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# Sustained Virological Response to Antiviral Therapy in a Randomized Trial of Cyclosporine versus Tacrolimus in Liver Transplant Patients with Recurrent Hepatitis C Infection

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**Background:** Choice of calcineurin inhibitor may influence response to antiviral therapy in liver transplant patients with hepatitis C virus (HCV) infection.

**Material/Methods:** In a randomized, multicenter, 80-week trial, liver transplant recipients (>6 months and ≤10 years post-transplant) with recurrent HCV infection received cyclosporine (n=50) or tacrolimus (n=42) with a 48-week course of pegylated interferon (peg-IFNα2a) and ribavirin. Twenty-three patients in each group completed the trial on study medication. The primary endpoint was sustained virological response (SVR) 24 weeks after the end of antiviral therapy, for which 43 patients were eligible for analysis.

**Results:** The rate of SVR was 60.0% (12/20) with cyclosporine and 43.5% (10/23) with tacrolimus (adjusted odds ratio 1.85; 95% CI 0.53–6.43; p=0.331). There were no significant intergroup differences for rapid or early virological response, relapse, HCV RNA viral load, or fibrosis progression. One cyclosporine-treated patient experienced acute rejection. One patient died in each group. Adverse events, treatment-related adverse events, and serious adverse events were similar between groups.

**Conclusions:** Since fewer patients were recruited than planned (92 versus 355), the study was underpowered and robust conclusions cannot be drawn regarding the effect of cyclosporine and tacrolimus on virological responses to antiviral treatment for recurrent HCV after liver transplantation. However, as reported in other trials, SVR was higher in cyclosporine-treated patients.

**MeSH Keywords:** Cyclosporine • Liver Transplantation • Tacrolimus

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## Background

Recurrence of HCV is virtually universal post-transplant, and up to 90% of patients subsequently develop chronic HCV disease [1,2]. HCV-related disease progresses to cirrhosis within 5 years in 30% of patients [3] and HCV infection is associated with a 44% increase in mortality risk [4]. As HCV-related cirrhosis is the most common indication for liver transplantation [4], improving outcomes in HCV-positive recipients is a clinical priority.

Achieving a sustained virological response (SVR) to antiviral therapy is associated with a survival advantage in non-transplanted patients with advanced HCV-related hepatic fibrosis [5,6]. Attenuated fibrosis progression and improved survival have also been demonstrated in liver transplant recipients who achieve SVR [7–11]. However, although the benefits of antiviral therapy are widely recognized, studies consistently report that only 28–40% of liver transplant patients achieve SVR [12–15], either due to non-response or discontinuation of treatment due to hematological intolerance or other toxicities [2,15].

Predictors of SVR include HCV genotype 2 or 3 [9,15], IL-28B genotype [16,17], compliance with the dose and duration of the antiviral regimen [7,9,11,15], baseline hemoglobin >14 g/dL [15], and absence of diabetes [10], but influencing them is impossible or difficult. In contrast, choice of calcineurin inhibitor (CNI) may represent a modifiable contributing factor. Cyclosporine (CsA) exerts its immunosuppressive effect via the protein target cyclophilin B [18], which is an essential cofactor for HCV replication [19]. *In vitro* data have consistently shown that CsA suppresses HCV RNA replication in replicon models and in human hepatocytes in a dose-dependent manner [20–24]. This effect is not observed with tacrolimus [20–22,24], for which the target is FK506-binding protein. There is clinical evidence which suggests that these *in vitro* data may translate to clinical benefits with CsA therapy in HCV-positive liver transplant patients [23,25–32]. Furthermore, IFN does not act via inhibition of cyclophilin, and *in vitro* data have shown that the inhibitory effect of CsA on HCV replication is independent of the IFN signalling pathways and, importantly, additive to the effect of IFN [23]. It is therefore possible that CsA-based immunosuppression may complement IFN/ribavirin antiviral therapy and enhance SVR in liver transplant patients treated with antiviral therapy. However, an effect of CNI choice on achievement of SVR remains to be confirmed. Trials comparing the efficacy of antiviral therapy with concomitant CsA versus tacrolimus are relatively sparse. To date, only 1 randomized pilot study [33] and several retrospective or observational analyses [34] have been published. A recent review of these reports concluded that the available data supports a potential benefit for CsA in HCV-positive liver transplant patients, but highlighted that additional controlled randomized studies are required to prove this definitively [35].

SUSTAIN was a randomized, multicenter trial in which liver transplant recipients with recurrent HCV infection received CsA or tacrolimus with peg-IFN and ribavirin therapy. The primary objective of the study was to determine whether patients treated with CsA versus tacrolimus would have a higher SVR at 24 weeks after the end of a 48-week course of antiviral therapy.

## Material and Methods

### Study design and conduct

This was a randomized, open-label, multicenter 80-week trial in which liver transplant patients with recurrent HCV infection received CsA or tacrolimus therapy with a 48-week course of antiviral treatment using peg-IFNα2a and ribavirin (NCT00938860). The study was undertaken at 41 centers during September 2009 to May 2013. The objective of the study was to show superiority for CsA versus tacrolimus in terms of SVR.

The sample size was calculated based on the initial objective of showing superiority for CsA versus tacrolimus in terms of the primary endpoint. The sample size calculation assumed that the proportion of patients with SVR would be 35% in the CsA arm and 20% in the tacrolimus arm. It was estimated that a sample size of 138 evaluable patients per group would provide 80% power to demonstrate superiority of CsA over tacrolimus using a 2-sided significance level of 5%. Assuming that sites potentially less experienced in the use of CsA would have greater discontinuation of patients in the CsA group, such that 30% of patients randomized to CsA and 10% of patients randomized to tacrolimus would be non-evaluable, randomization of 198 and 154 patients (1.3-1 ratio), respectively, would be required, with 355 patients in total.

The study was conducted in accordance with the principles of Good Clinical Practice and the Declaration of Helsinki following approval from the institutional review board at each center. Written information consent was obtained from all patients.

### Eligibility criteria

The study population comprised patients aged 18–70 years who had undergone liver transplantation >6 months and ≤10 years previously for whom the reason for transplant was end-stage liver disease due to HCV infection with genotypes 1 or 4 and who were receiving tacrolimus-based immunosuppression. Patients with hepatocellular carcinoma (HCC) within the Milan or University of California, San Francisco (UCSF) criteria were not excluded from the study. All patients were to have received tacrolimus for at least 6 months prior to randomization, have a diagnosis of HCV genotype 1 or 4 infection confirmed at screening, and have an indication for treatment with



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**Table 1.** Inclusion and exclusion criteria.

| Inclusion criteria | Exclusion criteria |
|---|---|
| • Male or female aged 18–70 years<br>• Recipients of a first liver transplant due to HCV cirrhosis<br>• Transplanted ≥6 months and up to 10 years prior to randomization<br>• Tacrolimus-based immunosuppressive regimen based on tacrolimus for ≥6 months prior to randomization<br>• HCV genotype 1 or 4 infection confirmed at screening<br>• Indication for treatment with peg-IFN and ribavirin due to histological evidence of chronic HCV infection defined as fibrosis stage ≥1 (Ishak-Knodell scoring system) on liver biopsy at screening or ≤4 months pre-randomization<br>• Written informed consent before any study assessment | • Serum creatinine >150 µmol/L or eGFR <50 mL/min (Cockcroft-Gault formula)<br>• Multi-organ transplant recipients<br>• Steroid-treated acute rejection <3 months prior to randomization, or >1 episode of steroid-treated or any steroid-resistant acute rejection in the last 6 months, including evidence of chronic rejection or ductopenia<br>• Conditions that could cause graft dysfunction other than HCV infection<br>• Signs of decompensated liver disease, defined as presence of ascites, variceal bleeding, encephalopathy, or deteriorated hepatic synthetic function (albumin <3.5 g/dL, direct bilirubin >2× upper limit of normal, or international normalized ratio (INR) >1.5)<br>• Co-infection with HIV or Hepatitis B (defined as HBsAg-positive)<br>• Use of everolimus or sirolimus <6 months prior to screening<br>• Antiviral treatment for HCV at any time post-transplant<br>• Corticosteroid dose >5 mg/day<br>• Fibrosing cholestatic hepatitis<br>• Platelet count <70,000/mm$^3$ or neutrophils <1500/mm$^3$<br>• Current diagnosis of malignancies, including lymphoproliferative disorders<br>• History of hepatocellular cancer outside Milan criteria (based on radiology) or University of California, San Francisco criteria (based on analysis of the explant)<br>• History of malignancy of any organ system within the past 5 years (other than non-metastatic basal or squamous cell carcinoma of the skin) |

peg-IFN and ribavirin due to histological evidence of chronic HCV infection, defined as fibrosis stage ≥1 (Ishak-Knodell scoring system [36,37]) on liver biopsy at screening or within 4 months prior to randomization. Patients were excluded if serum creatinine was >150 µmol/L or estimated GFR (eGFR) was <50 mL/min (Cockcroft-Gault formula [38]); if they had experienced steroid-treated acute rejection within 3 months prior to randomization (or more than 1 episode of steroid-treated acute rejection in the last 6 months) or steroid-resistant rejection in the last 6 months; used antiviral treatment for HCV infection since transplantation; or had fibrosing cholestatic hepatitis. Full inclusion and exclusion criteria are shown in Table 1.

**Immunosuppression**

Eligible patients were randomized centrally in a 1.3-to-1 ratio to CsA or tacrolimus via an interactive voice response system with stratification for: (i) use of mycophenolic acid (yes or no); (ii) Ishak-Knodell fibrosis score at study entry (1–2 or 3–6); and (iii) use of antiviral treatment pretransplant (yes or no). After randomization, patients receiving either CNI completed a run-in period of 4–8 weeks during which CNI blood concentrations were stabilized within target range, after which they continued CNI therapy for the remainder of the 80-week study.

Patients randomized to CsA received an initial dose of 5 mg/kg, adjusted to target $C_2$ blood levels of 800 (range 600–1000) ng/mL to month 12, and 600 (400–800 ng/mL) thereafter, or $C_0$ blood levels of 175 (150–200) ng/mL to month 12, and 125 (100–150) ng/mL thereafter. In the tacrolimus group, $C_0$ level was to be maintained within the range of 5–10 ng/mL. Blood levels of CsA and tacrolimus were to be adjusted according to local practice in patients receiving mycophenolic acid.

Concomitant everolimus or sirolimus therapy was prohibited, as was steroid dosing >5 mg/day.

**Antiviral therapy**

At the time the trial was initiated, current antiviral therapy for HCV genotype 1 comprised combined treatment with pegylated interferon (peg-IFN) and ribavirin for 48 weeks [2]. After a 4–8 week run-in period, a 48-week course of peg-IFNα2a and ribavirin was started once stable target CNI levels were reached,



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in all patients who re-qualified based on laboratory eligibility and who had not experienced biopsy-proven acute rejection (BPAR) or discontinued CNI therapy due to adverse events. All patients at a given center received the same antiviral regimen. The recommended dose of peg-IFNα2a was 180 µg/week. The recommended ribavirin starting dose was 600–1000 mg/day or 800–1200 mg/day for patients weighing <75 kg or ≥75 kg, respectively, with daily doses of 1000 mg/day or 1200 mg/day.

Treatment with peg-IFN was to be discontinued if the platelet count decreased to <25,000/mm$^3$, if the neutrophil count decreased to <500/mm$^3$, if the patient developed severe depression, or if there were signs of progressive liver dysfunction or evidence of hepatic decompensation. If at week 12 the patient had not achieved early virological response (defined as undetectable HCV RNA or a ≥2 log drop in HCV RNA titer), it was recommended that antiviral treatment should be discontinued unless treatment continuation was considered beneficial by the investigator. At week 24, detectable HCV RNA was considered treatment failure and antiviral treatment was to be stopped. No patient was to receive antiviral treatment during the study for longer than 48 weeks.

Use of erythropoietin and granulocyte colony-stimulating factor according to local practice was encouraged.

### Study endpoints

The primary endpoint was SVR (defined as HCV RNA below the lower limit of quantification) at 24 weeks after the end of antiviral therapy. In view of evidence that SVR at week 12 or week 24 post-treatment are concordant [39], a window of week 24±12 weeks was defined for the primary endpoint.

Secondary endpoints included: (i) rate of rapid virological response at week 4 after the start of antiviral therapy; (ii) the rate of early virological response at week 12 after the start of antiviral therapy; (iii) the end-of-treatment response; (iv) the non-responder rate (defined as failure to achieve at least a 2-log reduction of HCV RNA); (v) the relapse rate (defined as reappearance of detectable HCV RNA at 24±12 weeks after the end of antiviral therapy when HCV RNA was undetectable at the end of treatment); (vi) fibrosis progression (Ishak-Knodell scoring system) from study entry to study completion; (vii) a composite endpoint of BPAR, death, or graft loss (and of the individual components); (viii) adverse events; (ix) laboratory measurements; and (x) vital signs.

Protocol biopsies were performed at screening or up to 4 months pre-randomization and at the end of the study (week 80) and were assessed centrally in a blinded manner. HCV RNA viral load was measured centrally (Cobas® TaqMan® assay; Roche Molecular Diagnostics, Pleasanton, CA, USA [lower limit of quantification 43 IU/mL]).

### Statistical methods

The intent-to-treat (ITT) population comprised all randomized patients. The efficacy population comprised all randomized patients in whom both the randomized study drug and antiviral therapy was initiated. The safety population comprised all patients who received at least 1 dose of study drug and had at least 1 post-baseline safety assessment.

The primary variable, SVR at 24±12 weeks after the end of antiviral therapy, was compared between groups using logistic regression to account for the stratification factors applied at randomization (i.e., use of mycophenolic acid, Ishak-Knodell fibrosis score at study entry and use of antiviral treatment pre-transplant) as covariates, in addition to the treatment group (CsA or tacrolimus). Patients were excluded from the primary analysis if: (i) the last HCV RNA assessment was obtained prior to 24±12 weeks after the end of antiviral therapy; (ii) death, graft loss, withdrawal of consent or loss to follow-up occurred prior to week 24±12; (iii) antiviral therapy was not initiated; or (iv) there was <80% compliance with the randomized study drug, defined as the number of post-randomization days receiving study drug divided by the total number of post-randomization days to week 24 after the start of antiviral therapy. As a sensitivity analysis, the primary analysis was repeated regardless of study drug compliance.

HCV-RNA-related endpoints (SVR, rapid virological response, early virological response, end-of-treatment response, non-response, and relapse) and the incidence of an increase in Ishak-Knodell score were analyzed adjusting for randomization strata in a similar fashion to the primary endpoint. Odds ratios for treatment-group differences were computed and tested using Wald's chi-squared test. The incidence of BPAR, graft loss, and death was analyzed using a Kaplan-Meier time-to-event analysis. Treatment-group differences were assessed using a log-rank test. HCV RNA results below the lower limit of quantification were assigned a value equal to one-half of the lower limit of detection. Log-transformed values were used for analyses of HCV RNA viral load. Decrease in HCV viral load from start of antiviral treatment was compared between groups using the Wilcoxon rank-sum test. Laboratory parameters and vital signs were compared using a Wilcoxon rank-sum test.

## Results

### Study population

Recruitment to the trial was stopped early due to slow enrolment and the target of 355 patients was not reached. In total, 147 patients were screened, of whom 92 patients met the eligibility criteria and were randomized (50 CsA, 42 tacrolimus),



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**Table 2.** Baseline characteristics (ITT population).

| | CsA N=50 | | Tacrolimus N=42 | |
|---|---|---|---|---|
| Male sex, n (%) | 41 | (82.0) | 34 | (81.0) |
| Age, years | 54.2 | (6.30) | 55.0 | (6.84) |
| White race, n (%) | 38 | (76.0) | 33 | (78.6) |
| Body mass index (kg/m$^2$) | 27.0 | (4.4) | 25.8 | (3.8) |
| End-stage liver disease leading to transplantation, n (%) | | | | |
| Hepatitis C | 43 | (86.0) | 38 | (90.5) |
| Hepatocellular carcinoma | 5 | (10.0) | 3 | (7.1) |
| Other | 1 | (2.0) | 1 | (2.4) |
| Missing | 1 | (2.0) | 0 | (0.0) |
| Years since HCV diagnosis | 10.7 | (7.1) | 11.9 | (6.2) |
| Years since liver transplantation | 2.1 | (1.3) | 2.0 | (1.8) |
| HCV genotype, n (%) | | | | |
| 1a | 21 | (42.0) | 19 | (45.2) |
| 1b | 27 | (54.0) | 21 | (50.0) |
| 4 | 2 | (4.0) | 2 | (4.8) |
| HCV viral load, $\log_{10}$ U/L | 6.45 | (0.75) | 6.45 | (0.69) |
| Antiviral therapy for HCV infection pre-transplant, n (%) | 27 | (54.0) | 25 | (59.5) |
| Ishak-Knodell score | 2.7 | (1.1) | 2.6 | (1.0) |
| Ishak-Knodell score, n (%) | | | | |
| 1, n (%) | 0 | (0.0) | 0 | (0.0) |
| 2, n (%) | 29 | (58.0) | 25 | (59.5) |
| 3, n (%) | 12 | (24.0) | 10 | (23.8) |
| 4, n (%) | 5 | (10.0) | 4 | (9.5) |
| 5, n (%) | 1 | (2.0) | 0 | (0.0) |
| 6, n (%) | 3 | (6.0) | 2 | (4.8) |
| Missing, n (%) | 0 | (0.0) | 1 | (2.4) |
| Diabetes, n (%) | 15 | (83.3) | 19 | (90.5) |
| CMV-positive, n (%) | 29 | (58.0) | 28 | (66.7) |
| Estimated GFR (Cockcroft-Gault), mL/min | 101.5 | (30.8) | 87.3 | (30.6)* |
| Mycophenolic acid therapy, n (%) | 20 | (40.0) | 17 | (40.5) |

* p=0.043. Continuous variables are shown as mean (SD). CMV – cytomegalovirus; CsA – cyclosporine; GFR – glomerular filtration rate; HCV – hepatitis C virus; ITT – intent-to-treat.

forming the ITT and safety populations. Eighty-one patients (40 CsA, 41 tacrolimus) met the criteria for inclusion in the efficacy population. Forty-three patients (20 CsA, 23 tacrolimus) in the efficacy population met the criteria for inclusion in the analysis of the primary endpoint. Fifty patients (54.3%) completed the 80-week study, with 46 (50.0%) on study medication (Figure 1).



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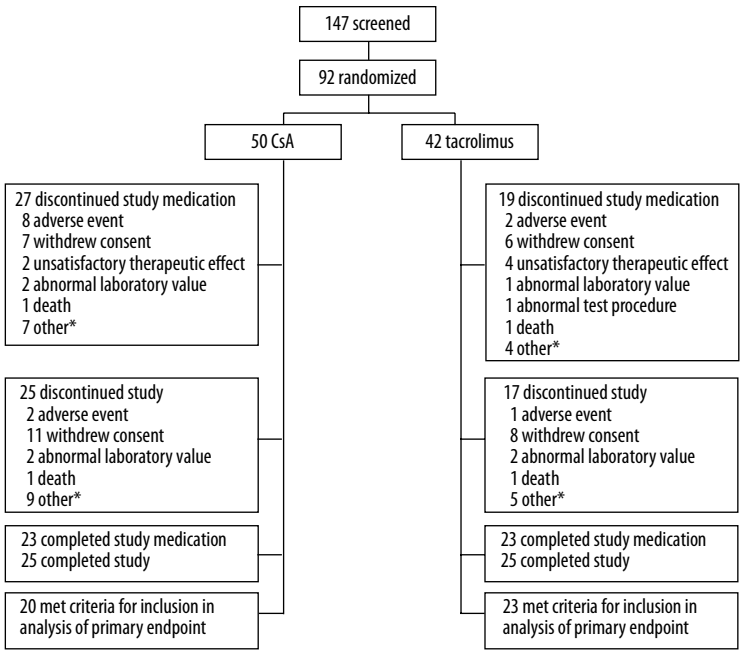


* Including loss to follow-up, admnistrative problems and protocol deviation

**Figure 1.** Patient disposition.

The baseline characteristics of the study population are shown in Table 2. The mean time since liver transplantation was approximately 2 years (mean [SD] 2.1 [1.3] years in the CsA group and 2.0 [1.8] years in the tacrolimus group). The primary cause of liver transplantation was HCV cirrhosis in 81 patients (88.0%) and HCC in 8 patients (8.7%), with the remaining 3 patients being transplanted for another cause or with missing data. All patients were HCV-positive at baseline (88 patients with genotype 1a or 1b, 4 patients with genotype 4). There were no marked differences between treatment groups other than higher eGFR (Cockcroft-Gault) in the CsA arm (mean 101.5 mL/min versus 87.3 mL/min in the tacrolimus group, p=0.043).

### Immunosuppression

Median CsA $C_0$ was in the range of 103–128 ng/mL, and median $C_2$ was in the range of 427–693 ng/mL, from the end of the run-in period up to week 80. Median tacrolimus $C_0$ level remained in the range of 5.2–7.7 ng/mL after the run-in period. By week 24, 4 patients in the CsA group and 3 patients in the tacrolimus group had compliance <80%.

### Antiviral therapy

The mean (SD) dose of peg-IFNα2a throughout the 48-week treatment period was similar in the 2 treatment groups (CsA 169 [22] µg/week, tacrolimus 168 [24] µg/week). During the study, 10/40 patients (25.0%) in the CsA group had a peg-IFNα2a dose reduction (3 as per protocol, 9 due to adverse events) compared to 11/41 patients (26.8%) in the tacrolimus group (4 as per protocol, 12 due to adverse events). The mean (SD) dose of ribavirin was 840 (362) mg/day (11.1 [5.6] mg/kg/day) and 914 (415) mg/day (11.9 [5.4] mg/kg/day) in the CsA and tacrolimus groups, respectively. The mean (SD) duration of antiviral therapy (peg-IFNα2a or ribavirin) was 31.6 (24.3) weeks in the CsA group and 26.7 (18.8) weeks in the tacrolimus group. In accordance with the pre-defined stopping rules (see Methods), 54.0% (27/50) and 52.4% (22/42) of patients in the CsA and tacrolimus groups, respectively, had discontinued both antiviral therapies by week 24; only 34.0% (17/50) and 28.6% (12/42) continued to receive at least 1 antiviral therapy for at least the full 48-week course. The proportion of patients requiring a dose reduction of peg-IFNα2a due to adverse events at any point in the study was 22.5% (9/40) in the CsA group and 29.3% (12/41) in the tacrolimus group (p=0.61); for ribavirin, the proportions were 40.0% (16/40) and 51.2% (21/41) (p=0.37) (data are based on patients still receiving antiviral therapy at week 12).

### Virological endpoints

The primary endpoint (SVR 24±12 weeks after the end of antiviral therapy) occurred in 60.0% (12/20) of patients in the CsA group and 43.5% (10/23) of patients in the tacrolimus group; odds ratio adjusted for randomization strata was 1.85 (95% CI 0.53–6.43) (p=0.331). A sensitivity analysis based on 50 evaluable patients in the ITT population showed an SVR rate of 13/24 (54.2%) in the CsA group and 12/26 (46.2%) in the tacrolimus group, yielding an adjusted odds ratio of 1.33 (95% CI 0.43–4.14) (p=0.624).



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**Table 3.** Efficacy endpoints.

| | CsA | | Tacrolimus | | Odds ratio (95% CI)* | P value* |
|---|---|---|---|---|---|---|
| | n/N | % | n/N | % | | |
| **Primary endpoint** | | | | | | |
| Sustained virological response at week 24 | | | | | | |
| Efficacy population (primary analysis) | 12/20 | 60.0 | 10/23 | 43.5 | 1.85 (0.53–6.43) | 0.331 |
| ITT population (sensitivity analysis) | 13/24 | 54.2 | 12/26 | 46.2 | 1.33 (0.43–4.14) | 0.624 |
| **Secondary endpoints (efficacy population)** | | | | | | |
| Rapid virological response rate at week 4 after start of antiviral therapy | 4/36 | 11.1 | 5/38 | 13.2 | 0.76 (0.18–3.16) | 0.705 |
| Early virological response at week 12 after start of antiviral therapy | 28/31 | 90.3 | 30/36 | 83.3 | 2.04 (0.44–9.38) | 0.358 |
| End of treatment response** | 24/35 | 68.6 | 27/39 | 69.2 | 0.97 (0.35–2.67) | 0.947 |
| Non-response¶ | 7/40 | 17.5 | 5/41 | 12.2 | 1.64 (0.46–5.87) | 0.448 |
| Relapse rate¶¶ | 5/23 | 21.7 | 7/21 | 33.3 | 0.70 (0.16–3.02) | 0.635 |
| Fibrosis progression from study entry to study completion§ | 3/13 | 23.1 | 5/17 | 29.4 | 0.68 (0.12–3.87) | 0.667 |
| **Secondary endpoints (ITT population)** | **CsA** | | **Tacrolimus** | | **P value§§** | |
| | n/N | KM survival rate (95% CI) | n/N | KM survival rate (95% CI) | | |
| Composite endpoint† | 2/50 | 94.3 (86.6–100.0) | 1/42 | 96.3 (89.2–100.0) | 0.544 | |
| Biopsy-proven acute rejection | 1/50 | 97.1 (91.4–100.0) | 0/42 | 100.0 (100.0–100.0) | 0.303 | |
| Death | 1/50 | 97.1 (91.6–100.0) | 1/42 | 96.3 (89.2–100.0) | 0.987 | |

n/N – number experiencing event/number of evaluable patients; * based on logistic regression modelling (Wald's chi-squared test); ** at end of antiviral therapy; ¶ defined as failure to achieve at least a 2-log reduction of HCV RNA; ¶¶ defined as reappearance of detectable HCV RNA at 24 weeks after the end of antiviral therapy when HCV RNA was undetectable at the end of treatment; § defined as increase of ≥1 level in Ishak-Knodell score; §§ log rank test; † BPAR, death or graft loss. BPAR – biopsy-proven acute rejection; CI – confidence interval; CsA – cyclosporine; ITT – intent-to-treat; KM – Kaplan-Meier.

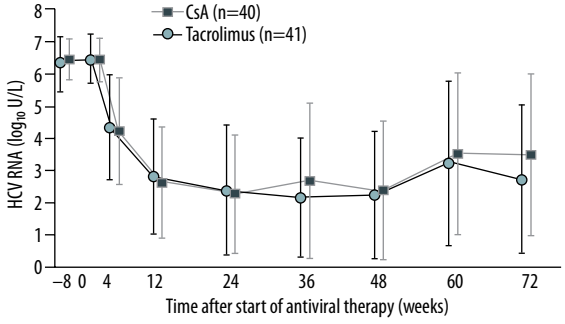


**Figure 2.** HCV RNA viral load over time (ITT population). Values are shown as mean (SD).

There were no significant differences between treatment groups for the secondary viral response endpoints, including rapid and early virological response rates and the relapse rate (Table 3). HCV RNA viral load over time in the ITT population was comparable between groups throughout the study (Figure 2). Mean (SD) values for HCV RNA in the CsA and tacrolimus groups, respectively, were 6.45 (0.75) and 6.45 (0.69) $\log_{10}$ U/L prior to the start of antiviral therapy, 2.40 (2.02) and 2.29 (1.83) $\log_{10}$ U/L at week 24 after the start of antiviral treatment, and 2.73 (2.29) and 3.48 (2.51) $\log_{10}$ U/L at week 72 after the start of antiviral treatment. Data on HCV RNA viral load were similar in the efficacy population.



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**Table 4.** Adverse events, n (%) (safety population).

| | CsA (n=50) | | Tacrolimus (n=42) | |
|---|---|---|---|---|
| Any adverse event | 47 | (94.0) | 42 | (100.0) |
| Any drug-related adverse event | 22 | (44.0) | 19 | (45.2) |
| Any serious adverse event | 18 | (36.0) | 17 | (40.5) |
| Any drug-related serious adverse event | 7 | (14.0) | 2 | (4.8) |
| **Adverse events occurring in ≥10% of either treatment group** | | | | |
| Anemia | 28 | (56.0) | 30 | (71.4) |
| Fatigue | 12 | (24.0) | 14 | (33.3) |
| Leukopenia | 11 | (22.0) | 10 | (23.8) |
| Diarrhea | 9 | (18.0) | 11 | (26.2) |
| Asthenia | 9 | (18.0) | 11 | (26.2) |
| Pyrexia | 9 | (18.0) | 7 | (16.7) |
| Hypertension | 9 | (18.0) | 4 | (9.5) |
| Headache | 8 | (16.0) | 8 | (19.0) |
| Depression | 7 | (14.0) | 4 | (9.5) |
| Neutropenia | 6 | (12.0) | 14 | (33.3) |
| Nausea | 6 | (12.0) | 12 | (28.6) |
| Insomnia | 6 | (12.0) | 6 | (14.3) |
| Pruritus | 6 | (12.0) | 6 | (14.3) |
| Cough | 6 | (12.0) | 4 | (9.5) |
| Dyspnea | 5 | (10.0) | 8 | (19.0) |
| Decreased appetite | 5 | (10.0) | 5 | (11.9) |
| Peripheral edema | 5 | (10.0) | 2 | (4.8) |
| Dizziness | 4 | (8.0) | 8 | (19.0) |
| Influenza-like illness | 3 | (6.0) | 7 | (16.7) |
| Muscle spasms | 1 | (2.0) | 7 | (16.7) |
| Irritability | 0 | (0.0) | 6 | (14.3) |

CsA – cyclosporine.

The proportion of patients exhibiting progression of fibrosis, defined as an increase of Ishak-Knodell score by at least 1 point from study entry, was also similar between the CsA and tacrolimus groups (23.1% versus 29.4%; adjusted odds ratio 0.68 [95% CI 0.12–3.87]; p=0.667) (Table 3).

### Immunosuppressive efficacy endpoints

Immunosuppressive efficacy was similar in both groups (Table 3). One patient in the CsA group experienced BPAR (Banff grade II) during the study, in whom the last reported CsA trough concentration was 150 ng/mL. One patient in the CsA group died due to sepsis, and 1 patient in the tacrolimus group died due to cerebral hemorrhage. Neither death was regarded by the investigator as being related to the study drug.

### Safety

The incidence of adverse events was 94.0% in the CsA group and 100.0% in the tacrolimus group. There was a similar rate of adverse events with a suspected relation to study drug, and a similar rate of serious adverse events (Table 4). There was no marked difference in the rate of serious adverse events between groups. Drug-related serious adverse events occurred in 7 patients randomized to CsA and 2 patients randomized to tacrolimus. Adverse events contributed to study



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**Table 5.** Laboratory results at week 80 (safety population).

| | CsA N=50 | Tacrolimus N=42 | P value |
|---|---|---|---|
| HbA1c | 5.6 (0.9) | 5.7 (0.8) | 0.585 |
| Hemoglobin, g/dL | 12.5 (2.0) | 13.6 (1.6) | 0.140 |
| Alanine transaminase, U/L | 32.7 (18.3) | 46.0 (50.4) | 0.812 |
| Aspartate transaminase, U/L | 33.2 (11.5) | 57.0 (67.7) | 0.890 |
| Total bilirubin, µmol/L | 16.1 (8.0) | 16.1 (8.8) | 0.890 |
| Total cholesterol, mmol/L | 4.8 (1.2) | 4.7 (1.1) | 0.990 |
| LDL-cholesterol, mmol/L | 2.9 (1.2) | 2.7 (0.9) | 0.836 |
| HDL-cholesterol, mmol/L | 1.1 (0.3) | 1.3 (0.4) | 0.301 |
| Triglycerides, mmol/L | 1.8 (0.9) | 1.6 (0.6) | 0.617 |
| eGFR (Cockcroft-Gault), mL/min | 80.2 (21.1) | 83.8 (27.1) | 0.626 |

Values are shown as mean (SD).

drug discontinuation in 10 patients (20.0%) in the CsA group and 5 patients in the tacrolimus group (11.9%) and were the primary reason for discontinuation in 8 patients (16.0%) and 2 patients (4.8%), respectively. No adverse event led to study drug discontinuation in more than 1 patient in either group other than anemia (2 tacrolimus patients).

Laboratory values were similar between treatment groups at week 80 (Table 5). There were no significant differences in body weight or blood pressure between groups at any time point.

## Discussion

In this prospective, randomized trial, the primary endpoint of SVR following treatment of recurrent HCV infection with peg-IFNα2a and ribavirin was numerically higher in patients receiving CsA versus tacrolimus (60.0% versus 43.5%). This finding is in agreement with our hypothesis and data from a recent meta-analysis [34]. Statistical significance was not reached in this small cohort of patients. The results of this trial should be interpreted cautiously in light of the fact that the number of patients recruited was only 26% of the planned population (92 compared to 355), precluding any definitive conclusion due to insufficient power. For the primary analysis, eligible patients represented only 16% of the expected number (43 versus 276).

The reasons for poor enrolment were not readily available, but were at least partly related to changes in the standard of care since study inception, recruitment to concurrent trials, and an unwillingness to consider changing a CNI-based regimen without a clinical imperative. The proportion of screened patients who met the criteria for enrolment was over 60%, suggesting that the criteria were not overly rigorous. Antiviral therapy was discontinued in more than 50% of patients by week 24, but this is consistent with protocol-specified stopping rules, notably that treatment was to be discontinued if early virological response was not achieved by week 12, or if HCV RNA was detected at week 24. There was no evidence to suggest greater intolerance to antiviral therapy with concomitant CsA compared to tacrolimus, as indicated by discontinuations or the need for dose reduction due to adverse events. The available literature also shows little evidence of a difference in terms of discontinuation of antiviral therapy due to intolerance in liver transplant patients receiving CsA or tacrolimus [34], although 1 randomized trial reported more dose reductions in CsA-treated patients versus those receiving tacrolimus [33].

During the 80-week study there was a high rate of CNI discontinuation (50.0% overall), for reasons that are not clear, although discontinuation of CsA did not reflect immunologic events such as rejection. In terms of efficacy, there was only 1 episode of BPAR following conversion from tacrolimus to CsA (in a patient with a typical CsA trough concentration [150 ng/mL]), suggesting that efficacy concerns should not discourage switch of liver transplant recipients with recurrent HCV from tacrolimus to CsA.

A recent meta-analysis by Rabie et al. compared SVR rates in liver transplant patients with recurrent HCV receiving CsA or tacrolimus and antiviral treatment with IFN and ribavirin, based on 1 randomized trial and several observational or retrospective studies (2309 patients in total) [34]. The pooled SVR rate was 42% in patients receiving CsA versus 35% with tacrolimus (relative risk 1.19, 95% CI 1.00–1.39), a difference of borderline significance (p=0.05). Excluding studies with fewer than



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40 patients (1634 patients in total), the difference became significant (relative risk 1.23, 95% CI 1.09–1.38; p<0.001). When comparing studies that did or did not show a benefit for CsA, there was no consistent difference in terms of time post-transplant, severity of fibrosis, or antiviral dosing. It was notable, however, that the 4 studies that reported outcomes specifically in patients with genotype 1 infection all reported higher SVR rates with CsA versus tacrolimus [23,33,40,41]. The only randomized trial to date is a single-center pilot study in 38 patients by Firpi et al. [33] in which the SVR rate was 39% with CsA and 35% with tacrolimus, but absolute numbers were low. In our study, the SVR rate in CsA-treated patients was higher according to the primary analysis, at 60.0%. It is not clear why the rates differed between the 2 trials. The rate of antiviral therapy discontinuation, a possible explanation, was not reported by Firpi et al. [33]. The time post-transplant and the proportion of patients with genotype 1 were not markedly different, nor were antiviral dosing or the definition of SVR (24 weeks after end of antiviral therapy), although the current trial included a large time window on either side of the 24-week time point (±12 weeks). It is possible that the relatively high proportion of patients (42/92, 46%) for whom data on SVR at month 24 was not available may have contributed to the relatively high observed SVR, since it is feasible that patients with a poor viral response were more likely to be withdrawn from the study prematurely.

As reported in non-transplant patients, early virological response in liver transplant recipients is, unsurprisingly, predictive of SVR [10, 15, 42]. In their meta-analysis, Rabie et al. did not observe a significant difference in early virological response between CsA and tacrolimus, although the relapse rate was lower with CsA (19% versus 26% with tacrolimus; p=0.02) [34]. In our population, there were no differences in early virological response or other secondary virological or fibrosis endpoints between the 2 groups. Similar findings were observed in the pilot study of 38 patients reported by Firpi et al. [33].

## Conclusions

This is the largest randomized controlled study to directly compare the 2 CNI therapies in terms of the efficacy of antiviral therapy for HCV after liver transplantation. The findings of this randomized trial coincide with those of an earlier pilot study [33] and are broadly consistent with those of a meta-analysis of studies published to date [34]. However, since only a fraction of the planned population was recruited, and since only half of the patients completed the study on the randomized study drug, robust conclusions cannot be drawn regarding the relative effect of CsA and tacrolimus on the virological response to antiviral treatment for recurrent HCV after liver transplantation. Further randomized clinical trials are required but are unlikely to be performed due to ongoing changes in the modalities for HCV treatment after liver transplantation.

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**Conflicts of interest**

C. Duvoux has received speaker's honoraria, research funding, educational grants, membership of advisory boards, travel grants from Novartis, and speaker's honoraria and travel grants from Astellas, been a member of a Data Safety Monitoring Board for Novartis, and has received educational grants and research funding from Astellas and Roche.
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E.L. Renner has received research grants and speaker's honoraria from Novartis and Astellas, and sat on advisory boards for Novartis and Astellas.
G.L. Grazi has no conflicts of interest to declare.
R.J. Firpi is a member of advisory boards for Gilead and Janssen, and has received research funding from Gilead, Boehringer, Idenix, Genfit, Salix, Daiichi Sankyo, Bayer, and Vertex.
G. Pageaux has been a member of advisory boards for Astellas and Novartis.
B. Mullhaupt has no conflicts of interest to declare.
F. Schirm is an employee of Datamap GmbH and received biostatistical support with funding from Novartis Pharma AG.
B. Rauer and P. Bernhardt are employees of Novartis Pharma AG.
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