ORIGINAL ARTICLE

HCC recurrence in HCV-infected patients after liver transplantation: SiLVER Study reveals benefits of sirolimus in combination with CNIs – a *post-hoc* analysis

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SUMMARY

Factors affecting outcomes in liver transplant (LTx) recipients with hepatocellular carcinoma (HCC) and hepatitis C viral (HCV) infection include the choice of immunosuppression. Here, we analyzed the HCV^+ subgroup of patients from the randomized controlled, international SiLVER Study. We performed a *post hoc* analysis of 166 HCV^+ SiLVER Study patients regarding HCC outcome after LTx. Control patients (group A: $n = 88$) received mTOR inhibitor (mTORi)-free, calcineurin inhibitor (CNI)-based versus sirolimus-based immunosuppression (group B: $n = 78$). We found no significant difference regarding HCV-RNA titers between group A and B. Since no effect in group B could be due to variable sirolimus dosing, we split group B into patients receiving sirolimus-based immunosuppression + CNIs for >50% (B1; $n = 44$) or <50% (B2; $n = 34$) of the time. While there remained no difference in HCV-RNA titer between groups, HCC recurrence-free survival in group B1 (81.8%) was markedly better versus both group A (62.7%; $P = 0.0136$) and group B2 (64.7%; $P = 0.0326$); Interestingly, further subgroup analysis revealed an increase ($P = 0.0012$) in liver enzyme values in group B2. Taken together, in HCV-infected patients with HCC and LTx, mTORi immunosuppression + CNIs yields excellent outcomes. Unexpectedly, higher levels of liver inflammation and poorer outcomes occur with mTORi monotherapy in the HCV^+ subgroup.

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Background

During the last two decades, hepatocellular carcinoma (HCC) has become the fastest rising cause of cancer-related deaths and has contributed to the increase in proportion of patients undergoing liver transplantation (LTx) for HCC [1]. LTx is a preferred treatment option since cirrhosis is often the underlying disease, and therefore, organ replacement offers a potential simultaneous cure for two otherwise fatal diseases [2]. Even with LTx, approximately one out of five recipients experience HCC recurrence post-transplantation, which is a

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leading cause of morbidity and mortality in these cases [3,4]. Application of strict rules for selection of LTx recipients with limited HCC (i.e., Milan criteria) reduce the likelihood of post-LTx HCC recurrence [5,6]. In addition, the use of immunosuppression with anti-cancer effects, namely mechanistic target of rapamycin inhibitors (mTORi), has further improved the outlook for certain patients, as demonstrated recently in the SiLVER Study [7]. The SiLVER Study was a prospective-randomized open-label multicenter international trial investigating whether sirolimus-based immunosuppression improves the outcome in LTx recipients with HCC. Results from this trial show that although flexible incorporation of sirolimus into an immunosuppressive regimen does not indefinitely improve long-term HCC recurrence-free (RFS) and overall survival (OS), outcomes are improved in the first 3–5 years after transplantation, especially in patients with tumors within Milan Criteria and on mTORi monotherapy [7].

Here, in the present analysis of HCV^+ patients from the SiLVER Study, we posed the question whether certain specific immunosuppressive regiments could otherwise benefit this special subpopulation of patients. To examine this question, we compared those patients that received an mTORi-free, calcineurin inhibitor (CNI)–based, immunosuppressive protocol (group A) to those that received sirolimus (mTORi)-based immunosuppression with CNI for either more (group B1) or less (group B2) than 50% percent of the visits over a 5–8 year period.

Materials & methods

Patient selection

For the SiLVER Study, 525 LTx recipients were recruited from 45 transplant centers in Europe (42), Canada (2), and Australia (1) in a multicenter, randomized, open-labeled, parallel group trial (EudraCT: 2005-005362-36; Clinicaltrials.gov: NCT00355862). The inclusion criteria for eligible patients (>18 years old) were histologically proven HCC before randomization and signed written informed consent. The main exclusion criteria were extrahepatic HCC manifestation and non-HCC malignancies within the past 5 years. The first patient was randomized in January 2006 with randomization completed in April 2009. All patients were followed up for at a minimum of 5 years post-LTx, with the last visit being conducted in March 2014.

Randomization

Patients were randomized into two groups. Group A was maintained on a center-specific mTORi-free, generally CNI-based, immunosuppressive protocol. In group B, sirolimus was incorporated into the regime (target range, 4–10 ng/ml) after 4 to 6 weeks, either as a monotherapy or as a combination therapy with non-mTORi–based drugs. More details of the protocol are published elsewhere [7,8].

Outcomes

As detailed in the original publication of the SiLVER Study [7], the primary endpoint of RFS was defined as HCC recurrence or patient death. Patients underwent a standardized tumor-specific follow-up at every visit. In the first year after LTx, all patients were followed up after month 1, 3, 6, 9, and 12; thereafter, patients were followed up every 6 months. OS was a secondary endpoint in the study.

Analysis

Within group A and B, 88 and 78 patients, respectively, were HCV-RNA positive at the time of randomization. HCV recurrence was determined by HCV viremia and elevated transaminases as per local center's practice/investigator discretion, in the absence of other reasons for graft dysfunction. Furthermore, liver inflammation was also assessed by measuring liver transaminases. For this *post hoc* analysis, five patients were excluded from group A due to mTORi application for more than one visit. To investigate the effect of mTORi monotherapy, group B was subdivided further based on the number of visits where the patients received mTORi in combination with CNIs. Based on an initial per protocol definition, patients in group B1 ($n = 44$) received mTORi-based immunosuppression with CNIs for more than 50% of the time, while patients in group B2 ($n = 34$) for less than 50% of the time. To confirm our observation, we calculated the median time of mTORi-based immunosuppression without CNIs (median 6 months, IQR 0–37.5 months) in the whole group B. Based on this calculation, we subdivided group B into B1 ($n = 38$) with mTORi-based immunosuppression without CNIs < 6 months and B2 ($n = 40$) with mTORi-based immunosuppression without CNIs ≥ 6 months.

Statistics

GraphPad Prism 7.0b (GraphPad Software, Inc., La Jolla, USA) was used for Chi-squared and Kruksall–Wallis tests, as well as for survival analysis and generating plots. All authors had access to the study data and critically reviewed and approved the final version of the manuscript.

Results

The HCV-positive subset ($n = 166$) of the complete SiLVER Study ($n = 525$) was analyzed to find a potential immunosuppressive regimen that might help to reduce HCC recurrence after LTx in patients with chronic HCV infection (Fig. 1). We compared those patients that received an mTORi-free, CNI-based, immunosuppressive protocol (control group A, $n = 88$) to those that received mTORi-based immunosuppression (group B; $n = 78$). Within group A, five patients were switched to mTORi for more than one visit and were therefore excluded from our analysis, leaving 83 patients in this group and 161 patients in total.

First, we wanted to determine whether mTORi-based immunosuppression had an effect on HCV replication, as previously reported from in vitro studies [9]. This analysis did not reveal any significant difference regarding HCV-RNA titers between group A and B patients (Figure S1). We then speculated that the lack of an effect in group B could be due to variable sirolimus dosing (e.g., early discontinuation or use mainly in combination with CNIs). Therefore, we split group B into patients receiving sirolimus-based immunosuppression with CNIs for either more than (group B1; n = 44), or less than (group B2; $n = 34$) 50% of the visits (Fig. 1).

A summary of the demographic data is given in Table 1 for all 161 analyzed patients. Notably, most patients were men (87.6%) and white (96.9%). The median age was 56.4 years, and the median time on the waiting list for LTx was 0.33 years. Overall, the three groups were well balanced with regard to the baseline demographic data. Pathological HCC specifics are also summarized in Table 1. A total of 115 patients (71.4%) were within Milan Criteria, whereas 46 patients (28.6%) had tumors outside Milan Criteria; this is a similar distribution that was published for the whole group [7]. For most patients (76.4%), the number of lesions was 1 or 2, whereas 23.6% of patients had 3 lesions or more. The maximum tumor size was < 3 cm for 56.5%, 3 to 5 cm for 37.9%, and greater than 5 cm for 5.6% of patients. Overall, the treatment groups were well balanced for HCC specifics. Regarding HCV characteristics, 92 patients (57.1%) had a genotype 1 and 35 (21.7%) a genotype 3 infection, while 25 patients (15.5%) were not tested for their respective genotype (Table 1). In this pre-DAA treatment era, 14 patients (8.7%) received IFNα and 71 patients (44.1%) were treated with pegIFNα plus ribavirin before LTx, while 76 patients (47.2%) remained treatment naïve.

While there remained no difference in the HCV-RNA titer (Fig. 2) between the three groups (A, B1 or B2), it was interesting to find that HCC RFS in group B1 (81.8%; $n = 36$) was substantially, and significantly,

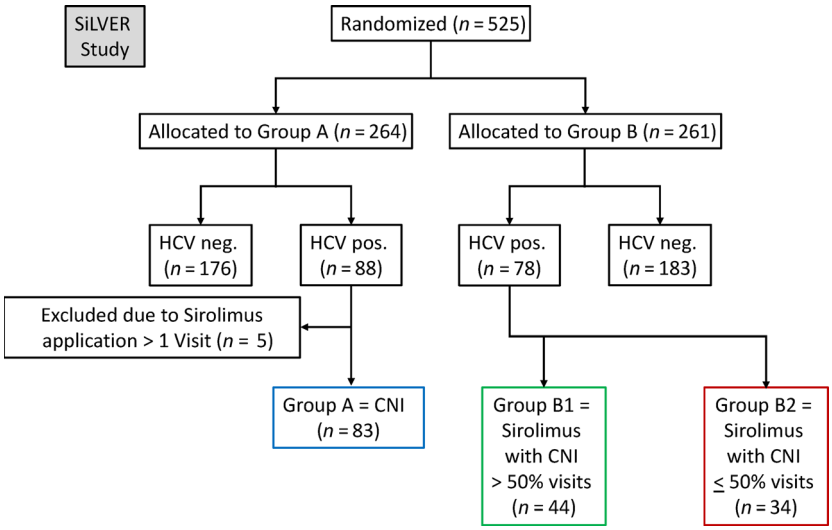

Figure 1 Patient disposition.

Table 1. Patient characteristics before LTx and Follow-up after LTx

	A (-mTORi)	B1 (>50% Visits mTORi + CNI)	B2 (≤50% Visits mTORi + CNI)	*P* value
Patient's demographics				
Number of patients	83	44	34	
Age at the time of consent, years				
Median	55.1	57	58.4	0.21**
(Q1–Q3)	51.8–63.7	52.3–61.8	54.1–63.6	
Gender				
Male	69 (83.1%)	41 (93.2%)	31 (91.2%)	0.20*
Female	14 (16.9%)	3 (6.8%)	3 (8.2%)	
Race				
White	80 (96.4%)	43 (97.7%)	33 (97.1%)	0.80*
African	1 (1.2%)		1 (2.9%)	
Asian	1 (1.2%)	1 (2.3%)		
Arabic	1 (1.2%)			
Time on waiting list, years				
Median	0.46	0.24	0.3	0.21***
(Q1–Q3)	0.17–0.92	0.14–0.64	0.07–1.01	
Patient's HCC characteristics before LTx				
Within Milan criteria				
Based on radiology report at listing	61 (73.5%)	31 (70.5%)	23 (76.5%)	0.84*
Based on pathology report after LTx	58 (70.7%)	32 (72.7%)	19 (55.9%)	0.22*
Maximum tumor size				
>5 cm	4 (4.8%)	2 (4.5%)	3 (8.8%)	0.63*
3-5 cm	28 (33.7%)	18 (40.9%)	15 (44.1%)	
<3 cm	51 (61.4%)	24 (54.5%)	16 (47.1%)	
Median tumor size in pathology report	2.5	3	3	0.96***
(Q1–Q3)	1.5–3.5	1.5–4	1–4	
Number of tumors				
1–2	62 (74.7%)	32 (72.7%)	29 (85.3%)	0.38*
>3	21 (25.3%)	12 (27.3%)	5 (14.7%)	
Patient's HCV characteristics before LTx				
Genotype				
1	46 (55.4%)	25 (56.8%)	21 (61.8%)	0.66*
2	1 (1.2%)	0 (0%)	2 (6.5%)	
3	19 (22.9%)	11 (23.4%)	5 (16.1%)	
4	3 (3.6%)	2 (4.3%)	0 (0%)	
5a	1 (1.2%)	0 (0%)	0 (0%)	
not tested	13 (15.7%)	6 (12.8%)	6 (19.4%)	
Pretreatment				
IFN alone	6 (7.2%)	3 (6.8%)	5 (14.7%)	0.72*
pegIFN + RBV	38 (45.8)	19 (43.2%)	14 (41.2%)	
naive	39 (47%)	22 (50%)	15 (44.1%)	
Patient's Follow-up after LTx				
Patients with acute rejection (AR)	24 (28.9%)	12 (27.3%)	11 (32.4%)	0.88*
Patients received treatment for AR	20 (83.3%)	9 (75.0%)	6 (54.5%)	0.19*
Increase in immunosuppression	7 (29.2%)	3 (25.0%)	1 (9.1%)	0.65*
Steroids pulse	14 (58.3%)	6 (50.0%)	5 (45.5%)	0.77*
CMV infection	9 (10.8%)	1 (2.3%)	2 (5.9%)	0.20*
HCV recurrence	52 (62.7%)	27 (61.4%)	27 (79.4%)	0.17*
HCV treatment				
pegIFN + RBV	31 (37.3%)	19 (43.2%)	15 (44.1%)	0.95*
pegIFN	3 (3.6%)	1 (2.3%)	1 (2.9%)	
RBV	1 (1.2%)	0 (0%)	0 (0%)	

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Table 1. Continued.

	A (-mTORi)	B1 (>50% Visits mTORi + CNI)	B2 (≤50% Visits mTORi + CNI)	*P* value
HCV treatment outcome				
SVR	15 (42.9%)	12 (60%)	6 (37.5%)	0.61*
Unknown	3 (8.6%)	0 (0%)	3 (18.8%)	
HCC recurrence	14 (16.9%)	5 (11.4%)	7 (20.6%)	0.53*

SVR, sustained virological response.

Statistic: *χ^2 test; **One-way ANOVA; ***Kruskal–Wallis test.

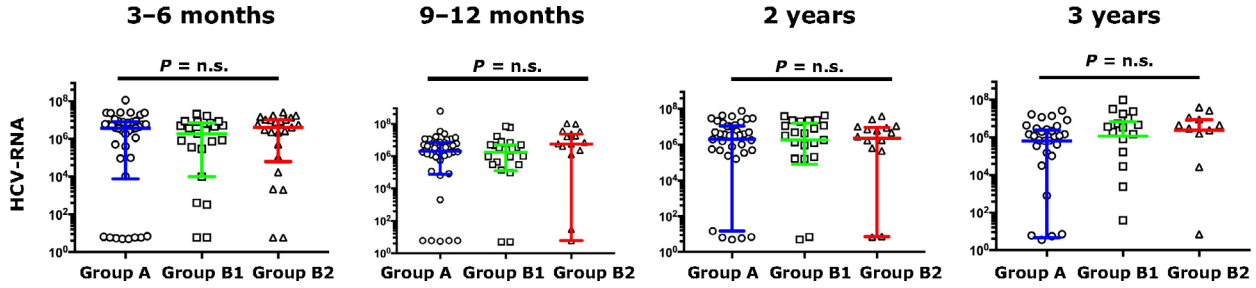

Figure 2 Patient follow-up during the first 3 years after randomization. HCV-RNA viral titer. Statistic: median +/– IQR; nonparametric Kruskal–Wallis test.

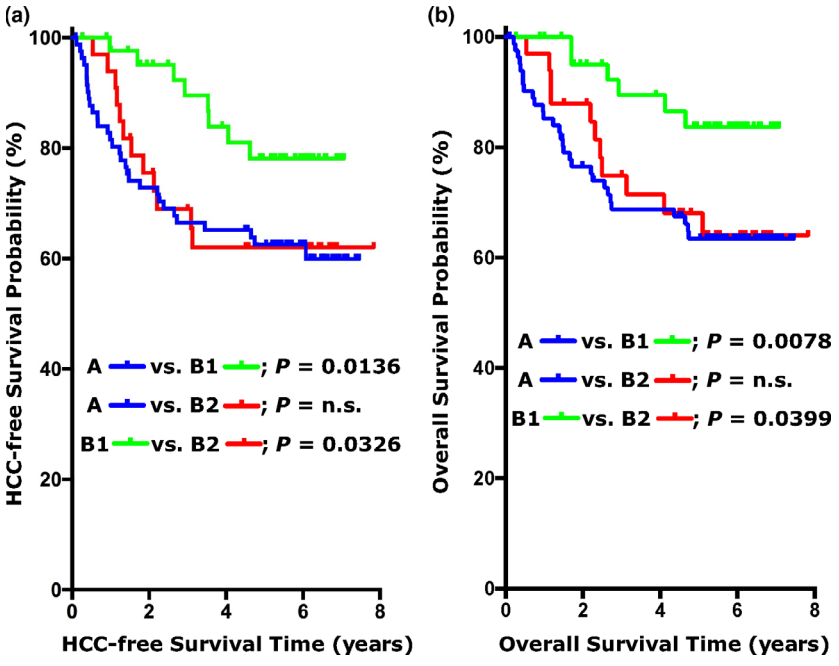

Figure 3 Patient outcome. (a) RFS and (b) OS. Statistic: Gehan–Breslow–Wilcoxon test

better compared with both group A (62.7%; $n = 52$, $P = 0.0136$) and group B2 (64.7%; $n = 22$, $P = 0.0326$) patients at study end (5–8 year follow-up) (Fig. 3a); similarly, the OS was better in group B1 (86.4%), compared with either group A (65.1%; $P = 0.0078$) or group B2 (67.6%; $P = 0.0399$) (Fig. 3b). The significantly better outcome in terms of RFS and OS was confirmed when group B was subdivided according to the

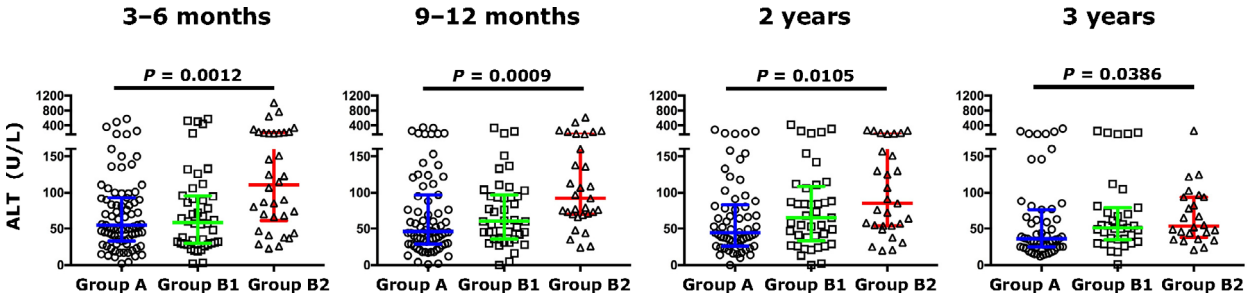

Figure 4 Patient follow-up during the first 3 years after randomization. ALT values in U/L. Statistic: median +/– IQR; nonparametric Kruskal–Wallis test.

median time of mTORi-based immunosuppression without CNIs (Figure S2).

Looking specifically at parameters that are known to have an impact on outcome in chronic HCV-infected patients during the follow-up after LTx (Table 1), there was no significant difference between the three groups with respect to acute rejection (n = 47; 29.2%), CMV infection (n = 12; 7.5%), HCV treatment (n = 71; 44.1%) or the number of patients that achieved a sustained virological response after treatment (n = 33; 46.5%). Although there was a difference in the time to HCC recurrence between group B1 and B2, this trend (P = 0.10) did not reach statistical significance over the time course (Figure S3). Likewise, there was no significant difference in the frequency of patients that experienced an HCC recurrence (n = 26; 16%) between the three groups (Table 1). However, looking at the laboratory workup at 3 to 6 months following LTx, there was a significant (P = 0.0012) increase in ALT values in group B2 (111; 61.3–205.8 (Median; Q1–Q3)) compared with group A (55; 33–93) and group B1 (58.5; 30–95.8). Interestingly, this significant increase in ALT values in group B2 persisted throughout the first three years after LTx (Fig. 4).

Discussion

In this subgroup analysis of HCV-positive patients within the SiLVER Study, we found an improved HCC RFS and OS for patients that received mTORi-based immunosuppression in combination with CNIs for more than 50% of the visits (group B1), as compared to patients that were on mTORi without CNIs for more than 50% of the time (group B2) or on CNIs only (group A). Remarkably, this higher RFS and OS in the group B1 receiving CNIs interestingly contrasts with results from the intention-to-treat SiLVER Study analysis dataset [7], which showed a survival benefit particularly for those patients receiving mTORi monotherapy (like group B2 patients). Therefore, caution should be taken when broadly applying the intention-to-treat SiLVER Study conclusions to all LTx recipients with HCC, since certain patient subgroups may be better served by the application of mTORi in combination with other treatments.

The question then becomes why the HCV subgroup of patients appear to benefit from a combination of mTORi with CNIs. We hypothesized that too little immunosuppression with mTORi monotherapy could possibly be related to increased liver transaminases, which might lead to serious morbidity and poorer outcomes in this HCV-infected patient population [10,11]. Indeed, we present data that suggest patients on CNIs plus mTORi had lower liver transaminases than mTORi monotherapy patients. Therefore, we suggest that in HCV-infected patients with HCC, CNIs help to keep inflammation under control, but do not interfere with the anti-tumor effects of mTORi. A balance between anti-tumor activities and reduced liver inflammation could be the reason for the excellent long-term outcomes in this subgroup of patients on combined mTORi/CNI therapy [12]. However, the reasons why the HCV^+ patients on CNIs plus mTORi had better outcomes are complex and might also be attributed to limitations of this secondary analysis that need to be considered. First, the SiLVER Study was not designed or powered to explore efficacy of CNIs alone vs. CNIs plus mTORi vs. mTORi monotherapy. Secondly, the retrospective nature of our *post hoc* analysis and the limited number of patients need to be emphasized. Furthermore, in the pre-DAA era of the SiLVER Study conduct, treatment regimens for HCV infection varied widely and were not controlled in the study protocol, so we do not know how this may have impacted outcomes, especially in an internationally conducted trial. Besides, measuring of HCV-related parameters such as

viral titer was not centrally performed, leaving our knowledge of the HCV status of patients in question. We must also recognize that besides strictly controlling for the presence of mTORi use in the two study arms, immunosuppressive regimens were largely left to the discretion of the investigators, requiring here that we design *post hoc* groups of patients on regimens within certain selected parameters for this analysis. Furthermore, rejection rates and subsequent adjustments of the immunosuppression such as steroid pulses or other confounding factors cannot be ruled out as contributing factors [13]. While we acknowledge these limitations, the SiLVER Study is nonetheless the largest prospective randomized controlled trial of LTx patients with HCC, so no comparable HCV study cohort exists to examine the questions we posed this analysis.

While these results from HCV-infected patients in the SiLVER Study are interesting, it is important to consider that treatment for HCV infection has dramatically changed since this trial was performed, with the advent of DAA therapy [14-16]. Interestingly, although treatment for HCV has now been revolutionized by successful DAA treatments, we propose that our findings could provide important insight into new options for optimizing outcomes in those patients with HCC receiving a LTx. We propose that since our data indicate that reducing inflammation benefits these patients, elimination of the virus by DAAs will also eliminate the probable cause of inflammation. Therefore, an important hypothesis coming from our current study is that combining DAAs with mTORi monotherapy in this special scenario could substantially improve long-term outcomes. That benefit could be realized in situations where HCV is eliminated before LTx, or when it is necessary to treat with DAAs after LTx. While this hypothesis predicts that mTORi monotherapy would be particularly effective today in the DAA therapy era, the concept will need to be proven in a randomized controlled trial.

In summary, HCV-infected patients in the pre-DAA (SiLVER Study) therapy era with HCC and a LTx benefited from mTORi immunosuppression when used in combination with limited CNIs to reduce virally related inflammation, yielding excellent survival outcomes. While higher levels of liver inflammation and poorer outcomes were observed in the mTORi monotherapy patients of the SiLVER Study, we suggest that reduced viral-associated inflammation now possible in the era of DAA HCV treatment might prove in future clinical trials to optimize outcomes in this patient subpopulation by combining synergistically with the anti-cancer activities of mTORi monotherapy.

Authorship

HJS and EKG: involved in conception and design of the study. JMW, RK, MG, and AAS: involved in data acquisition. JMW, MH, MG, and EKG: involved in analysis and interpretation of the data. JMW and MH: drafted the manuscript. HJS, EKG, and JMW: revised the manuscript. All authors had access to the study data and critically reviewed and approved the final version of the manuscript.

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Conflict of interest

The authors declare that no competing interests exist. HJS receives consulting and lecture fees. The remaining authors receive no external income.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Patient follow-up during the first 2 years after randomization. HCV RNA viral titer. Statistic: Median +/− IQR; nonparametric Kruskal–Wallis test.

Figure S2. Patient outcome. (A) RFS and (B) OS. Statistic: Gehan-Breslow-Wilcoxon test.

Figure S3. Patient outcome. Time to HCC recurrence. Statistic: Gehan-Breslow-Wilcoxon test.

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