American Journal of Transplantation 2013; 13: 1734–1745
Wiley Periodicals Inc.

doi: 10.1002/ajt.12280

Renal Function at Two Years in Liver Transplant Patients Receiving Everolimus: Results of a Randomized, Multicenter Study

F. Saliba[1,*], P. De Simone[2], F. Nevens[3], L. De Carlis[4], H. J. Metselaar[5], S. Beckebaum[6,7], S. Jonas[8], D. Sudan[9], L. Fischer[10], C. Duvoux[11], K. D. Chavin[12], B. Koneru[13], M. A. Huang[14], W. C. Chapman[15], D. Foltys[16], G. Dong[17], P. M. Lopez[18], J. Fung[19] and G. Junge[18], for the H2304 Study Group[†]

[1]*Hepatobiliary Center, AP-HP Hôpital Paul Brousse, Université Paris-Sud, Villejuif, France*
[2]*General Surgery and Liver Transplantation, Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy*
[3]*Department of Hepatology, University Hospital KU Leuven, Leuven, Belgium*
[4]*Department of General Surgery and Transplantation, Azienda Ospedaliera Niguarda Cá Granda, Milan, Italy*
[5]*Department of Gastroenterology and Hepatology, Erasmus MC, University Hospital Rotterdam, Rotterdam, the Netherlands*
[6]*Department of General, Visceral and Transplantation Surgery, University Hospital Essen, Essen, Germany*
[7]*Department of Transplant Medicine, University Hospital Münster, Münster, Germany*
[8]*Department of Visceral, Transplantation, Thoracic and Vascular Surgery, University Medical Center Leipzig, Leipzig, Germany*
[9]*Division of Transplant Surgery, Department of General Surgery, Duke University Medical Center, Durham, NC*
[10]*Department of Hepatobiliary Surgery and Transplantation, University Medical Center Eppendorf, Hamburg, Germany*
[11]*Liver Transplant Unit, AP-HP Hôpital Henri Mondor, Créteil, France*
[12]*Division of Transplant Surgery, Medical University of South Carolina, Charleston, SC*
[13]*Department of Surgery, University of Medicine and Dentistry—New Jersey Medical School, Newark, NJ*
[14]*Division of Gastroenterology, Department of Internal Medicine, Henry Ford Hospital, Detroit, MI*
[15]*Department of Surgery, Washington University School of Medicine, St Louis, MO*
[16]*Department of Transplant Surgery, University Medical Center, Johannes Gutenberg University, Mainz, Germany*
[17]*Novartis Pharmaceuticals, East Hanover, NJ*
[18]*Novartis Pharma AG, Basel, Switzerland*
[19]*Transplantation Center, Cleveland Clinic, Cleveland, OH*
**Corresponding author: Faouzi Saliba, faouzi.saliba@pbr.aphp.fr*
Trial registration number: NCT00622869.
[†]H2304 study investigators are listed in the Appendix.

In a 24-month prospective, randomized, multicenter, open-label study, *de novo* liver transplant patients were randomized at 30 days to everolimus (EVR) + Reduced tacrolimus (TAC; n = 245), TAC Control (n = 243) or TAC Elimination (n = 231). Randomization to TAC Elimination was stopped prematurely due to a significantly higher rate of treated biopsy-proven acute rejection (tBPAR). The incidence of the primary efficacy endpoint, composite efficacy failure rate of tBPAR, graft loss or death postrandomization was similar with EVR + Reduced TAC (10.3%) or TAC Control (12.5%) at month 24 (difference −2.2%, 97.5% confidence interval [CI] −8.8%, 4.4%). BPAR was less frequent in the EVR + Reduced TAC group (6.1% vs. 13.3% in TAC Control, p = 0.010). Adjusted change in estimated glomerular filtration rate (eGFR) from randomization to month 24 was superior with EVR + Reduced TAC versus TAC Control: difference 6.7 mL/min/1.73 m^2 (97.5% CI 1.9, 11.4 mL/min/1.73 m^2, p = 0.002). Among patients who remained on treatment, mean (SD) eGFR at month 24 was 77.6 (26.5) mL/min/1.73 m^2 in the EVR + Reduced TAC group and 66.1 (19.3) mL/min/1.73 m^2 in the TAC Control group (p < 0.001). Study medication was discontinued due to adverse events in 28.6% of EVR + Reduced TAC and 18.2% of TAC Control patients. Early introduction of everolimus with reduced-exposure tacrolimus at 1 month after liver transplantation provided a significant and clinically relevant benefit for renal function at 2 years posttransplant.

Key words: Everolimus, glomerular filtration rate, mTOR inhibitors, renal function, tacrolimus

Abbreviations: ALT, alanine aminotransferase; ANCOVA, analysis of covariance; AST, aspartate aminotransferase; CI, confidence interval; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CNI, calcineurin inhibitor; eGFR, estimated glomerular filtration rate; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; ITT, intent-to-treat; MDRD4, four-variable Modification of Diet in Renal Disease; mTOR, mammalian target of rapamycin; RAI, rejection activity index; SE, standard error; tBPAR, treated biopsy-proven acute rejection.

Received 28 November 2012, revised 06 February 2013 and accepted 11 February 2013

Introduction

Minimizing exposure to calcineurin inhibitors (CNIs) following solid organ transplantation is a well-established clinical objective. Use of mammalian target of rapamycin (mTOR) inhibitors to support CNI minimization is a highly promising approach, but it remains unclear whether long-term maintenance of low-exposure CNI therapy or CNI elimination is a preferable strategy. Data from kidney transplantation suggest that although CNI reduction can be undertaken without loss of efficacy in the presence of the mTOR inhibitor everolimus (1), tubulointerstitial and glomerular damage continues in the presence of reduced CNI exposure (2). Experience with the use of everolimus to minimize CNI exposure following liver transplantation is more limited and is largely related to CNI-free regimens. One prospective (3) and one retrospective (4) study in maintenance liver transplant patients have suggested that the benefit of conversion to everolimus from CNI therapy appears to be concentrated in patients with greater residual renal function at the time of conversion (3,4). Two randomized trials have explored early switch from CNI to everolimus therapy in liver transplantation (5,6). Masetti et al. (5) observed significantly higher renal function in patients maintained on everolimus monotherapy from day 30 versus cyclosporine with mycophenolate mofetil. In the PROTECT study, conversion from tacrolimus or cyclosporine to everolimus (with or without steroids) over an 8-week period starting on day 30 resulted in higher renal function at 12 months posttransplant based on the four-variable Modification of Diet in Renal Disease (MDRD4) formula without loss of efficacy (6).

In a prospective trial, 719 patients were randomized at 1 month after liver transplantation to continue a standard tacrolimus-based regimen or start everolimus with reduced-exposure tacrolimus or tacrolimus elimination. The primary results at month 12 posttransplant have been reported previously (7). Following completion of the entire 24-month study, the current manuscript focuses on the evolution of renal function from randomization to month 24 posttransplant.

Methods

Study design

This was a 24-month prospective, randomized, multicenter, three-arm, parallel-group, open-label study in *de novo* liver transplant recipients undertaken at transplant centers in 19 countries in Europe, North America and South America and other countries including Australia, Russia and Israel during January 2008 to April 2012.

After a run-in period during which the immunosuppression regimen was identical for all groups, patients were randomized at 30 (±5) days posttransplant in a 1:1:1 ratio to one of three treatment groups: (i) EVR + Reduced TAC; (ii) TAC Control or (iii) TAC Elimination. Patients were stratified prior to randomization according to pretransplant hepatitis C virus (HCV) status and quartiles of renal function (based on estimated glomerular filtration rate [eGFR] according to the MDRD4 formula (8)). Randomization to the TAC Elimination arm was stopped in April 2010, when approximately 690 patients had been randomized in total. This followed a recommendation from the independent Data Monitoring Committee based on a significantly higher rate of acute rejections leading to study discontinuation clustered around the time of tacrolimus withdrawal versus the other two treatment arms. The study protocol was amended to stop recruitment to the TAC Elimination arm, and eligible patients were subsequently randomized equally between the EVR + Reduced TAC and TAC Control groups. Patients already randomized to TAC Elimination who were $\leq$180 days postrandomization were converted to standard treatment, whereas those who were >180 days postrandomization with stable graft function could continue on their assigned TAC Elimination regimen.

The trial was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines and all patients provided written informed consent.

Study endpoints

The primary efficacy endpoint was the composite efficacy failure rate of treated biopsy-proven acute rejection (tBPAR), graft loss or death since randomization. tBPAR was defined as treated acute rejection with rejection activity index (RAI) $\geq$3 according to Banff 1997 criteria (9). The key secondary endpoint was the change in renal function from randomization as assessed by eGFR using the MDRD4 formula (10). These endpoints were revised from the original endpoints after implementation of the protocol amendment. The original co-primary endpoints were noninferior composite efficacy failure rate of death, graft loss or loss to follow-up, and superior renal function (as assessed by eGFR using the MDRD4 formula).

Inclusion and exclusion criteria

Adult (18–70 years) recipients of a primary full-size liver transplant from a deceased donor who had received tacrolimus and corticosteroids (with or without mycophenolic acid) from time of transplant were eligible to enter the run-in period. Key inclusion criteria at the time of randomization comprised (i) eGFR (MDRD4) $\geq$30 mL/min/1.73 m^2; (ii) acceptable graft function (aspartate aminotransferase [AST], alanine aminotransferase [ALT] and total bilirubin levels $\leq$3 times the upper limit of normal, with alkaline phosphatase $\leq$5 times the upper limit of normal) and (iii) tacrolimus trough concentration $\geq$8 ng/mL in the week prior to randomization. Key exclusion criteria included hepatocellular carcinoma (HCC) that did not fulfill Milan criteria (11,12) as per explant histology, and receipt of antibody induction therapy. To enter the run-in period, patients were also excluded if urine protein excretion was $\geq$1.0 g/day. At the point of randomization, key additional inclusion criteria were: Doppler ultrasound showing the patency of hepatic artery, hepatic and portal veins; confirmation of eGFR $\geq$30 mL/min/1.73 m^2; and absence of acute rejection requiring T cell depleting antibody therapy or $\geq$1 episode of steroid-sensitive rejection during the run-in period.

Immunosuppression

In the EVR + Reduced TAC arm, everolimus therapy was initiated at a dose of 1.0 mg b.i.d. within 24 h of randomization, adjusted from day 5 to target a trough (C_0) concentration in the range 3–8 ng/mL. Once everolimus trough concentration was within this range, tacrolimus dose was tapered to achieve a trough concentration of 3–5 ng/mL by week 3 after randomization. In the TAC Control arm, the target tacrolimus trough concentration was 8–12 ng/mL until month 4 posttransplant and 6–10 ng/mL thereafter. In the TAC Elimination arm, everolimus was administered as in the EVR + Reduced TAC group until month 4 posttransplant, when the target trough concentration range had to be increased to 6–10 ng/mL. Tacrolimus elimination was then started and was to be completed by the end of month 4 posttransplant.

Corticosteroids were to be initiated in all patients at the time of transplant and administered according to local practice (including peri-operative intravenous corticosteroids), with a minimum oral dose of 5 mg

prednisolone/day after randomization to be continued until at least month 6 posttransplant. Mycophenolic acid, if used, was administered as per local practice but had to be discontinued by the time of randomization.

Statistical analysis

No inferential statistical comparisons were undertaken for the TAC Elimination group due to premature discontinuation of recruitment and extensive conversion of patients in this arm to standard therapy.

The primary endpoint of composite efficacy failure at month 12 was analyzed based on a noninferiority test with a prespecified 12% noninferiority margin at a one-sided 0.0125 significance level. The incidence of the composite efficacy failure endpoint was estimated using the Kaplan–Meier product-limit formula and standard error (SE) based on Greenwood's formula. The incidence rates of the composite efficacy failure endpoint at month 24, as well as its components at months 12 and 24, were also estimated based on the Kaplan–Meier method. The key secondary endpoint of change in eGFR (MDRD4) from randomization to month 12 was analyzed using an analysis of covariance (ANCOVA) model, with treatment group, pretransplant HCV status and eGFR (MDRD4) at randomization as covariates. Based on this model, a noninferiority test was performed with a noninferiority margin of -6 mL/min/1.73 m^2 at a one-sided 0.0125 significance level. A similar ANCOVA analysis was performed for the change in eGFR from randomization to month 24.

Efficacy and renal function analyses were based on the intent-to-treat (ITT) population, comprising all randomized patients. On-treatment renal function (eGFR) analyses were carried out as supportive analyses, and included eGFR values taken up to 2 days after study drug discontinuation. Safety analyses other than renal function were performed on the safety population, which included all randomized patients who received at least one dose of randomized study drug.

Results

Patient population

The ITT population comprised all 719 randomized patients (EVR + Reduced TAC 245, TAC Elimination 231, TAC Control 243). Study medication was discontinued before month 24 in 104 patients (42.4%) in the EVR + Reduced TAC group and 79 patients (32.5%) in the TAC Control group (Figure 1). In the TAC Elimination group, 166 patients

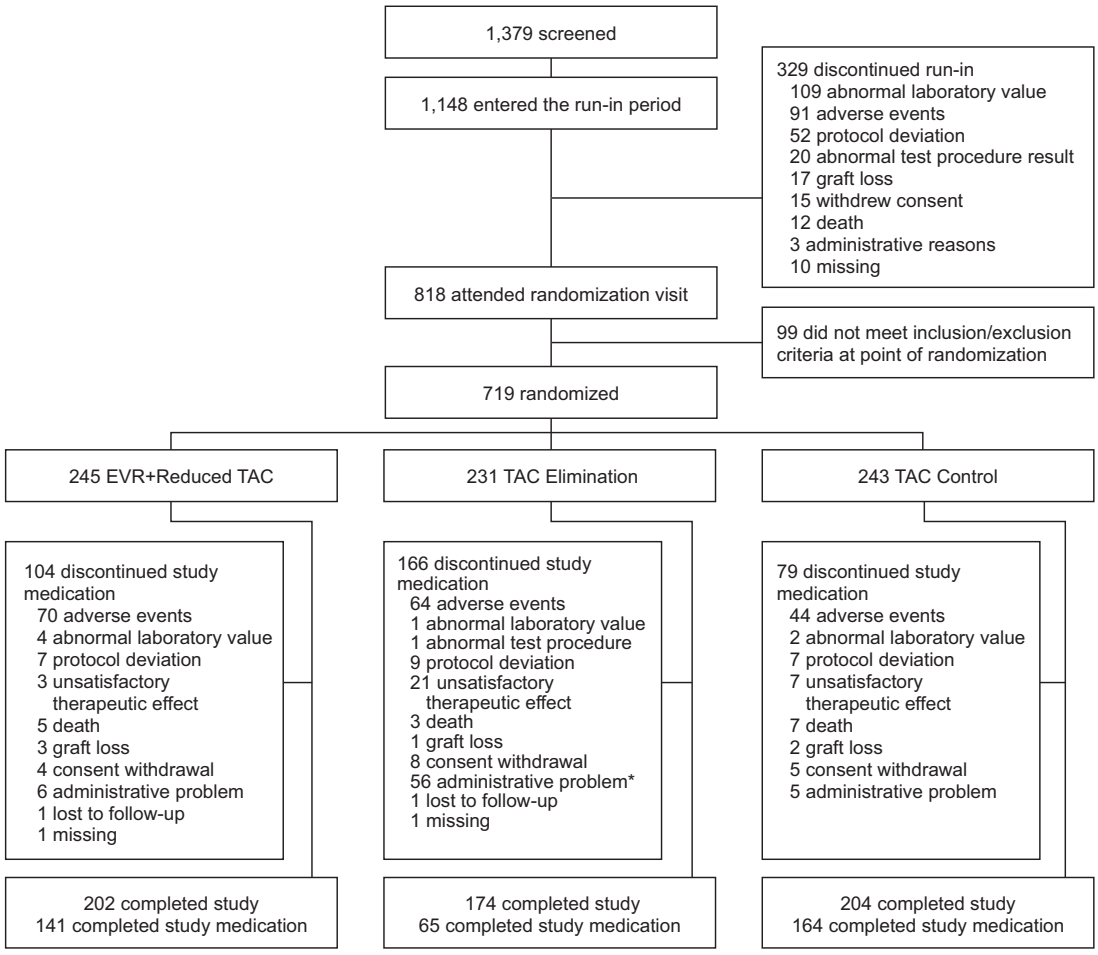


Figure 1: Patient disposition.

(71.9%) discontinued study medication prematurely, including patients up to 180 days postrandomization who converted to standard treatment according to the protocol amendment. In total, 202, 204 and 174 patients in the EVR + Reduced TAC, TAC Control and TAC Elimination groups, respectively, completed the 24-month study (141, 164 and 65 on study medication at 24 months; Figure 1). The treatment groups were well balanced in terms of risk factors for chronic renal failure, including age, gender, pretransplant HCV infection and the incidence of diabetes (Table 1).

Immunosuppression

In the EVR + Reduced TAC group, tacrolimus tapering began after everolimus whole blood trough levels were confirmed to be in the target range (3–8 ng/mL). Immediately after initiation of everolimus (month 1), median C_0 tacrolimus levels declined, reaching 5.7 and 5.2 ng/mL, respectively, at months 2 and 3. From month 6 onwards median C_0 tacrolimus trough levels remained below 5.0 ng/mL reaching 4.5 ng/mL at month 12 and 3.8 ng/mL at month 24 (Figure S1).

Table 1: Demographics and baseline characteristics (ITT population)

	EVR + Reduced TAC N = 245	TAC Elimination N = 231	TAC Control N = 243
Age (years)	53.6 (9.2)	53.2 (10.8)	54.5 (8.7)
Male gender, n (%)	180 (73.5)	164 (71.0)	179 (73.7)
Race, n (%)			
Caucasian	211 (86.1)	196 (84.8)	195 (80.2)
Black	4 (1.6)	6 (2.6)	9 (3.7)
Asian	4 (1.6)	8 (3.5)	5 (2.1)
Other	21 (8.6)	17 (7.4)	29 (11.9)
Missing	5 (2.0)	4 (1.7)	6 (2.5)
Body mass index (kg/m^2)[1]	25.2 (4.2)	25.3 (4.3)	24.5 (4.2)
HCV positive at randomization, n (%)	79 (32.2)	72 (31.2)	76 (31.3)
Diabetes at randomization, n (%)[1]	87 (35.5)	79 (34.2)	97 (39.9)
Primary disease leading to liver transplantation, n (%)			
Alcoholic cirrhosis	70 (28.6)	49 (21.2)	51 (21.0)
Hepatitis C	61 (24.9)	55 (23.8)	56 (23.0)
Hepatocellular carcinoma	44 (18.0)	32 (13.9)	36 (14.8)
Hepatitis B	16 (6.5)	17 (7.4)	15 (6.2)
Sclerosing cholangitis	8 (3.3)	20 (8.7)	12 (4.9)
Primary biliary cirrhosis	8 (3.3)	11 (4.8)	8 (3.3)
Metabolic disease	5 (2.0)	4 (1.7)	6 (2.5)
Cryptogenic cirrhosis	7 (2.9)	11 (4.8)	18 (7.4)
Autoimmune hepatitis	4 (1.6)	7 (3.0)	6 (2.5)
Acute hepatic failure	2 (0.8)	2 (0.9)	3 (1.2)
Other	20 (8.2)	23 (10.0)	32 (13.2)
MELD score[2]	19.2 (9.0)	19.6 (7.5)	19.0 (7.6)
Donor age (years)	48.8 (18.2)	50.0 (18.2)	48.7 (17.4)
Cold ischemia time (h)	8.4 (4.4)	7.5 (2.7)	8.0 (5.2)
Acute rejection prior to randomization, n (%)			
tBPAR	15 (6.1)	10 (4.3)	13 (5.3)
BPAR	20 (8.2)	16 (6.9)	20 (8.2)
Acute rejection[3]	21 (8.6)	20 (8.7)	24 (9.9)
Cystatin C (mg/L) at randomization	1.7 (0.5)	1.7 (0.5)	1.7 (0.5)
eGFR (MDRD4) at randomization, mL/min/1.73 m^2			
Mean (SD)	81.1 (32.6)	82.6 (37.2)	78.0 (27.5)
Median (range)	77.1 (25.4–247.7)	74.9 (21.2–259.1)	75.2 (21.1–193.2)
eGFR (MDRD4) at randomization, n (%)			
<30 mL/min/1.73 m^2	5 (2.0)	4 (1.7)	4 (1.7)
30 to <45 mL/min/1.73 m^2	24 (9.8)	22 (9.5)	23 (9.5)
45 to <60 mL/min/1.73 m^2	42 (17.1)	35 (15.2)	34 (14.0)
≥60 mL/min/1.73 m^2	174 (71.0)	170 (73.6)	182 (74.9)

[1]At randomization.
[2]MELD score based on laboratory values only.
[3]Clinically suspected acute rejection regardless of biopsy confirmation.
Continuous variables are shown as mean (SD) unless otherwise stated.
BPAR, biopsy-proven acute rejection; tBPAR, treated biopsy-proven acute rejection; eGFR, estimated GFR; HCV, hepatitis C virus; MDRD4, four-variable Modification of Diet in Renal Disease; MELD, model for end-stage liver disease.

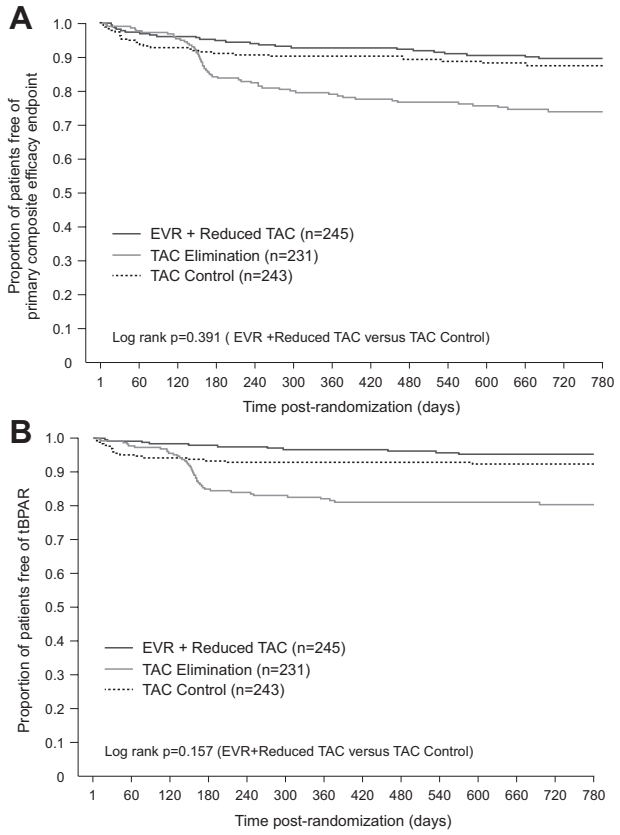


Figure 2: Kaplan–Meier plots for the proportion of patients free from (A) the primary composite efficacy endpoint of tBPAR, graft loss or death and (B) tBPAR (ITT population).

Mean everolimus C_0 concentration was within target range for the EVR + Reduced TAC and TAC Elimination groups throughout the study. At month 12, 22.9% (56/245), 34.3% (83/242) and 20.5% (47/229) of patients in the EVR + Reduced TAC, TAC Control and TAC Elimination groups were receiving corticosteroids; corresponding values at month 24 were 11.8% (29/245), 22.3% (54/242) and 9.6% (22/229). The mean (SD) dose of corticosteroids from randomization to month 24 in the EVR + Reduced TAC arm, the TAC Control arm and the TAC Elimination group was 0.19 (0.61) mg/kg/day, 0.12 (0.08) mg/kg/day and 0.14 (0.16) mg/kg/day, respectively.

Efficacy

As reported previously (7), primary efficacy failure (composite of tBPAR, graft loss or death) at month 12 occurred in 6.5% (16/245) of EVR + Reduced TAC patients and 9.5% (23/243) of TAC Control patients (Kaplan–Meier incidence rate 6.7% vs. 9.7%, respectively, with a difference of −3.0%, 97.5% confidence interval [CI] −8.7%, 2.6%; $p < 0.001$ for a noninferiority test with a noninferiority margin of 12%). The incidence of the primary efficacy failure endpoint was similar between the two groups during months 12–24 (Figure 2A). Comparability for the primary efficacy endpoint was maintained at month 24 (10.3% in the EVR + Reduced TAC group, 12.5% in the TAC Control group; difference −2.2%, 97.5% CI −8.8%, 4.4% in favor of EVR + Reduced TAC; Table 2). The incidence of BPAR at month 24 was significantly lower in the EVR + Reduced TAC group versus the TAC Control group (Kaplan–Meier incidence rate 6.1% vs. 13.3%, $p = 0.010$; Table 2). By month 24, moderate or severe BPAR had occurred in no EVR + Reduced TAC patients and in 10 TAC Control patients.

The incidence of tBPAR (Figure 2B) was numerically lower in the EVR + Reduced TAC group. Graft loss and/or death was similar in the two treatment groups (Table 2).

Estimated GFR

Mean eGFR (MDRD4) was similar between the EVR + Reduced TAC and TAC Control groups at the point of randomization (Table 1). eGFR in the EVR + Reduced TAC

Table 2: Primary efficacy endpoint and selected secondary efficacy endpoints at month 24 (ITT population)

	EVR + Reduced TAC, N = 245	TAC Control, N = 243	TAC Elimination, N = 231	EVR + Reduced TAC vs. TAC Control	
				Difference (97.5% CI)	p value[1]
Primary efficacy endpoint[2,3]					
n	24	29	55		–
KM incidence rate, %	10.3	12.5	26.0	−2.2 (−8.8, 4.4)	0.452
Secondary end points					
Graft loss or death	17 (7.3)	14 (6.2)	18 (8.6)	1.1 (−4.2, 6.4)	0.638
Graft loss, n (KM %)	9 (3.9)	7 (3.2)	6 (2.8)	0.8 (−3.2, 4.7)	0.661
Death, n (KM %)	12 (5.2)	10 (4.4)	15 (7.3)	0.8 (−3.7, 5.2)	0.701
tBPAR, n (KM %)[4]	11 (4.8)	18 (7.7)	42 (19.9)	−2.9 (−7.9, 2.2)	0.203
BPAR, n (KM %)[4]	14 (6.1)	30 (13.3)	52 (26.4)	−7.2 (−13.5, −0.9)	0.010

[1]Z-test (for non-different test).
[2]Treated BPAR (tBPAR), graft loss or death.
[3]Noninferiority test with noninferiority margin of 12%; $p < 0.001$.
[4]BPAR episodes occurring prior to randomization were excluded.
KM, Kaplan–Meier.

arm was statistically superior to the TAC Control arm from 1 month after randomization until the end of the 24-month study, although the difference in favor of the EVR + Reduced TAC arm reduced slightly from months 12 to 24 (Figure 3A; Table S1). At month 12, mean eGFR (SD) in the EVR + Reduced TAC and TAC Control groups was 80.6 (27.5) and 70.3 (23.1) mL/min/1.73 m^2, respectively ($p < 0.001$); at month 24, the corresponding values were 74.7 (26.1) and 67.8 (21.0) mL/min/1.73 m^2 ($p = 0.007$; Table 3).

The evolution of renal function from randomization to month 24 was better in the EVR + Reduced TAC group versus TAC Control when estimated using the MDRD4, CKD-EPI, Nankivell and Cockcroft-Gault formulae (Figure 4). The key secondary endpoint, adjusted change in eGFR (MDRD4) from randomization to month 12 posttransplant, was superior in the EVR + Reduced TAC group versus TAC Control, with a difference of 8.50 mL/min/1.73 m^2 (97.5% CI 3.74, 13.27 mL/min/1.73 m^2, $p < 0.001$ [ANCOVA]). The change from randomization remained significantly superior in the EVR + Reduced TAC group at month 24 (difference 6.7 mL/min/1.73 m^2, 97.5% CI 1.9, 11.4 mL/min/1.73 m^2, $p = 0.002$). More patients in the EVR + Reduced TAC group versus the TAC Control group improved at least one stratum by month 24 among those with poor renal function (30 to <45 mL/min/1.73 m^2) or moderate renal function (45 to <60 mL/min/1.73 m^2; Table S2).

The evolution of eGFR over 2 years posttransplant was similar in the TAC Elimination group to the EVR + Reduced TAC group in the ITT population (Figure 3A). Based on ANCOVA analysis, the difference in adjusted change in eGFR (MDRD4) from randomization for the TAC Elimination group versus TAC Control was 9.22 mL/min/1.73 m^2 (97.5% CI 4.39, 16.1 mL/min/1.73 m^2 [ANCOVA]) to month 12, and 10.4 mL/min/1.73 m^2 (97.5% CI 5.6, 15.3 mL/min/1.73 m^2, $p = 0.002$) to month 24.

Patients were stratified according to HCV serology status at time of randomization. Among patients for whom eGFR data were available at randomization and month 24, the mean (SD) change in the EVR + Reduced TAC group was −8.1 (24.5) mL/min/1.73 m^2 in HCV-negative patients versus −1.6 (33.8) mL/min/1.73 m^2 in HCV-positive patients. For the TAC Control group, the corresponding values were −15.1 (23.9) mL/min/1.73 m^2 and −4.3 (23.9) mL/min/1.73 m^2. For the TAC Elimination group the mean change to month 24 was −8.8 (36.5) mL/min/1.73 m^2 in HCV-negative recipients and 1.5 (38.8) mL/min/1.73 m^2 in HCV-positive recipients.

Estimated GFR: on-treatment analysis

In a pre–planned analysis, on-treatment eGFR was also evaluated using eGFR values up to 2 days after study drug discontinuation. As in the ITT population, eGFR in the EVR + Reduced TAC group was significantly higher than in the TAC Control arm by 1 month after randomization and thereafter (all $p < 0.001$; Figure 3B). At month 24, mean (SD) eGFR (MDRD4) for patients who remained on-treatment was 77.6 (26.5) mL/min/1.73 m^2 in the EVR + Reduced TAC group versus 66.1 (19.3) mL/min/1.73 m^2 in the TAC Control group ($p < 0.001$) and 86.2 (28.0) mL/min/1.73 m^2 in the TAC Elimination group. As mentioned above, the difference in favor of the EVR + Reduced TAC arm reduced slightly by month 24 in the ITT population, but in the on-treatment population the mean (SD) change from months 12 to 24 was similar in the EVR + Reduced TAC group and the TAC Control group (−3.0 [16.2] vs. −2.8 [15.2] mL/min/1.73 m^2).

Other renal function parameters

Mean (SD) serum creatinine level was similar between the EVR + Reduced TAC and TAC Control groups at randomization, but significantly lower in the EVR + Reduced TAC cohort at month 24 (Table 3). During the period from randomization to month 24, renal replacement therapy was required by seven patients in the EVR + Reduced TAC arm, eight patients in the TAC Control group and four patients in the TAC Elimination arm (2.9%, 3.3% and 1.7%, respectively).

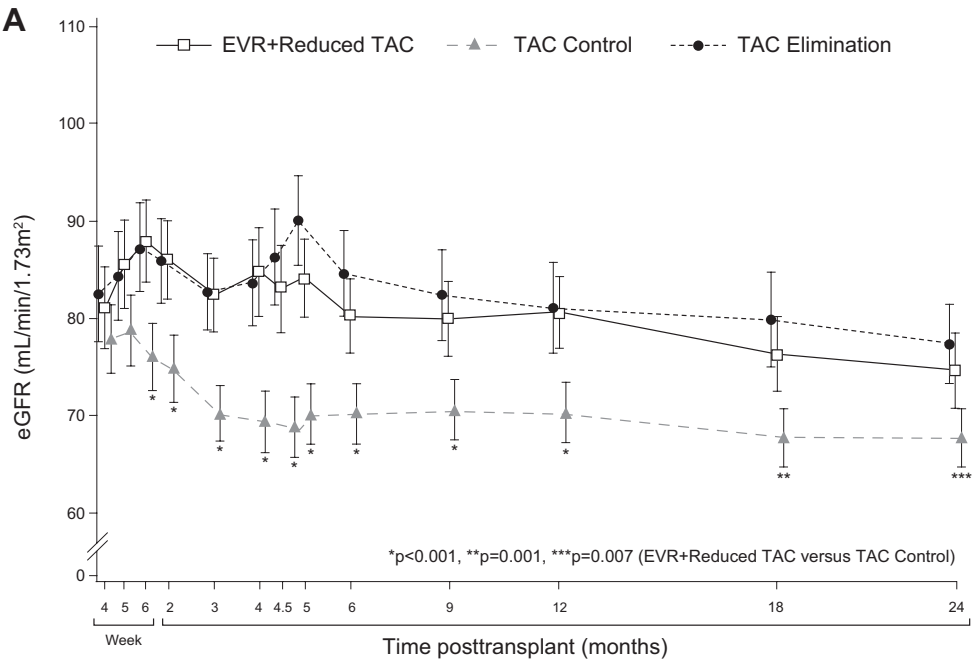
Proteinuria (defined as 1.0 to <3.0 g/day) was present in four EVR + Reduced TAC patients and one TAC Control patient at randomization and in five and three patients, respectively, at month 24. Proteinuria was reported as an adverse event in nine and two patients, respectively ($p = 0.063$). Nephrotic syndrome (≥3.0 g/day) was present in no EVR + Reduced TAC patients and one TAC Control patient at randomization, and in no patients at month 24.

The urine protein to creatinine ratio became slightly higher in the EVR + Reduced TAC group versus TAC Control by month 4 in the ITT population (Figure S2A), but for patients on-treatment the difference was not significant (Figure S2B). Values peaked at month 6 in the EVR + Reduced TAC group (mean [SD] 290 (607) mg/g, median [range] 105 [33–4143] mg/g) and at month 2 in the TAC Control arm (mean [SD] 264 (914) mg/g, median [range] 108 [39–10 370]) but remained below 300 mg/g in both treatment arms at all times after randomization. Of the 10 patients in the EVR + Reduced TAC group who had urine protein to creatinine ratio ≥500 mg/g at randomization, six showed a subsequent decline, three did not provide a postrandomization value, and the remaining patient had a ratio of 2254 mg/g at baseline and 3646 mg/g at month 18 with no value recorded at month 24.

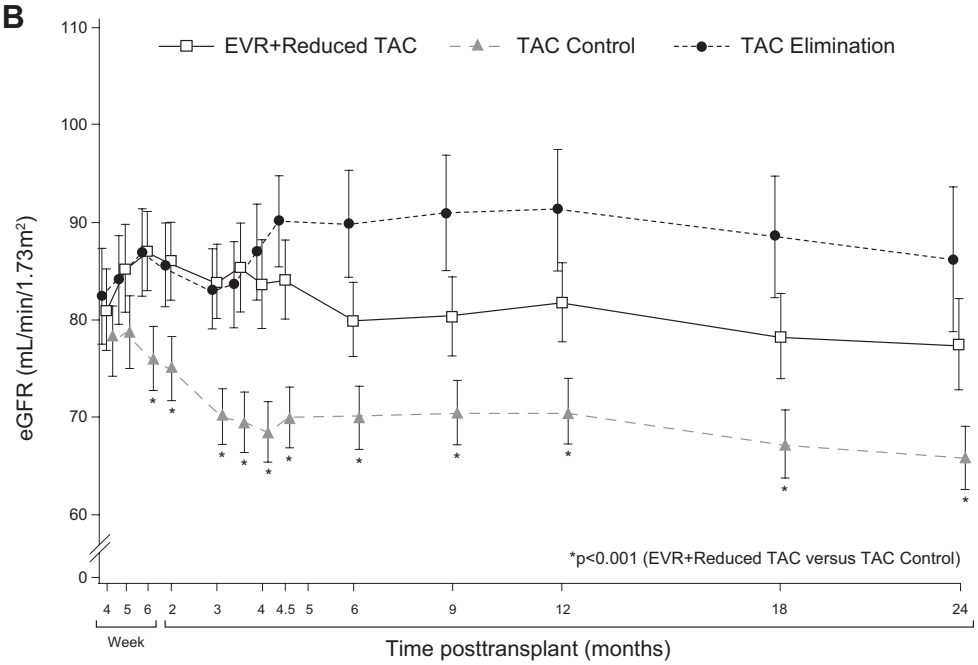
Cystatin C levels were similar at randomization (Table 1) and at month 24, when mean (SD) values were 1.3 (0.4) mg/L in both the EVR + Reduced TAC and TAC Control groups.

Safety

Over the 24-month study period, the overall rates of adverse events (96.3% in the EVR + Reduced TAC group and 97.9% in the TAC Control group) and serious adverse events (56.3% and 54.1%, respectively) were similar



No. patients	W4	M2	M3	M4	M4.5	M5	M6	M9	M12	M18	M24
TAC Elimination	218	218	213	170	148	163	202	196	195	175	163
EVR+Reduced TAC	233	223	221	189	168	191	204	216	216	203	184
TAC Control	227	227	229	192	177	197	218	215	209	197	186



No. patients	W4	M2	M3	M4	M4.5	M5	M6	M9	M12	M18	M24
TAC Elimination	218	212	190	163	138	154	136	106	195	67	57
EVR+Reduced TAC	233	216	205	183	166	188	177	179	216	149	124
TAC Control	227	225	218	191	177	197	193	188	209	163	147

Figure 3: eGFR (MDRD4) according to treatment group (A) ITT population (B) on-treatment patients. Values are shown as mean and 95% CI.

Table 3: Renal endpoints at month 24 according to treatment group (ITT population)

	Randomization				Month 24			
	EVR + Reduced TAC	TAC Control	TAC Elimination	p value[1]	EVR + Reduced TAC	TAC Control	TAC Elimination	p value[1]
MDRD4 (mL/min/1.73 m^2), mean (SD)	81.1 (32.6) (n = 233)	78.0 (27.5) (n = 227)	82.6 (37.2) (n = 218)	0.553	74.7 (26.1) (n = 184)	67.8 (21.0) (n = 186)	77.5 (26.2) (n = 163)	0.007
eGFR, CKD-EPI (mL/min/1.73 m^2), mean (SD)	78.2 (25.3) (n = 233)	76.5 (23.9) (n = 227)	78.6 (26.8) (n = 218)	0.455	74.1 (24.4) (n = 184)	67.5 (20.0) (n = 186)	76.5 (23.3) (n = 163)	0.006
eGFR, Nankivell (mL/min/1.73 m^2), mean (SD)	91.3 (27.6) (n = 223)	87.2 (23.7) (n = 209)	91.6 (28.2) (n = 208)	0.275	88.9 (23.3) (n = 169)	82.2 (18.6) (n = 164)	90.7 (22.6) (n = 148)	0.003
Creatinine clearance Cockcroft-Gault (mL/min), mean (SD)	87.3 (31.9) (n = 233)	81.3 (27.7) (n = 227)	88.6 (39.2) (n = 218)	0.069	88.3 (35.5) (n = 184)	80.0 (27.9) (n = 186)	90.3 (36.0) (n = 163)	0.033
Serum creatinine (μmol/L), mean (SD)	96 (35) (n = 233)	98 (34) (n = 227)	95 (35) (n = 218)	0.563	101 (36) (n = 184)	106 (29) (n = 186)	96 (34) (n = 163)	0.014

[1]Wilcoxon rank sum test for EVR + Reduced TAC vs. TAC Control.

CKD-EPI, chronic kidney disease epidemiology collaboration; eGFR, estimated glomerular filtration rate; MDRD4, four-variable Modification of Diet in Renal Disease; SD, standard deviation.

between groups. Hyperlipidemia, neutropenia, peripheral edema, stomatitis/mouth ulceration and thrombocytopenia were more frequent in the EVR + Reduced TAC arm, while renal failure excluding proteinuria was more frequent in the TAC Control group (Table 4). Discontinuation of study medication due to adverse events was more frequent in the EVR + Reduced TAC arm versus TAC Control (70/245 [28.6%] vs. 44/242 [18.2%]). The most frequent adverse events leading to discontinuation of study medication were proteinuria (eight EVR + Reduced TAC patients and one TAC Control), hepatitis C recurrence (six and five patients) and renal failure (two and six patients). Pancytopenia, leukopenia and thrombocytopenia led to discontinuation of three, two and two patients, respectively, in the EVR + Reduced TAC group.

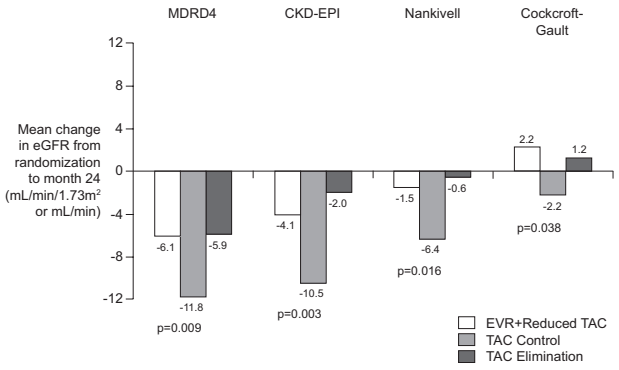


Figure 4: Change in eGFR from randomization to month 24 according to treatment group by different formulae (ITT population). p values refer to EVR + Reduced TAC vs. TAC Control (Wilcoxon's Rank sum test).

Infections occurred in 56.3% (138/245) of patients in the EVR + Reduced TAC group, with bacterial, viral and fungal infections reported in 19.6%, 18.4% and 3.3%, respectively. The incidence of infection in the TAC Control arm was 51.7% (125/242; 13.2% bacterial infection, 18.2% viral infections and 6.2% fungal infections). There were no significant differences in infection rates overall or for each category of infection (Table 4), although there was a trend to more bacterial infections in the EVR + Reduced TAC group (p = 0.067, risk difference 6.4%, 95% CI −0.2, 12.9). Cytomegalovirus infection was reported as an adverse event in 12 EVR + Reduced TAC patients (4.9%) and 13 TAC Control (5.4%; Table 4).

Discussion

This is the first trial to report outcomes at 2 years in liver transplant recipients randomized to reduced-exposure TAC or TAC Elimination versus a standard TAC Control group. The results demonstrate that renal function, as assessed by eGFR, was superior at 2 years after liver transplantation in patients randomized to EVR + Reduced TAC at 1 month posttransplant versus those receiving a standard tacrolimus regimen. The superior renal function in the EVR + Reduced TAC arm was achieved without a penalty in terms of efficacy: Comparable efficacy results versus the TAC Control group at month 24 were confirmed despite very low TAC concentrations (3–5 ng/mL). Indeed, significantly fewer BPAR events were observed with EVR + Reduced TAC versus the TAC Control arm. Although enrollment into the TAC Elimination arm was discontinued prematurely and many patients were converted back to standard treatment, the relatively smaller number of patients who remained on a

Table 4: Adverse events and infections of clinical interest at month 24, N (%; safety population)

				EVR + Reduced TAC vs. TAC Control	
	EVR + Reduced TAC, N = 245	TAC Elimination, N = 229	TAC Control, N = 242	p value[1]	Risk difference, % (95% CI)
Any adverse event	236 (96.3)	216 (94.3)	237 (97.9)	0.42	−1.6 (−4.6, 1.4)
Anemia	24 (9.8)	29 (12.7)	25 (10.3)	0.88	−0.5 (−5.9, 4.8)
Angioedema	6 (2.4)	4 (1.7)	5 (2.1)	1.00	0.4 (−2.3, 3.0)
Ascites	11 (4.5)	14 (6.1)	11 (4.5)	1.00	−0.1 (−3.7, 3.6)
Cytomegalovirus infection	12 (4.9)	17 (7.4)	13 (5.4)	0.84	−0.5 (−4.4, 3.4)
Cardiovascular event	10 (4.1)	4 (1.7)	15 (6.2)	0.31	−2.1 (−6.0, 1.8)
Gastrointestinal ulcers	5 (2.0)	3 (1.3)	8 (3.3)	0.42	−1.3 (−4.1, 1.6)
Hepatocellular carcinoma recurrence	3 (1.2)	4 (1.7)	3 (1.2)	1.00	−0.0 (−2.0, 1.9)
Hyperlipidemia	66 (26.9)	63 (27.5)	28 (11.6)	<0.001	15.4 (8.5, 22.2)
Incisional hernia	24 (9.8)	15 (6.6)	19 (7.9)	0.52	1.9 (−3.1, 7.0)
Interstitial lung disease	2 (0.8)	1 (0.4)	2 (0.8)	1.00	−0.0 (−1.6, 1.6)
Malignancy	19 (7.8)	16 (7.0)	17 (7.0)	0.86	0.7 (−3.9, 5.4)
Neutropenia	38 (15.5)	31 (13.5)	19 (7.9)	0.011	7.7 (2.0, 13.3)
New onset diabetes mellitus	51 (20.8)	53 (23.1)	40 (16.5)	0.25	4.3 (−2.6, 11.2)
Peripheral edema	55 (22.4)	45 (19.7)	36 (14.9)	0.036	7.6 (0.7, 14.5)
Pleural effusion	15 (6.1)	7 (3.1)	13 (5.4)	0.85	0.8 (−3.4, 4.9)
Proteinuria	9 (3.7)	11 (4.8)	2 (0.8)	0.063	2.8 (0.2, 5.5)
Renal failure (excluding proteinuria)	52 (21.2)	40 (17.5)	74 (30.6)	0.023	−9.4 (−17, −1.6)
Stomatitis/mouth ulceration	26 (10.6)	10 (4.4)	3 (1.2)	<0.001	9.4 (5.3, 13.5)
Thrombocytopenia	20 (8.2)	21 (9.2)	7 (2.9)	0.016	5.3 (1.2, 9.3)
Thrombotic and thromboembolic events	18 (7.3)	13 (5.7)	14 (5.8)	0.58	1.6 (−2.8, 6.0)
Thrombotic microangiopathy	0 (0.0)	1 (0.4)	0 (0.0)	—	—
Wound healing complications	27 (11.0)	25 (10.9)	20 (8.3)	0.36	2.8 (−2.5, 8.0)
Any infection	138 (56.3)	134 (58.5)	125 (51.7)	0.32	4.7 (−4.2, 13.5)
Bacterial infection	48 (19.6)	45 (19.7)	32 (13.2)	0.067	6.4 (−0.2, 12.9)
Viral infection	45 (18.4)	45 (19.7)	44 (18.2)	1.00	0.2 (−6.7, 7.1)
Fungal infection	8 (3.3)	17 (7.4)	15 (6.2)	0.14	−2.9 (−6.7, 0.8)

[1]1Fisher's exact test.

tacrolimus-free regimen showed strikingly good renal function at month 24.

The effect of treatment on renal function was apparent early after randomization, with the difference reaching significance by the first month (7) and peaking around month 4. In the first 3–4 months after randomization, eGFR remained largely unchanged in the EVR + Reduced TAC group but declined in the standard tacrolimus arm until reaching a plateau at a level approximately 10 mL/min/1.73 m^2 lower than in the EVR + Reduced TAC cohort. Although this difference narrowed somewhat during months 12–24 in the ITT population, the difference between the two groups in adjusted change of eGFR from randomization to month 24 was 6.7 mL/min/1.73 m^2, a benefit that can be considered clinically relevant. Interestingly, the difference in eGFR at month 24 for on-treatment patients was 11.5 mL/min/1.73 m^2 in favor of EVR + Reduced TAC, reflecting the impact of patients being discontinued from study medication in the EVR + Reduced TAC arm and then receiving higher doses of CNI. In the TAC Control arm the reverse was observed: A greater proportion of patients with tacrolimus exposure below the recommended target range appeared beneficial to renal function in the ITT population (Figure 3B). Impaired renal function at 1 year after liver transplantation is predictive of subsequent deterioration and poor kidney function at 5 years (13–15), so it would seem likely that a benefit in renal function might be sustained long term although this remains speculative.

It was notable that among those patients who remained on their randomized treatment, the difference in mean eGFR between the EVR + Reduced TAC group versus the control group (11.5 mL/min/1.73 m^2) was more marked than in the overall ITT population (Figure 3B). Results of this preplanned on-treatment analysis provide a more accurate assessment of the potential renal effects of an everolimus-based low-CNI regimen.

A clear separation in tacrolimus exposure and a substantial overall reduction of 39% was achieved in the EVR + Reduced TAC arm compared to TAC Control. Median C_0 tacrolimus trough levels were below 6.0 ng/mL as early as month 1 after randomization and were consistently below 5.0 ng/mL from month 5 postrandomization onward, suggesting that the recommended target ranges for both everolimus 3–8 ng/mL and tacrolimus 3–5 ng/mL were

achieved in the majority of EVR + Reduced TAC patients. Given the very low rate of BPAR in the EVR + Reduced TAC arm, further reduction to even lower tacrolimus exposure ranges might be feasible after introduction of everolimus and could be justified in order to minimize CNI-related toxicity.

Premature discontinuation of recruitment to the TAC Elimination arm, and switching of patients less than 6 months postrandomization to standard therapy, necessarily limits interpretation of renal function in this group. However, eGFR data were provided at month 24 by 57 patients who continued to receive their assigned tacrolimus-free treatment regimen throughout the study. It is striking that in a preplanned analysis of on-treatment patients, eGFR values for the TAC Elimination and EVR + Reduced TAC groups diverged after CNI discontinuation (from month 4 posttransplant), with the TAC Elimination group showing a sustained improvement in renal function. At month 24, eGFR was approximately 10 mL/min/1.73 m^2 higher in the tacrolimus-free arm versus the EVR + Reduced TAC group, and approximately 20 mL/min/1.73 m^2 higher than the standard TAC group. Although relatively fewer patients were maintained on the CNI-free regimen up to month 24 compared to the number of patients in the other two treatment groups who completed the 2-year study, this marked renal benefit does suggest that tacrolimus elimination with everolimus introduction at month 1 after liver transplantation could be a potentially valuable strategy for preserving renal function. Further characterization of patients who may be suitable candidates for an everolimus-based, CNI-free regimen is required, as is exploration of different partner drugs (e.g. mycophenolic acid) and the optimal procedure for conversion based on the current findings and those of previous trials of CNI elimination (5,6).

There was no significant difference in the rate of wound healing events between the EVR + Reduced TAC and the TAC Control arms (11.0% vs. 8.3%, respectively), although it should be borne in mind that the study was not powered to detect differences in relatively infrequent adverse events. In kidney transplantation, recent studies which have employed a similar regimen of concentration-controlled everolimus with reduced-exposure CNI therapy to that used here have reported no association with an increased rate of wound healing complications (1,16). Moreover, in the current trial introduction of everolimus was delayed until day 30, avoiding the period of highest risk for healing complications related to the initial transplant procedure. Bacterial infections, while numerically higher in the EVR + Reduced TAC arm, did not differ significantly and the overall rate of infection was similar between the two groups.

Nephrotic proteinuria was rare. The mean urine protein to creatinine ratio was slightly higher in the EVR + Reduced TAC arm versus the TAC Control group, a difference that was significant in the ITT population but not among patients who remained on treatment. It remained below the subnephrotic range throughout the study with only 1 of the 10 patients with overt proteinuria ($\geq$500 mg/day) at randomization experiencing worsening proteinuria during the study. Overall, aggravation of proteinuria in maintenance liver (17) and kidney (18,19) transplant patients after conversion to mTOR inhibitors, particularly in patients with significant existing proteinuria, does not seem as concerning in the *de novo* liver transplant setting. Nevertheless, careful monitoring of proteinuria in future studies, over longer follow-up periods, is important.

The study design included several features that merit consideration. Use of an open-label design was mandated by the need for careful adjustments in everolimus and tacrolimus exposure. The absence of blinding, however, would not have influenced laboratory measurements of renal function. Stratification according to HCV status and renal function at the time of randomization helped to achieve balance between the treatment arms in terms of the likely evolution of renal function (20,21). The control regimen—tacrolimus with steroids either continued or withdrawn not earlier than 6 months posttransplant—represents a standard immunosuppressive regimen after liver transplantation, although addition of mycophenolic acid (particularly during the early posttransplant period to facilitate lower tacrolimus exposure) has become increasingly widespread since the study protocol was developed. Introduction of everolimus at day 30 and exclusion of patients without patent hepatic vasculature by hepatic Doppler ultrasound were considered appropriate strategies to minimize the possible risk of transplant-related wound healing events and hepatic artery thrombosis reported using sirolimus in *de novo* liver transplant recipients. For the assessment of renal function, eGFR is known to be less reliable than direct measurements (22,23) but multiple methods of estimating GFR were used to confirm the findings obtained with the MDRD4 formula and showed consistent results. In terms of generalizability, the study population showed similar eGFR values at the time of randomization to those seen in the general liver transplant population (13).

In conclusion, results from this randomized trial demonstrate that introduction of everolimus with reduced-exposure tacrolimus at 1 month after liver transplantation achieves robust preservation of renal function at 2 years posttransplant with no compromise in efficacy. These results mirror findings at 1 year (7), with no marked changes in efficacy or safety profile during months 12–24. While elimination of tacrolimus at 4 months posttransplant was associated with an increased rate of acute rejection, those patients who remained on the tacrolimus-free regimen at 2 years exhibited excellent renal function, with mean eGFR approximately 20 mL/min/1.73 m^2 higher than in the standard-tacrolimus group.

Acknowledgments

The study was supported by Novartis Pharma AG, Switzerland. A draft manuscript was prepared by a medical writer with funding by Novartis Pharma AG, which was then critically reviewed and revised by all authors. The final decision to submit was made by the authors.

Disclosure

F.S. is a member of an Advisory Committee for Novartis, Astellas, Roche and Genzyme and has received speaker's fees from Schering Plough and Gambro; P.D.S. acts as a consultant to Novartis; F.N. has received grant support from Ipsen, Roche, M.S.D. and Boston Scientific; L.F. is a member of an Advisory Committee for Novartis, has received grant support from Novartis and Astellas and has received speaker's fees from Gilead Sciences; C.D. 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; M.A.H. has received speaker's fees from Vertex and is a member of an Advisory Committee for Bayer and Onyx; J.F. is a member of an Advisory Committee for BMS, has received speaker's fees from GSK and acts as a consultant to Vital Therapies; G.D., P.M.L. and G.J. are employees of Novartis; L.D.C., H. J.M., S.B., S.J., D.S., K.D.C., B.K., W.C.C. and D.F. have no conflicts of interest to declare.

References

1. Tedesco Silva H Jr., Cibrik D, Johnston T, et al. Everolimus plus reduced-exposure CsA versus mycophenolic acid plus standard-exposure CsA in renal-transplant recipients. Am J Transplant 2010; 10: 1401–1413.
2. Flechner SM, Kobashigawa J, Klintmalm G. Calcineurin inhibitor-sparing regimens in solid organ transplantation: Focus on improving renal function and nephrotoxicity. Clin Transplant 2008; 22: 1–15.
3. De Simone P, Metselaar HJ, Fischer L, et al. Conversion from a calcineurin inhibitor to everolimus therapy in maintenance liver transplant recipients: A prospective, randomized, multicenter trial. Liver Transpl 2009; 15: 1262–1269.
4. Saliba F, Dharancy S, Lorho R, et al. Conversion to everolimus in maintenance liver transplant patients: A multicenter, retrospective analysis. Liver Transpl 2011; 17: 905–913.
5. Masetti M, Montalti R, Rompianesi G, et al. Early withdrawal of calcineurin inhibitors and everolimus monotherapy in *de novo* liver transplant recipients preserves renal function. Am J Transplant 2010; 10: 2252–2262.
6. Fischer L, Klempnauer J, Beckebaum S, et al. A randomized, controlled study to assess the conversion from calcineurin-inhibitors to everolimus after liver transplantation—PROTECT. Am J Transplant 2012; 12: 1855–1865.
7. De Simone P, Nevens F, De Carlis L, et al. Everolimus with reduced tacrolimus improves renal function in *de novo* liver transplant recipients: A randomized controlled trial. Am J Transplant 2012; 12: 3008–3020.
8. Poggio ED, Wang X, Weinstein DM, et al. Assessing glomerular filtration rate by estimation equations in kidney transplant recipients. Am J Transplant 2006; 6: 100–108.
9. Banff schema for grading liver allograft rejection: An international consensus document. Hepatology 1997; 25: 658–663.
10. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med 2009; 150: 604–612.
11. Mazzaferro V, Regalia E, Doci R, et al. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. N Engl J Med 1996; 334: 693–699.
12. Shetty K, Timmins K, Brensinger C, et al. Liver transplantation for hepatocellular carcinoma validation of present selection criteria in predicting outcome. Liver Transpl 2004; 10: 911–918.
13. Burra P, Senzolo M, Masier A, et al. Factors influencing renal function after liver transplantation. Results from the MOST, an international observational study. Dig Liver Dis 2009; 41: 350–356.
14. Herlenius G, Fistouris J, Olausson M, Felldin M, Bäckman L, Friman S. Early renal function post-liver transplantation is predictive of progressive chronic kidney disease. Scand J Gastroenterol 2008; 43: 344–349.
15. O'Riordan A, Wong V, McCormick PA, Hegarty JE, Watson AJ. Chronic kidney disease post-liver transplantation. Nephrol Dial Transplant 2006; 21: 2630–2636.
16. Albano L, Berthoux F, Moal MC, et al. Incidence of delayed graft function and wound healing complications after deceased-donor kidney transplantation is not affected by *de novo* everolimus. Transplantation 2009; 88: 69–76.
17. Wadei HM, Zaky ZS, Keaveny AP, et al. Proteinuria following sirolimus conversion is associated with deterioration of kidney function in liver transplant recipients. Transplantation 2012; 93: 1006–1012.
18. Lebranchu Y, Thierry A, Thervet E, et al. Efficacy and safety of early cyclosporine conversion to sirolimus with continued MMF-four-year results of the Postconcept study. Am J Transplant 2011; 11: 1665–1675.
19. Sánchez-Fructuoso AI, Ruiz JC, Calvo N, et al. Everolimus as primary immunosuppression in kidney transplantation: Experience in conversion from calcineurin inhibitors. Transplantation 2012; 93: 398–405.
20. Lee JP, Heo NJ, Joo KW, et al. Risk factors for consequent kidney impairment and differential impact of liver transplantation on renal function. Nephrol Dial Transplant 2010; 25: 2772–2785.
21. Fabrizi F, Dixit V, Martin P, Messa P. Chronic kidney disease after liver transplantation: Recent evidence. Int J Artif Organs 2010; 33: 803–811.
22. Gonwa TA, Jennings L, Mai ML, Stark PC, Levey AS, Klintmalm GB. Estimation of glomerular filtration rates before and after orthotopic liver transplantation: Evaluation of current equations. Liver Transpl 2004; 10: 301–309.
23. Cantarovich M, Yoshida EM, Peltekian KM, et al. Poor prediction of the glomerular filtration rate using current formulas in *de novo* liver transplant patients. Transplantation 2006; 82: 433–436.

Appendix: The H2304 Study Investigators

Argentina: A.C. Gadano, Buenos Aires; L. McCormack, Buenos Aires; L. Toselli, San Martin; S. Yantorno, Buenos Aires; F. Zingalie, Rosario. Australia: J. Chen, Bedford Park; G. Jeffrey, Nedlands; R. Jones, Heidelberg; G. McCaughan,

Camperdown. Brazil: G. Cantisani, Porto Alegre; O. Castro, Ribeirao Preto; L.F. Moreira, Rio de Janerio; J.C. Wiederkehr, Blumenau. Belgium: O. Detry, Liege; F. Nevens, Leuven; X. Rogiers, Gent. Canada: N. Kneteman, Edmonton; P. Marotta, London; G. Therapondros, Toronto; E. Yoshida, Vancouver. Colombia: L. Caicedo, Cali; G. Mejía, Bogotá. Czech Republic: P. Trunecka, Praha. France: E. Boleslawski, Lille; F. Durand, Clichy; C. Duvoux, Creteil; J. Hardwigsen, Marseilles; M. Neau-Cransac, Bordeaux; G. Pageaux, Montpellier; F. Saliba, Villejuif. Germany: L. Fischer, Hamburg; D.B. Foltys, Mainz; S. Jonas, Leipzig; S. Beckebaum, Essen; P. Neuhaus, Berlin; J. Schmidt, Heidelberg; A. Schnitzbauer, Regensburg. Hungary: J. Jaray, Budapest. Ireland: A. McCormick, Dublin. Israel: R. Nakache, Tel-Aviv. Italy: L. De Carlis, Milan; F. Filopponi, Pisa; G.E. Gerunda, Modena; M. Rossi, Rome; M. Salizzoni, Turin. The Netherlands: H.J. Metselaar, Rotterdam. Russia: S. Gautier, Moscow; A.V. Zhao, Moscow. Spain: B. Bilbao Aguirre, Barcelona; J. Fabregat, Llobregat; I. Herero, Pamplona; V. Cuervas Mons, Majadanonda; A. Moya, Valencia; M. Navasa, Barcelona; J. Ortiz de Urbina, Baracaldo; M. Salcedo, Madrid. Sweden: B-G Ericzon, Stockholm. United Kingdom: N. Heaton, London; A. MacGilchrist, Edinburgh. USA: A. Alsina, Tampa; R. Brown, New York; S. Gordon Burroughs, Houston; W.C. Chapman, St Louis; K. Chavin, Charleston; S.S. Cheng, Dallas; S.D. Coloquhoun, Los Angeles; C. Doria, Philadelphia; G. Everson, Aurora; S. Feng, San Francisco; J.J. Fung, Cleveland; R. Gedaly, Lexington; S. Geevarghese, Nashville; J. Goss, Houston; J. Hong, Los Angeles; M.A. Huang, Detroit; L. Johnson, Washington; M. Johnson, Atlanta; G.B. Klintmalm, Dallas; V. Kohli, Oklahoma City; B. Koneru, Newark; T. Kozlowski, Chapel Hill; H. Merhav, Houston; S. Orloff, Portland; L. Sher, Los Angeles; D. Sudan, Durham; L.W. Teperman, New York; G. Testa, Chicago; K. Watt, Rochester.

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Figure S1: Tacrolimus C_0 concentration according to treatment group from time of randomization (ITT population). Values are shown as median with 5th and 95th percentiles.

Figure S2: Median urinary protein to creatinine ratio according to treatment group in (a) ITT population (b) on-treatment population. P values refer to comparisons of EVR + Reduced TAC versus TAC Control.

Table S1: eGFR (MDRD4, mL/min/1.73 m^2) according to treatment group (ITT population). Values are shown as mean (SD)

Table S2: Shift in eGFR (MDRD4, mL/min/1.73 m^2) strata from randomization to month 24 according to treatment group. Percentage values at month 24 calculated using the number of patients in that category at randomization as the denominator