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Association of Clinical Events With Everolimus Exposure in Kidney Transplant Patients Receiving Low Doses of Tacrolimus

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A key objective in the use of immunosuppression after kidney transplantation is to attain the optimal balance between efficacy and safety. In a phase 3b, multicenter, randomized, open-label, noninferiority study, the incidences of clinical events, renal dysfunction, and adverse events (AEs) were analyzed at 12 months in 309 *de novo* renal transplant recipients receiving everolimus (EVR), low-dose tacrolimus (LTac), and prednisone. Cox proportional hazard regression modeling was used to estimate the probability of clinical events at specified combinations of time-normalized EVR and Tac trough concentrations. At 12 months, the highest incidence of treated biopsy-proven acute rejection (tBPAR) and graft loss occurred most often in patients with EVR trough concentration <3 ng/mL (64.7% and 10.5%, respectively). At 1 month and 12 months, increasing EVR levels were associated with fewer tBPAR events (both $p < 0.0001$). Low estimated glomerular filtration rate (eGFR) and decreased eGFR occurred more often in patients with lower EVR and higher Tac levels. AEs were most often observed in patients with EVR levels <3 ng/mL. This study supports maintaining an EVR trough concentration of 3–8 ng/mL, when combined with LTac, to achieve balanced efficacy and safety in renal transplant recipients. Trial registration: NCT01025817.

Abbreviations: AE, adverse event; CMV, cytomegalovirus; CNI, calcineurin inhibitor; CsA, cyclosporine; eGFR, estimated glomerular filtration rate; EVR, everolimus; HLA, human leukocyte antigen; ITT, intention-to-treat; LTac, low-dose tacrolimus; MDRD, Modification of Diet in Renal Disease; mTOR, mammalian target of rapamycin; NODM, new-onset diabetes mellitus; PK, pharmacokinetics; PRA, panel reactive antibody; sTac, standard-dose tacrolimus; Tac, tacrolimus; tBPAR, treated biopsy-proven acute rejection; UPC, urine protein:creatinine ratio

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Introduction

Long-term graft survival in renal transplant patients relies significantly on immunosuppressive agents. Achieving balance between a low rate of acute rejections and avoidance of immunosuppression-related toxicities is a goal in kidney transplantation. The mammalian target of rapamycin (mTOR) inhibitors bind to the immunophilin FKBP12 to block the activity of mTOR, a serine threonine protein kinase involved in the proliferation and clonal expansion of antigen-activated T cells (1,2), the main mechanism underlying acute transplant rejection. Kidney transplant patients receiving mTOR inhibitors can benefit from reduced exposure to calcineurin inhibitors (CNIs), which are known to be associated with nephrotoxicity (3), with no loss of immunosuppressive efficacy (4–8).

The 24-month CRAD001A2309 study in 833 *de novo* kidney transplant patients showed that treatment with the mTOR inhibitor everolimus (EVR), particularly at a trough concentration of 3–8 ng/mL, allowed for reduced exposure to the CNI cyclosporine (CsA), while maintaining renal function and a reduced risk of treated biopsy-proven acute rejection (tBPAR) (7,9). Analyses of the exposure–response relationship provide further support for maintaining an EVR trough concentration of 3–8 ng/mL, when combined with reduced-exposure CsA (10). The 2013 Organ Procurement and Transplantation Network and Scientific Registry of Transplant Recipients annual report demonstrated that tacrolimus (Tac), and not CsA, was by far the most frequently used CNI-based *de novo* regimen for all solid organ transplantation (11).

Previous studies have shown the combination of EVR and Tac to be safe and well tolerated in renal transplant patients (5,12,13). An open-label exploratory study assessed EVR and Tac combination treatment in maintenance renal patients and found no pharmacokinetic interactions when these treatments were given simultaneously (14). The exploratory, open-label, randomized CRAD001AUS09 study also found no pharmacokinetic interactions between concentration-controlled EVR and low- or standard-dose Tac (LTac or sTac, respectively) in 92 *de novo* renal transplant patients (5). EVR trough levels >3 ng/mL were necessary to achieve low tBPAR and excellent graft function at 6 months posttransplantation (12).

Results from the phase 3b, multicenter, randomized, open-label noninferiority study CRAD001AUS92 demonstrated that EVR with LTac was associated with an increased rejection rate (15). To correlate efficacy and safety events with EVR and Tac concentrations, we assessed the incidence of posttransplantation tBPAR, graft loss, and other adverse events (AEs) associated with EVR and Tac exposure at 12 months in the CRAD001AUS92 study. Because renal dysfunction at 1 year posttransplantation is known to be associated with poor graft outcome, we also assessed posttransplantation renal function in this population.

Materials and Methods

Study design and conduct

The US92 study methodology and inclusion/exclusion criteria have been described in detail previously (15). Briefly, in a 12-month, multicenter, randomized, open-label, noninferiority trial, adult recipients of a primary, *de novo* kidney transplant were randomized (1:1) to one of two treatment arms within 24 h posttransplantation: EVR 0.75 mg twice daily (1.5 mg/day; C0: 3–8 ng/mL) plus induction therapy plus LTac, or mycophenolate mofetil 1 g twice daily (2 g/day) plus induction therapy plus sTac. Only patients randomized to receive EVR and LTac (EVR plus LTac; n = 309) were included in the current analysis. The study was conducted in accordance with the Declaration of Helsinki following approval from the institutional review board at each center and written informed consent was obtained from all patients. Enrollment commenced in January 2010, and the study was completed in March 2013.

Key inclusion criteria included all patients aged 18–70 years and a recipient of a kidney from a deceased donor (including expanded-criteria donor organs and donor organs after cardiac death), living unrelated donor, or non-human leukocyte antigen (HLA) identical living related donor kidney. Key exclusion criteria included cold ischemic time >30 h; ABO incompatible transplants or T- or B-cell cross-match–positive transplant; platelet count <100 000/mm^3, neutrophil count <1500/mm^3, or white blood cell count <3000/mm^3 at randomization; history of malignancy; and HIV or hepatitis C or B virus infection or any other systemic infections.

Immunosuppression

Patients received EVR 0.75 mg twice daily as a starting dose, which was adjusted from day 5 to maintain a trough level of 3–8 ng/mL. Patients received Tac and prednisone per local guidelines, with Tac dosing adjusted from day 3 onward to achieve trough levels of 4–7 ng/mL (0–2 months), 3–6 ng/mL (2–6 months), and 2–5 ng/mL (6–12 months). Patients also received induction therapy according to their immunologic risk status: basiliximab 20 mg for those with low to moderate risk (panel reactive antibody [PRA] <20%) or thymoglobulin for high-risk patients (PRA ≥20% or recipients of extended-criteria donor organs or deceased donor organs after cardiac death), as per local guidelines.

Study objectives

The primary objective of US92 was to evaluate the noninferiority of a composite efficacy failure rate (tBPAR, graft loss, death, loss to follow-up) at month 12 between the treatment groups. In order to investigate the optimal EVR exposure when used in combination with LTac, we present *post hoc* exploratory analyses to correlate efficacy, renal function, and safety events with EVR and LTac concentrations.

Statistical methods

All efficacy analyses were assessed in the full analysis set (i.e. all randomized patients; n = 309). Renal and safety analyses were assessed in patients in the safety set (i.e. patients who received at least one dose of study drug and provided a postbaseline safety assessment; n = 306). Only on-treatment renal and safety events were included in the analyses. As there were too few on-treatment efficacy events to be considered for analysis alone, all efficacy events were included in the analyses. Definitions of renal events are as follows: low eGFR (eGFR <30 mL/min/1.73 m^2), decreased eGFR (decrease in eGFR by 30% compared to month 1), and proteinuria (urinary protein:creatinine ratio of ≥500 mg/g). Rates of BK virus and cytomegalovirus (CMV) were reported incidences based on AEs and were not gathered prospectively.

No formal testing of differences between patient subpopulations was done. Such testing was not considered statistically valid because patients were not randomized to the drug exposure subpopulations.

Data are based on centrally measured, time-normalized mean trough concentrations for EVR and Tac. EVR and Tac concentrations were determined centrally by using LC-MS/MS. Time-normalized mean trough concentrations were calculated as $\Sigma A^i/(D^k - D^0)$ where A^i is the trapezoid area $(½)[(C^{i-1} + C^i)*(D^i - D^{i-1})]$ under the concentrations C^{i-1} and C^i, D^i is the blood sampling day for C^i, i = 1,…, k. $D^0 = 7$ or the first trough concentration examination date when a trough was obtained after day 7. Concentrations below the lower limit of quantification (0.4 ng/mL) were set to 0.2 ng/mL. Time-normalized mean EVR and Tac trough concentrations were calculated up to the occurrence of an event or for patients without event, the last on-treatment value.

The influence of EVR or Tac exposure on efficacy, renal, and safety events was assessed by dividing the time-normalized mean trough concentrations into predefined ranges (<3, 3–<6, 6–<8, 8–<12, and ≥12 ng/mL for EVR and <2, 2–<5, 5–<8, and ≥8 ng/mL for Tac).

Cox proportional hazard regression modeling was used to estimate the probability of tBPAR, renal dysfunction events, and high urinary protein: creatinine ratio at specified combinations of time-normalized EVR and Tac trough concentrations in the pharmacokinetics (PK) efficacy population (n = 299), that is, the intention-to-treat (ITT) group minus those patients for whom EVR or Tac levels were not measured.

Results

Patient population

The full analysis set randomized to the EVR + LTac arm consisted of 309 patients. Three individuals were

Table 1: Demographics and baseline characteristics (safety set)

	EVR+LTac N = 306
Age, mean ± SD	50.0 ± 13.3
Male, n (%)	205 (67.0)
Race, n (%)	
White	196 (64.1)
Black	70 (22.9)
Asian	17 (5.6)
Other	23 (7.5)
Patients with diabetes mellitus	111 (36.3)
Primary disease leading to transplantation, n (%)	
Diabetes mellitus	83 (27.1)
Glomerulonephritis/glomerular disease	68 (22.2)
Hypertension/nephrosclerosis	59 (19.3)
Polycystic disease	40 (13.1)
HLA mismatches ≥3,* n (%)	261 (85.3)
Two mismatches at loci DR	118 (38.6)
PRA ≥20% most current evaluation, n (%)	40 (13.1)
Mean UPC ratio (mg/g)	3022.41
Donor demographics	
Age (years)	
Mean ± SD	39.6 ± 14.9
Gender, n (%)	
Male	150 (49.0)
Extended-criteria donor, n (%)	34 (11.1)
Donor characteristics	
Deceased	170 (55.5)
Deceased heart beating	136 (44.4)
Living	136 (44.4)
Living related	68 (22.2)
CMV-positive serology, n (%)	185 (60.5)

EVR, everolimus; LTac, low-dose tacrolimus; HLA, human leukocyte antigen; PRA, panel reactive antibody; UPC, urine protein:creatinine ratio; CMV, cytomegalovirus.
*$p < 0.05$.

randomized but did not receive study medication and were excluded from the safety set (n = 306). The mean age was 50 years and 67% of patients were male (Table 1). Of these 309 patients, 293 (94.8%) completed the 12-month study period. A total of 105 (34.0%) patients discontinued the study medication, with over half of these being due to AEs.

Efficacy events

tBPAR: At 12 months posttransplantation, tBPAR had occurred in 59 (19.1%) of 309 patients receiving EVR + LTac (Table 2). The highest rates of tBPAR occurred in patients with EVR trough concentration <3 ng/mL (n = 22/34; 64.7% vs. 14.0% at EVR ≥3 ng/mL), while the lowest tBPAR rates were associated with EVR 6–<8 ng/mL (n = 5/64; 7.8%) (Table 2). Of the four patients with Tac <2 ng/mL, two cases of tBPAR were reported (50.0% vs. 19.3% at ≥2 ng/mL).

A Cox proportional hazard regression model was used to estimate the probability of tBPAR at specified combinations of time-normalized EVR and Tac concentrations in the PK efficacy population (n = 299), that is, the ITT group minus those patients for whom EVR or Tac levels were not measured. The lowest EVR and Tac concentrations (EVR <3 ng/mL and Tac <2 ng/mL) showed the highest estimated probability of tBPAR at 1 month (Figure 1A) and 12 months (Figure 1B) at 19.8% and 58.3%, respectively. According to the Cox model, at 1 month posttransplantation, fewer tBPAR events occurred with increasing EVR levels ($p < 0.0001$) and with increasing Tac levels ($p = 0.0018$). At 12 months posttransplantation, increasing

Table 2: Incidence of clinical events at month 12 in patients randomized to the US92 EVR plus LTac arm

		Tac (ng/mL)				
Event, n (%)	EVR (ng/mL)	<2	2–<5	5–<8	≥8	All[1]
tBPAR	<3	1/2 (50.0)	11/14 (78.6)	8/14 (57.1)	2/4 (50.0)	22/34 (64.7)
	3–<6	1/2 (50.0)	8/66 (12.1)	18/117 (15.4)	4/8 (50.0)	31/193 (16.1)
	6–<8	0	0/20 (0.0)	5/39 (12.8)	0/5 (0.0)	5/64 (7.8)
	8–<12	0	0	0/4 (0.0)	1/4 (25.0)	1/8 (12.5)
	≥12	0	0	0	0	0
	All[1]	2/4 (50.0)	19/100 (19.0)	31/174 (17.8)	7/21 (33.3)	59/309 (19.1)
Graft loss	<3	1/1 (100.0)	1/6 (16.7)	0/10 (0.0)	0/2 (0.0)	2/19 (10.5)
	3–<6	1/1 (100.0)	0/73 (0.0)	0/123 (0.0)	0/8 (0.0)	1/205 (0.5)
	6–<8	0	0/21 (0.0)	0/38 (0.0)	0/5 (0.0)	0/64 (0.0)
	8–<12	0	0/2 (0.0)	0/5 (0.0)	0/4 (0.0)	0/11 (0.0)
	≥12	0	0	0	0	0
	All[1]	2/2 (100.0)	1/102 (1.0)	0/176 (0.0)	0/19 (0.0)	4/309 (1.3)
Death	<3	0/1 (0.0)	0/6 (0.0)	0/10 (0.0)	0/2 (0.0)	0/19 (0.0)
	3–<6	0/1 (0.0)	0/73 (0.0)	3/123 (2.4)	1/8 (12.5)	4/205 (2.0)
	6–<8	0	1/21 (4.8)	1/38 (2.6)	0/5 (0.0)	2/64 (3.1)
	8–<12	0	0/2 (0.0)	0/5 (0.0)	0/4 (0.0)	0/11 (0.0)
	≥12	0	0	0	0	0
	All[1]	0/2 (0.0)	1/102 (1.0)	4/176 (2.3)	1/19 (5.3)	6/309 (1.9)

EVR, everolimus; LTac, low-dose Tac; Tac, tacrolimus; tBPAR, treated biopsy-proven acute rejection.
[1]Data for which EVR or Tac values were unknown are not represented.

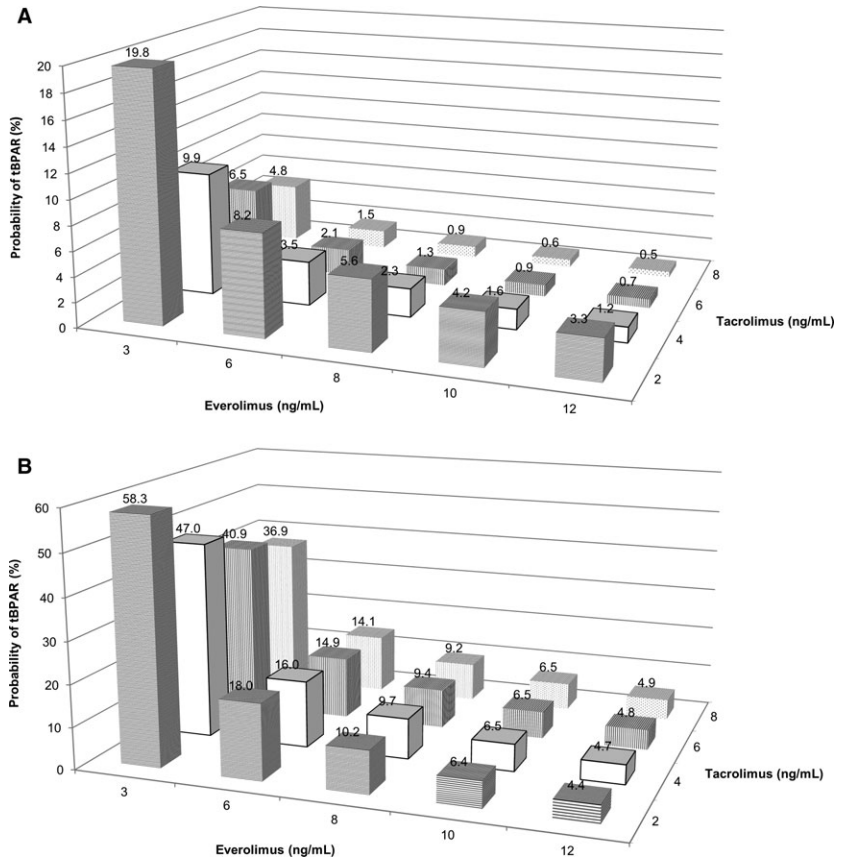

Figure 1: Estimated probability of tBPAR as a function of time-normalized everolimus and tacrolimus trough levels (pharmacokinetics efficacy population) at (A) 1 month and (B) 12 months posttransplantation. tBPAR, treated biopsy-proven acute rejection.

EVR levels were associated with fewer events ($p < 0.0001$), while there was no association between Tac levels and tBPAR ($p = 0.5211$).

During the first 2 weeks posttransplantation, a higher proportion of the patients with tBPAR were below the EVR target range of 3–8 ng/mL than those who did not have tBPAR (Day 7: 65.3% of patients with tBPAR vs. 53.7% without tBPAR, Day 14: 38.0% of patients with tBPAR vs. 32.4% without tBPAR). The mean EVR trough level at Day 7 was also lower for patients who experienced tBPAR than those that did not (2.62 ng/mL vs. 3.35 ng/mL, respectively).

Graft loss, deaths and loss to follow-up: Graft loss had occurred in 4 of 309 patients (1.3%) at 12 months following transplantation. The highest rate of graft loss occurred at the lowest EVR and Tac concentrations: EVR <3 ng/mL (n = 2/19; 10.5% vs. 0.4% at ≥3 ng/mL) and Tac <2 ng/mL (n = 2/2; 100.0% vs. 0.3% at ≥2 ng/mL) (Table 2). All 4 graft losses occurred during the first month posttransplantation and none were due to rejection: one case each of infarcted kidney, renal artery stenosis, renal vein thrombosis, and acute tubular necrosis.

Death was reported for 6 patients (1.9%) with no obvious associations to treatment trough concentrations (Table 2). Deaths were evenly distributed across each induction cohort based on immunological risk: 3 deaths occurred in the basiliximab induction cohort and 3 in the thymoglobulin induction cohort. Deaths were due to one case each of myocardial infarction, *Pneumocystis jiroveci* pneumonia, sepsis, malignant neoplasm, pulmonary embolism, and renal failure. Death in the latter patient occurred on Day 159 posttransplantation and was attributed to renal failure, electrolyte imbalance and arrhythmia. The patient was being treated for a number of medical conditions before undergoing renal transplant, namely hypertension, insulin-dependent diabetes mellitus, hyperlipidemia, hyperphosphatemia, secondary hyperparathyroidism, vitamin D deficiency, hypomagnesemia and fluid retention.

Renal function

Low eGFR and decreased eGFR: At 12 months posttransplantation, of 306 patients, low eGFR was reported in 27 (8.8%) and decreased eGFR in 55 (18.0%) receiving EVR+LTac (Table 3). The highest incidence

Table 3: Incidence of renal dysfunction parameters at month 12 in patients randomized to the US92 EVR plus LTac arm

		Tac (ng/mL)				
Parameter, n (%)	EVR (ng/mL)	<2	2–<5	5–<8	≥8	All[1]
Low eGFR[2]	<3	0/1 (0.0)	2/8 (25.0)	0/10 (0.0)	0/2 (0.0)	2/21 (9.5)
	3–<6	0/1 (0.0)	3/67 (4.5)	16/128 (12.5)	2/10 (20.0)	21/206 (10.2)
	6–<8	0	0/21 (0.0)	3/36 (8.3)	0/5 (0.0)	3/62 (4.8)
	8–<12	0	0/2 (0.0)	0/4 (0.0)	0/3 (0.0)	0/9 (0.0)
	≥12	0	0	0	1/1 (100.0)	1/1 (100.0)
	All[1]	0/2 (0.0)	5/98 (5.1)	19/178 (10.7)	3/21 (14.3)	27/306 (8.8)
Decreased eGFR[3]	<3	0/1 (0.0)	0/5 (0.0)	3/13 (23.1)	0/2 (0.0)	3/21 (14.3)
	3–<6	0/1 (0.0)	9/66 (13.6)	35/129 (27.1)	0/8 (0.0)	44/204 (21.6)
	6–<8	0	0/20 (0.0)	5/37 (13.5)	1/5 (20.0)	6/62 (9.7)
	8–<12	0	0/2 (0.0)	0/5 (0.0)	2/5 (40.0)	2/12 (16.7)
	≥12	0	0	0	0	0
	All[1]	0/2 (0.0)	9/93 (9.7)	43/184 (23.4)	3/20 (15.0)	55/306 (18.0)

EVR, everolimus; LTac, low-dose Tac; Tac, tacrolimus; eGFR, estimated glomerular filtration rate.
[1]Data for which EVR or Tac values were unknown are not represented.
[2]Defined as eGFR (Modification of Diet in Renal Disease [MDRD]) <30 mL/min/1.73 m^2.
[3]Defined as decrease in eGFR (MDRD) by 30% compared to month 1.

rates of both renal dysfunction measures occurred in patients with EVR <6 ng/mL (low eGFR: n = 23/227; 10.1% vs. 5.6% at EVR ≥6 ng/mL; decreased eGFR: n = 47/225; 20.9% vs. 10.8% at EVR ≥6 ng/mL) and Tac ≥5 ng/mL (low eGFR: n = 22/199; 11.1% vs. 0.5% at Tac <5 ng/mL; decreased eGFR: n = 46/204; 22.6% vs. 9.5% at Tac <5 ng/mL).

A Cox proportional hazard regression model was used to estimate the probability of low eGFR (Figure 2A) and decreased eGFR (Figure 2B) at 12 months in the PK efficacy population. According to the Cox model, the estimated probability of low eGFR and decreased eGFR at 12 months were both highest at lower EVR trough concentrations (p = 0.0006 and p = 0.0016, respectively). By contrast, higher Tac levels were associated with an increase in the estimated probability of low eGFR and decreased eGFR at 12 months (p = 0.0085 and p = 0.0376, respectively).

Analysis of eGFR rates in those patients who did not have graft rejection demonstrated that patients with lower EVR levels had lower eGFR values (p = 0.0109 for low eGFR and p = 0.0035 for decreased eGFR).

Proteinuria: According to the Cox model, the estimated probability of proteinuria (urinary protein: creatinine ratio of ≥500 mg/g) at 12 months was highest with decreasing EVR concentration (p = 0.0024) (Figure 2C). By contrast, the estimated probability of proteinuria was higher with increasing Tac trough levels (p < 0.0001).

Safety

Incidence rates of AEs at month 12 posttransplantation stratified by EVR and Tac trough concentration are shown in Table 4. Of the 306 patients assessed in the safety set, 68 patients (22.2%) discontinued study medication due to an AE, with the most common reason for discontinuation being BK viral infection (n = 7; 2.3%).

Of 306 patients, wound healing events occurred in 89 individuals (29.1%), with the most frequent occurrence in those with EVR <3 ng/mL (n = 35/49; 71.4% vs. 19.3% at ≥3 ng/mL) and Tac <2 ng/mL (n = 4/5; 80.0% vs. 27.2% at ≥2 ng/mL). Peripheral edema (n = 116/306 [37.9%]) also occurred most often in the same patient population, with highest incidence at EVR <3 ng/mL (n = 52/68; 76.5% vs. 25.7% at ≥3 ng/mL) and Tac <2 ng/mL (n = 10/12; 83.3% vs. 35.5% at ≥2 ng/mL). The incidence of new onset diabetes (NODM) (n = 25/306 [8.2%]) showed a similar pattern, occurring most often in patients with EVR <3 ng/mL (n = 6/24; 25.0% vs. 5.2% at ≥3 ng/mL) and Tac <2 ng/mL (n = 3/5; 60.0% vs. 5.9% at ≥2 ng/mL). Interestingly, although incidence was highest for these AEs at the lowest EVR and Tac levels, the second highest rates of both wound-healing events and peripheral edema were at EVR and Tac levels ≥8 ng/mL (Table 4).

At 12 months posttransplantation, BK viral infection occurred in 31 of 306 patients (10.1%), and was most frequent at EVR <3 ng/mL (n = 4/21; 19.0% vs. 9.7% at ≥3 ng/mL) and Tac ≥8 ng/mL (n = 6/23; 26.1% vs. 9.1% at <8 ng/mL). The incidence of stomatitis or oral ulcers (n = 10/306 [3.3%]) was also highest at Tac ≥8 ng/mL (n = 2/19; 10.5% vs. 2.9% at <8 ng/mL) but did not show any discernible association with EVR levels.

CMV infection was reported in only 7 of 306 patients (2.3%), with the highest incidence rate in those with Tac ≥5 ng/mL (n = 6/196; 3.1% vs. 1.0% at <5 ng/mL).

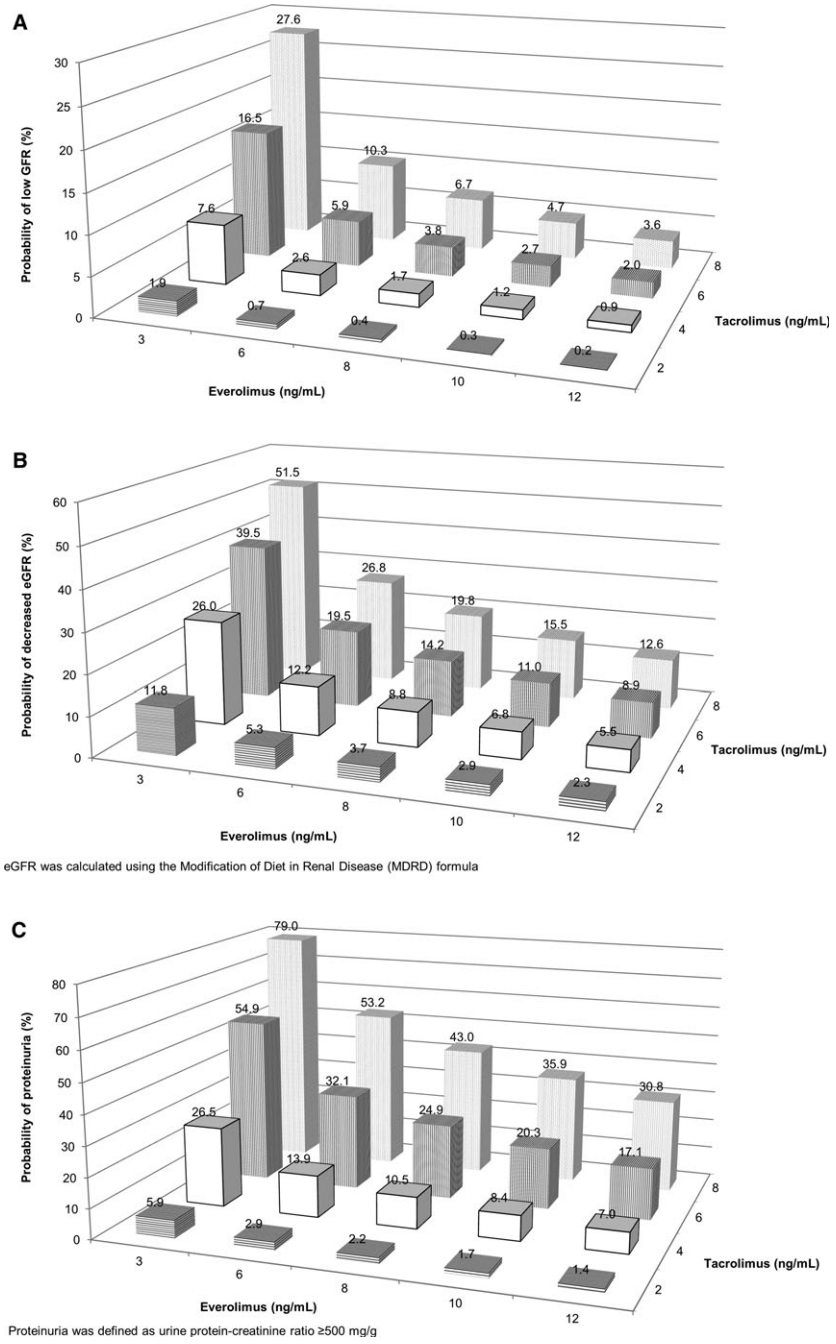

Figure 2: Estimated probability of (A) low eGFR (B) decreased eGFR, and (C) high urinary protein:creatinine ratio (≥500 mg/g) at 12 months posttransplantation. eGFR, estimated glomerular filtration rate.

Hypertriglyceridemia (n = 41/306 [13.4%]) also occurred most often in the Tac ≥5 ng/mL group (n = 38/203; 18.7% vs. 3.1% at <5 ng/mL) but did not show any discernible association with EVR levels.

Hypercholesterolemia occurred in 175 of 306 patients (57.2%) and incidence rates did not clearly associate with increased or decreased levels of EVR or Tac.

Discussion

Management of immunosuppression after solid organ transplantation is associated with the well-known difficulty of finding the right balance between appropriate efficacy to prevent organ rejection and optimal safety (16–19). Kidney transplant patients receiving immunosuppressive therapy with mTOR inhibitors can benefit from less

Table 4: Incidence of AEs at month 12 in patients randomized to the US92 EVR plus LTac arm

		Tac (ng/mL)				
AE, n (%)	EVR (ng/mL)	<2	2–<5	5–<8	≥8	All[1]
Wound healing	<3	4/4 (100.0)	20/24 (83.3)	9/17 (52.9)	2/4 (50.0)	35/49 (71.4)
	3–<6	0/1 (0.0)	7/58 (12.1)	24/113 (21.2)	6/9 (66.7)	37/181 (20.4)
	6–<8	0	1/18 (5.6)	3/27 (11.1)	3/7 (42.9)	7/52 (13.5)
	8–<12	0	0/2 (0.0)	1/3 (33.3)	2/5 (40.0)	3/10 (30.0)
	≥12	0	0	0	0	0
	All[1]	4/5 (80.0)	28/102 (27.5)	37/160 (23.1)	13/25 (52.0)	89/306 (29.1)
Peripheral edema	<3	9/10 (90.0)	20/24 (83.3)	17/26 (65.4)	6/8 (75.0)	52/68 (76.5)
	3–<6	1/2 (50.0)	7/50 (14.0)	27/102 (26.5)	8/12 (66.7)	43/166 (25.9)
	6–<8	0	2/13 (15.4)	6/29 (20.7)	3/8 (37.5)	11/50 (22.0)
	8–<12	0	1/2 (50.0)	0/2 (0.0)	3/6 (50.0)	4/10 (40.0)
	≥12	0	0	0	0	0
	All[1]	10/12 (83.3)	30/89 (33.7)	50/159 (31.4)	20/34 (58.8)	116/306 (37.9)
NODM	<3	2/3 (66.7)	2/7 (28.6)	0/10 (0.0)	2/4 (50.0)	6/24 (25.0)
	3–<6	1/2 (50.0)	2/70 (2.9)	4/116 (3.4)	3/11 (27.3)	10/199 (5.0)
	6–<8	0	0/18 (0.0)	3/39 (7.7)	1/6 (16.7)	4/63 (6.3)
	8–<12	0	0/2 (0.0)	0/3 (0.0)	0/3 (0.0)	0/8 (0.0)
	≥12	0	0	0	0	0
	All[1]	3/5 (60.0)	4/97 (4.1)	7/168 (4.2)	6/24 (25.0)	25/306 (8.2)
BK viral infection	<3	0/1 (0.0)	1/7 (14.3)	3/11 (27.3)	0/2 (0.0)	4/21 (19.0)
	3–<6	0/1 (0.0)	2/65 (3.1)	13/123 (10.6)	6/13 (46.2)	21/202 (10.4)
	6–<8	0	2/21 (9.5)	4/41 (9.8)	0/5 (0.0)	6/67 (9.0)
	8–<12	0	0/1 (0.0)	0/5 (0.0)	0/3 (0.0)	0/9 (0.0)
	≥12	0	0	0	0	0
	All[1]	0/2 (0.0)	5/94 (5.3)	20/180 (11.1)	6/23 (26.1)	31/306 (10.1)
Stomatitis/oral ulcers	<3	0/1 (0.0)	0/5 (0.0)	1/11 (9.1)	0/2 (0.0)	1/19 (5.3)
	3–<6	0/1 (0.0)	1/72 (1.4)	5/123 (4.1)	1/9 (11.1)	7/205 (3.4)
	6–<8	0	0/21 (0.0)	1/39 (2.6)	1/4 (25.0)	2/64 (3.1)
	8–<12	0	0/2 (0.0)	0/5 (0.0)	0/4 (0.0)	0/11 (0.0)
	≥12	0	0	0	0	0
	All[1]	0/2 (0.0)	1/100 (1.0)	7/178 (3.9)	2/19 (10.5)	10/306 (3.3)
CMV infection	<3	0/1 (0.0)	0/6 (0.0)	0/10 (0.0)	0/2 (0.0)	0/19 (0.0)
	3–<6	0/1 (0.0)	1/72 (1.4)	4/123 (3.3)	0/8 (0.0)	5/204 (2.5)
	6–<8	0	0/21 (0.0)	2/39 (5.1)	0/5 (0.0)	2/65 (3.1)
	8–<12	0	0/2 (0.0)	0/5 (0.0)	0/4 (0.0)	0/11 (0.0)
	≥12	0	0	0	0	0
	All[1]	0/2 (0.0)	1/101 (1.0)	6/177 (3.4)	0/19 (0.0)	7/306 (2.3)
Hypertriglyceridemia	<3	0/1 (0.0)	1/7 (14.3)	3/13 (23.1)	0/2 (0.0)	4/23 (17.4)
	3–<6	0/1 (0.0)	1/67 (1.5)	18/128 (14.1)	5/12 (41.7)	24/208 (11.5)
	6–<8	0	1/18 (5.6)	7/34 (20.6)	5/9 (55.6)	13/61 (21.3)
	8–<12	0	0/2 (0.0)	0/3 (0.0)	0/2 (0.0)	0/7 (0.0)
	≥12	0	0	0	0	0
	All[1]	0/2 (0.0)	3/94 (3.2)	28/178 (15.7)	10/25 (40.0)	41/306 (13.4)
Hypercholesterolemia	<3	2/3 (66.7)	15/20 (75.0)	18/27 (66.7)	5/7 (71.4)	40/57 (70.2)
	3–<6	1/1 (100.0)	16/41 (39.0)	72/123 (58.5)	22/24 (91.7)	111/189 (58.7)
	6–<8	0	1/11 (9.1)	13/28 (46.4)	6/8 (75.0)	20/47 (42.6)
	8–<12	0	0/1 (0.0)	0/1 (0.0)	3/3 (100.0)	3/5 (60.0)
	≥12	0	0	0	0	0
	All[1]	3/4 (75.0)	32/73 (43.8)	103/179 (57.5)	36/42 (85.7)	175/306 (57.2)

AE, adverse event; EVR, everolimus; Tac, tacrolimus; NODM, new-onset diabetes mellitus; CMV, cytomegalovirus.
[1]Data for which EVR or Tac values were unknown are not represented.

nephrotoxicity by reducing their exposure to CNIs (3–8). There is a paucity of published data on the use of EVR in combination with Tac in *de novo* renal transplant recipients. Here, we present *post hoc* exploratory analyses from the US92 study to correlate efficacy, renal function, and safety events with specific EVR and LTac trough concentrations. For all patients receiving EVR+LTac in this study, EVR whole blood trough levels were targeted to be maintained between 3 and 8 ng/mL, with Tac trough concentrations adjusted in fixed intervals to

reduce from 4 to 7 ng/mL at day 28 to 2–5 ng/mL at month 12.

Here we demonstrate a low incidence of tBPAR and graft loss at an EVR trough level of ≥3 ng/mL with Tac concentration above 2 ng/mL at 12 months in *de novo* kidney transplant patients. These findings indicate that EVR trough levels ≥3 ng/ml are most highly associated with reduced incidence of tBPAR, meaning that underexposing *de novo* kidney transplant patients to EVR may lead to an increased rate of graft rejection.

At 12 months posttransplantation, the estimated probability of renal dysfunction was highest at decreasing EVR trough concentrations, regardless of whether this outcome was assessed by low eGFR, decreased eGFR, or proteinuria. By contrast, as Tac levels increased, so did the estimated probability of low eGFR and decreased eGFR at 12 months, showing that the renal dysfunction was predominantly driven by increased exposure to Tac. These findings demonstrate that an increased dose of EVR in combination with reduced exposure to Tac is the optimal regimen to preserve renal function in this patient cohort following kidney transplantation. This finding is not consistent with analyses of US09 trial data that showed renal function at 6 months posttransplantation was unaffected by EVR or Tac levels (12).

Analysis of eGFR rates in those patients who did not have graft rejection demonstrated that patients with lower EVR levels still had lower eGFR values, suggesting that the lower eGFR observed in patients with EVR trough concentration <3 ng/mL was not attributable to the increased rejection rate in this population.

Wound-healing events and peripheral edema occurred least frequently at the target EVR range of 3–8 ng/mL, in agreement with findings on a similar patient cohort receiving EVR and reduced CsA (6). A lower incidence of other AEs was generally observed in patients with EVR concentrations ≥6 ng/mL and with Tac concentrations <5 ng/mL. The highest rates of wound-healing events, peripheral edema, NODM, BK viral infection, and stomatitis or oral ulcers occurred at the lowest EVR trough concentration <3 ng/mL. Factors other than EVR or Tac levels, such as surgical techniques, baseline diabetes status, and body mass index, may have played a role in the increased rate of wound healing events. Investigators may have kept the drug levels lower, knowing these phenotypical characteristics.

There were no events that were unexpected for this disease indication and patient population. Overall, the safety findings were consistent with the known safety profile of both EVR and Tac.

Several limitations to the current *post hoc* analysis need to be considered. Of note, many categories had few patients in the denominator, making interpretation of the results difficult. For example, because EVR levels were targeted to be maintained between 3 and 8 ng/mL, the highest and lowest trough concentrations were represented by fewer patients than the targeted range. In addition, renal dysfunction and AEs are less time dependent than rejection, so while increased renal and safety events can be attributed to rising Tac exposure, it is less clear for rejections, because Tac levels were higher in the first period of this study by design. Finally, the need to adjust EVR and Tac trough levels throughout the study required an unblinded design, which introduces the potential for bias in how AEs were reported.

In summary, the US92 study provides evidence that a regimen combining EVR 3–8 ng/mL and a low dose of Tac allows for good efficacy, excellent renal function, and a favorable safety profile at 12 months posttransplantation. Underexposing patients to EVR may lead to an increased rate of rejection and an increased incidence of AEs. Renal dysfunction was higher with increased Tac trough concentrations and with decreased EVR trough concentrations. Collectively, these findings further support the importance of maintaining an EVR trough concentration of 3–8 ng/mL, when combined with LTac, to achieve balanced efficacy and safety in renal transplant recipients.

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Disclosure

The authors of this manuscript have conflicts of interest to disclose as described by the *American Journal of Transplantation*. F.S. has served as a speaker and/or advisor for Alexion, Mallinckrodt, and Novartis. Y.Q. has served as a speaker and/or advisor for Alexion, Astellas, BMS, and Mallinckrodt. S.M. and V.R.P. have served as advisors for Novartis. S.M., V.R.P., and D.S. have received research support from Novartis. K.M. and D.P. are employees of Novartis Pharmaceuticals Corporation.

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