

Association of clinical events with everolimus exposure in kidney transplant patients receiving reduced cyclosporine

Shihab FS, Cibrik D, Chan L, Kim YS, Carmellini M, Walker R, Zibari G, Pattison J, Cornu-Artis C, Wang Z, Tedesco-Silva H Jr. Association of clinical events with everolimus exposure in kidney transplant patients receiving reduced cyclosporine.

Abstract: Background: The association between clinical events and everolimus exposure in patients receiving reduced-exposure calcineurin inhibitor therapy is poorly explored.

Methods: In a pre-planned, descriptive analysis of data from a randomized controlled trial, events were correlated with everolimus trough concentrations in 556 newly transplanted kidney transplant patients receiving everolimus with reduced-exposure cyclosporine (CsA) and steroids. Influence of everolimus exposure on clinical events was stratified according to predefined time-normalized concentrations.

Results: The incidence of treated biopsy-proven acute rejection and graft loss at month 12 was highest in patients with everolimus <3 ng/mL (36.4% and 28.6%, respectively, vs. 9.1–15.3% and 0.9–5.0% with higher concentration ranges). A higher mortality rate was observed in patients with an everolimus trough concentration ≥ 12 ng/mL (10.0% vs. 1.7–5.6% with lower concentration ranges). The lowest rates of renal dysfunction (defined as poor renal function [estimated GFR, serum creatinine] or proteinuria), wound healing events, peripheral edema, new-onset diabetes mellitus, hypercholesterolemia and hypertriglyceridemia were generally observed with everolimus trough concentration in the range 3–8 ng/mL and CsA <100 ng/mL. Proteinuria occurred most frequently in patients with very low or very high everolimus trough concentrations.

Conclusions: These results support an exposure–response relationship between clinical events and everolimus trough concentrations in kidney transplant patients receiving reduced-exposure CsA.

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The mammalian target of rapamycin (mTOR) inhibitors exert their immunosuppressive effect by blocking growth factor-driven T-cell proliferation (1, 2), complementing the early inhibition of IL-2 gene transcription by calcineurin inhibitors (CNIs). This synergism between mTOR inhibitors and CNIs permits CNI dose/exposure reduction (2) and a series of studies has confirmed that use of an mTOR inhibitor with reduced-exposure CNI regimen incurs no loss of efficacy following kidney transplantation (3–8).

Analyses of pharmacokinetic data from earlier studies in which kidney transplant patients received a fixed dose of the mTOR inhibitor everolimus with standard-exposure cyclosporine (CsA) showed a clear association between the everolimus trough concentration and incidence of biopsy-proven acute rejection (BPAR) (9, 10). An increased risk of BPAR was observed for everolimus trough concentration below 3 ng/mL (9, 10). In contrast, everolimus trough concentration >3 ng/mL prevented more than 80% of BPAR episodes (10).

Similar analyses of the association between clinical events and everolimus exposure when used in combination with reduced CNI dose/exposure are rare (11). The A2309 study was a 24-month trial in which 833 *de novo* kidney transplant patients were randomized to everolimus trough concentrations between 3 and 8 ng/mL or between 6 and 12 ng/mL with reduced CsA exposure or to standard CsA with mycophenolic acid (MPA) (5). A pre-planned, descriptive analysis supported by *post hoc* exploratory analyses was undertaken to correlate efficacy, renal dysfunction, and safety events with everolimus and CsA concentrations and the results are reported here.

Materials and methods

Study design and conduct

The study methodology has been described in detail previously (5) and additional information is

shown in SDC. Briefly, in a 24-month, multicenter, parallel-group, international, open-label trial, adult recipients of a primary, *de novo* kidney transplant were randomized to one of three treatment arms: (i) everolimus 0.75 mg b.i.d. targeting a trough concentration of 3–8 ng/mL with reduced CsA (ii) everolimus 1.5 mg b.i.d. targeting a trough concentration of 6–12 ng/mL with reduced CsA or (iii) MPA as enteric-coated mycophenolate sodium (myfortic[®], Novartis Pharma AG, Basel, Switzerland) 720 mg b.i.d. with standard CsA. Only patients randomized to receive everolimus and reduced CsA 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 the patients. The study started in October 2005 with the last patient visit in August 2009.

Immunosuppression regimens

Reduced CsA exposure targeted a CsA trough concentration of 100–200 ng/mL starting at day 5 visit; 75–150 ng/mL starting at the month 2 visit; 50–100 ng/mL starting at the month 4 visit; and 25–50 ng/mL starting at the month 6 visit and maintained thereafter. All patients received basiliximab induction and corticosteroids were administered according to local practice and patients were low to moderate immunological risk.

Primary endpoint

The primary endpoint was efficacy failure at month 12 defined as treated BPAR (tBPAR), graft loss, death, or loss to follow-up.

Definitions

Poor renal function or proteinuria at months 12 and 24 was defined as: (i) a low estimated glomerular filtration rate (eGFR) defined as eGFR

<30 mL/min/1.73 m² (MDRD formula (12)) (ii) a decreased eGFR defined as a decrease in renal function by >30% as compared to month 1 as assessed by either eGFR (MDRD and Nankivell (13) equations) or creatinine clearance (CrCl) (Cockcroft-Gault (14) formula) (iii) a high serum creatinine defined as $\geq 200 \mu\text{M}$ (2.28 mg/dL) after month 1 and (iv) proteinuria defined as urinary protein:creatinine ratio $\geq 300 \text{ mg/g}$ (30 mg/mmol) after month 1.

New-onset diabetes mellitus (NODM) (i.e., occurring in patients who were non-diabetics at the time of transplant) was defined as (i) diabetes reported as an adverse event >14 d after the date of transplantation or (ii) a random glucose $\geq 11 \text{ mM}$ (200 mg/dL) 14 d after transplant (excluding elevated glucose levels that resolved within 14 d) or (iii) diabetes recorded as the reason for diabetic medication given 14 d after transplantation and lasting for 14 d or more. Hypercholesterolemia was defined as total cholesterol $\geq 6.2 \text{ mM}$ (239 mg/dL), hypertriglyceridemia as triglycerides $\geq 5.6 \text{ mM}$ (496 mg/dL), and low testosterone in male patients as testosterone <10 nM if age <50 yr or <7 nM if age ≥ 50 yr.

Statistical methods

No formal testing of differences between patient subpopulations was undertaken. Such testing was not considered statistically valid because (i) patients were randomized either to the everolimus 3–8 or 6–12 ng/mL treatment groups and not to the drug exposure subpopulations used in this analysis and (ii) different numbers of patients were in the trough concentration ranges.

All efficacy events were included in the analysis as there were too few on-treatment efficacy events to be considered for analysis alone. Renal and safety events were included only in patients remaining on treatment. Efficacy events (tBPAR, graft loss and death) and renal events were assessed at 12 and 24 months. Safety events (adverse events, selected hematological events, and lipid and hormone changes) were selected for analysis if they occurred with an incidence $\geq 5\%$ in the everolimus group or were more frequent in the everolimus treatment group than in the MPA cohort (p value ≤ 0.1 , chi-square test). Few safety events occurred from month 12 to month 24, preventing a meaningful interpretation of data, so safety analyses are restricted up to month 12.

Data are based on centrally measured time-normalized mean trough concentrations for everolimus and CsA. Time-normalized mean trough concentrations were calculated as $\Sigma A_i / (D_k - D_0)$

where A_i is the trapezoid area $(1/2)[(C_{i-1} + C_i) * (D_i - D_{i-1})]$ under the concentrations C_{i-1} and C_i , D_i is the blood sampling day for C_i , $i = 1, \dots, k$. $D_0 = 7$ 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. Data were pooled for both everolimus treatment groups. Time-normalized mean everolimus trough concentrations and time-normalized mean CsA trough concentrations were calculated up to the occurrence of an event or for patients without event, the last on-treatment value and for change in eGFR from month 1 to month 12 or to the last on-treatment sampling date.

The influence of everolimus or CsA 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 everolimus and <50, 50–100, 100–200, and ≥ 200 ng/mL for CsA). To assess the influence of everolimus trough concentrations on efficacy and renal events, median-effect analyses (15) were performed. *Post hoc* Cox proportional hazard regression modeling was used to estimate the probability of tBPAR and high urinary protein:creatinine ratio ($\geq 300 \text{ mg/g}$ [30 mg/mmol]) at specified combinations of time-normalized everolimus and CsA trough concentrations, i.e., the predicted incidence of tBPAR and high urinary protein:creatinine ratio was estimated for specific everolimus concentrations (3, 6, 8, 10, and 12 ng/mL) and CsA concentrations (50, 100, 150 and 200 ng/mL). The linear regression model was applied to evaluate the predicted change in eGFR from month 1 to month 12, using the same specified values of everolimus and CsA concentrations as for tBPAR. The last observation carry forward (LOCF) method was used for missing eGFR values at month 1 or month 12. The interaction between everolimus and CsA trough concentration was investigated in these regression models.

Analyses of the relationship between everolimus exposure and efficacy or renal events were performed on the pharmacokinetic efficacy population, comprising all intent-to-treat patients (i.e., all randomized patients) who provided any everolimus or CsA trough concentration measurement. Analyses of everolimus exposure and safety events were performed on all patients in the safety population (i.e., patients who received at least one dose of study drug and provided a post-baseline safety assessment) who provided any everolimus or CsA trough concentration measurement after randomization. As only those patients with exposure data available at the time of the event or who were

event-free at the specified time point were included, the total number of patients available for analysis varies among events. Time-normalized values of CsA concentration were calculated up to an event or up to the last on-treatment value for event-free patients. As the occurrence of acute rejection is expected to be concentrated in the early post-transplant period before protocol-mandated CsA exposure reductions occurred, CsA exposure values will be heavily skewed upward in patients who experienced tBPAR. To account for this, an additional analysis was performed that uses data up to day 45 (the end of the month 1 visit window) prior to CsA exposure reduction.

Results

Patient population

Five hundred fifty-six patients were randomized to receive everolimus (277 at 1.5 mg/d, 279 at 3.0 mg/d). The mean age was 45.5 yr and 66.2% of patients were male with no marked differences in demographics or baseline characteristics between treatment groups (Table 1). At months 12 and 24, respectively, 490 (88.1%) and 470 (84.5%) everolimus-treated patients remained in the study; 354 (63.7%) and 328 (59.0%) remained on the study medication.

Table 1. Demographics and patient characteristics by treatment group

	Everolimus 1.5 mg/d N = 277	Everolimus 3.0 mg/d N = 279	All everolimus patients N = 556
Age, yr	45.7 (12.7)	45.3 (13.4)	45.5 (13.0)
Male, %	177 (63.9)	191 (68.5)	368 (66.2)
White, %	193 (69.7)	180 (64.5)	373 (67.1)
Cause of end-stage renal disease, n (%)			
Hypertension/ nephrosclerosis	50 (18.1)	56 (20.1)	106 (19.1)
Glomerular disease	44 (15.9)	55 (19.7)	99 (17.8)
Diabetes mellitus	39 (14.1)	29 (10.4)	68 (12.2)
Polycystic disease	36 (13.0)	29 (10.4)	65 (11.7)
Unknown	33 (11.9)	37 (13.3)	70 (12.6)
Other	75 (27.1)	73 (26.2)	148 (26.6)
HLA mismatches	3.5 (1.54)	3.3 (1.59)	3.4 (1.57)
PRA (peak evaluation) ≥ 20%, n (%)	17 (6.1)	13 (4.7)	30 (5.4)
Donor age, yr	41.4 (13.9)	41.1 (13.0)	41.2 (13.4)
Deceased donor (heart beating), (n %)	128 (46.2)	126 (45.2)	254 (45.7)
CMV serology status, n (%)			
D+/R+	140 (50.5)	139 (49.8)	279 (50.2)
D+/R-	30 (10.8)	28 (10.0)	58 (10.4)
D-/R+	36 (13.0)	44 (15.8)	80 (14.4)
D-/R-	62 (22.4)	53 (19.0)	115 (20.7)

Continuous variables are shown as mean (SD).

Efficacy events

Among the patients receiving everolimus, tBPAR had occurred in 86 patients (15.5%), graft loss in 26 patients (4.7%), and death in 17 patients (3.1%) by month 12. At month 24, the corresponding numbers were 97 (17.4%), 33 (5.9%), and 19 (3.4%). The incidence of tBPAR was markedly higher in patients with an everolimus trough concentration <3 ng/mL (36.4% at month 12, 47.6% at month 24) but no difference in the incidence of tBPAR was observed above 3 ng/mL except at everolimus trough concentrations ≥ 12 ng/mL where the incidence of tBPAR was lower (9.1% at month 12, 10.0% at month 24) (Fig. 1A, Table 2). Kaplan–Meier analysis of the proportion of patients free from tBPAR in the <3, 3–6, 6–8, 8–12, and ≥ 12 ng/mL groups were 55.9%, 85.2%, 84.0%, 86.2%, and 89.7%, respectively, at month 12 and 39.9%, 83.2%, 81.8%, 85.3%, and 89.7% at month 24. Fig. 2A shows a three-dimensional representation of the estimated probability of tBPAR at month 1 at specified combinations of time-normalized everolimus and CsA trough concentration based on data up to day 45 (i.e., the last day of the month 1 study window prior to CsA dose reduction) from the Cox model. Patients with everolimus concentrations below 3 ng/mL at month 1 showed higher incidences of tBPAR, which were reduced with increasing concentrations of CsA. In contrast, in patients with everolimus concentrations ≥ 8 ng/mL at month 1, the incidence of tBPAR was not strongly influenced by CsA concentrations. Although the predicted probabilities in Fig. 2A indicate visually the exposure-response relationship between the incidence of tBPAR, everolimus concentration and CsA concentration, this could not be demonstrated statistically in the current analysis. The effect of everolimus trough concentrations, CsA trough concentrations, and their interaction on the incidence of tBPAR was statistically non-significant ($p = 0.259, 0.295$ and 0.176 , respectively). However, the study was not powered for this analysis and the number of included events was small because of the limited time frame of 45 d, which was selected because CsA concentrations were expected to be constant during this period as per the study design.

The incidence of graft loss at months 12 and 24 was similar within the everolimus range of 3–12 ng/mL (Table 2), but below 3 ng/mL, the incidence of graft loss greatly increased (28.6% at month 12, 42.1% at month 24) (Fig. 1B). Eight graft losses in patients with everolimus trough concentrations <3 ng/mL by month 24 were due to

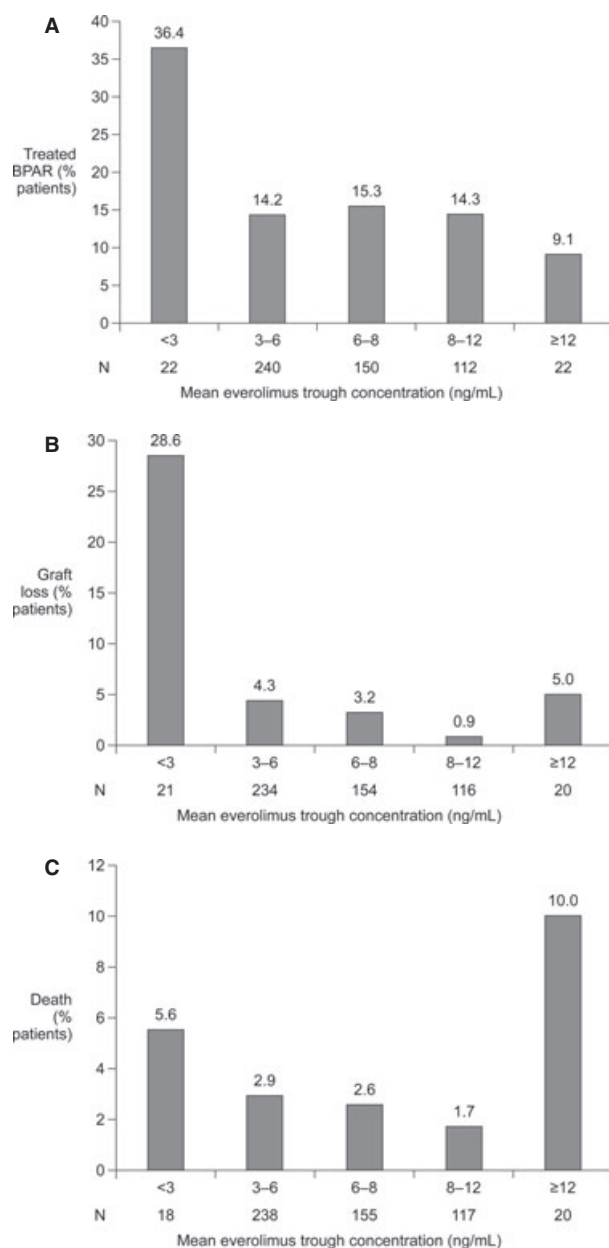


Fig. 1. Incidence of (A) treated biopsy-proven acute rejection (BPAR) (B) graft loss (C) death according to predefined range of mean time-normalized everolimus trough concentration at month 12 post-transplant (pharmacokinetic efficacy population).

acute vascular rejection (two), chronic rejection (three including one presumed), renal artery thrombosis (two) and non-compliance (one). A single graft loss occurred at an everolimus trough concentration ≥ 12 ng/mL due to acute rejection. A similar pattern was observed for Kaplan–Meier estimates of the proportion of patients free from graft loss at month 12 (65.8%, 95.5%, 96.6%, 99.2%, and 94.7% in the <3, 3–6, 6–8, 8–12 and ≥ 12 ng/mL groups, respectively) and month 24 (49.3%, 94.5%, 95.9%, 97.5%, and 94.7%, respectively). Mortality was similar across the everolimus

exposure ranges except a higher incidence of mortality was observed in patients with a trough concentration ≥ 12 ng/mL but the number of events in this range was small (Fig. 1C, Table 2). The two deaths that occurred at an everolimus trough concentration ≥ 12 ng/mL were caused by acute myocardial infarction and sepsis. The only death in the lowest everolimus range (<3 ng/mL) was the result of sepsis.

Exploratory exposure–event analyses of everolimus trough concentrations vs. efficacy failure events showed a significant inverse relationship between everolimus exposure at months 12 and 24 and the incidence of graft loss (median–effect logistic regression for combined everolimus groups: month 12, $p = 0.007$; month 24, $p = 0.004$). This trend was mainly driven by a higher incidence of graft loss below an average everolimus trough concentration of 4 ng/mL. No clear relationship was observed between the everolimus trough concentration and tBPAR at month 12 (the analysis was not performed for tBPAR at month 24) or for death at months 12 and 24, although the number of events was low.

Renal events

Regardless of whether renal function was assessed by eGFR, CrCl, or serum creatinine, more patients had poor renal function at months 12 and 24 when the everolimus exposure was <3 ng/mL (Table S1). In contrast, proteinuria defined as >300 mg protein per gram of creatinine was more frequent in patients either under-exposed (<3 ng/mL) or over-exposed (≥ 12 ng/mL) to everolimus (SCD, Table 1). The numbers of patients with nephrotic-range proteinuria (≥ 3000 mg/g [339 mg/mmol]) at month 12 ($n = 8$) and month 24 ($n = 7$) were too low to permit a meaningful analysis by everolimus exposure. A significant correlation was observed between everolimus trough concentrations and the incidence of proteinuria (higher trough level leading to a higher rate of events) at months 12 and 24 ($p = 0.004$ and $p = 0.010$ from logistic regression, respectively) but no correlation was observed for other definitions of renal dysfunction.

In general, as CsA concentrations increased, more patients had poor renal function measured by either low eGFR, decreased eGFR, decreased CrCl, or high serum creatinine level (SDC, Table 1). Poor renal function was observed in a higher proportion of patients when CsA concentrations were above 100 ng/mL regardless of everolimus concentrations up to 12 ng/mL. A few patients with very low everolimus concentrations

Table 2. Incidence of efficacy parameters according to time-normalized cyclosporine (CsA) and everolimus (EVR) trough concentrations achieved to month 12 and month 24 (pharmacokinetic efficacy population)

Event, n (%)	EVR (ng/mL)	Month 12					Month 24				
		CsA concentration (ng/mL)					CsA concentration (ng/mL)				
		<50	50 to <100	100 to <200	≥ 200	All	<50	50 to <100	100 to <200	≥ 200	All
Treated BPAR	<3	1/4 (25.0)	4/10 (40.0)	1/4 (25.0)	2/4 (50.0)	8/22 (36.4)	2/5 (40.0)	5/9 (55.6)	1/3 (33.3)	2/4 (50.0)	10/21 (47.6)
	3-6	1/39 (2.6)	7/111 (6.3)	23/76 (30.3)	3/14 (21.4)	34/240 (14.2)	1/32 (3.1)	11/117 (9.4)	23/75 (30.7)	3/14 (21.4)	38/238 (16.0)
	6-8	1/29 (3.4)	3/61 (4.9)	15/49 (30.6)	4/11 (36.4)	23/150 (15.3)	1/36 (2.8)	4/58 (6.9)	17/44 (38.6)	4/12 (33.3)	26/150 (17.3)
	8-12	0/9 (0.0)	2/62 (3.2)	11/31 (35.5)	3/10 (30.0)	16/112 (14.3)	0/15	3/57 (5.3)	11/35 (31.4)	3/10 (30.0)	17/117 (14.5)
	≥ 12	0/0	0/10 (0.0)	1/8 (12.5)	1/4 (25.0)	2/22 (9.1)	-	0/9 (0.0)	1/8 (12.5)	1/3 (33.3)	2/20 (10.0)
	All	3/81 (3.7)	16/254 (6.3)	51/168 (30.4)	13/43 (30.2)	83/546 (15.2)	4/88 (4.5)	23/250 (9.2)	53/165 (32.1)	13/43 (30.2)	93/546 (16.8)
Graft loss	<3	1/3 (33.3)	2/10 (20.0)	0/3 (0.0)	3/5 (60.0)	6/21 (28.6)	2/3 (66.7)	2/8 (25.0)	0/2 (0.0)	4/6 (66.7)	8/19 (42.1)
	3-6	0/39 (0.0)	4/121 (3.3)	4/60 (6.7)	2/14 (14.3)	10/234 (4.3)	0/32 (0.0)	6/127 (4.7)	4/57 (7.0)	2/14 (14.3)	12/230 (5.2)
	6-8	0/29 (0.0)	2/70 (2.9)	3/43 (7.0)	0/12 (0.0)	5/154 (3.2)	0/36 (0.0)	2/67 (3.0)	3/38 (7.9)	1/14 (7.1)	6/155 (3.9)
	8-12	0/10 (0.0)	0/70 (0.0)	1/26 (3.8)	0/10 (0.0)	1/116 (0.9)	0/15 (0.0)	2/68 (2.9)	1/28 (3.6)	0/11 (0.0)	3/122 (2.5)
	≥ 12	0/0	0/10 (0.0)	1/7 (14.3)	0/3 (0.0)	1/20 (5.0)	0/0	0/9 (0.0)	1/8 (12.5)	0/2 (0.0)	1/19 (5.3)
	All	1/81 (1.2)	8/281 (2.8)	9/139 (6.5)	5/44 (11.4)	23/545 (4.2)	2/85 (2.4)	12/279 (4.3)	9/133 (6.8)	7/47 (14.9)	30/459 (6.5)
Death	<3	0/3 (0.0)	1/9 (11.1)	0/3 (0.0)	0/3 (0.0)	1/18 (5.6)	0/3 (0.0)	1/8 (12.5)	0/2 (0.0)	0/3 (0.0)	1/16 (6.3)
	3-6	0/39 (0.0)	2/119 (1.7)	4/66 (6.1)	1/14 (7.1)	7/238 (2.9)	0/32 (0.0)	3/124 (2.4)	4/65 (6.2)	1/14 (7.1)	8/235 (3.4)
	6-8	0/29 (0.0)	0/70 (0.0)	3/44 (6.8)	1/12 (8.3)	4/155 (2.6)	0/36 (0.0)	1/67 (1.5)	3/39 (7.7)	1/13 (7.7)	5/155 (3.2)
	8-12	0/10 (0.0)	0/70 (0.0)	1/27 (3.7)	1/10 (10.0)	2/117 (1.7)	0/15 (0.0)	0/67 (0.0)	1/30 (3.3)	1/11 (9.1)	2/123 (1.6)
	≥ 12	0/0	1/11 (9.1)	0/6 (0.0)	1/3 (33.3)	2/20 (10.0)	0/0	1/10 (10.0)	0/7 (0.0)	1/2 (50.0)	2/19 (10.5)
	All	0/81 (0)	4/279 (1.4)	8/146 (5.5)	4/42 (9.5)	16/548 (2.9)	0/86 (0)	6/276 (2.1)	8/143 (5.6)	4/43 (9.3)	18/548 (3.3)

BPAR, biopsy-proven acute rejection.

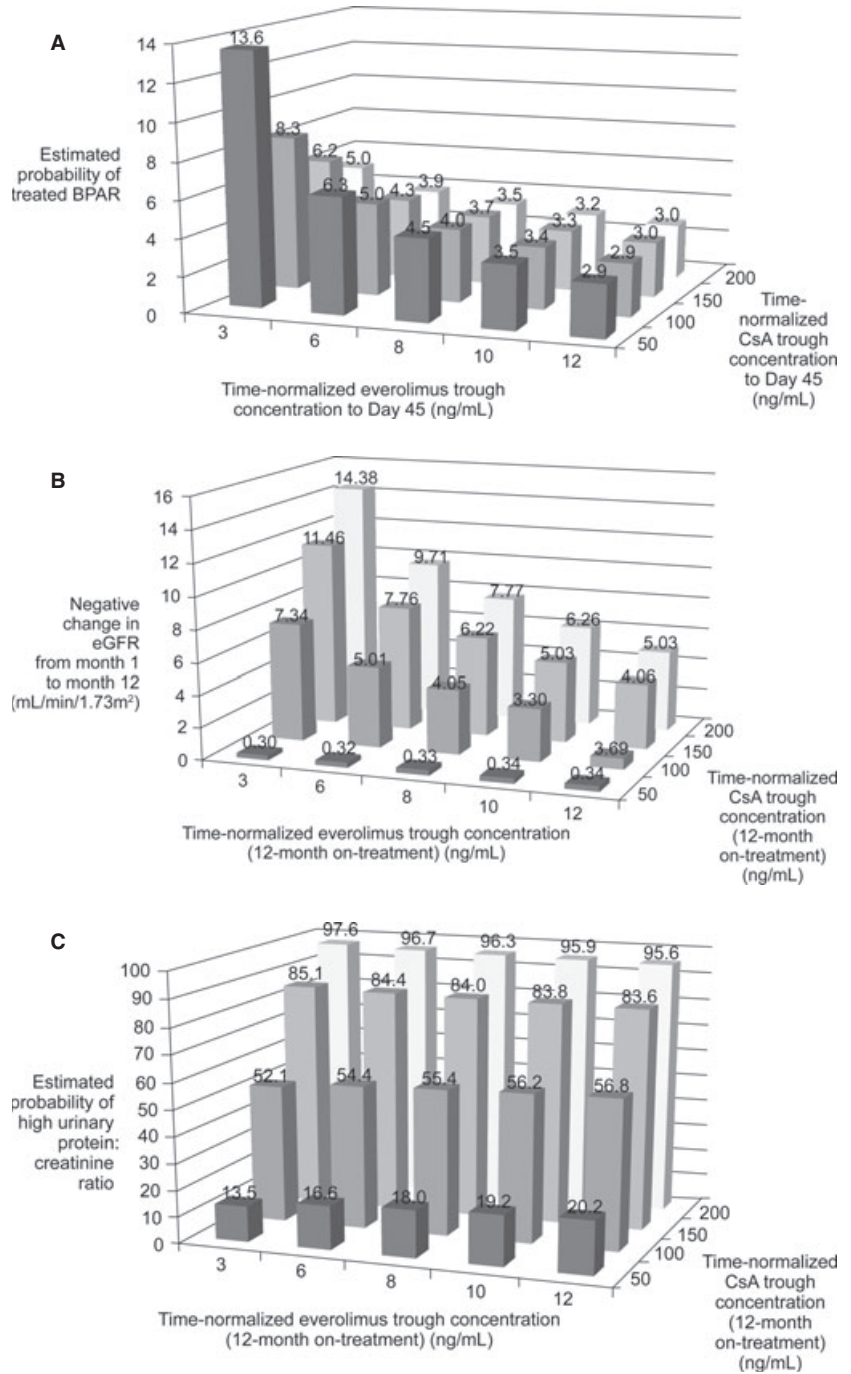


Fig. 2. (A) Estimated probability of treated biopsy-proven acute rejection (tBPAR) at month 1 from Cox proportional hazards model based on data to day 45 (pharmacokinetic efficacy population) (B) the negative change in estimated glomerular filtration rate (eGFR) (MDRD) from month 1 to month 12 at specified combinations of time-normalized everolimus and cyclosporine (CsA) trough concentration from the linear regression model (pharmacokinetic efficacy population – 12-month on-treatment analysis) (C) estimated probability of high urinary protein:creatinine ratio (≥ 300 mg/g after month 1) from Cox proportional hazards model at specified combinations of time-normalized everolimus and CsA trough concentration (pharmacokinetic efficacy population – 12-month on-treatment analysis). All graphs showed predicted values at specified combinations of time-normalized everolimus trough concentration (3, 6, 8, 10 and 12 ng/mL) and CsA trough concentration (50, 100, 150 and 200 ng/mL) in the pharmacokinetic efficacy population. For example, in Fig. 2A, a patient with time-normalized everolimus trough concentration of 8 ng/mL and time-normalized everolimus trough concentration of 100 ng/mL would have a 4.0% predicted probability of tBPAR.

(<3 ng/mL) and high CsA concentrations had worse renal function. Of note, many categories had very few patients in the denominator making interpretation of the results difficult. In addition, more patients with high everolimus and CsA concentrations had proteinuria (everolimus concentrations ≥ 12 ng/mL with CsA concentrations >100 ng/mL) (SDC, Table 1). Fig. 2B represents a three-dimensional representation of the negative change in eGFR from month 1 to month 12 at specified combinations of time-normalized everolimus and

CsA trough concentrations from the linear regression model. Increasing concentrations of CsA were significantly associated with a substantially greater decline in eGFR ($p = 0.077$). In contrast, higher everolimus concentrations did not show a significant association with the change in eGFR ($p = 0.351$). At low CsA concentrations (50 ng/mL), a negligible change in eGFR was observed over the first year post-transplant regardless of the everolimus concentration. No statistically significant everolimus and CsA trough concentration

interaction ($p = 0.288$) contributed to the change in eGFR. The estimated probability of high urinary protein:creatinine at specified combinations of time-normalized everolimus and CsA trough concentrations from the Cox model, as depicted in Fig. 2C, showed no significant association with everolimus exposure ($p = 0.751$), but a clear effect could be attributed to increasing CsA exposure ($p < 0.001$). No significant interaction between everolimus and CsA on proteinuria was observed ($p = 0.335$).

Safety events

Safety events that met the criteria for analysis (i.e., incidence $\geq 5\%$ of patients in the everolimus groups or higher incidence with everolimus than MPA) were wound healing events, peripheral edema, stomatitis/oral ulcers, NODM, hypercholesterolemia, hypertriglyceridemia, and low testosterone in male subjects. The lowest rates of wound healing events, peripheral edema, NODM, hypercholesterolemia and hypertriglyceridemia were generally observed with everolimus trough concentration in the range 3–8 ng/mL and CsA < 100 ng/mL (Table 3). When data from all patients were analyzed, regardless of CsA exposure, the incidence of wound healing events and peripheral edema was lowest when everolimus concentrations were in the range 3–8 ng/mL (Table 3). No clear correlation was observed between everolimus exposure on stomatitis/oral ulcers or NODM. Hypercholesterolemia and hypertriglyceridemia were frequent events across all everolimus ranges (Table 3). Low testosterone levels did not show any association with everolimus exposure. In general, the incidence of wound healing events, peripheral edema, stomatitis/oral ulcers, NODM, and lipid disorders increased as CsA exposure increased over most everolimus ranges. The highest rates of hypercholesterolemia and hyperlipidemia were observed in patients with high exposure to both CsA and everolimus. No trend was observed for low testosterone levels.

Discussion

These results support an exposure–response relationship between efficacy and safety and everolimus trough concentration within the range 3–12 ng/mL in low-to-moderate immunological risk kidney transplant patients. Increasing everolimus exposure above 3 ng/mL was associated with some additional benefit in reducing the risk of tBPAR. An inverse relationship was observed

between everolimus exposure and graft loss at months 12 and 24, with a higher graft loss rate at everolimus trough concentrations below 4 ng/mL but as the absolute number of events was low, particularly at higher everolimus concentrations, this finding should be interpreted cautiously.

The decrease in eGFR over the first year post-transplant was driven by rising CsA concentration but not by increasing everolimus concentration. At low CsA exposure (50 ng/mL), eGFR results were excellent regardless of exposure to everolimus (Fig. 2B). A previous analysis in kidney transplant patients has observed only a minor effect of everolimus on serum creatinine in patients receiving standard-exposure CsA (10). The lowest rate of proteinuria was observed in patients with everolimus trough concentration in the range 3–8 ng/mL.

Wound healing events and peripheral edema occurred less frequently in the everolimus exposure range 3–8 ng/mL than at higher concentrations. Although no consistent correlation between everolimus exposure and NODM was apparent, the high incidence of NODM in patients with mean everolimus concentration ≥ 12 ng/mL in the presence of CsA concentration ≥ 200 ng/mL was noteworthy although absolute numbers were small (4/6 patients). Hypercholesterolemia and hypertriglyceridemia were most frequent in patients with CsA trough concentration ≥ 100 ng/mL and everolimus trough concentration > 8 ng/mL. In summary, an increased risk of certain adverse events was observed with everolimus trough concentrations above 8 ng/mL, and particularly above 12 ng/mL. This is consistent with findings from a recent study of 285 *de novo* kidney transplant patients randomized to everolimus 3–8 or 8–12 ng/mL, in which a higher rate of discontinuations due to adverse events occurred in the 8–12 ng/mL arm (16).

Several limitations of the current report need to be considered. Although this study was a largely pre-planned (only the Cox modeling of everolimus and CsA exposure correlations with efficacy and safety events was performed *post hoc*) analysis of a randomized trial, it is of descriptive nature; statistical comparisons were not valid due to potential bias imposed by the different number of patients in each subpopulation. In addition, only a few patients had either very high or very low everolimus trough concentrations. For example, in the highest everolimus exposure group (≥ 12 ng/mL), the rate of tBPAR (9.1% at month 12) was lower than in any other group, but only 22 patients were in this cohort. Also, graft failure and death occurred rarely, so the results become less reliable.

Table 3. Incidence of adverse events according to time-normalized cyclosporine (CsA) and everolimus mean trough concentration achieved to month 12, n (%) (pharmacokinetic safety population)

Event, n (%)	Mean everolimus concentration (ng/mL)	Month 12				
		Mean CsA concentration (ng/mL)				
		<50	50 to <100	100 to <200	≥ 200	All
Wound healing events	<3	1/4 (25.0)	3/11 (27.3)	10/11 (90.9)	3/4 (75.0)	17/30 (56.7)
	3-6	1/4 (25.0)	8/98 (8.2)	27/80 (33.8)	22/29 (75.9)	58/211 (27.5)
	6-8	0/2 (0.0)	6/57 (10.5)	23/59 (39.0)	10/22 (45.5)	39/140 (27.9)
	8-12	0/3 (0.0)	2/46 (4.3)	20/44 (45.5)	24/29 (82.8)	46/122 (37.7)
	≥ 12	0/0	0/3 (0.0)	7/12 (58.3)	9/10 (90.0)	16/25 (64.0)
	All	2/13 (15.4)	19/215 (8.8)	87/206 (42.2)	68/94 (72.3)	176/528 (33.3)
Peripheral edema	<3	4/6 (66.7)	4/10 (40.0)	6/8 (75.0)	4/5 (80.0)	18/29 (62.1)
	3-6	7/9 (77.8)	16/83 (19.3)	47/89 (52.8)	24/31 (77.4)	94/212 (44.3)
	6-8	0/3 (0.0)	9/51 (17.6)	26/46 (56.5)	22/30 (73.3)	57/130 (43.8)
	8-12	0/2 (0.0)	9/35 (25.7)	24/42 (57.1)	22/26 (84.6)	55/105 (52.4)
	≥ 12	0/0	1/4 (25.0)	6/11 (54.5)	10/11 (90.9)	17/26 (65.4)
	All	11/20 (55.0)	39/183 (21.3)	109/196 (55.6)	82/103 (79.6)	241/502 (48.0)
Stomatitis/oral ulcers	<3	0/3 (0.0)	0/9 (0.0)	0/2 (0.0)	0/3 (0.0)	0/17 (0)
	3-6	0/3 (0.0)	1/119 (0.8)	8/85 (9.4)	5/18 (27.8)	14/225 (6.2)
	6-8	0/6 (0.0)	5/76 (6.6)	10/62 (16.1)	1/18 (5.6)	16/162 (9.9)
	8-12	0/3 (0.0)	1/65 (1.5)	7/40 (17.5)	2/13 (15.4)	10/121 (8.3)
	≥ 12	0/0	0/10 (0.0)	0/9 (0.0)	0/2 (0.0)	0/21 (0)
	All	0/15 (0)	7/279 (2.5)	25/198 (12.6)	8/54 (14.8)	40/546 (7.3)
New-onset diabetes mellitus	<3	0/3 (0.0)	0/8 (0.0)	0/2 (0.0)	0/3 (0.0)	0/16 (0)
	3-6	1/3 (33.3)	2/125 (1.6)	10/85 (11.8)	3/14 (21.4)	16/227 (7.0)
	6-8	0/5 (0.0)	6/77 (7.8)	9/59 (15.3)	2/17 (11.8)	17/158 (10.8)
	8-12	0/3 (0.0)	0/57 (0.0)	10/42 (23.8)	5/14 (35.7)	15/116 (12.9)
	≥ 12	0/0	0/9 (0.0)	1/10 (10.0)	4/6 (66.7)	5/25 (20.0)
	All	1/14 (7.1)	8/276 (2.9)	30/198 (15.2)	14/54 (25.9)	53/542 (9.8)
Hypercholesterolemia	<3	1/4 (25.0)	15/18 (83.3)	10/11 (90.9)	7/10 (70.0)	33/43 (76.7)
	3-6	0/1 (0.0)	9/42 (21.4)	86/121 (71.1)	46/55 (83.6)	141/219 (64.4)
	6-8	0/3 (0.0)	7/27 (25.9)	47/67 (70.1)	32/38 (84.2)	86/135 (63.7)
	8-12	0/1 (0.0)	3/15 (20.0)	50/59 (84.7)	34/38 (89.5)	87/113 (77.0)
	≥ 12	0/0	0/2 (0.0)	9/11 (81.8)	18/19 (94.7)	27/32 (84.4)
	All	1/9 (11.1)	34/104 (32.7)	202/269 (75.1)	137/160 (85.6)	374/542 (69.0)
Hypertriglyceridemia	<3	0/3 (0.0)	1/9 (11.1)	2/3 (66.7)	2/5 (40.0)	5/20 (25.0)
	3-6	0/2 (0.0)	5/108 (4.6)	27/95 (28.4)	8/21 (38.1)	40/226 (17.7)
	6-8	0/6 (0.0)	2/66 (3.0)	15/58 (25.9)	8/23 (34.8)	25/153 (16.3)
	8-12	0/4 (0.0)	2/55 (3.6)	15/46 (32.6)	10/17 (58.8)	27/122 (22.1)
	≥ 12	0/0	0/10 (0.0)	4/11 (36.4)	4/6 (66.7)	8/27 (29.6)
	All	0/15 (0)	10/248 (4.0)	63/213 (29.6)	32/72 (44.4)	105/548 (19.2)
Low testosterone (males)	<3	0/0	0/2 (0.0)	0/0	0/0	0/2 (0)
	3-6	0/2 (0.0)	13/68 (19.1)	13/39 (33.3)	2/3 (66.7)	28/112 (25.0)
	6-8	0/4 (0.0)	13/44 (29.5)	7/26 (26.9)	2/7 (28.6)	22/81 (27.2)
	8-12	0/3 (0.0)	6/47 (12.8)	9/25 (36.0)	0/4 (0.0)	15/79 (18.9)
	≥ 12	0/0	0/9 (0.0)	1/3 (33.3)	0/0	1/12 (8.3)
	All	0/9 (0)	32/170 (18.8)	30/93 (32.3)	4/14 (28.6)	66/286 (23.1)

The ability to assess the impact of CsA exposure based on this model was limited due to the nature of the study design, i.e., CsA exposure was highest in the early post-transplant period, when most acute rejection events occurred. Thus, as CsA exposure values were calculated up to the occurrence of an event, values would tend to be heavily skewed upward in patients with BPAR. Renal dysfunction and adverse events are less time-dependent than rejection, so renal and safety events showed the expected pattern of increasing inci-

dences with rising CsA exposure (Table 3). Lastly, the study was not blinded due to the need to adjust everolimus and CsA exposure, and the analyses based on adverse event-reporting may reflect investigator bias.

In conclusion, we observed inferior efficacy in patients with low everolimus trough concentrations and an increased rate of wound healing, peripheral edema, and hypercholesterolemia at higher exposure levels. These findings are consistent with a relationship between everolimus

exposure and efficacy and safety during the first two yr post-transplant in *de novo* kidney transplant patients receiving reduced CsA.

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Authors' contributions

FSS, DC, LC, YSK, MC, RW, GZ, JP and HT-S recruited patients and collected study data. ZW provided biostatistical support. CCA contributed to the design of the current analyses. All authors contributed to the interpretation of the data, reviewed the manuscript critically, and approved the final version for submission.

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Supporting Information

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

Table S1. Incidence of renal dysfunction parameters according to time-normalized cyclosporine (CsA) and everolimus (EVR) trough concentrations achieved to month 12 and month 24 (pharmacokinetic efficacy population).