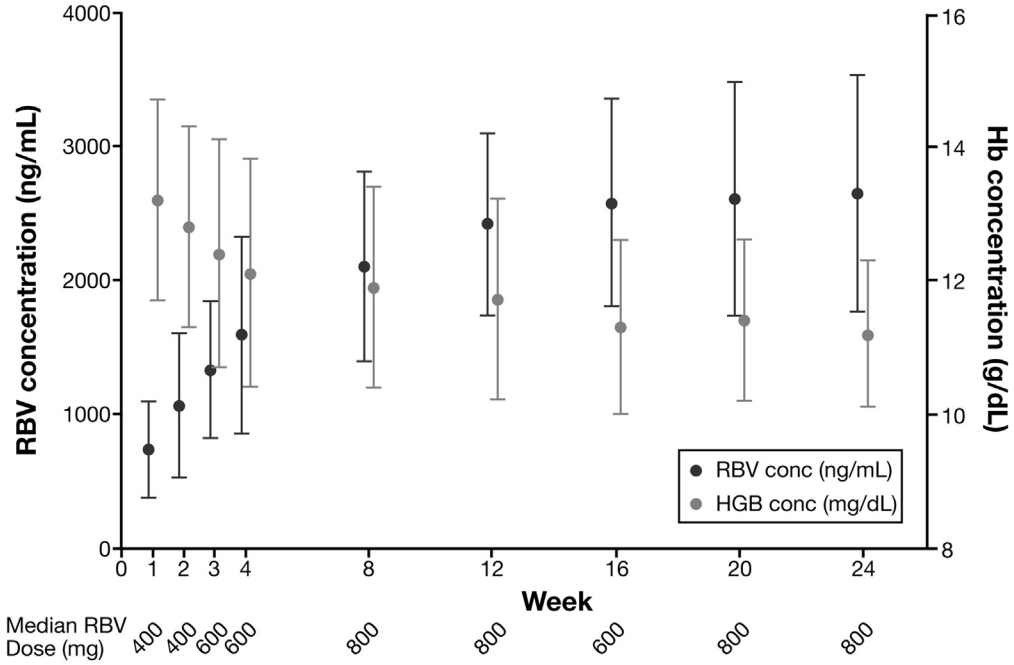

Figure 3. Ribavirin (RBV) concentrations and hemoglobin levels by study visit, mean (SD).

The NS5B S282T variant, which is known to have reduced susceptibility to sofosbuvir, was not observed in this study, confirming the high genetic barrier to resistance of this drug. Low levels of V321A (3.95%), a sofosbuvir treatment-emergent variant with no phenotypic resistance to sofosbuvir, were detected in one patient infected with HCV genotype 1a. Phenotypic analysis of the NS5B gene of 10 relapsers for whom data are available did not show reduced susceptibility to sofosbuvir or ribavirin.

The kinetics of HCV RNA decline in this study were also notable because the impact of immunosuppression and impairment of host adaptive immunity could not be predicted. Sofosbuvir led to rapid and substantial declines in HCV RNA plasma concentrations in all patients, such that 100% of patients had HCV RNA plasma concentrations <25 IU/mL by week 4 of treatment. The rapid decline in HCV RNA plasma concentrations was comparable with that seen with sofosbuvir-based therapy in nontransplantation patients, of which 97%−100% had undetectable HCV RNA plasma concentration by week 4 of therapy.[13,14] Despite some impairment of host immunity, brought about with the immunosuppressive agents used in this study, there did not appear to be a discernible effect on HCV kinetics. In this study, no resistance to sofosbuvir or viral breakthrough was detected.

Liver transplant recipients have consistently exhibited a high frequency of side effects during treatment with interferon, ribavirin, boceprevir, or telaprevir. These side effects, especially cytopenias, limit the utility of these agents for treating recurrent HCV infection. The second important observation of this study is that the combination of sofosbuvir and ribavirin therapy had a favorable safety profile in this population, with no deaths, graft losses, episodes of rejection or immunological dysfunction observed. In contrast, interferon-based treatments have been associated with post-treatment immunologic dysfunction (particularly plasma cell hepatitis) and even hepatic decompensation in liver transplant recipients.[25–27] The safety profile of sofosbuvir and ribavirin is in contrast with that recently reported in 2 multicenter studies of telaprevir or boceprevir with peginterferon and ribavirin, with both studies reporting near universal need for dose reductions of interferon and ribavirin related to adverse events and treatment-related mortality (3%−8%).[10,28] Notably, sofosbuvir exposure was minimally altered in the post-transplantation setting in the context of concomitant cyclosporine-based therapy in contrast to the results of a prior drug–drug interaction study, where a high dose of cyclosporine (600 mg) produced a 4.5-fold increase in sofosbuvir exposure, which was not considered clinically meaningful.[17] These results provide context to the potential for a drug–drug interaction between sofosbuvir and cyclosporine in a clinical setting and support their co-administration. Increases in GS-331007 levels (<2-fold compared with prior phase 2/3 study population) were observed in this study and are explained by impaired renal function in the study population. Because the metabolism of tacrolimus and cyclosporine are dependent on hepatic function, which may be affected by eradication of HCV infection, levels of tacrolimus and cyclosporine may be dynamic during and after successful treatment of HCV infection. We advocate vigilant monitoring of trough levels of calcineurin inhibitors (eg, weekly or every other week) during and after treatment with direct-acting antiviral agents.

We had anticipated substantial reductions in hemoglobin levels during treatment, which are nearly universal in liver transplant recipients treated with peginterferon and ribavirin-based therapies. To mitigate this, a low initial dose (400 mg/d) of ribavirin was utilized, with doses increased according to hemoglobin levels. Using this approach, the

median ribavirin dose was increased from 600 mg/d at week 4 to 800 mg/d at weeks 8 to 24 (with the exception of week 16, where median dose was 600 mg/d) of treatment; However, despite this slow and deliberate dose-escalation protocol, one quarter of patients still required ribavirin dose reductions, and anemia precluded full ribavirin dosing in the majority of patients. There were no substantial differences in ribavirin AUC_{24} between patients achieving SVR12 and those experiencing relapse. Renal insufficiency has emerged as a common event during treatment with telaprevir or boceprevir with peginterferon and ribavirin, despite stable whole trough levels of calcineurin inhibitors.[7,24] Creatinine levels remained stable throughout treatment with sofosbuvir and ribavirin.

Finally, it is important to consider the impact of sofosbuvir and ribavirin on co-administered immunosuppressive agents. Both cyclosporine and tacrolimus are substrates of cytochrome P450 3A and P-glycoprotein, neither of which are inhibited or induced by sofosbuvir. No net directional changes in trough levels of cyclosporine or tacrolimus were observed during the study. The lack of effect of sofosbuvir on the metabolism of immunosuppressive agents is an important factor for the tolerability, safety and, hence, efficacy of sofosbuvir in the post-transplantation setting.

There are several limitations that should be considered in the interpretation of this study. The main limitation is sample size, which is insufficiently large to allow rigorous subgroup comparisons and characterization of efficacy in certain subpopulations (eg, black patients, HCV genotype 3). In addition, no patients infected with HCV genotype 2 were enrolled. In addition, our population consisted of patients with well-compensated liver disease. A recent study in which 12 patients with severe recurrent hepatitis C after liver transplantation (including 3 with fibrosing cholestatic hepatitis and 9 with cirrhosis) received sofosbuvir and daclatasvir with and without ribavirin concluded that optimal outcomes require initiation of treatment before decompensation.[29] Finally, the array of direct-acting antiviral agents approved for the treatment of HCV infection is evolving rapidly. The likely approval of new direct-acting antiviral therapies is inevitable. The impending availability of newer agents does not negate the importance of this study, as we anticipate that sofosbuvir will remain a cornerstone of post-transplantation antiviral therapy. In addition, the pharmacokinetic and drug–drug interaction aspects of this study are likely to be of enduring relevance.

In summary, treatment with the all-oral regimen of sofosbuvir and ribavirin for 24 weeks resulted in a SVR rate of 70% among patients who experienced recurrence of HCV infection after liver transplantation. This population, which includes a high proportion of patients with characteristics that have historically been difficult to cure with interferon-based regimens—HCV genotype 1, prior treatment experience, advanced fibrosis and cirrhosis, and concurrent immunosuppression—may benefit from this all-oral therapy. In addition, the addition of other potent oral antiviral agents to sofosbuvir-based regimens may allow for even higher efficacy rates with shorter total durations of therapy, even in immunosuppressed liver transplant recipients. Such studies are currently underway.

Supplementary Materials

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.2014.10.001.

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Received July 29, 2014. Accepted October 2, 2014.

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Acknowledgments
The authors thank the patients and their families, as well as the investigators and site personnel.

In addition, the authors thank Meredith Gonzalez and Kimberly Boynton of Gilead Sciences for their contributions to study conduct. Writing assistance was provided by Jennifer King of August Editorial and David McNeel of Gilead Sciences.

Conflicts of interest
These authors disclose the following: Michael Charlton has received grant and research support from Gilead, Merck, Janssen, and Novartis and is a consultant for Gilead, Janssen, AbbVie, BMS, and Sanofi-Aventis. Edward Gane is on the advisory boards of Gilead, Novartis, Merck, Achillion, Idenix, and AbbVie and is a speaker for Gilead, Achillion, Novartis, and AbbVie. Robert S. Brown Jr has received grant and research support from Gilead and is a consultant for Gilead. Michael P. Curry has received grant and research support from Gilead, Salix, Merck, and MassBiologics, is on the advisory board of Gilead, and is a consultant for Gilead. Paul Y. Kwo has received grant and research support from AbbVie, BMS, Conatus, Janssen, Merck, Roche, and Vertex and is a consultant for AbbVie, BMS, Boehringer Ingelheim, GSK, Janssen, Merck, Novartis, and Vertex. Robert J. Fontana has received grant and research support from Vertex, Gilead, BMS, and Janssen. Richard Gilroy has been a speaker for Gilead, NPS, and Salix. Andrew J. Muir has received grant and research support from AbbVie, Achillion, BMS, Gilead, Merck, Roche, Santarus, and Vertex and is on the advisory boards of AbbVie, BMS, Gilead, and Merck. Didier Samuel is a consultant for Astellas, BMS, Gilead, Janssen-Cilag, LFB, MSD, Novartis, Roche, and Biotest. Xavier Forns has received grant and research support from Merck, Roche and is a consultant for AbbVie, Gilead, and Janssen. Norah A. Terrault has received grant and research support from Gilead and is on the advisory board at Gilead. All other authors are current employees of Gilead Sciences.

Funding
This work was supported by Gilead Sciences, Inc.