An open-label, randomized trial indicates that everolimus with tacrolimus or cyclosporine is comparable to standard immunosuppression in *de novo* kidney transplant patients

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This is a randomized trial (ATHENA study) in *de novo* kidney transplant patients to compare everolimus versus mycophenolic acid (MPA) with similar tacrolimus exposure in both groups, or everolimus with concomitant tacrolimus or cyclosporine (CsA), in an unselected population. In this 12-month, multicenter, open-label study, *de novo* kidney transplant recipients were randomized to everolimus with tacrolimus (EVR/TAC), everolimus with CsA (EVR/CsA) or MPA with tacrolimus (MPA/TAC), with similar tacrolimus exposure in both groups. Non-inferiority of the primary end point (estimated glomerular filtration rate [eGFR] at month 12), assessed in the per-protocol population of 338 patients, was not shown for EVR/TAC or EVR/CsA versus MPA/TAC. In 123 patients with TAC levels within the protocol-specified range, eGFR outcomes were comparable between groups. The mean increase in eGFR during months 1 to 12 post-transplant, analyzed *post hoc*, was similar with EVR/TAC or EVR/CsA versus MPA/TAC. The incidence of treatment failure (biopsy proven acute rejection, graft loss or death) was not significant for EVR/TAC but significant for EVR/CsA versus MPA/TAC. Most biopsy-proven acute rejection events in this study were graded mild (BANFF IA). There were no differences in proteinuria between groups. Cytomegalovirus and BK virus infection were significantly more frequent with MPA/TAC. Thus, everolimus with TAC or CsA showed comparable efficacy to MPA/TAC in *de novo* kidney transplant patients. Non-inferiority of renal function, when pre-specified, was not shown, but the mean increase in eGFR from month 1 to 12 was comparable to MPA/TAC.

Kidney International (2019) **96,** 231–244; https://doi.org/10.1016/j.kint.2019.01.041

KEYWORDS: cyclosporine; efficacy; everolimus; kidney transplantation; mycophenolate mofetil [MMF]; mycophenolic acid; randomized; renal function; tacrolimus

Immunosuppression based on calcineurin inhibitor (CNI) therapy is almost universal after kidney transplantation,[1,2] but the dose-dependent complications of chronic CNI therapy—most notably nephrotoxicity—are widely recognized.[2] One strategy for minimizing CNI exposure is to use a mammalian target of rapamycin inhibitor instead of MPA, with the aim of reducing CNI-related kidney lesions and avoiding the hematological and gastrointestinal adverse events associated with MPA.[3,4] Randomized trials have confirmed that coadministration of everolimus with reduced-exposure CNI therapy does not compromise immunosuppressive efficacy.[5-8] In addition, there are nonimmunosuppressive benefits associated with everolimus therapy, including a possible reduction in the risk of posttransplant malignant neoplasm[9–11] and a reduced risk of cytomegalovirus (CMV) infection.[12,13]

CNI dosing has declined in the last 15 years, and the question arises whether additional reductions in CNI

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Received 6 August 2018; revised 25 January 2019; accepted 31 January 2019; published online 27 February 2019

exposure are still necessary in patients receiving everolimus compared to MPA. A conservative approach would be to apply similar CNI exposure levels regardless of whether everolimus or MPA was given concomitantly. In addition, previous studies in this setting have used the original CNI agent CsA[6–8] or included recipients given either CsA or tacrolimus.[5] Because tacrolimus has mostly replaced CsA as the CNI of choice after kidney transplantation,[1] it would be informative to compare everolimus with MPA in tacrolimus-treated kidney transplant patients. Furthermore, the majority of these trials excluded high-risk patients; only a few enrolled broader populations including extended criteria donation and old-for-old recipients. Thus, we undertook the challenge of studying everolimus with 2 candidate CNI partners in a direct comparison in a real-world population. This comes with certain challenges: As 1-year survival and patient outcomes have largely improved since the recent decade and hence superiority testing in the transplant setting is no longer applicable, an important question remains how to set the right noninferiority margin, especially when studying nonhomogeneous populations.[14,15]

The ATHENA study was therefore undertaken to randomize *de novo* kidney transplant patients to 1 of 3 treatment arms—EVR/TAC, EVR/CsA, or a control regimen comprising MPA/TAC—with similar tacrolimus exposure in the EVR/TAC and MPA/TAC groups. Because TAC/MPA reflects the current standard of care for combination therapy with CNI and MPA, the addition of a further group, CsA/MPA, was not considered relevant. The objective was to demonstrate noninferiority of renal function with EVR/TAC and/or EVR/CsA versus MPA/TAC. The results of this 1-year study are reported here.

RESULTS

Study population

In total, 659 patients were screened, of whom 655 met the eligibility criteria and were randomized. Six hundred twelve patients received at least 1 dose of the study drug and formed the intent-to-treat (ITT) population. In error, 1 patient randomized to the EVR/TAC group received MPA/TAC, 2 patients randomized to the EVR/CsA group received EVR/TAC, and 1 patient randomized to the MPA/TAC group received EVR/CsA and another was given EVR/TAC. Thus, the safety population also included 612 patients but with a different distribution across randomized groups (Figure 1). The study was discontinued prematurely by 19.0%, 20.7%, and 11.3% of patients ($P = 0.026$) in the EVR/TAC, EVR/CsA, and MPA/TAC groups, respectively, with the study drug discontinued prematurely in 45.2%, 57.1%, and 24.0% of patients ($P < 0.001$) (Figure 1). Rates of study drug discontinuation varied widely between centers (0%−100%, 0%−100%, and 0%−60% in the EVR/TAC, EVR/CsA, and MPA/TAC groups, respectively).

The per-protocol population included 338 of 612 patients (51.6%), with premature discontinuation of the study drug (n = 256) and the absence of a post-baseline estimated glomerular filtration rate (eGFR) value (n = 131) being the most frequent reasons for exclusion.

Sixty-nine recipients (11.3%) were transplanted at German centers as part of the Eurotransplant Senior Program, in

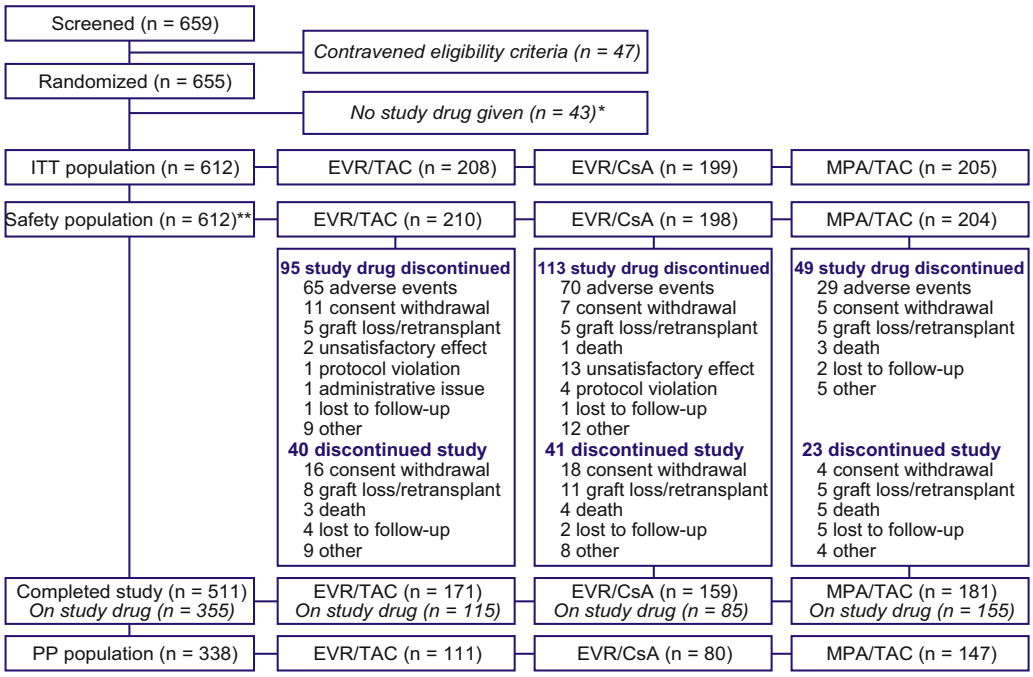

Figure 1 | Patient disposition (safety population). *EVR/TAC, n = 17; EVR/CsA, n = 13; MPA/TAC, n = 13. **Safety population reflects actual treatment received. EVR/CsA, everolimus with cyclosporine; EVR/TAC, everolimus with tacrolimus; ITT, intent-to-treat; MPA/TAC, mycophenolic acid with tacrolimus; PP, per protocol.

which donors aged ≥65 years are allocated regionally to recipients aged ≥65 years without human leukocyte antigen matching.[14] Overall, 83.8% (n = 512) and 16.2% (n = 99) of patients received a graft from a deceased or living donor, respectively. One patient in the EVR/TAC group was not transplanted. Baseline characteristics were similar between treatment groups other than more frequent older recipients and donors in the EVR/CsA group and a higher proportion of patients with 2 human leukocyte antigen-DR mismatches in the EVR/TAC and EVR/CsA groups (Table 1).

Immunosuppression

In the EVR/TAC and EVR/CsA groups, the mean everolimus level was in the range 5.5 to 6.4 ng/ml throughout the study (Figure 2a), with >70% of patients in both groups within the target range (3−8 ng/ml) at any study visit. The mean tacrolimus level at months 1, 6, and 12 exceeded target throughout the study (Figure 2b). At different study visits, the proportion of patients with tacrolimus concentrations above the target range was in the range 42% to 77% in the EVR/TAC group and in the range 57% to 89% in the MPA/TAC group (Supplementary Table S1). The mean CsA level in the EVR/CsA group exceeded the target range at month 1 and was close to the upper limit subsequently (Figure 2c). The CsA level was above the target range in 29% to 58% of patients at different study visits (Supplementary Table S1).

Renal function

The mean eGFR at month 12 was 62.2 ml/min per 1.73 m^2 in the EVR/TAC group, 58.4 ml/min per 1.73 m^2 in the EVR/CsA group, and 67.8 ml/min per 1.73 m^2 in the MPA/TAC group at month 12 in the per-protocol population (least-squares mean values). Noninferiority of the primary end point—eGFR (Nankivell formula) at month 12—was not shown for the EVR/TAC or EVR/CsA groups versus the MPA/TAC group because the lower limit of the 95% confidence

Table 1 | Baseline characteristics (ITT population)

Characteristic	EVR/TAC (n = 208)	EVR/CsA (n = 199)	MPA/TAC (n = 205)
Recipient characteristics			
Age			
Mean ± SD (yr)	54.3 ± 13.5	55.1 ± 12.6	55.3 ± 12.1
≥65 yr	54 (26.0)	60 (30.2)	49 (23.9)
Male	138 (66.3)	133 (66.8)	140 (68.3)
White	197 (95.2)	196 (98.5)	199 (97.5)
Body mass index (kg/m^2)	26.5 ± 4.4	26.6 ± 4.3	26.4 ± 4.1
Panel reactive antibodies			
0	187 (95.9)	179 (94.7)	191 (96.0)
≤10	7 (3.6)	7 (3.7)	6 (3.0)
>10 and ≤20	1 (0.5)	0 (0.0)	1 (0.5)
>20	0 (0.0)	3 (1.6)	1 (0.5)
Missing	13	10	6
Previous kidney transplant	10 (4.8)	2 (1.0)[a]	5 (2.4)
HLA-A mismatches			
0	54 (26.1)	55 (27.6)	61 (29.8)
1	102 (49.3)	92 (46.2)	96 (46.8)
2	51 (24.6)	52 (26.1)	48 (23.4)
HLA-B mismatches			
0	43 (20.8)	40 (20.1)	48 (23.4)
1	92 (44.4)	80 (40.2)	78 (38.0)
2	72 (34.8)	79 (39.7)	79 (38.5)
HLA-DR mismatches			
0	58 (28.0)	66 (33.2)	75 (36.6)
1	98 (47.3)	83 (41.7)	103 (50.2)
2	51 (24.6)	50 (25.1)	27 (13.2)
Cold ischemia time (h)	11.0 ± 6.2	11.8 ± 5.9	11.3 ± 5.8
Participant in the Eurotransplant Senior Program	24 (11.5)	23 (11.6)	22 (10.7)
Donor characteristics[b]			
Age			
Mean ± SD (yr)	53.3 ± 15.7	56.3 ± 14.4	55.0 ± 14.9
≥65 yr (all patients)	51 (24.6)	59 (29.6)	51 (24.9)
≥65 yr (deceased donor)	27/173 (15.6)	34/166 (20.5)	24/173 (13.9)
Male	103 (49.8)	112 (56.3)	102 (49.8)
White	40 (97.6)	41 (95.3)	36 (97.3)
Deceased heart beating	173 (83.6)	166 (83.4)	173 (84.4)
Living related	24 (11.6)	22 (11.1)	23 (11.2)
Living unrelated	10 (4.8)	11 (5.5)	9 (4.4)

EVR/CsA, everolimus with cyclosporine; EVR/TAC, everolimus with tacrolimus; HLA, human leukocyte antigen; ITT, intent-to-treat; MPA/TAC, mycophenolic acid with tacrolimus.
[a]Including 1 patient with 2 previous transplants.
[b]Donor type was not applicable in 1 EVR/TAC patient who did not receive a transplant.
Data are n (%) unless otherwise specified.

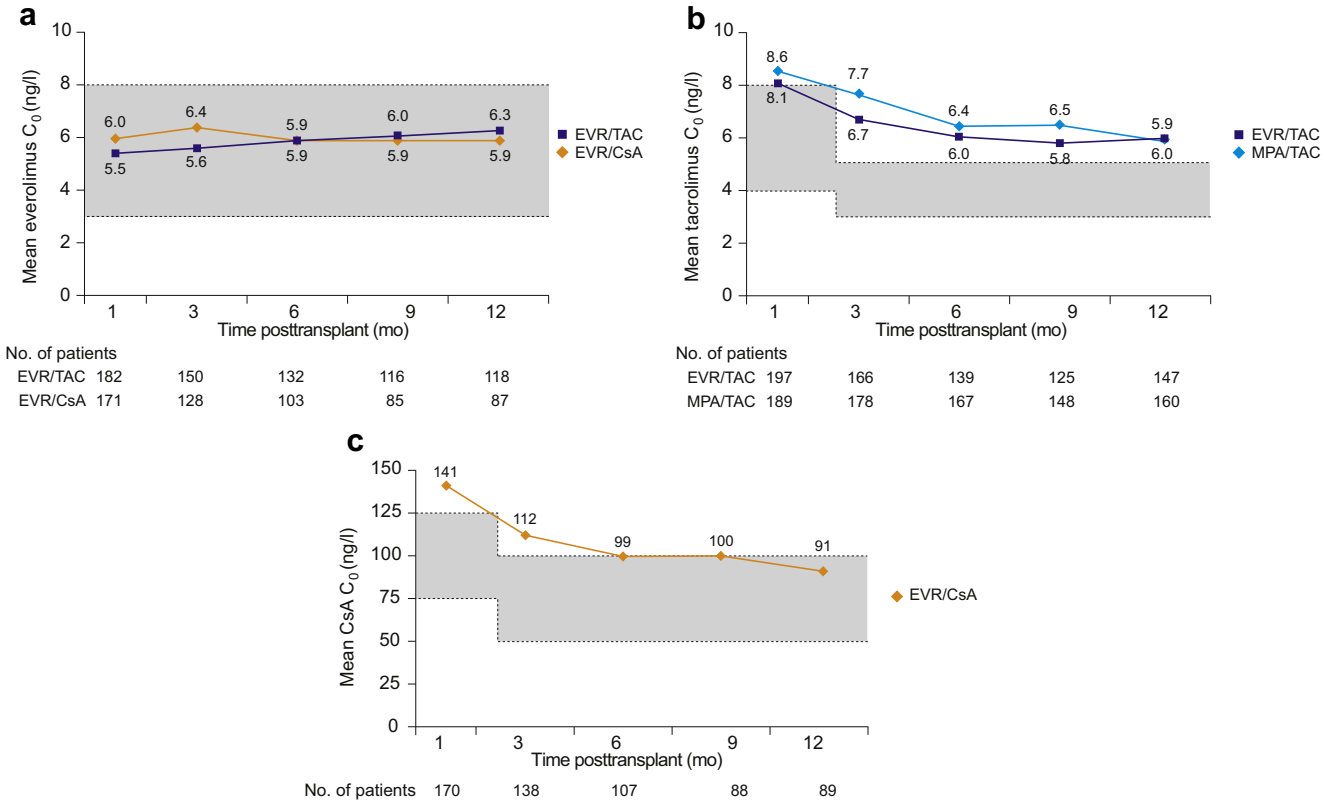

Figure 2 | Mean trough concentrations of (a) everolimus, (b) tacrolimus, and (c) cyclosporine (CsA) at month 12. Shaded areas indicate target ranges. C_0, trough level; EVR/CsA, everolimus with cyclosporine; EVR/TAC, everolimus with tacrolimus; MPA/TAC, mycophenolic acid with tacrolimus.

interval was above the *a priori* defined noninferiority margin of −7 ml/min per 1.73 m^2 ($P = 0.239$ and $P = 0.151$ for noninferiority, respectively) (Table 2). A *post hoc* analysis revealed that for the subgroup of patients in the per-protocol population who had tacrolimus trough levels ≤5 ng/ml on 3 consecutive visits after month 3 (i.e., within the tacrolimus target range specified in the protocol), eGFR at month 12 was not significantly different between the EVR/TAC and MPA/TAC groups (least-squares mean, 64.9 ml/min per 1.73 m^2 [n = 62] and 70.3 ml/min per 1.73 m^2 [n = 61], respectively; $P = 0.080$) (Table 2).

When eGFR was compared between groups by using a *post hoc* matched-pair analysis, that is, for patients with eGFR values available at both month 1 and month 12 (n = 473), the observed mean eGFR at month 1 was 59.1, 56.2, and 61.7 ml/min per 1.73 m^2 in the EVR/TAC, EVR/CsA, and MPA/TAC groups, respectively, compared with 62.2, 60.5, and 68.2 ml/min per 1.73 m^2 at month 12 (Figure 3). The change in eGFR from month 1 to month 12 was comparable between groups, with 3.1, 4.3, and 6.5 ml/min per 1.73 m^2 in the EVR/TAC, EVR/CsA, and MPA/TAC groups, respectively ($P = 0.069$ for EVR/TAC vs. MPA/TAC; $P = 0.276$ for EVR/CsA vs. MPA/TAC). When the primary analysis was repeated with eGFR at month 1 added as a factor, the least-squares mean values were 63.2, 60.5, and 68.2 ml/min per 1.73 m^2 in the EVR/TAC, EVR/CsA, and MPA/TAC groups, respectively. Applying a noninferiority margin of −9 ml/min per 1.73 m^2 *post hoc*, noninferiority was shown for EVR/TAC versus MPA/TAC ($P = 0.009$) in the per-protocol population and for EVR/TAC and EVR/CsA versus MPA/TAC ($P = 0.007$ and $P = 0.009$, respectively) in the ITT population by using multiple imputation for missing values (least-squares mean, 59.9, 59.8, and 64.8 ml/min per 1.73 m^2 in the EVR/TAC, EVR/CsA, and MPA/TAC group, respectively) (Table 2).

Other sensitivity and *post hoc* analysis of renal function are shown in Supplementary Table S2.

The incidences of delayed graft function and slow graft function were comparable between groups (Table 2).

Renal function according to donor age

Analysis of covariance revealed that donor age among recipients of a deceased donor graft had a significant effect on change in eGFR from month 1 to month 12 ($P < 0.001$) regardless of the treatment group. Patients who received a graft from a deceased donor aged ≥65 years had a lower mean eGFR at month 1 than did recipients of a graft from a deceased donor aged 50 to 64, 35 to 49, or <35 years ($P < 0.001$ for each pairwise comparison), as well as a lower mean eGFR at month 12 ($P = 0.003$, $P < 0.001$, and $P < 0.001$ for respective comparisons) (Table 3). This difference between deceased donor

Table 2 | Renal end points at month 12

Characteristic	EVR/TAC (n = 208)	EVR/CsA (n = 199)	MPA/TAC (n = 205)	*P* value across groups
eGFR (Nankivell formula) (ml/min per 1.73 m^2)				
PP population (primary end point)	*n = 111*	*n = 80*	*n = 147*	–
Mean ± SD	***63.3 ± 17.0***	***61.5 ± 16.9***	***68.3± 17.8***	
LS mean, observed cases (95% CI)	*62.2 (58.0 to 66.4)*	*58.4 (53.8 to 63.0)*	*67.8 [63.7; 71.8)*	
Difference vs. MPA/TAC, LS mean	*−5.6 (−9.6 to −1.6)*	*−9.4 (−13.8 to −4.9)*	–	
P values for noninferiority vs. MPA/TAC	*0.239*[b]	*0.151*[b]	–	
P values for difference to MPA/TAC	*0.007*[b]	*<0.001*[b]	–	–
LS mean, observed cases (95% CI)[a]	63.2 (59.7 to 66.7)	60.5 (56.6 to 64.5)	68.2 (64.8 to 71.5)	
Difference vs. MPA/TAC, LS mean	−5.0 (−8.3 to −1.6)	−7.6 (−11.4 to 3.8)		
P values for noninferiority vs. MPA/TAC	0.116[a]	0.625[a]		
P values for noninferiority vs. MPA/TAC	0.009[a,c]	0.236[a,c]		
P values for difference to MPA/TAC	0.004[a]	<0.001[a]		
PP population deceased donors aged <65 yr	n = 76	n = 51	n = 96	–
LS mean, observed cases (95% CI)[a]	**67.4 (63.8 to 70.9)**	**63.1 (58.8 to 67.4)**	**70.9 (67.6 to 74.3)**	–
Difference vs. MPA/TAC, LS mean	−3.6 (−7.7 to 0.5)	−7.9 (−12.6 to −3.1)		
P values for noninferiority vs. MPA/TAC	0.050[a]	0.637[a]		
P values for noninferiority vs. MPA/TAC	0.005[a,c]	0.317[a,c]		
P values for difference to MPA/TAC	0.088[a]	0.001[a]		
PP population with tacrolimus C_0 ≤5 ng/ml[d]	n = 62	–	n = 61	–
LS mean (95% CI)	**64.9 (59.4 to 70.4)**		**70.3 (64.7 to 75.8)**	
Difference vs. MPA/TAC, LS mean	−5.4 (−11.4 to 0.64)		–	
P values for difference to MPA/TAC	0.080		–	
eGFR (Nankivell formula) (ml/min per 1.73 m^2)				
ITT population	n = 208	n = 199	n = 205	
No. of observed cases at month 12	161	149	171	–
Missing values at month 12	47	50	34	
Mean ± SD (observed cases)	**61.9 ± 17.1**	**60.9 ± 16.8**	**68.3 ± 17.8**	
LS mean, multiple imputation (95% CI)	58.3 (54.6 to 62.0)	57.1 (53.3 to 60.8)	66.2 (62.4 to 69.9)	
Difference vs. MPA/TAC, LS mean	−7.8 (−11.7 to −4.0)	−9.1 (−13.0 to −5.2)	–	–
P values for difference to MPA/TAC	<0.001[b]	<0.001[b]	–	
LS mean (95% CI)[a]	59.9 (56.6 to 63.1)	59.8 (56.6 to 63.1)	64.8 (61.6 to 68.1)	
Difference vs. MPA/TAC, LS mean	−4.98 (−8.2 to −1.8)	−5.0 (−8.3 to −1.7)	–	
P values for noninferiority vs. MPA/TAC	0.110[a]	0.119[a]	–	
P values for noninferiority vs. MPA/TAC	0.007[a,c]	0.009[a,c]	–	
P values for difference to MPA/TAC	0.002[a]	0.003[a]	–	
ITT population deceased donor aged ≥65 yr	n = 27	n = 32	n = 23	–
LS mean, LOCF (95% CI)	**47.6 (41.4 to 53.7)**	**45.3 (40.2 to 50.5)**	**54.2 (48.2 to 60.1)**	
Difference vs. MPA/TAC, LS mean	−6.6 (−14.5 to 1.3)	−8.8 (−16.3 to −1.4)	–	
P values for difference to MPA/TAC	0.100	0.021	–	
ITT population deceased donor aged <65 yr	n = 119	n = 108	n = 121	–
LS mean, LOCF (95% CI)	**63.2 (59.9 to 66.4)**	**60.6 (57.2 to 64.1)**	**67.3 (63.9 to 70.6)**	
Difference vs. MPA/TAC, LS mean	−4.2 (−8.2 to −0.2)	−6.6 (−10.7 to −2.5)	–	
P values for difference to MPA/TAC	0.042	0.002	–	
Delayed graft function, n/M (%)	38/187 (20.3)	38/172 (22.1)	35/197 (17.8)	0.577[e]
Slow graft function, n/M (%)	91/187 (48.7)	85/171 (49.7)	90/195 (46.2)	0.780[e]

C_0, trough level; CI, confidence interval; eGFR, estimated glomerular filtration rate; EVR/CsA, everolimus with cyclosporine; EVR/TAC, everolimus with tacrolimus; ITT, intent-to-treat; LOCF, last observation carried forward; LS, least-squares; M, number of assessable patients; MPA/TAC, mycophenolic acid with tacrolimus; PP, per protocol.
[a]Analysis of variance with treatment, center, donor type, and eGFR at month 1.
[b]Analysis of variance with treatment, center, and donor type.
[c]The shifted 1-sided *P* value for null hypothesis: LS mean for difference ≤ −9 ml/min per 1.73 m^2.
[d]At any 3 consecutive visits after month 3.
[e]Pearson's chi-square test.
The primary endpoint is shown in italic text. LS mean values are shown in bold text.

age groups was seen regardless of the treatment group ($P <$ 0.001 for the comparison of deceased donor age groups ≥65 years vs. <35 years) (Figure 4).

In addition to deceased donor age, eGFR at month 1 showed a significant association with eGFR at month 12 ($P < 0.001$ for both). An analysis of variance of the primary variable was repeated by adding eGFR at month 1 as a cofactor in the per-protocol population of patients who received an organ from a deceased donor aged <65 years. The difference was not significant between the EVR/TAC and MPA/TAC groups ($P =$ 0.088), and the lower limit of the 95% confidence interval was within the prespecified noninferiority margins ($P = 0.050$ for −7 ml/min per 1.73 m^2 and $P = 0.005$ for −9 ml/min per 1.73 m^2) for EVR/TAC compared to MPA/TAC (Table 2).

Proteinuria

The incidence of proteinuria reported as an adverse event was 14.3% (30 of 210), 15.2% (30 of 198), and 11.8% (24 of 204) in the EVR/TAC, EVR/CsA, and TAC/MPA groups, respectively, and led to permanent discontinuation of the study drug

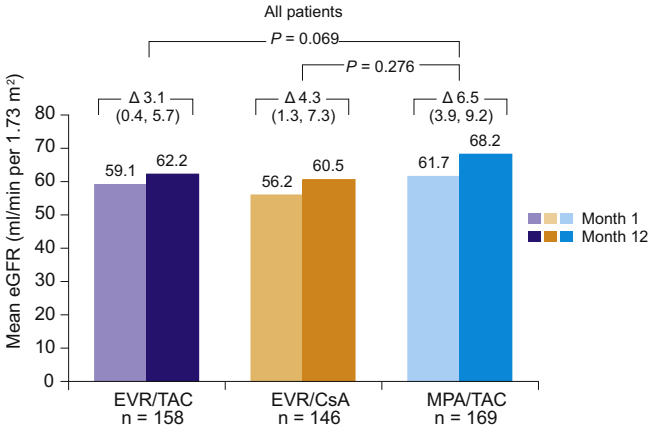

Figure 3 | Observed mean estimated glomerular filtration rate (eGFR; Nankivell formula) values at months 1 and 12 in patients with eGFR data available at both time points (intent-to-treat [ITT] population). The change from month 1 to month 12 was not significantly different between the everolimus with tacrolimus (EVR/TAC) and mycophenolic acid with tacrolimus (MPA/TAC) groups ($P = 0.069$) or between the everolimus with cyclosporine (EVR/CsA) and MPA/TAC groups ($P = 0.276$).

in 0.5% (1 of 210), 1.5% (3 of 198), and 0.0% (0 of 204) of patients. Figure 5 illustrates the incidence of mild, subnephrotic, and nephritic proteinuria at months 3 and 12 in patients for whom data were available. Nephrotic proteinuria was rare in all 3 groups, but numerically higher in the EVR/TAC group at month 3 and in the MPA/TAC group at month 12.

Efficacy

The incidence of treatment failure (biopsy-proven acute rejection [BPAR], graft loss, or death) was comparable between the EVR/TAC and MPA/TAC groups, with Kaplan-Meier estimates being 13.0%, 24.6%, and 9.8% in the EVR/TAC, EVR/CsA, and MPA/TAC groups, respectively (log-rank, $P = 0.260$ for EVR/TAC and $P < 0.001$ for EVR/CsA vs. MPA/TAC), with the difference between the EVR/CsA and MPA/TAC groups arising from variations in the rate of BPAR (7.2%, 19.1%, and 4.9%, respectively; log-rank, $P = 0.291$ for EVR/TAC vs. MPA/TAC and $P < 0.001$ for EVR/CsA vs. MPA/TAC) (Table 4). Rates of BPAR varied widely between centers (0%−75.0%, 0%−66.7%, and 0%−50.0% in the EVR/TAC, EVR/CsA and MPA/TAC groups, respectively). In total, there were 316, 345, and 261 biopsies performed in the EVR/TAC, EVR/CsA, and MPA/TAC groups, representing a mean of 1.52, 1.73, and 1.27 per patient.

In addition, 7 episodes of BPAR (3 BANFF IA, 1 BANFF IIA, 1 BANFF III, and 2 other) in the everolimus groups occurred only after study drug discontinuation (2 EVR/TAC and 5 EVR/CsA) (log-rank for BPAR on treatment: $P = 0.488$ for EVR/TAC and $P < 0.001$ for EVR/CsA vs. MPA/TAC).

Treated BPAR was more frequent under EVR/CsA treatment (17.6%; log-rank, $P < 0.001$), largely because of a higher incidence of BPAR grade 1A, but occurred at a similar rate in the EVR/TAC and MPA/TAC groups (6.7% vs. 3.9%; log-rank, $P = 0.183$), with no differences across all BANFF categories (Table 4).

The rate of graft loss was 4.8%, 6.5%, and 2.9% in the EVR/TAC, EVR/CsA, and MPA/TAC groups, respectively (log-rank, $P = 0.294$ for EVR/TAC vs. MPA/TAC and $P = 0.082$ for EVR/CsA vs. MPA/TAC) (Table 4). Mortality rates were similar between groups (Table 4). Pulmonary embolism was the most frequent cause of death in the MPA/TAC group (n = 4) (Table 4).

Table 3 | eGFR according to donor age in recipients of a deceased donor graft (ITT population)

Variable	LS mean eGFR (95% CI)	P[a]
Month 1		
Donor age ≥65 yr	44.5 (39.3–49.6)	Reference
Donor age 50−64 yr	55.4 (51.6–59.2)	<0.001
Donor age 35−49 yr	61.7 (56.9–66.5)	<0.001
Donor age <35 yr	70.6 (64.4–76.8)	<0.001
Month 12		
Donor age ≥65 yr	49.7 (45.0–54.3)	Reference
Donor age 50−64 yr	57.7 (54.4–61.0)	0.003
Donor age 35−49 yr	67.0 (62.8–71.1)	<0.001
Donor age <35 yr	79.8 (74.3–85.2)	<0.001

CI, confidence interval; eGFR, estimated glomerular filtration rate; ITT, intent-to-treat; LS, least-squares.
[a]Analysis of variance with treatment, center, and donor type; last observation carried forward method.

Left ventricular hypertrophy

In an exploratory analysis, left ventricular hypertrophy was assessed. There were no significant differences between groups in the proportion of patients with concentric left ventricular hypertrophy, eccentric left ventricular hypertrophy, or abnormal relative wall thickness either at baseline or at the end-of-treatment visit (Supplementary Table S3).

Safety

Rates of adverse events as well as adverse events with a suspected relation to study drug (regimen) were comparable between groups (Table 5). Serious adverse events were reported in 76.7%, 72.7%, and 66.2% of patients in the EVR/TAC, EVR/CsA, and MPA/TAC groups, respectively.

Adverse events led to study drug discontinuation in 31.0% of EVR/TAC patients, 35.4% of EVR/CsA patients, and 14.2% of MPA/TAC patients ($P < 0.001$). In contrast, dose reduction or temporary interruptions of the study drug due to adverse events occurred more frequently in the MPA/TAC group than in either EVR group (EVR/TAC, 25.7%; EVR/CsA, 20.7%; MPA/TAC, 45.1%) ($P < 0.001$), most commonly because of infections (7.1%, 4.5%, and 23.5%, respectively) and leukopenia (1.9%, 2.5%, and 10.8%, respectively) (Table 5).

Infections, a prespecified secondary end point, were less frequent under everolimus treatment (EVR/TAC, 73.3%; EVR/CsA, 71.7%; MPA/TAC, 81.9%; $P = 0.038$), a difference arising mainly from fewer viral infections (25.7%, 11.6%, and 40.7% respectively). There were significantly fewer CMV infections as detected locally by reverse transcription polymerase chain reaction ($P < 0.001$), including lower rates in high-risk patients with a CMV-positive donor (Table 5), in the EVR/TAC and EVR/CsA groups than in the MPA/TAC group (Figure 6a). Eight patients in the MPA/TAC group had recurrent CMV infection (up to 4 events per patient), and

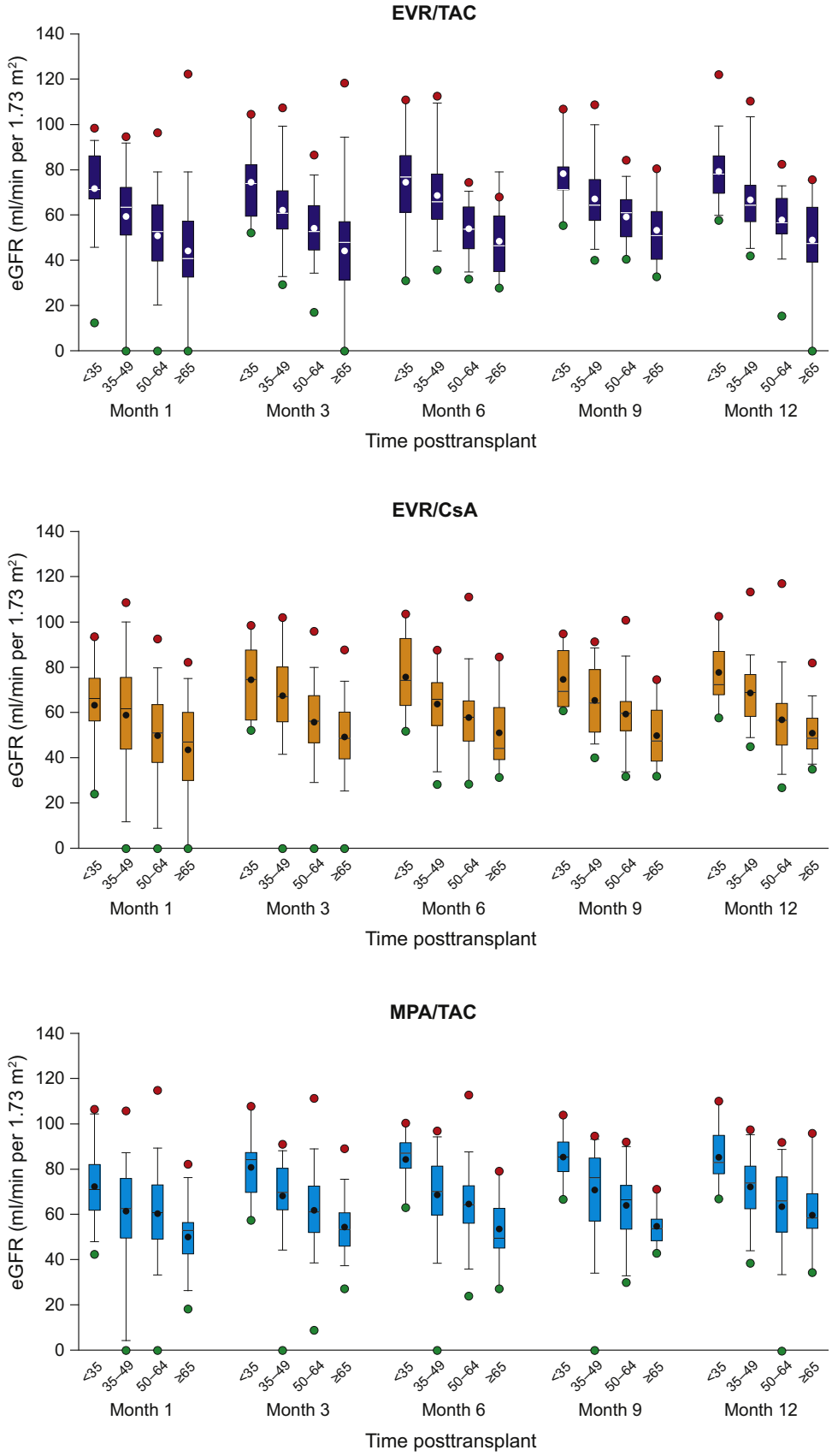

Figure 4 | Box and whisker plots of the observed estimated glomerular filtration rate (eGFR) values by deceased donor age category (<35, 35−49, 50−64, ≤65 years) according to the treatment group (intent-to-treat [ITT] population). EVR/CsA, everolimus with cyclosporine; EVR/TAC, everolimus with tacrolimus; MPA/TAC, mycophenolic acid with tacrolimus.

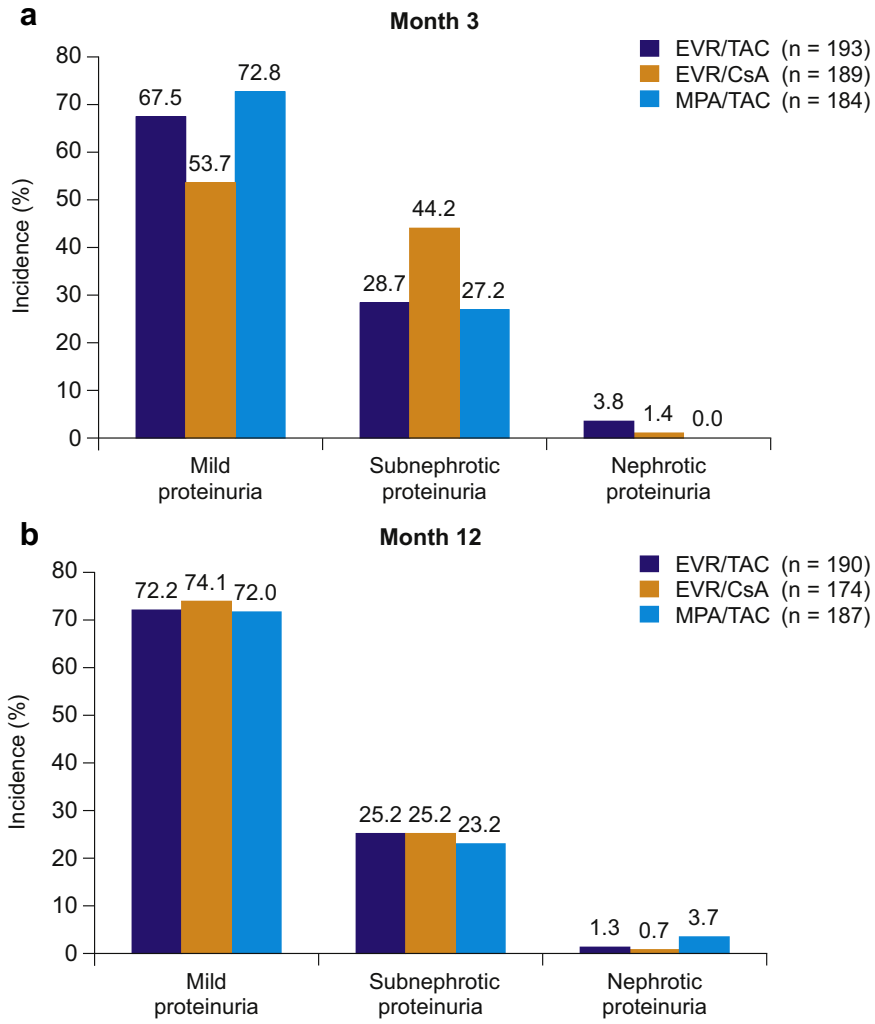

Figure 5 | Urinary protein excretion at (a) month 3 and (b) month 12. *Mild proteinuria* was defined as a urinary protein excretion of 3.39 to <33.9 mg/mmol, *subnephrotic proteinuria* was defined as a urinary protein excretion of 33.9 to <339 mg/mmol, and *nephrotic proteinuria* was defined as a urinary protein excretion of ≥339 mg/mmol. EVR/CsA, everolimus with cyclosporine; EVR/TAC, everolimus with tacrolimus; MPA/TAC, mycophenolic acid with tacrolimus.

none under EVR/TAC or EVR/CsA treatment. CMV syndrome occurred in 1 patient under EVR/TAC treatment, and 5 MPA/TAC patients developed CMV disease including organ involvement. There were significantly fewer BK virus (BKV) infections in the EVR/TAC and EVR/CsA groups than in the MPA/TAC group ($P = 0.001$) (Figure 6b) as assessed from urine or blood samples by local reverse transcription polymerase chain reaction analysis ($>10^3$ copies/ml) and clinical assessments and histology, where available. BKV infection with organ involvement was reported in 3 EVR/TAC patients, 1 EVR/CsA patient, and 6 MPA/TAC patients.

Laboratory data at month 12 are presented in Supplementary Table S4.

DISCUSSION

The primary objective of this trial was to demonstrate noninferiority of renal function with EVR/TAC and/or EVR/CsA versus MPA/TAC at month 12 posttransplant. This objective was not achieved. In a matched-pair analysis of observed values, the change in eGFR from month 1 to month 12 was comparable between groups. In addition, in patients in whom the tacrolimus level was maintained at ≤5 ng/ml, the protocol-specified upper limit after month 2, GFR was comparable between the EVR/TAC and MPA/TAC groups. Immunosuppressive efficacy was similar in the EVR/TAC and MPA/TAC groups, as indicated by comparable low rates of BPAR, but mild BPAR episodes were more frequent under EVR/CsA treatment. The interpretation of the results was complicated by high rates of study drug discontinuation and withdrawal from the study.

The study protocol used a conservative approach whereby CNI levels were not further reduced in everolimus-treated patients compared with the control group of MPA-treated patients. Historically, tacrolimus trough levels were adjusted to 8 to 12 ng/ml after kidney transplantation. This altered after publication of the ELITE-Symphony study in 2007, which demonstrated that kidney allograft outcomes were improved with tacrolimus levels in the range 3 to 7 ng/ml

Table 4 | Kaplan-Meier estimates for efficacy end points at month 12 (ITT population)

Variable	EVR/TAC (n = 208)	EVR/CsA (n = 199)	MPA/TAC (n = 205)	P[a]	
				EVR/TAC vs. MPA/TAC	EVR/CsA vs. MPA/TAC
Treatment failure[b]	27 (13.0)	49 (24.6)	20 (9.8)	0.260	<0.001
BPAR	15 (7.2)	38 (19.1)	10 (4.9)	0.291	<0.001
Treated BPAR	14 (6.7)	35 (17.6)	8 (3.9)	0.183	<0.001
Grade IA	4	18	3	0.693	<0.001
Grade IB	1	1	0	0.324	0.310
Grade IIA	4	3	1	0.173	0.259
Grade IIB	2	1	1	0.557	0.954
Grade III	1	3	0	0.308	0.063
Missing	2	9	3	0.698	0.047
Graft loss[c]	10 (4.8)	13 (6.5)	6 (2.9)	0.294	0.082
Death[d]	5 (2.4)	2 (1.0)	6 (2.9)	0.817	0.894

BPAR, biopsy-proven acute rejection; EVR/CsA, everolimus with cyclosporine; EVR/TAC, everolimus with tacrolimus; ITT, intent-to-treat; MPA/TAC, mycophenolic acid with tacrolimus.
[a]Log-rank test.
[b]BPAR, graft loss, or death.
[c]Causes of graft loss: EVR/TAC: primary nonfunction, n = 3; poor organ quality/cold ischemia time lesions, n = 1; no graft function, n = 1; renal artery/vein thrombosis, n = 2; acute transplant failure, n = 1; postbiopsy bleeding, n = 1; not identified, n = 1. EVR/CsA: primary nonfunction, n = 5; renal artery/vein thrombosis, n = 2; acute rejection, 1; chronic rejection, 1; acute + chronic rejection, n = 1; infection, n = 1; rupture of kidney capsule/parenchyma, n = 2. MPA/TAC: primary nonfunction, n = 1; infarcted kidney, n = 1; noncompliance, n = 1; renal artery stenosis/thrombosis, n = 2; acute renal failure, n = 1.
[d]Causes of death: EVR/TAC: septic shock, n = 2; Kaposi sarcoma, n = 1; cardiogenic shock, n = 1; unknown, n = 1. EVR/CsA: sepsis, n = 1; multiorgan dysfunction syndrome, n = 2; unknown, n = 2. MPA/TAC: pulmonary embolism, n = 4; malignant lung neoplasm, n = 1; intestinal ischemia, n = 1.
Data are n (%).

when given in combination with mycophenolate mofetil.[16] Because most transplant centers worldwide now use the immunosuppressive regimen established by the ELITE-Symphony trial, the ATHENA study aimed to maintain tacrolimus exposure within this range to allow a direct comparison of the effects of everolimus and MPA in combination with tacrolimus. This decision was also driven by the fact that further reduction in tacrolimus trough levels, for example, during episodes of malabsorption, may lead to temporary under-immunosuppression that in the long term could be associated with induction of donor-specific antibodies. The third regimen, EVR/CsA, was included in ATHENA to provide a direct comparison with the results of previous everolimus-based CNI-minimization trials such as ZEUS[17] and HERAKLES[18] that used EVR/CsA immunosuppressive regimens.

Modern dosing regimens of tacrolimus, in particular, are associated with a reduced nephrotoxic effect as compared with earlier higher-dose CsA regimens.[15] Previous randomized trials that examined renal function with everolimus and reduced-exposure CNI therapy have reported comparable levels of eGFR compared to standard CNI with MPA.[6,7,19,20] The large randomized A2309 study demonstrated noninferiority for eGFR using EVR/CsA versus MPA with standard-exposure CsA.[6] More recently, in the randomized TRANSFORM trial of >2000 *de novo* kidney transplant patients, patients given everolimus with either reduced-exposure tacrolimus or reduced-exposure CsA had a similar mean eGFR and equivalent efficacy at month 12 to that of a standard MPA/CNI regimen.[19] As per the study protocol, CNI exposure levels were markedly higher in the present study than in these trials. Indeed, tacrolimus exposure was above the planned maximum throughout the study. At month 12, the mean tacrolimus level in the EVR/TAC group was 6.0 ng/ml compared to 4.1 ng/ml in the TRANSFORM study,[19] whereas the mean CsA level at month 12 (98 ng/ml) was noticeably higher than that in previous trials (50–75 ng/ml).[7,15,19] CsA levels exceeded those recommended in the license for everolimus.[20] Compared with sirolimus, everolimus given in combination with CNI does not appear to demonstrate nephrotoxic effects.[21] The effect of tacrolimus exposure in everolimus-treated patients was explored in the randomized ASSET trial, where the lower-exposure group (mean tacrolimus level, 3.4 ng/ml at month 12) showed a trend to superior renal function compared with the higher-exposure group (5.5 ng/ml at month 12).[22] Increasing concentrations of CNI have been shown to be associated with a greater decline in eGFR from month 1 to month 12 in everolimus-treated kidney transplant recipients.[23] The failure to show noninferiority with the prespecified noninferiority margin of −7 ml/min per 1.73 m^2 indicates that the CNI exposure levels in our study might have not been sufficiently different between treatment groups, or too high when compared to the standard treatment arm, in which the protocol specified low exposure levels. Renal function was comparable between the EVR/TAC and MPA/TAC groups in the subpopulation in whom the tacrolimus trough concentration was ≤5 mg/ml after month 3, indicating that the protocol *per se* may have been acceptable but adherence to the specified thresholds was inadequate. Two different noninferiority margins were tested when comparing eGFR. According to recommendations,[24,25] an adequate noninferiority margin should span half of the SD of means from the corresponding outcome variable in a specified population. Adopting a strict and conservative approach, a noninferiority margin of −7 ml/min per 1.73 m^2 was defined *a priori* for the present trial,[26] slightly less than the margin selected for the recent international A2309 study.[6] However, the study population in ATHENA was less

Table 5 | Adverse events (safety population)

Variable	EVR/TAC (n = 210)	EVR/CsA (n = 198)	MPA/TAC (n = 204)
Adverse events			
Any adverse event	209 (99.5)	198 (100.0)	204 (100.0)
With suspected relation to the study drug	174 (82.9)	154 (77.8)	167 (81.9)
Leading to study drug discontinuation	65 (31.0)	69 (34.8)	33 (16.2)
Leading to study drug dose reduction or temporary interruption	54 (25.7)	41 (20.7)	92 (45.1)
Adverse events occurring in ≥15% of patients in any group			
Peripheral edema	85 (40.5)	100 (50.5)	62 (30.4)
Urinary tract infection	87 (41.4)	80 (40.4)	84 (41.2)
Constipation	70 (33.3)	81 (40.9)	71 (34.8)
Wound complication	64 (30.5)	56 (28.3)	68 (33.3)
Hyperkalemia	56 (26.7)	59 (29.8)	69 (33.8)
Hypokalemia	37 (17.6)	41 (20.7)	33 (16.2)
Hypophosphatemia	37 (17.6)	30 (15.2)	24 (11.8)
Hypertension	63 (30.0)	57 (28.8)	52 (25.5)
Complications of the transplanted kidney	56 (26.7)	59 (29.8)	44 (21.6)
Anemia	54 (25.7)	60 (30.3)	44 (21.6)
Infectious diarrhea	44 (21.0)	34 (17.2)	58 (28.4)
Nausea	44 (21.0)	42 (21.2)	47 (23.0)
Increased blood creatinine	46 (21.9)	38 (19.2)	34 (16.7)
Lymphocele	38 (18.1)	49 (24.7)	30 (14.7)
Insomnia	39 (18.6)	24 (12.1)	41 (20.1)
BK virus infection	37 (17.6)	18 (9.1)	46 (22.5)
CMV infection	13 (6.2)	5 (2.5)	42 (20.6)
Diabetes mellitus	37 (17.6)	28 (14.1)	26 (12.7)
Flatulence	35 (16.7)	25 (12.6)	24 (11.8)
Leukopenia	18 (8.6)	13 (6.6)	39 (19.1)
Adverse events leading to permanent discontinuation of the study drug in ≥5% of patients in any group	0 (0)	0 (0)	0 (0)
Adverse events leading to pausing or adjustment of the study drug in ≥5% of patients in any group			
BK virus infection	2 (1.0)	3 (1.5)	13 (6.4)
CMV infection	0 (0)	0 (0)	13 (6.4)
Infectious diarrhea	1 (0.5)	0 (0)	10 (4.9)
Leukopenia	4 (1.9)	5 (2.5)	22 (10.8)
Serious adverse events			
Any serious adverse event	161 (76.7)	144 (72.7)	135 (66.2)
With suspected relation to the study drug	93 (44.3)	74 (37.4)	64 (31.4)
Leading to discontinuation of the study drug	45 (21.4)	45 (22.7)	19 (9.3)
Leading to death	5 (2.4)	5 (2.5)	6 (2.9)
Serious adverse events occurring in ≥10% of patients in any group			
Urinary tract infection	27 (12.9)	16 (8.1)	21 (10.3)
Complications of the transplanted kidney	22 (10.5)	18 (9.1)	18 (8.8)
Lymphocele	19 (9.0)	24 (12.1)	12 (5.9)
Kidney transplant rejection	19 (9.0)	22 (11.1)	8 (3.9)
Infections			
Any infection	154 (73.3)	142 (71.7)	167 (81.9)
Bacterial	93 (44.3)	85 (42.9)	85 (41.7)
Viral	54 (25.7)	23 (11.6)	83 (40.7)
CMV infection	13 (6.2)	5 (2.5)	42 (20.6)
D+/R+	1.4	0.5	4.4
D+/R−	3.8	1.5	8.3
D−/R+	0.5	0.0	4.9
D−/R−	0.5	0.5	2.4
BK virus infection	36 (17.1)	18 (9.1)	46 (22.5)
Fungal	22 (10.5)	12 (6.1)	6 (2.9)

CMV, cytomegalovirus; EVR/CsA, everolimus with cyclosporine; EVR/TAC, everolimus with tacrolimus; MPA/TAC, mycophenolic acid with tacrolimus.
Data are n (%).

homogeneous than has been typical in similar trials—notably, recipients of a graft from a deceased donor aged >65 years were not excluded and represented ∼17% of all deceased donor recipients—and in this "real-world" population the SD of the mean eGFR in the per-protocol population was as high as 18 ml/min per 1.73 m^2. With this insight, we *post hoc* adjusted the statistical model to apply the noninferiority margin of −9 ml/min per 1.73 m^2, which was approximately one-half the observed SD, to assess study outcomes within an adequate analysis setting. Using this margin, noninferiority of

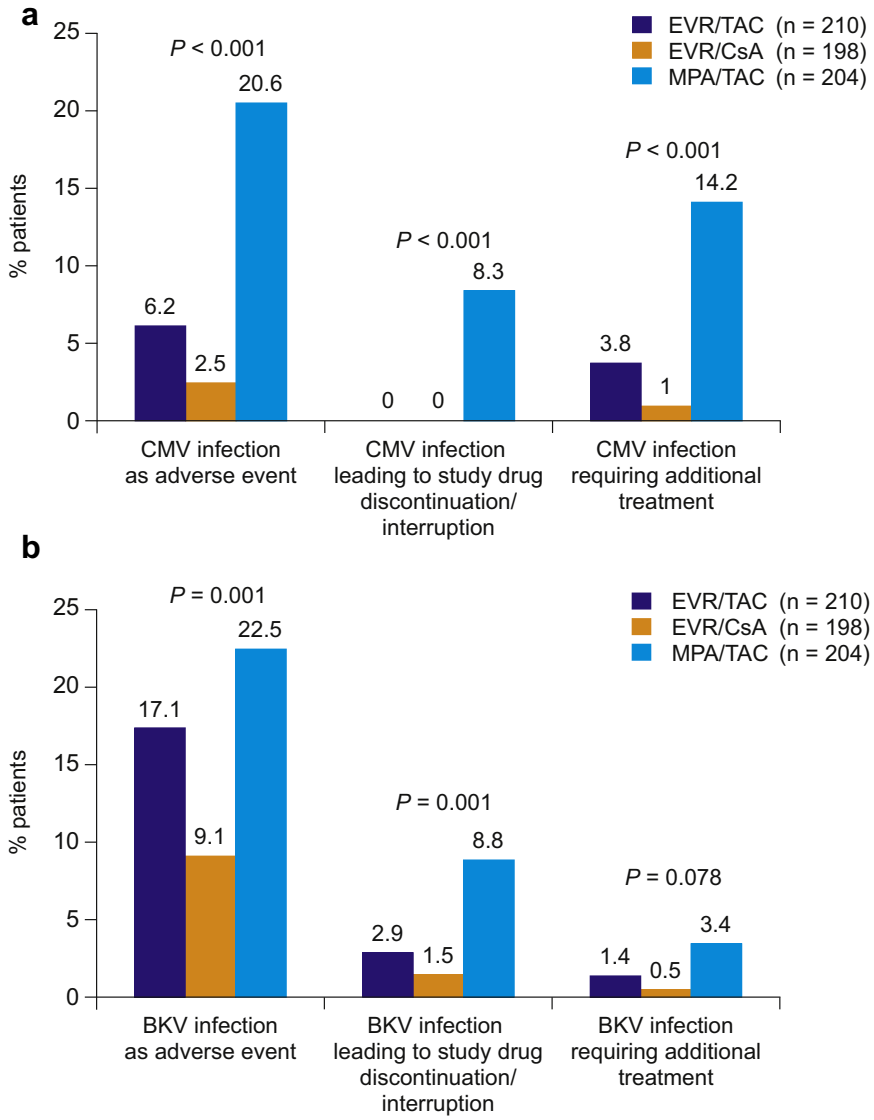

Figure 6| Incidence of (a) cytomegalovirus (CMV) and (b) BK virus (BKV) events by month 12. EVR/CsA, everolimus with cyclosporine; EVR/TAC, everolimus with tacrolimus; MPA/TAC, mycophenolic acid with tacrolimus.

renal function at month 12 was shown for EVR/CNI versus MPA/TAC.

Pooled analyses of randomized trials in kidney transplant patients have found the incidence of proteinuria to be higher with mammalian target of rapamycin inhibitors than with MPA therapy.[13,27,28] Here, encouragingly, urinary protein levels were similar across all groups and may relate to the fact that everolimus was used *de novo*, a setting in which the effect is dose dependent.[29]

Efficacy was similar between the EVR/TAC and MPA/TAC groups. The higher incidence of BPAR in the EVR/CsA group versus the MPA/TAC group was largely due to additional mild episodes, an observation not made in other randomized trials that compared everolimus with reduced-exposure CsA and MPA with standard-exposure CsA given from the time of transplantation.[6,7] Higher rates of biopsy in the 2 everolimus groups may have contributed to this phenomenon. In addition, local reading of biopsies and the high rate of missing BANFF categories complicate the interpretation of the results. It is also possible that population differences may have played a role, including the higher proportions of older recipients and donors as well as greater human leukocyte antigen-DR mismatch in the EVR/CsA group than in the MPA/TAC group.[30,31]

The study drug was discontinued prematurely because of adverse events twice as often in the everolimus-based groups than in the MPA/TAC group despite a similar rate of drug-related adverse events across all groups. Conversely, dose reductions or temporary interruptions due to adverse events were approximately twice as frequent under MPA/TAC treatment. Given the wide discrepancies in the rate of study drug discontinuation between centers—with some centers discontinuing all patients and some none—it seems likely that faced with intolerance in this open-label study some centers

showed a bias toward immediately switching from the "novel" regimens to standard MPA/CNI therapy whereas adverse events under the conventional regimen were managed by amending or pausing dosing.

The adverse events in all 3 treatment groups were consistent with known safety profiles. Wound healing complications did not differ between treatment arms, in line with previous experience that such complications are not increased when everolimus levels are in the range 3 to 8 ng/ml without concomitant MPA.[23,31,32] In addition, no differences were observed in delayed graft function.[30] The higher incidence of leukopenia in the MPA/TAC group is a known side effect of MPA therapy and is a frequent cause of MPA dose reductions.[3,33] The significant benefit of everolimus-based regimens in reducing risk of CMV infections, demonstrated in previous analyses in kidney[12] and heart transplantation,[34] was confirmed here by regular CMV monitoring and a prespecified end point, with more than a 3-fold reduction in the everolimus groups compared to the control arm. A similar strong benefit was seen for everolimus with a reduced occurrence of BKV infections compared to MPA/TAC.

Interestingly, adverse events in the MPA/TAC arm less frequently led to discontinuation of the study drug compared to patients receiving EVR/CNI regimens—regardless of their frequency and severity—whereas in almost every second patient in the MPA/TAC arm, adverse events led to drug adjustments or pausing. Discontinuation in the EVR/CNI groups could not be ascribed to a specific cluster of adverse events but showed considerable variety between centers. Similar observations were made regarding the rates of BPAR. Taken together, these clinical variations may reflect individual bias toward certain immunosuppressive drugs or regimens, which cannot be ruled out in open-label studies.

The study benefitted from a randomized, multicenter design. The major limitation was the high rate of dropouts from the per-protocol population because of discontinuation of the study drug and from both the per-protocol and ITT populations because of patients withdrawing from the study. Missing post-baseline eGFR values were therefore common. The *post hoc* matched-pair analysis of eGFR in ITT patients with data provided at both month 1 and month 12 sought to maximize the interpretation of the available data on the basis of the observed values, that is, without imputation of missing values. A further limitation was that mean tacrolimus levels exceeded the target range throughout the study in both EVR- and MPA-treated patients, with CsA levels above the target or close to the planned maximum. The reluctance on the part of investigators to adhere to tacrolimus target ranges appears to reflect caution about lowering exposure in patients given a mammalian target of rapamycin inhibitor; it is possible that evidence from the recent TRANSFORM study[19] will widen acceptance that efficacy is maintained when everolimus is given with reduced-exposure CNI therapy. A further point to consider is that there were numerical imbalances between groups in terms of proportion of patients receiving a graft from a deceased donor aged ≥65 years, with a numerically higher proportion in the EVR/CsA group. Deceased donor age was found to have a significant effect on change in eGFR regardless of the treatment group. Patients who received an organ from a deceased donor aged ≥65 years showed significantly worse outcomes in terms of graft function as compared with other deceased donor age categories over the duration of the study. In view of these points, we recommend carefully planning statistical model assumptions when studying broader patient populations and we advise stratifying for deceased donor age.

Even though noninferiority for renal function was not shown for everolimus versus MPA with comparable CNI exposure levels for the reasons discussed above, the regimens were both efficacious and safe in this population of *de novo* kidney transplant patients. The study provides further evidence for a significantly lower risk of CMV and BKV infections under everolimus therapy. Of particular interest, and in contrast to studies that did not predefine such events as study end points, there were no differences in reported rates of wound healing complications or proteinuria with everolimus therapy compared to MPA/TAC.

METHODS

ATHENA was a 12-month, multicenter, randomized, open-label study undertaken in *de novo* kidney transplant recipients (ClinicalTrials.gov identifier: NCT01843348; EudraCT number: 2011-005238-21). There were 3 parallel groups: (i) EVR/TAC, (ii) EVR/CsA, and (iii) MPA/TAC. The primary objective was to demonstrate noninferiority in renal function, on the basis of eGFR (Nankivell formula[35]), in at least one of the everolimus arms versus the MPA/TAC arm at month 12 posttransplant.

The study was conducted from December 2012 through March 2016 at 15 transplant centers in Germany and 12 centers in France. The study protocol was approved by the national institutional review board or independent ethics committee at each center. Written informed consent was obtained from all patients. The clinical study was designed and conducted in accordance with the ethical principles laid down in the Declaration of Helsinki.

Study population

De novo adult recipients of a primary or secondary kidney transplant from a deceased or living donor were eligible for the study unless the first graft loss was due to immunological reasons. Key exclusion criteria were an ABO-incompatible transplant, preexisting donor-specific antibodies, or cold ischemia time >30 hours. Detailed inclusion and exclusion criteria have been published previously.[26]

Immunosuppression and concomitant intervention

Eligible patients were randomized pretransplant (1:1:1 ratio) using an automated, validated system, stratified by living donor, deceased donor, and participation of the recipient in the Eurotransplant Senior Program (Germany only).

All patients received basiliximab induction therapy (20 mg on days 0 and 4). In the EVR/TAC and EVR/CsA groups, the target everolimus trough concentration was in the range 3 to 8 ng/ml throughout the study period, with CNI therapy initiated ≤24 hours posttransplant. The target tacrolimus trough concentration in the EVR/TAC and MPA/TAC groups was in the range 4 to 8 ng/ml until the end of month 2 and in the range 3 to 5 ng/ml thereafter. In the

EVR/CsA group, the CsA target was in the range 75 to 125 ng/ml until the end of month 2 and in the range 50 to 100 ng/ml thereafter. In the MPA/TAC group, MPA was given as enteric-coated mycophenolate sodium (1.44 g/d) or mycophenolate mofetil (2 g/d). All patients received steroids (≥5 mg/d) until month 12.

CMV prophylaxis was mandatory for ≥3 months in the case of a CMV-positive donor. Trimethoprim/sulfamethoxazole prophylaxis for *Pneumocystis jirovecii* pneumonia was given for 6 months.

Study end points and assessments

The primary end point was eGFR (Nanikvell formula[35]) at month 12. The key secondary end points were *treatment failure*, defined as a composite of BPAR, graft loss, or death, at month 12 posttransplant and outcomes for infections, with a focus on CMV and BKV infection. Other secondary end points are listed in Supplementary Table S5.

Statistical analysis

The ITT population consisted of all patients who received at least 1 dose of the study drug. The per-protocol population comprised all ITT patients without any major deviations from the protocol procedures that could affect the study outcomes. The safety set consisted of all ITT patients with at least 1 post-baseline safety assessment.

For the primary end point, the null hypothesis was that the treatment difference in mean eGFR at month 12 was equal to or greater than the noninferiority margin of −7 ml/min per 1.73 m^2 (i.e., closer to zero than the noninferiority margin), based on least-squares mean values, versus the alternative that the treatment difference was lower than the noninferiority margin (i.e., further from zero than the noninferiority margin). Two null hypotheses were tested confirmatively—EVR/TAC versus MPA/TAC and EVR/CsA versus MPA/TAC—using analysis of variance with treatment as well as center and donor type (living vs. cadaveric) as factors. The null hypothesis of noninferiority was to be accepted if the lower limit of the 2-sided 95% confidence interval for the treatment difference included the noninferiority margin of −7 ml/min per 1.73 m^2.

The primary analysis was based on the per-protocol population in accordance with recommendations.[36] As specified in the *a priori* statistical plan for the study, sensitivity analyses were performed by repeating the primary analysis in the ITT population with missing eGFR values imputed by multiple imputation and by the last observation carried forward method (applying the last post-baseline value). The multiple imputation rules are summarized in Supplementary Table S5. If a patient was on dialysis at the time of eGFR assessment, the eGFR value was set to 0. In addition, a mixed model for repeated measures was developed using treatment group and visit (as a categorical time variable) as fixed variables and subject as a random variable and fitted to the per-protocol and ITT populations. *Post hoc*, analysis of variance was repeated with the addition of eGFR at month 1 as a cofactor to test the null hypothesis that the treatment difference in mean eGFR at month 12 was equal to or greater than the noninferiority margin of −7 or −9 ml/min per 1.73 m^2 in the per-protocol and ITT populations. A margin of −9 ml/min per 1.73 m^2 was applied *post hoc* on the basis of recommendations that clinically relevant noninferiority margins should span one-half of the SD from the observed mean eGFR values within a specified population.[24,36]

Further information on statistical methods is given in Supplementary Table S5.

APPENDIX

List of the ATHENA Study Group

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DISCLOSURE

CS's institution has received research funding from Chiesi and Novartis. BS's institution has received research funding and/or travel grants honoraria from Chiesi, Novartis, Neovii, and Astellas. DD has received research funds, travel grants, or speakers' honoraria from Novartis, Astellas, Chiesi, Sanofi, and Hexal. PS has received research funds, travel grants, and/or honoraria from Astellas, Hexal, Neovii, and Novartis. IAH has received research funds, travel grants, and/or honoraria from Alexion, Astellas, Chiesi, Hexal, Novartis, Roche, Sanofi, and Teva. OW has received research funds and/or honoraria from Alexion, Astellas, Bristol-Myers Squibb, Chiesi, Janssen-Cilag, MSD, Novartis, Pfizer, Roche, and Shire. CH has received research funds and/or honoraria from Alexion, Amgen, Astellas, AstraZeneca, Berlin-Chemie, Boehringer Ingelheim, Fresenius, Otsuka, Novartis, and Sanofi. NK has received speakers' fees and participated in advisory boards for Abbvie, Amgen, Astellas, Chiesi, Fresenius, Gilead, Medical Care, Merck Sharp & Dohme, Neovii, Novartis, Roche, Sanofi, and Shire. PM has received research funds and travel grants from Astellas, Novartis, and Chiesi and participated in advisory boards for Bristol-Myers Squibb, Fresenius, and Sanofi. MJ is an employee of Novartis. FT's institution has received study honoraria from Novartis, Sanofi, Astellas, Alexion, Hexal, Chiesi, and Pfizer. BN has been a member of the advisory board and received speakers' honoraria from Astellas, Chiesi, and Novartis.

ACKNOWLEDGEMENTS

The study was funded by Novartis Pharma GmbH, Nürnberg, Germany. A medical writer (C. Lamont) was funded by Novartis Pharma GmbH, German. The funders of the study (Novartis Pharma GmbH) contributed to the study design and data analysis and reviewed drafts of the manuscript. All authors approved the final manuscript for publication. The authors thank Ayan Das Gupta for support in programming and statistical analyses.

SUPPLEMENTARY MATERIAL

Table S1. Exposure to calcineurin inhibitor therapy (safety population).
Table S2. Estimated glomerular filtration rate at month 12 (sensitivity and *post hoc* analyses).
Table S3. Left ventricular hypertrophy (LVH).
Table S4. Laboratory values at month 12.
Table S5. Primary and secondary study end points.
Supplementary material is linked to the online version of the paper at www.kidney-international.org.

REFERENCES

1. United States Renal Data System 2015 Annual Data Report. Available at: https://www.usrds.org/2015/view/. Accessed March 20, 2019.
2. Rush R. The impact of calcineurin inhibitors on graft survival. *Transplant Rev (Orlando)*. 2013;27:93–95.
3. Vanhove T, Kuypers D, Claes KJ, et al. Reasons for dose reduction of mycophenolate mofetil during the first year after renal transplantation and its impact on graft outcome. *Transpl Int*. 2013;26:813–821.
4. Knoll GA, MacDonald I, Khan A, Van Walraven C. Mycophenolate mofetil dose reduction and the risk of acute rejection after renal transplantation. *J Am Soc Nephrol*. 2003;14:2381–2386.
5. Pascual J, Srinivas TR, Chadban S, et al. TRANSFORM: a novel study design to evaluate the effect of everolimus on long-term outcomes after kidney transplantation. *Open Access J Clin Trials*. 2014;6:45–54.
6. 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.

7. Takahashi K, Uchida K, Yoshimura N, et al. Efficacy and safety of concentration-controlled everolimus with reduced-dose cyclosporine in Japanese de novo renal transplant patients: 12-month results. *Transplant Res*. 2013;2:14.
8. Santos SM, Carlos CM, Cabanayan-Casasola CB, Danguilan RA. Everolimus with reduced-dose cyclosporine versus full-dose cyclosporine and mycophenolate in de novo renal transplant patients: a 2-year single-center experience. *Transplant Proc*. 2012;44:154–160.
9. Euvrard S, Boissonnat P, Roussoulières A, et al. Effect of everolimus on skin cancers in calcineurin inhibitor-treated heart transplant recipients. *Transpl Int*. 2010;23:855–857.
10. Holdaas H, De Simone P, Zuckermann A. Everolimus and malignancy after solid organ transplantation: a clinical update. *J Transplant*. 2016;2016:4369574.
11. Piselli P, Serraino D, Segoloni GP, et al. Immunosuppression and Cancer Study Group. Risk of de novo cancers after transplantation: results from a cohort of 7217 kidney transplant recipients, Italy 1997-2009. *Eur J Cancer*. 2013;49:336–344.
12. Nashan B, Gaston R, Emery V, et al. Review of cytomegalovirus infection findings with mammalian target of rapamycin inhibitor-based immunosuppressive therapy in de novo renal transplant recipients. *Transplantation*. 2012;93:1075–1085.
13. Mallat SG, Tanios BY, Itani HS, et al. CMV and BKPyV infections in renal transplant recipients receiving an mTOR inhibitor-based regimen versus a CNI-based regimen: a systematic review and meta-analysis of randomized, controlled trials. *Clin J Am Soc Nephrol*. 2017;12:1321–1336.
14. Eurotransplant. Eurotransplant Senior Program. Available at: https://www.eurotransplant.org/cms/index.php?page=esp. Accessed January 23, 2018.
15. Chapman JR. Chronic calcineurin inhibitor nephrotoxicity—lest we forget. *Am J Transplant*. 2011;11:693–697.
16. Ekberg H, Tedesco-Silva H, Demirbas A, et al. Reduced exposure to calcineurin inhibitors in renal transplantation. *N Engl J Med*. 2007;357:2562–2575.
17. Budde K, Becker T, Arns W, et al. for ZEUS Study Investigators. Everolimus-based, calcineurin-inhibitor-free regimen in recipients of de-novo kidney transplants: an open-label, randomised, controlled trial. *Lancet*. 2011;377:837–847.
18. Budde K, Zeier M, Witzke O, et al., HERAKLES Study Group. Everolimus with cyclosporine withdrawal or low-exposure cyclosporine in kidney transplantation from month 3: a multicentre, randomized trial. *Nephrol Dial Transplant*. 2017;32:1060–1070.
19. Pascual J, Berger SP, Witzke O, et al. Everolimus with reduced calcineurin inhibitor exposure in renal transplantation. *J Am Soc Nephrol*. 2018;29:1979–1991.
20. Certican® (Everolimus) Summary of Product Characteristics. Basel, Switzerland: Novartis Pharma AG.
21. Klawitter J, Nashan B, Christians U. Everolimus and sirolimus in transplantation-related but different. *Expert Opin Drug Saf*. 2015;14:1055–1070.
22. Langer RM, Hené R, Vitko S, et al. Everolimus plus early tacrolimus minimization: a phase III, randomized, open-label, multicentre trial in renal transplantation. *Transplant Int*. 2012;25:592–602.
23. Shihab FS, Cibrik D, Chan L, et al. Association of clinical events with everolimus exposure in kidney transplant patients receiving reduced cyclosporine. *Clin Transplant*. 2013;27:217–226.
24. Witte S, Schmidli H, O'Hagan A, Racine A. Designing a non-inferiority study in kidney transplantation: a case study. *Pharm Stat*. 2011;10:427–432.
25. Sis B, Mengel M, Haas M, et al. Banff '09 meeting report: antibody mediated graft deterioration and implementation of Banff working groups. *Am J Transplant*. 2010;10:464–471.
26. Sommerer C, Suwelack B, Dragun D, et al. Design and rationale of the ATHENA study—a 12-month, multicentre, prospective study evaluating the outcomes of a de novo everolimus-based regimen in combination with reduced cyclosporine or tacrolimus versus a standard regimen in kidney transplant patients: study protocol for a randomised controlled trial. *Trials*. 2016;17:92.
27. de Paula MI, Medina Pestana JO, Nicolau Ferreira A, et al. Long-term follow-up of de novo use of mTOR and calcineurin inhibitors after kidney transplantation. *Ther Drug Monit*. 2016;38:22–31.
28. Murakami N, Riella LV, Funakoshi T. Risk of metabolic complications in kidney transplantation after conversion to mTOR inhibitor: a systematic review and meta-analysis. *Am J Transplant*. 2014;14:2317–2327.
29. Wiseman AC, McCague K, Kim Y, et al. The effect of everolimus versus mycophenolate upon proteinuria following kidney transplantation and relationship to graft outcomes. *Am J Transplant*. 2013;13:442–449.
30. Halleck F, Khadzhynov D, Liefeldt L, et al. Immunologic outcome in elderly kidney transplant recipients: is it time for HLA-DR matching? *Nephrol Dial Transplant*. 2016;31:2143–2149.
31. Albano L, Berthoux F, Moal MC, et al. RAD A2420 Study Group. 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.
32. Nashan B, Citterio F. Wound healing complications and the use of mammalian target of rapamycin inhibitors in kidney transplantation: a critical review of the literature. *Transplantation*. 2012;94:547–561.
33. Pascual J, Ocaña J, Marcén R, et al. Mycophenolate mofetil tolerability and dose changes in tacrolimus-treated renal allograft recipients. *Transplant Proc*. 2006;38:2398–2399.
34. Kobashigawa J, Ross H, Bara C, et al. Everolimus is associated with a reduced incidence of cytomegalovirus infection following de novo cardiac transplantation. *Transpl Infect Dis*. 2013;15:150–156.
35. Nankivell BJ, Gruenewald SM, Allen RD, Chapman JR. Predicting glomerular filtration rate after kidney transplantation. *Transplantation*. 1995;59:1683–1689.
36. European Medicines Agency. ICH Topic E 9: Statistical Principles for Clinical Trials. Step 5: Note for Guidance on Statistical Principles for Clinical Trials (CPMP/ICH/363/96). Available at: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002928.pdf. Accessed August 20, 2018.