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Everolimus With Reduced Tacrolimus Improves Renal Function in *De Novo* Liver Transplant Recipients: A Randomized Controlled Trial

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In a prospective, multicenter, open-label study, *de novo* liver transplant patients were randomized at day 30±5 to (i) everolimus initiation with tacrolimus elimination (TAC Elimination) (ii) everolimus initiation with reduced-exposure tacrolimus (EVR+Reduced TAC) or (iii) standard-exposure tacrolimus (TAC Control). Randomization to TAC Elimination was terminated prematurely due to a higher rate of treated biopsy-proven acute rejection (tBPAR). EVR+Reduced TAC was noninferior to TAC Control for the primary efficacy endpoint (tBPAR, graft loss or death at 12 months posttransplantation): 6.7% versus 9.7% (−3.0%; 95% CI −8.7, 2.6%; $p<0.001$ for noninferiority [12% margin]). tBPAR occurred in 2.9% of EVR+Reduced TAC patients versus 7.0% of TAC Controls ($p = 0.035$). The change in adjusted estimated GFR from randomization to month 12 was superior with EVR+Reduced TAC versus TAC Control (difference 8.50 mL/min/1.73 m^2, 97.5% CI 3.74, 13.27 mL/min/1.73 m^2, $p<0.001$ for superiority). Drug discontinuation for adverse events occurred in 25.7% of EVR+Reduced TAC and 14.1% of TAC Controls (relative risk 1.82, 95% CI 1.25, 2.66). Relative risk of serious infections between the EVR+Reduced TAC group versus TAC Controls was 1.76 (95% CI 1.03, 3.00). Everolimus facilitates early tacrolimus minimization with comparable efficacy and superior renal function, compared to a standard tacrolimus exposure regimen 12 months after liver transplantation.

Key words: Efficacy, everolimus, liver transplantation, reduced, tacrolimus, withdrawal

Abbreviations: ALT, alanine aminotransferase; ANCOVA, analysis of covariance; AST, aspartate aminotransferase; BPAR, biopsy-proven acute rejection; CKD EPI, Chronic Kidney Disease Epidemiology Collaboration; CMV, cytomegalovirus; CNI, calcineurin inhibitor; DMC, Data Monitoring Committee; EGFR, estimated glomerular filtration rate; HAT, hepatic artery thrombosis; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; ITT, intent-to-treat; MDRD, modification of diet in renal disease; MELD, model for end-stage liver disease; MPA, mycophenolic acid; MTOR, mammalian target of rapamycin; RAI, rejection activity index; RR, relative risk; SD, standard deviation; SE, standard error; TBPAR, treated biopsy-proven acute rejection.

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Introduction

Calcineurin inhibitors (CNIs) remain the mainstay of immunosuppression following liver transplantation (1) but are associated with significant long-term complications including nephrotoxicity, which induces progressive, dose-related histological and functional renal deterioration (2,3). With more than 10% of liver transplant recipients progressing to severe chronic kidney disease by 5 years posttransplant (4,5), there is a pressing need to minimize CNI-related nephrotoxicity in the liver transplant population.

Immunosuppressants of the mammalian target of rapamycin (mTOR) inhibitor class act synergistically with CNIs (6), offering an opportunity to lower CNI exposure. However, evidence as to whether conversion from CNI- to mTOR inhibitor-based immunosuppression improves kidney function in patients with renal insufficiency after liver transplantation is conflicting (7–10), highlighting the need for early reduction or elimination of CNI exposure before irreversible renal deterioration has developed. No randomized trial has compared early introduction of everolimus combined with reduced CNI exposure to standard CNI therapy in a *de novo* liver transplant population.

This study was undertaken to evaluate the efficacy and safety of using everolimus to eliminate or reduce tacrolimus compared to a standard tacrolimus regimen in *de novo* liver transplant recipients.

Methods

Study design and conduct

A 24-month prospective, randomized, multicenter, three-arm, parallel-group and open-label study of adult *de novo* liver transplant recipients was undertaken at transplant centers in Europe, North/South America and Australia during the period from January 2008 to April 2011. The 12-month study period comprised a run-in period, with randomization performed 30 (±5) days posttransplant followed by an 11-month treatment period.

Patients

Adult (18–70 years) recipients of a primary full-size liver transplant from a deceased donor, who had been initiated on an immunosuppressive regimen containing tacrolimus and corticosteroids (with or without mycophenolic acid [MPA]), were eligible to enter the run-in period. Key inclusion criteria for randomization comprised (i) acceptable graft function (aspartate aminotransferase [AST], alanine aminotransferase [ALT] and total bilirubin levels ≤3 times the upper limit of normal, with alkaline phosphatase ≤5 times the upper limit of normal), (ii) estimated GFR (eGFR) ≥30 mL/min/1.73 m^2 (MDRD4) and (iii) tacrolimus trough concentration ≥8 ng/mL in the week prior to randomization. Key exclusion criteria included HCC that did not fulfill Milan criteria (11,12) at time of transplant as per explant histology, and receipt of antibody induction therapy. To enter the run-in period, patients were also excluded if urine protein to creatinine ratio indicated proteinuria (≥1.0 g/24 h). At the point of randomization (day 30), key additional inclusion criteria were Doppler ultrasound evidence showing the patency of hepatic artery, hepatic and portal veins; confirmation of eGFR ≥30 mL/min/1.73 m^2; and the absence of acute rejection requiring antibody therapy or ≥1 episode of steroid-sensitive rejection during the run-in period.

Randomization

Patients were stratified according to pretransplant hepatitis C (HCV) status (based on the presence/absence of anti-HCV antibodies) and quartiles of renal function at the time of randomization (based on eGFR [MDRD4]) in order to balance these risk factors for graft and patient survival, then randomized in a 1:1:1 ratio to (i) TAC Elimination, (ii) EVR+Reduced TAC, (iii) TAC Control.

In April 2010, the independent Data Monitoring Committee (DMC) recommended to stop the enrollment to the TAC Elimination arm due to a significantly higher rate of tBPAR in this group versus the other two treatment arms, which appeared to be clustered after tacrolimus withdrawal during days 120–180 after randomization. Randomization to the TAC Elimination arm was stopped, patients up to 180 days after randomization were converted to standard treatment, those who were more than 180 days postrandomization could continue on their assigned regimen, and a protocol amendment was implemented. At this point, approximately 690 patients had been randomized and eligible patients completing the run-in phase were randomized equally between the EVR+Reduced TAC and TAC Control groups.

Intervention and concomitant medication

In the TAC Elimination arm, everolimus was initiated at a dose of 1.0 mg b.i.d. within 24 h of randomization with the dose adjusted from day 5 onward to maintain a trough (C_0) concentration in the range 3–8 ng/mL until month 4 posttransplant, after which the target range increased to 6–10 ng/mL. Once everolimus trough concentration was in the range 3–8 ng/mL, tacrolimus dose was tapered to achieve tacrolimus trough concentration of 3–5 ng/mL by week 3 after randomization, then tacrolimus elimination was started after everolimus trough concentration achieved 6–10 ng/mL at the start of month 4 posttransplant and if liver function was confirmed to be adequate (see the "Patients" section). Tacrolimus elimination was to be completed by the end of month 4 after transplantation. In the EVR+Reduced TAC arm, everolimus therapy was initiated and monitored as for the TAC Elimination group, but the initial target range of 3–8 ng/mL was maintained throughout the study. Once everolimus trough concentration was within this range, tacrolimus dose was tapered to achieve a trough concentration by week 3 after randomization of 3–5 ng/mL, which remained unchanged for the remainder of the study. In the TAC Control arm, tacrolimus trough concentration was to be maintained in the range 8–12 ng/mL until month 4, after which the target range was 6–10 ng/mL until the end of the study.

For all patients, corticosteroids were to be initiated at the time of transplant and administered according to local practice (including perioperative intravenous corticosteroids), with a minimum oral dose of 5 mg prednisolone/day after randomization to be continued until at least month 6 posttransplant. MPA, if used, was administered as per local practice but had to be discontinued by the time of randomization.

Study endpoints

The primary efficacy endpoint was the composite efficacy failure rate of treated biopsy-proven acute rejection (tBPAR), graft loss or death at 12 months posttransplantation (excluding events before randomization). tBPAR was defined as acute rejection with a locally confirmed rejection activity index (RAI) ≥3 according to Banff 1997 criteria (13) treated with antirejection therapy. All suspected cases of BPAR were to be assessed by biopsy and assessed locally. The key secondary endpoint was the change in renal function from randomization to month 12 posttransplant as assessed by estimated

glomerular filtration rate (eGFR) using the four-variable modification of diet in renal disease (MDRD4) formula [14]). These endpoints were revised from the original endpoints after implementation of the protocol amendment to discontinue the TAC Elimination arm, in accordance with the EMA guideline on clinical investigation of immunosuppressants for solid organ transplantation (15). The original coprimary 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) at month 12 posttransplant.

The current analysis reports 12-month endpoints (intent-to-treat [ITT] population).

Statistical analysis

A sample size of 242 patients per arm was calculated to provide (i) at least 80% power at the one-sided 0.0125 level for noninferiority of the EVR+Reduced TAC group versus the TAC Control arm in the proportion of patients with tBPAR, graft loss or death, assuming that both groups each have a true proportion of tBPAR, graft loss or death of 24% and a noninferiority margin of 12% (ii) at least 90% power at the one-sided 0.0125 level for noninferiority of the EVR+Reduced TAC group versus TAC Control for mean change in eGFR from randomization to month 12, assuming a noninferiority margin of the difference in eGFR is −6.0 mL/min with a standard deviation (SD) of 20 mL/min and a correlation coefficient with prerandomization eGFR of 0.5, using an analysis of covariance (ANCOVA) model.

Efficacy and renal function analyses were based on the ITT population, comprising all randomized patients. Safety analyses except renal function were based on the safety population, which included all randomized patients who received at least one dose of study medication.

Results

Efficacy, renal function and safety data were reported for the EVR+Reduced TAC and TAC Control groups, but only limited safety data were presented for the TAC Elimination arm due to extensive conversion of patients from TAC Elimination to standard treatment after implementation of the DMC recommendation to stop randomization to this group. Statistical comparisons of the TAC Elimination group versus the other two treatment arms were not considered meaningful and are not shown.

Patients

A total of 1147 patients underwent liver transplantation and entered the run-in period. Seven hundred and nineteen patients were eligible for randomization at day 30 and formed the ITT population (EVR+Reduced TAC 245, TAC Elimination 231, TAC Controls 243) (Figure 1). Three patients did not receive study medication (1 TAC Elimination, 2 TAC Controls), such that the safety population comprised 716 patients. The treatment groups were well balanced (Table 1).

Immunosuppression

At the time of randomization, 171 (70%), 151 (66%) and 168 (70%) patients in the EVR+Reduced TAC, TAC Elimination and TAC Control groups, respectively, were receiving mycophenolate mofetile, which was discontinued according to protocol.

At days 3–7 posttransplant, mean (SD) tacrolimus concentration was 6.1 (3.0) ng/mL and 6.0 (3.0) ng/mL in the EVR+Reduced TAC group and the TAC Control group, respectively; corresponding values at week 2 posttransplant were 8.5 (4.2) ng/mL and 8.9 (4.3) ng/mL. Supporting Figure S1 illustrates tacrolimus C_0 concentrations from the time of randomization to month 12. In the EVR+Reduced TAC group, mean (SD) tacrolimus C_0 concentration was 6.5 (5.2) ng/mL, 5.8 (5.8) ng/mL and 5.6 (6.3) ng/mL at months 3, 6 and 12 posttransplant, respectively, i.e. slightly above the target range. Corresponding values in the TAC Control group were 9.8 (3.2) ng/mL, 8.4 (3.8) ng/mL and 7.6 (2.8) ng/mL, all of which were within target range. The reduction in tacrolimus C_0 concentration in the EVR+Reduced TAC group versus the TAC Control group varied from 26.3% to 38.4% at different timepoints during the study. Mean (SD) everolimus C_0 concentration was within target ranges throughout the study in the EVR+Reduced TAC arm. After initial uptitration, the mean everolimus C_0 concentration in the EVR+Reduced TAC group remained stable, within the range 5.5–6.3 ng/mL during months 3–12, with a maximum value observed at month 4.5 (6.3 [4.7] ng/mL). The mean (SD) dose of corticosteroids from randomization to month 12 was 0.15 (0.21) mg/kg/day in the TAC Elimination group, 0.20 (0.65) mg/kg/day in the EVR+Reduced TAC group and 0.13 (0.08) mg/kg/day in TAC Control.

The study was completed on-treatment to month 12 by 179 patients (73.1%) in the EVR+Reduced TAC group, 102 patients (44.2%) in the TAC Elimination group and 189 (77.8%) in the TAC Controls group (Figure 1).

Efficacy

In the ITT population, the primary efficacy endpoint of tBPAR, graft loss or death at month 12 occurred in 45/231 patients (19.5%) in the TAC Elimination arm, 16/245 (6.5%) EVR+Reduced TAC subjects and 23/243 (9.5%) TAC Controls. To allow for censoring of the patients who were lost to follow-up, Kaplan–Meier incidence rates were calculated. The Kaplan–Meier incidence rate of the primary efficacy endpoint was statistically noninferior for EVR+Reduced TAC compared to TAC Controls: 6.7% versus 9.7%, respectively, with a difference of −3.0% (97.5% CI −8.7%, 2.6%) ($p<0.001$ for the noninferiority test with a noninferiority margin of 12%) (Table 2, Figure 2a). The incidence of graft loss or death, or either event individually, did not differ between the EVR+Reduced TAC group and TAC Controls, but the incidence of tBPAR (excluding events that occurred prior to randomization, i.e. tBPAR episodes between day 30 and month 12) was significantly lower in the EVR+Reduced TAC arm (Table 2, Figure 2b). No episodes of tBPAR in the EVR+Reduced TAC group were graded higher than RAI 4–5 (mild), compared to 9 episodes in the TAC Control group which were graded 6–7 (moderate) or 8–9 (severe) (Table 2). None of the graft losses in the EVR+Reduced TAC and TAC Control groups were related to rejection.

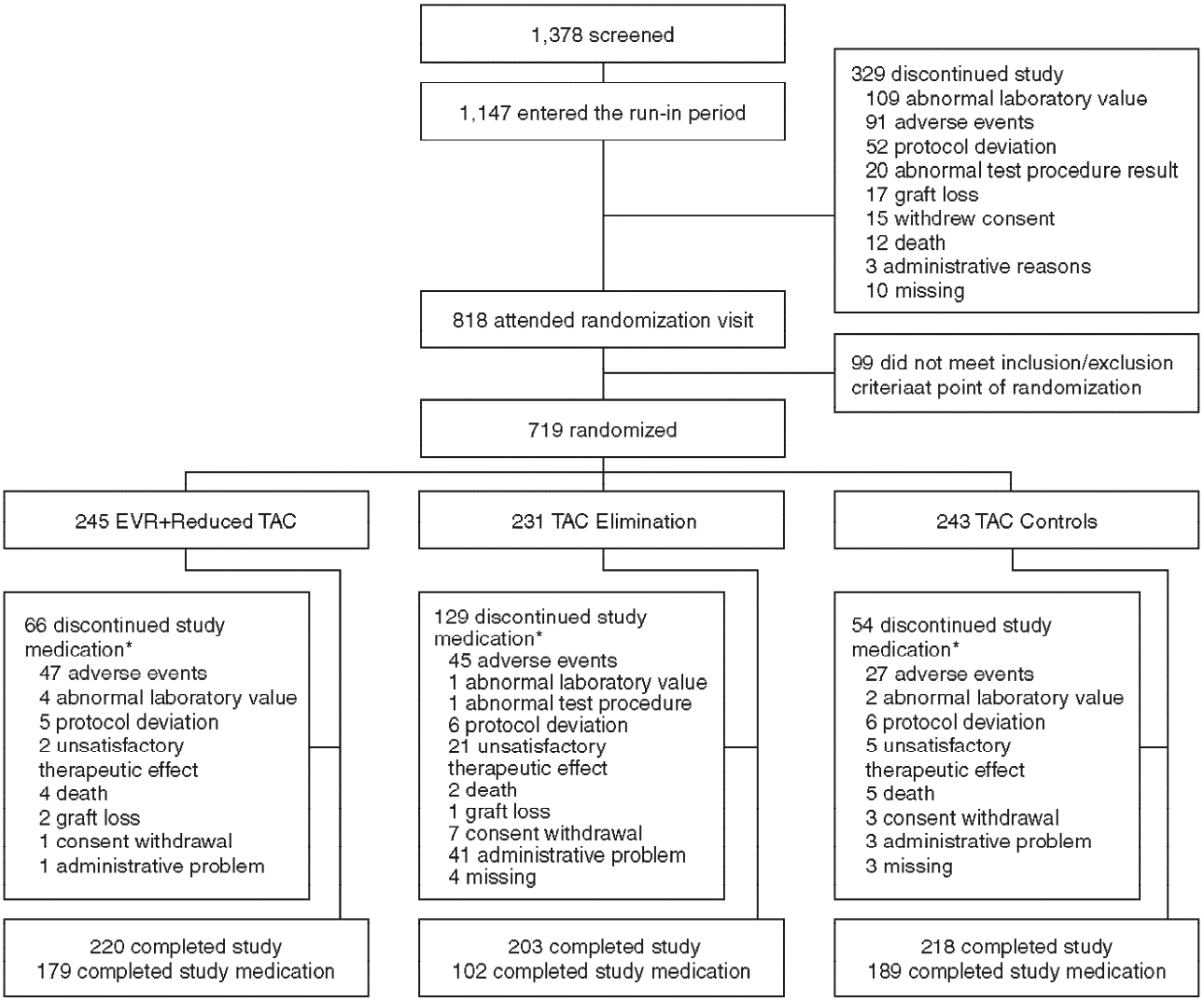

Figure 1: Patient disposition. All randomized patients were included in the intent-to-treat (ITT) population (n = 719). The safety population excluded patients who were randomized but did not receive at least one dose of study medication (one TAC elimination patient and two TAC Control patients).

Renal function

The change in adjusted eGFR (MDRD4) from randomization to month 12 was superior in the EVR+Reduced TAC group over 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, $p < 0.001$).

A significant between-group difference in eGFR at month 12 was observed using MDRD4 and other formulae (Table 2). The difference in eGFR (MDRD4) values between the two groups was significant at all time points from week 6 posttransplant onward (all $p < 0.001$) (Figure 3). Urinary protein to creatinine ratio peaked at month 6 in the EVR+Reduced TAC group (median 105 mg/g, range 33–4143 mg/g), and at month 2 (median 108 mg/g, 39–10,370 mg/g) in the TAC Control arm. Mean values remained below 300 mg/g in both treatment arms at all time points. None of the nine patients in the EVR+Reduced TAC group who had a preexisting urinary protein to creatinine ratio ≥500 mg/g (but lower than 1.0 g/24 h) showed an increase at month 12. Renal replacement therapy was required in six, three and four patients in the EVR+Reduced TAC, TAC Elimination and TAC Control arms, respectively. No patient remained on renal replacement therapy at month 12. Of these, three, two and four cases, respectively, occurred in patients who were in critical care and subsequently died.

Safety

The proportion of patients experiencing one or more adverse event, or serious adverse event, was similar between

Table 1: Demographics and baseline characteristics

	EVR+reduced TAC N = 245	TAC elimination N = 231	TAC controls 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 (8.2)	17 (7.4)	28 (0.7)
Missing	5 (2.0)	4 (1.7)	6 (2.5)
Body mass index (kg/m^2)[1]	25.1 ± 4.2	25.3 ± 4.3	24.5 ± 4.2
HCV positive, n (%)	78 (31.8)	72 (31.2)	76 (31.3)
eGFR (MDRD4) (mL/min/1.73^2)[1]	80.8 ± 32.7	82.9 ± 37.2	78.9 ± 27.7
Diabetic n (%)[1]	95 (38.8)	83 (35.9)	101 (41.6)
Primary disease leading to liver transplantation, n (%)			
Alcoholic cirrhosis	71 (29.0)	49 (21.2)	51 (21.0)
Hepatitis C	62 (25.3)	56 (24.2)	57 (23.5)
Hepatocellular carcinoma	42 (17.1)	31 (13.4)	35 (14.4)
Hepatitis B	17 (6.9)	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	19 (7.8)	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)

[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).
BPAR = biopsy-proven acute rejection; tBPAR = treated biopsy-proven acute rejection; eGFR = estimated GFR; HCV = hepatitis C virus; MDRD4 = abbreviated modification of diet in renal disease; MELD = model for end-stage liver disease.

the EVR+Reduced TAC group and the TAC Control arm (Table 3). The incidence of individual adverse events did not differ between the two groups other than a higher risk of peripheral edema and leukopenia in the EVR+Reduced TAC patients. The incidence of leukopenia, thrombocytopenia and anemia in the EVR+Reduced TAC patients was 11.8%, 5.3% and 7.8%, respectively, compared to 5.0%, 1.7% and 8.3% in the TAC Controls. Interstitial lung disease was reported for one patient in each of the three treatment groups.

During the randomized treatment period, hepatic artery thrombosis (HAT) was reported for one EVR+Reduced TAC patient. This was a second episode of HAT in the same patient, with the first having occurred during the run-in period, requiring reanastomosis of the hepatic artery and stent placement. A late and temporary hepatic artery occlusion without graft loss was reported for one TAC Elimination patient that resolved under heparin. This compares to 14 patients with HAT during the prerandomization run-in phase.

Wound healing complications were reported in a similar proportion of patients in each group: 11.0% (n = 27), 9.6% (n = 22) and 7.9% (n = 19) of the EVR+Reduced TAC, TAC Elimination and TAC Control patients (RR 1.40, 95% CI 0.80, 2.45 between EVR+Reduced TAC and TAC Control groups).

The overall incidence of infections was similar between groups (Table 3), as was the incidence of viral infections (17.1% [n = 42] of EVR+Reduced TAC patients, 13.3% [n = 32] of TAC Controls (RR 1.29, 95% CI 0.84, 1.97)). Cytomegalovirus (CMV) viremia was detected at a similar rate between groups (EVR+Reduced TAC 6.5%, TAC Controls 6.6%). The relative risk of serious infections in the EVR+Reduced TAC group versus TAC Controls was 1.76 (95% CI 1.03, 3.00). The incidence of pneumonia as

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