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# CLINICAL—LIVER

## Sofosbuvir and Ribavirin Prevent Recurrence of HCV Infection After Liver Transplantation: An Open-Label Study

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**BACKGROUND & AIMS:** Patients with detectable hepatitis C virus (HCV) RNA at the time of liver transplantation universally experience recurrent HCV infection. Antiviral treatment before transplantation can prevent HCV recurrence, but existing interferon-based regimens are poorly tolerated and are either ineffective or contraindicated in most patients. We performed a trial to determine whether sofosbuvir and ribavirin treatment before liver transplantation could prevent HCV recurrence afterward. **METHODS:** In a phase 2, open-label study, 61 patients with HCV of any genotype and cirrhosis (Child–Turcotte–Pugh score, $\leq$7) who were on waitlists for liver transplantation for hepatocellular carcinoma, received up to 48 weeks of sofosbuvir (400 mg) and ribavirin before liver transplantation. The primary end point was the proportion of patients with HCV-RNA levels less than 25 IU/mL at 12 weeks after transplantation among patients with this HCV-RNA level at their last measurement before transplantation. **RESULTS:** Sixty-one patients received sofosbuvir and ribavirin, and 46 received transplanted livers. The per-protocol efficacy population consisted of 43 patients who had HCV-RNA level less than 25 IU/mL at the time of transplantation. Of these 43 patients, 30 (70%) had a post-transplantation virologic response at 12 weeks, 10 (23%) had recurrent infection, and 3 (7%) died (2 from nonfunction of the primary graft and 1 from complications of hepatic artery thrombosis). Of all 61 patients given sofosbuvir and ribavirin, 49% had a post-transplantation virologic response. Recurrence was related inversely to the number of consecutive days of undetectable HCV RNA before transplantation. The most frequently reported adverse events were fatigue (in 38% of patients), headache (23%), and anemia (21%). **CONCLUSIONS:** Administration of sofosbuvir and ribavirin before liver transplantation can prevent post-transplant HCV recurrence. ClinicalTrials.gov: NCT01559844.

*Keywords:* Hepatitis C Virus; Liver Transplantation; HCV Recurrence; Direct-Acting Antiviral Agents.

Liver disease resulting from chronic hepatitis C virus (HCV) infection is the leading indication for liver transplantation in the United States, Europe, and Japan.[1,2] Between 1995 and 2010 there were 126,862 new registrants for primary liver transplantation in the United States, of which more than 52,000 (41%) had HCV-associated liver disease, primarily cirrhosis and hepatocellular carcinoma (HCC).[3] For patients with detectable HCV-RNA levels at the time of transplantation, postoperative recurrence of HCV infection was "immediate and universal."[4] Recurrent HCV infection follows an aggressive course: 10%–30% of patients with recurrent HCV after transplantation develop cirrhosis within 5 years, and more than 40% develop cirrhosis within 10 years.[5,6] Rates of graft loss and patient mortality also are markedly higher for patients with recurrent HCV than for uninfected patients,[5] and retransplantation frequently is associated with a poor outcome.[7]

***Abbreviations used in this paper:*** **HCC, hepatocellular carcinoma; HCV, hepatitis C virus; LLOQ, lower limit of quantification; MELD, model for end-stage liver disease; pTVR, post-transplantation virologic response.**

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There is currently no safe and broadly effective treatment to prevent recurrence of HCV infection after liver transplantation. Antiviral therapy either before or immediately after liver transplantation has been studied, but results from clinical studies have been mixed.[8] Trials of pretransplantation antiviral therapy with interferon and ribavirin have prevented HCV recurrence in only 20%–28% of patients.[9–13] Moreover, interferon-based treatment is poorly tolerated, and is associated with life-threatening infections and decompensation. Up to a third of patients discontinue interferon-based treatment because of adverse events.[13–15]

Sofosbuvir is a nucleotide polymerase inhibitor of NS5B-directed HCV-RNA replication. In clinical trials, administration of sofosbuvir with ribavirin was associated with rapid decreases of HCV RNA to undetectable levels in patients with HCV genotype 1, 2, 3, 4, and 6 infections.[16,17] In more than 3000 patients treated to date, sofosbuvir has been shown to be safe, viral breakthrough during treatment has been rare (and associated with nonadherence), and few drug interactions have been observed.[18,19] In addition, patients with hepatic impairment do not require modification of sofosbuvir dosage.[20]

We conducted this open-label study to determine if the administration of up to 48 weeks of sofosbuvir and ribavirin to HCV-infected patients with liver cancer before liver transplantation could prevent post-transplant recurrence of HCV infection.

## Materials and Methods

### Patients

Patients were enrolled at 13 centers in the United States, 1 in New Zealand, and 1 in Spain. Eligible patients were at least 18 years old with a body mass index of $\geq$18 kg/m$^2$ and documented HCV infection of any genotype with an HCV-RNA value greater than $10^4$ IU/mL. Patients who had failed previous treatment for HCV were eligible. Patients were required to be on the waiting list for liver transplantation (with anticipated time until transplantation of $<$1 y) from a deceased donor. Patients had HCC meeting the Milan criteria,[21] with a biological model for end-stage liver disease (MELD) score of less than 22, and a Child–Turcotte–Pugh score of 7 or less, and had to be eligible for a MELD exception score as per the policy of the United Network for Organ Sharing. We chose to study patients with HCC because they could be expected to undergo liver transplantation within 1 year.

### Study Design

This phase 2, open-label, pilot study had 2 phases: a pretransplant treatment phase and a post-transplant follow-up phase. During the pretransplant treatment phase, patients received sofosbuvir (Gilead Sciences, Foster City, CA) administered orally at a dose of 400 mg once daily, along with ribavirin (Ribasphere; Kadmon, Warrendale, PA) administered orally as a divided dose according to body weight (1000 mg/day in patients with a body weight of $<$75 kg, and 1200 mg/day in patients with a body weight of $\geq$75 kg). According to the original study protocol, treatment lasted up to 24 weeks or until time of transplant, whichever occurred first. Patients who completed treatment before transplantation also were assessed for sustained virologic response. Patients who relapsed after stopping the study drug during the pretransplant treatment phase and who were not found to have the S282T NS5B mutation (which is associated with resistance to sofosbuvir) were allowed to restart treatment and continue for an additional 24 weeks or until transplant. In a subsequent amendment, the study design was changed to allow all patients who had not reached 24 weeks of treatment at the time of the amendment to continue treatment uninterrupted to 48 weeks or transplant. This change was made after observing virologic relapse in 3 patients before transplantation in patients who stopped treatment after completing 24 weeks.

For patients still receiving treatment at the time of transplantation, dosing was discontinued within 24 hours before transplant. During the post-transplant follow-up phase, patients were followed up for 48 weeks for evidence of recurrent HCV infection. All participating sites planned to use a standard post-transplantation immunosuppressive regimen of solumedrol/prednisone, tacrolimus, and/or mycophenolate mofetil (up to 2 g/day) for the first 12 weeks after transplantation. Antibody induction was prohibited during the study.

### Study Assessments

The primary efficacy end point was post-transplantation virologic response (pTVR), defined as HCV-RNA level less than the lower limit of quantification (LLOQ, 25 IU/mL) at 12 weeks post-transplant in patients who had HCV-RNA levels less than the LLOQ at their last assessment before transplantation. According to the original study analysis plan, only patients who received at least 12 weeks of treatment before transplantation were to be included in the efficacy analysis. However, this restriction was not used in the analysis, therefore the efficacy population includes patients who received any duration of treatment (Table 2 shows the overall results for both populations). Other secondary efficacy end points included an evaluation of safety and tolerability. Plasma HCV-RNA levels were measured with the COBAS TaqMan HCV Test, version 2.0, for use with the High Pure System (Roche Molecular Systems, Branchburg, NJ).

### Resistance Testing

Population sequencing of the HCV NS5B-encoding region of the viral polymerase was performed using standard sequencing technology on all baseline (pretreatment) viral samples. Deep sequencing with an assay cut-off value of 1% was performed for all patients who qualified for resistance testing as a result of an incomplete virologic response on treatment, post-treatment relapse, post-transplant recurrence, or early termination with HCV-RNA levels greater than 1000 IU/mL. Nucleoside inhibitor-associated variants were defined as N142T, L159F, L230F, and V321A, and any substitutions at position S282 of NS5B. Drug susceptibility testing was performed using a replicon system with either patient population samples or site-directed mutants.

### Statistical Methods

Assuming an observed week 12 pTVR rate of 50%, we calculated that a sample size of 31 would be sufficient to show that the 1-sided 95% upper bound of the confidence interval (using a normal approximation of the binomial) for the

**Table 1.** Baseline Characteristics

| Characteristic | All patients dosed (n = 61) | All patients transplanted with HCV-RNA level <25 IU/mL at time of transplant (N = 43) |
|---|---|---|
| Median age, *y* (range) | 59 (46–73) | 59 (50–73) |
| Male sex, n (%) | 49 (80) | 32 (74) |
| Race, n (%) | | |
| White | 55 (90) | 40 (93) |
| Black | 6 (10) | 3 (7) |
| Ethnicity, n (%) | | |
| Hispanic or Latino | 12 (20) | 8 (19) |
| Non-Hispanic | 49 (80) | 35 (81) |
| Median body mass index, $kg/m^2$ (range) | 27.4 (20.0–58.7) | 27.1 (20.0–58.7) |
| Prior HCV treatment—no. (%) | | |
| No | 15 (25) | 9 (21) |
| Yes | 46 (75) | 34 (79) |
| Prior null response | 11/46 (24) | 6/34 (18) |
| Prior partial response | 11/46 (24) | 9/34 (26) |
| Prior breakthrough | 3/46 (7) | 3/34 (9) |
| Prior relapse | 9/46 (20) | 7/34 (21) |
| Unknown prior response | 12/46 (26) | 9/34 (26) |
| Median baseline HCV-RNA level, $\log_{10}$ IU/mL (range) | 6.2 (4.1–7.2) | 6.3 (4.1–7.0) |
| Baseline viral load, n (%) | | |
| <6 $\log_{10}$ IU/mL | 20 (33) | 14 (33) |
| >6 $\log_{10}$ IU/mL | 41 (67) | 28 (67) |
| HCV genotype, n (%) | | |
| 1a | 24 (39) | 15 (35) |
| 1b | 21 (34) | 16 (37) |
| 2 | 8 (13) | 6 (14) |
| 3a | 7 (11) | 5 (12) |
| 4a | 1 (2) | 1 (2) |
| IL28B genotype, n (%) | | |
| CC | 13/60 (22) | 10 (23) |
| CT | 39/60 (65) | 28 (65) |
| TT | 8/60 (13) | 5 (12) |
| Baseline ALT level > 1.5 upper limit of normal, n (%) | 26 (43) | 17 (40) |
| Median glomerular filtration rate, *mL/min* (range) | 113 (63–211) | 111 (63–209) |
| Baseline Child–Turcotte–Pugh score, n (%) | | |
| 5 | 26 (43) | 18 (42) |
| 6 | 18 (30) | 15 (35) |
| 7 | 14 (23) | 9 (21) |
| 8[a] | 3 (5) | 1 (2) |
| Baseline MELD score, n (%) | | |
| 6 | 5 (8) | 4 (9) |
| 7 | 18 (30) | 14 (33) |
| 8 | 12 (20) | 6 (14) |
| 9 | 9 (15) | 7 (16) |
| 10 | 6 (10) | 4 (9) |
| 11 | 8 (13) | 7 (16) |
| 13 | 2 (3) | 1 (2) |
| 14 | 1 (2) | N/A |

ALT, alanine aminotransferase.
[a]These patients were deemed by the site to have a Child–Turcotte–Pugh score of 7 during screening, but after the initiation of dosing the central laboratory found them to have a Child–Turcotte–Pugh score of 8.

recurrence rate would be 65%. See the Supplementary Appendix for a detailed description of the statistical methods.

## Study Oversight

The study was approved by the institutional review board or independent ethics committees at 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 according to protocol by the sponsor (Gilead) in collaboration with the principal investigators. 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 reviewed and approved the final manuscript.

**Table 2.** Post-Transplant Virologic Response by Visit for Patients With HCV-RNA Level Less Than the LLOQ at the Last Measurement Before Liver Transplantation

| | Sofosbuvir-ribavirin for ≥12 weeks (N = 32) | Sofosbuvir-ribavirin for any duration (N = 43) |
|---|---|---|
| Post-transplant week 1 | | |
| <LLOQ, n/N (%) | 28 (88%) | 37 (86%) |
| 90% CI | 74%–96% | 74%–94% |
| Post-transplant week 2 | | |
| <LLOQ, n/N (%) | 26 (81%) | 35 (81%) |
| 90% CI | 66%–92% | 69%–90% |
| Post-transplant week 4 | | |
| <LLOQ, n/N (%) | 24 (75%) | 31 (72%) |
| 90% CI | 59%–87% | 59%–83% |
| Post-transplant week 8 | | |
| <LLOQ, n/N (%) | 24 (75%) | 31 (72%) |
| 90% CI | 59%–87% | 59%–83% |
| Post-transplant week 12 | | |
| <LLOQ, n/N (%) | 24 (75%) | 30 (70%) |
| 90% CI | 59%–87% | 56%–81% |

NOTE. HCV RNA was analyzed using the Roche TaqMan version 2.0 assay for use with the High Pure system with a limit of quantification of 25 IU/mL.
CI, confidence interval.

## Results

### *Study Patients and Disposition*

Supplementary Figure 1 shows the disposition of patients throughout the study. Of the 92 patients screened, 63 were enrolled in the study, and 61 received at least 1 dose of study drug (Supplementary Table 1). Of the 61 patients who received at least 1 dose of study drugs, 46 underwent a transplantation and 15 discontinued the study before transplantation. Of the 46 patients who underwent transplantation, 43 had HCV-RNA level less than the LLOQ at the time of transplantation. These 43 patients had been on the waiting list for liver transplantation for a mean of 295 days (median, 128 days). Baseline demographic characteristics for the 61 patients who received study drugs and the 43 patients who underwent transplantation and had HCV-RNA level less than the LLOQ are shown in Table 1. Of the 61 dosed, more than 70% were infected with genotype 1 HCV, and the majority (79%) previously received treatment for their HCV infection. This article describes the efficacy results in 43 patients who underwent transplantation with an HCV-RNA level less than the LLOQ and safety and resistance results from the entire treated population.

### *Efficacy*

**Pretransplant treatment phase.** In the 61 subjects who received study drug. the median duration of exposure to study drugs was 21 weeks (range, 2.3–52.3 wk). Treatment with sofosbuvir and ribavirin resulted in rapid suppression of circulating virus with a median decrease in HCV-RNA level of 3.93 $\log_{10}$ IU/mL after 1 week of treatment. By the fourth week of treatment, 54 of the 58 patients (93%) receiving treatment had an HCV-RNA level less than the LLOQ. The rate and amount of decrease in HCV-RNA levels did not differ by prior HCV treatment history or Child–Turcotte–Pugh class.

**Post-transplant follow-up phase.** Of the 46 patients who underwent transplantation, 43 had HCV RNA less than the LLOQ at the time of transplantation and represent the prespecified group for which we determined treatment efficacy. The median donor age for the 38 of 43 grafts for whom donor information was available was 38 years (range, 19–75 y). Of the 43 patients with an HCV-RNA level less than the LLOQ at the time of transplantation, 30 (70%) achieved pTVR12 (Table 2). For all 30 patients with pTVR, HCV-RNA level was undetectable (target not detected) at post-transplant week 12. Of the 13 patients not achieving pTVR12, 10 patients had confirmed HCV recurrence (Supplementary Table 2) and 3 patients died immediately after transplant (details later). When expressed as a percentage of the total population who received study treatment, 49% (30 of 61) achieved pTVR.

Rates of pTVR12 in various subgroups are shown in Supplementary Table 3. In univariate logistic regression analysis, pTVR was associated positively with HCV genotypes other than HCV1b infection and a greater number of consecutive days with undetectable HCV-RNA level before transplantation. However, in multivariate logistic regression analysis, the only statistically significant predictor of pTVR was the number of consecutive days a patient's HCV-RNA level was undetectable (target not detected) before transplantation (Figure 1, Supplementary Table 4). This result was supported by a separate analysis, which found that the median number of consecutive days with undetectable HCV-RNA level before transplantation was 5.5 days (range, 0–88 days) for patients with observed recurrence compared with 99.5 days (range, 1–473 days) for patients with pTVR ($P < .001$, 2-sided Wilcoxon rank sum test). Outcomes did not appear to correlate with donor age or other donor characteristics, although given the small numbers of patients with recurrence and incomplete donor information for all patients, this observation is preliminary.

**Resistance analyses.** Baseline population sequencing detected the presence of 2 variants associated with resistance to nucleotide inhibitors: L159F in 4 patients and N142T in 1 patient. Resistance analysis by deep sequencing was performed for 29 of 61 patients who showed virologic failure before transplantation or recurrence after transplantation with HCV-RNA level greater than 1000 IU/mL. No NS5B mutant S282T was detected in any patient samples analyzed. Twelve of 29 patients developed other nucleoside inhibitor resistance–associated variants and only as minor subpopulations (<10% of population) in 11 of 12 patients (Table 3). All 4 patients with L159F at baseline relapsed and had the L159F variant at the time of relapse. The patient with N142T at baseline achieved sustained virological response 12. Phenotypic testing of the patient samples and site-directed mutants of the variants (N142T, L159F, V321A,

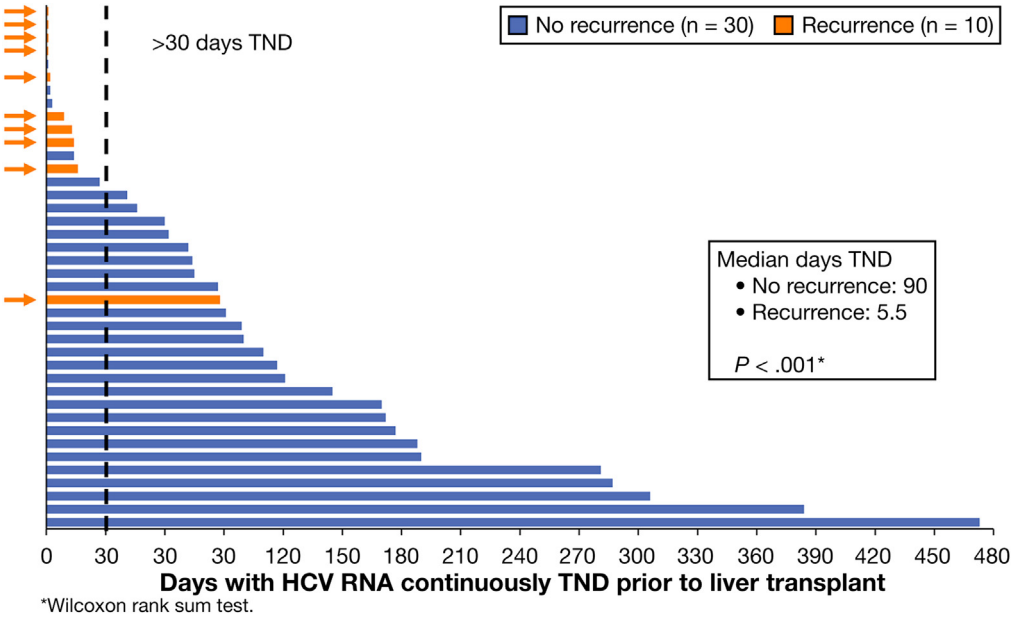


**Figure 1.** No recurrence/recurrence by days HCV RNA continuously target not detected before liver transplantation. TND, Target not detected.



and L320F) did not show any change in susceptibility to sofosbuvir (sofosbuvir fold-change, <2.0; data not shown). Observed minor variants, S282R and S282G, also were introduced by site-directed mutagenesis in replicons but failed to replicate in vitro precluding phenotypic analysis. No ribavirin treatment-associated mutations, M390I or F415Y, developed in patients who qualified for resistance testing.

### *Overall Safety*

Eighty-nine percent of the 61 patients receiving at least 1 dose of drug reported an adverse event (Table 4). The most common events were fatigue (38% of patients), headache (23% of patients), anemia (21% of patients), nausea (16% of patients), and rash (15% of patients). Two subjects discontinued treatment because of adverse events (pneumonitis and sepsis/acute renal failure). Eleven patients (18%) experienced serious adverse events; 3 of those events

**Table 3.** Development of Nucleoside Inhibitor Treatment–Associated Minor Variants in NS5B

| NS5B nucleoside inhibitor treatment–associated variants | Number of patients | Viral population with developed variant, % |
|---|---|---|
| N142T | 2/61 | 1.0, 1.3 |
| L159F | 5/61 | 2.1, 3.2, 6.1, 9.5, 79.1 |
| S282G | 1/61 | 6.1 |
| L320F | 3/61 | 1.6, 2.9, 7.3 |
| L159F, S282R, L320F, V321A[a] | 1/61 | 1.1–3.0 |

[a]One patient with incomplete response to treatment had detectable L159F, S282R, L320F, and V321A.

**Table 4.** Adverse Events and Hematologic Abnormalities

| Event | Sofosbuvir-ribavirin (N = 61) |
|---|---|
| Median duration of exposure to study regimen, *wk* (range) | 21.0 (2.3–52.3) |
| Any adverse event | 54 (89) |
| Any serious adverse event | 11 (18) |
| Any ≥grade 3 adverse event | 11 (18) |
| Hematologic event | |
| Hemoglobin level, n (%) | |
| Patients with at least 1 postbaseline hemoglobin value <10 g/dL | 18 (30) |
| Patients with at least 1 postbaseline hemoglobin value <8.5 g/dL | 3 (5) |
| Absolute lymphocyte count, n (%) | |
| 350 to <500 per $mm^3$ | 5 (8) |
| <350 per $mm^3$ | 5 (8) |
| Platelets | |
| 25,000 to <50,000 per $mm^3$ | 4 (7) |
| Absolute neutrophil count, n (%) | |
| 500 to <750 per $mm^3$ | 2 (3) |
| Common adverse events, n (%)[a] | |
| Fatigue | 23 (38) |
| Headache | 14 (23) |
| Anemia | 13 (21) |
| Nausea | 10 (16) |
| Rash | 9 (15) |
| Cough | 7 (11) |
| Dyspnea | 7 (11) |
| Insomnia | 7 (11) |
| Constipation | 6 (10) |
| Pruritus | 6 (10) |

[a]Events occurring in ≥10% of patients.

occurred in more than 1 patient: progression of hepatocellular carcinoma, obstructive umbilical hernia, and pyrexia (Supplementary Table 5 shows the full list of treatment-emergent serious adverse events). One treatment-emergent death as a result of sepsis occurred 15 days after the last dose of study drug. Four additional non–treatment-emergent deaths occurred as a result of pneumonitis, liver graft failure, cardiogenic shock, and sepsis. None were attributed by the investigators to study treatment.

Laboratory findings at baseline were consistent with decompensated cirrhosis (thrombocytopenia, increased total bilirubin, and prolonged prothrombin time). Twenty-one patients (34%) experienced grade 3 laboratory abnormalities and 7 patients (11%) experienced grade 4 laboratory abnormalities. The most common grade 3 or 4 laboratory abnormalities were a grade 3 decrease in hemoglobin level ($\geq$4.5 g decrease from baseline or absolute value of 7.0–8.9 g/dL) in 15% of patients and grade 3 hyperglycemia (251–500 mg/dL) in 11% of patients. A mean increase of 0.26 mg/dL in total bilirubin level was seen at week 12 of treatment; 5 patients had grade 3 hyperbilirubinemia (2.6–5.0 $\times$ upper limit of normal) and 1 patient had grade 4 hyperbilirubinemia ($>$5.0 $\times$ upper limit of normal). During treatment, alanine aminotransferase level decreased from a baseline median of 76 IU/L to a median alanine aminotransferase level of 30 IU/L or less by week 2, which was sustained throughout treatment. Hemoglobin values also decreased during treatment (consistent with the known effects of ribavirin treatment), with a mean decrease from baseline (baseline mean, 13.5 g/dL) to week 24 of 1.5 g/dL; 18 (30%) patients had at least 1 hemoglobin measurement of less than 10 g/dL and 3 patients (5%) had a hemoglobin measurement of less than 8.5 g/dL. Twelve (20%) patients had ribavirin dose reductions during treatment. No patients received blood products or epoetin during the study. Platelet counts increased from a baseline mean of 107 $\times$ $10^3/\mu$L to 120 $\times$ $10^3/\mu$L at week 24. MELD scores remained stable before transplant. Three patients experienced progression of liver cancer that placed them outside the Milan criteria, and as a result were removed from the waiting list for liver transplantation. Two of these patients stopped treatment at week 24 and relapsed, and the other patient, who received 48 weeks of treatment, reached sustained virological response 12.

## Discussion

In this pilot study, sofosbuvir and ribavirin before liver transplantation prevented recurrence of HCV infection in 70% of patients with chronic HCV infection and liver cancer who achieved an HCV-RNA level less than 25 IU/mL before transplantation and in almost half of the total patients in the study. This population of patients with compensated or mildly decompensated cirrhosis included patients with characteristics historically associated with lower rates of response to antiviral therapy: high viral load, non-CC genotype, and prior nonresponse to interferon therapy. The rate of discontinuation owing to adverse events was low, and most observed events were those associated commonly with ribavirin therapy—fatigue, anemia, headache, and nausea—as were the laboratory abnormalities of decreased hemoglobin and increased bilirubin levels.

This study provides proof of concept that virologic suppression without interferon significantly can reduce the rate of recurrent HCV after liver transplantation. The presumed absence of extrahepatic reservoirs of viral replication, the potency of the antiviral regimen, and host immune response all are possible determinants of clinical outcome. Although the reservoir of HCV replication largely is limited to the liver, HCV RNA has been detected in peripheral blood, suggesting possible sites of "occult infection."[22–25] In this study, we have shown that removal of the infected liver in the setting of undetectable levels of HCV RNA in the blood is associated with low rates of recurrence, suggesting that other possible reservoirs of infection may not be as important as previously thought. The rapid decrease in HCV-RNA level with direct-acting antiviral therapy, including sofosbuvir, has been modeled using a multiscale age-structured approach,[26,27] indicating a triphasic pattern of serum viral load decrease. The model suggests that 6–8 weeks of suppression of HCV RNA (continuously undetectable) is required for complete virologic clearance. The magnitude of HCV-RNA decrease in these patients also is similar to that observed with sofosbuvir in phase 3 studies, reflecting the enhanced rates of loss of intracellular viral RNA, replication templates, and infected cells.

The results from this trial compare favorably with those observed in other trials of pretransplantation antiviral therapy.[9–13] In prior small, mostly single-center, studies using regimens containing peginterferon and ribavirin, rates of post-transplant virologic response ranged from 20% to 28%.[14,15] Treatment was associated with high rates of discontinuations for adverse events and high rates of serious, often life-threatening, complications. In the only randomized controlled trial of pretransplantation antiviral treatment conducted to date, patients with MELD scores of 20 or less received a low accelerating dose regimen of peginterferon alfa-2b and ribavirin or no treatment.[13] Of the 44 patients who underwent treatment in that study, 26 (59%) achieved an undetectable HCV-RNA level by the time of transplantation. The rate of post-transplant response among treated patients was 22% in patients with HCV genotype 1, 4, or 6 infection, and 29% in patients with genotype 2 or 3 infection. The response rate was associated with duration of treatment—no patients who received fewer than 8 weeks of treatment achieved a sustained response, compared with 18% among patients who received 8–16 weeks of treatment and 50% among those who received more than 16 weeks of treatment with peginterferon-ribavirin. Forty-six percent of treated patients also had serious adverse events during pretransplantation treatment.

Deep sequencing analysis of patients with pretransplant virologic failure or recurrence post-transplant showed no evidence of the S282T mutant in NS5B. These results are consistent with the low prevalence of this NS5B mutant after relapse after sofosbuvir treatment as previously described.[16,17,28–30] However, minor subpopulations of other nucleoside inhibitor treatment–associated variants were observed in multiple patients. The observed

enrichment of these minor variants suggests that they may encode for marginal reductions in susceptibility to sofosbuvir that cannot be measured with current in vitro systems. It is possible that there is ongoing low-level replication during treatment in some patients, perhaps owing to the presence of the HCC lesions, resulting in an enrichment of these mutants relative to wild-type and then transient detection at relapse/recurrence before wild-type dominates again. The clinical significance of the appearance of these minor variants remains to be determined.

Because of the small size of this study, any conclusions must be considered preliminary in nature and require further evaluation in larger studies. Extrapolation of these results to all patients with HCV awaiting liver transplant is limited by the fact that the population studied comprised patients with compensated or mildly decompensated liver disease undergoing transplantation for hepatocellular carcinoma. At the time the study was designed, the safety of sofosbuvir had not been evaluated in decompensated liver disease, and we therefore chose patients with a diagnosis of hepatocellular carcinoma meeting the Milan criteria so that the efficacy of the regimen for preventing post-transplant recurrence could be evaluated in patients with lower MELD scores, but who would be expected to undergo liver transplantation within 1 year. Studies of sofosbuvir regimens in patients with more advanced disease pretransplant are underway. The lack of a control arm to define efficacy and tolerability of the regimen was another shortcoming, although ascertainment bias is unlikely given the universal recurrence of HCV in untreated patients.

The majority of patients in this study had an undetectable viral load at the time of transplant and achieved pTVR. However, nonresponse and relapse were observed in a substantial proportion of patients, which led to re-infection of the allograft. It is unknown whether continuation of sofosbuvir and ribavirin through the post-transplant period in patients with a shorter duration of virologic suppression before transplantation could reduce rates of recurrence. Alternatively, higher rates of pTVR may be possible through the addition of another direct-acting antiviral to pretransplant sofosbuvir and ribavirin.

In conclusion, therapy with sofosbuvir and ribavirin before liver transplantation prevented the recurrence of HCV infection after transplantation in 70% of patients who had undetectable levels of HCV RNA before transplantation. Given the burden of disease owing to HCV recurrence post-transplantation—the increased morbidity, mortality, and costs—these results provide hope for patients in need.

## 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.2014.09.023.

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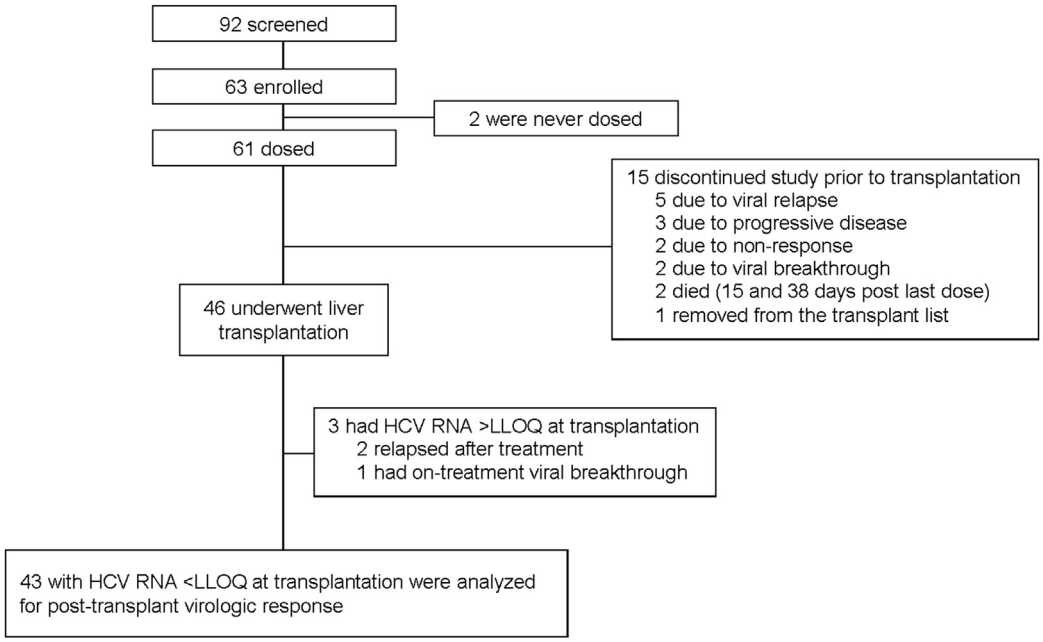


**Supplementary Figure 1.** Patient disposition.