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ORIGINAL ARTICLE

Efficacy and safety of conversion from cyclosporine to everolimus in living-donor kidney transplant recipients: an analysis from the ZEUS study

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Conflicts of interest
FL has received consultancy and speaker's fees from Novartis Pharma, Astellas Pharma and Roche AG. KB has consultancy agreements with Bristol-Myers Squibb, Hexal, LifeCycle Pharma, Novartis Pharma, TCL Pharma and Pfizer and has received research grants for clinical studies, speaker's fees, honoraria, travel expenses and payment for development

Summary

Conversion of living-donor kidney transplant patients from calcineurin inhibitor therapy to an mTOR inhibitor is poorly documented. In the prospective, multicentre ZEUS study, 300 kidney transplant recipients without prior rejection (Banff grade >1) and serum creatinine ≤265 μmol/l were randomized to continue cyclosporine or convert to everolimus at 4.5 months post-transplant. In a *post hoc* analysis of 80 living-donor recipients, adjusted estimated GFR (Nankivell) at month 12 (the primary endpoint) was 74.3 (95% CI [70.7, 77.9]) ml/min/1.73 m^2 with everolimus versus 63.8 (95% CI [60.0, 67.7]) ml/min/1.73 m^2) with cyclosporine, a difference of 10.5 ml/min/1.73 m^2 in favour of everolimus ($P < 0.001$). From randomization to month 12, adjusted estimated GFR increased by a mean of 9.8 (95% CI [6.2, 13.4]) ml/min/1.73 m^2 with everolimus versus −0.7 (95% CI [−4.6, 3.1]) ml/min/1.73 m^2) ($P < 0.001$) with cyclosporine. There were six biopsy-proven acute rejection episodes in everolimus-treated patients (five Banff grade I) and one episode in cyclosporine-treated patients (Banff grade 1). Overall safety profile was similar between groups. Discontinuation due to adverse events occurred in three everolimus patients (7.1%) and five cyclosporine patients (13.2%) between randomization and month 12. Initiation of everolimus with early elimination of calcineurin therapy is associated with a significant renal

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benefit at 12 months post-transplant that is observed in both living and deceased-donor recipients. (clinicaltrials.gov NCT00154310)

Introduction

Living-donor kidney transplantation continues to offer markedly superior outcomes to deceased-donor transplants [1], while incurring no extra financial cost [2]. Data from the US Organ Procurement Network indicate graft survival to be 10% higher, and patient survival to be 6% higher, in living-donor recipients at 3 years post-transplant compared with deceased-donor transplants [1]. Early results showing superior kidney graft survival rates in living-related transplants versus deceased-donor grafts [3] were quickly followed by evidence that both unrelated [4] and older [5,6] living donors also offer superior outcomes. The consequent expansion of potential of the living-donor pool, together with other factors such as adoption of laparoscopic donor nephrectomy, led to a substantial increase in living donation during the early 2000s, although the rate has declined more recently [1,7].

Various factors contribute to the improved outcomes observed in living-donor transplants, including a healthy

donor, better matching, shorter duration of pretransplant dialysis, brief cold ischaemia time and lower risk of delayed graft function and acute rejection. These favourable conditions lead to improved early renal function compared with deceased-donor grafts [8]. Once transplanted, however, living-donor and deceased-donor grafts are both subjected to similar immunologic and nonimmune insults, such as calcineurin inhibitor (CNI)-related nephrotoxicity. An analysis from the United States Renal Data System has shown that although the rate of decline in estimated GFR (eGFR) in living-donor recipients was historically less steep than in deceased-donor patients, by 2008, the slope of deterioration in eGFR at 1 year post-transplant had become similar for both donor types [8]. Consistent with data from kidney transplantation overall [9,10], poor renal function at 1 year shows a strong association with subsequent death-censored graft survival in living-donor recipients, [11] and preservation of renal function following living donation is a key clinical objective.

Immunosuppression is one of the few modifiable risk factors for poor renal function after kidney transplantation. CNI-related nephrotoxicity, in particular, has been well-documented, and this has led to various strategies to minimize CNI exposure. The mammalian target of rapamycin (mTOR) inhibitor class has proven effective in facilitating low-exposure CNI regimens [12–17] or CNI elimination after the initial high-risk post-transplant period [18–23]. However, few randomized trials or subanalyses of randomized studies have compared mTOR inhibitor-based regimens versus CNI regimens specifically in living-donor recipients [24–27], and the available data in this population focus primarily on entirely CNI-free or reduced-CNI maintenance regimens.

In the prospective, multicentre ZEUS study, 300 kidney transplant recipients were randomized to continue receiving cyclosporine (CsA) or to convert to the mTOR inhibitor everolimus at 4.5 months after transplantation. Results from the full study population have been published previously [28,29]. We report here the outcomes of a *post hoc* analysis of the living-donor subpopulation, with a particular focus on renal function at 1 year post-transplant. Data from deceased-donor recipients are presented for comparison.

Methods

Study design

ZEUS was a randomized, multicentre, open-label, parallel-group trial undertaken at 13 transplant centres in Germany and Switzerland during June 2005–September 2007, in which patients were randomized at 4.5 months after kidney transplantation to switch to everolimus or to continue CsA-based therapy (clinicaltrials.gov, NCT00154310). Study visits took place at baseline and at months 1, 2, 3, 4.5, 6, 9 and 12, with additional weekly visits during the switch period (months 4.5–6) as necessary. The study was conducted in compliance with Good Clinical Practice and the Declaration of Helsinki.

Patients

The current *post hoc* analysis studied the living-donor recipient population of the overall study population. Patients were adult (18–65 years) recipients of a kidney transplant. Key exclusion criteria were more than one previous renal transplantation; loss of previous graft due to immunological reasons in the first year after transplantation; multiple organ transplantation (e.g. kidney–pancreas transplant); organ donated after cardiac death; donor age <5 years or >65 years; historical or current peak panel reactive antibodies (PRA) >25%; platelets <75 000/mm³ with an absolute neutrophil count of <1500/mm³ or leucocytes <2500/mm³; haemoglobin <6 g/dl; and severe liver disease. At the time of randomization (4.5 months post-transplant), patients were required to be receiving treatment with CsA, enteric-coated mycophenolate sodium (EC-MPS) at a dose of ≥720 mg/day, and corticosteroids, and serum creatinine level was to be no higher than 265 μmol/l. Key exclusion criteria at the time of randomization were graft loss, rejection that was Banff grade ≥II, recurrent or steroid-resistant rejection, dialysis dependency and proteinuria >1 g/day.

Immunosuppression

All patients received basiliximab induction therapy (Simulect®, Novartis Pharma, Nürnberg, Germany). From the time of transplantation, the immunosuppression regimen comprised CsA microemulsion (Sandimmun Optoral®, Novartis Pharma) dosed according to prespecified target ranges for trough concentration (C_0) and concentration at 2 h postdose (C_2). CsA C_0 target range to month 4.5 was 150–200 ng/ml, while CsA C_2 target range was 1100–1400 ng/ml during month 1, 950–1300 ng/ml during month 2 and 800–1200 ng/ml during months 3–4.5 onwards.

All patients received EC-MPS (1440 mg/day; *myfortic*®, Novartis) and corticosteroids according to local practice. At month 4.5 post-transplant, patients were randomized in a 1:1 ratio to start everolimus or continue receiving CsA using an automated, validated system, with patients stratified according to living or deceased donor. In the everolimus group, CsA was withdrawn in a stepwise manner over a maximum of 4 weeks, with an everolimus target C_0 concentration of 6–10 ng/ml following CsA discontinuation. In the CsA arm, target CsA C_0 (C_2) concentration was 120–

180 ng/ml (700–1000 ng/ml) during months 4.5–6 and 100–150 ng/ml (500–800 ng/ml) from month 6 onwards.

Study endpoints

The primary efficacy endpoint was adjusted eGFR (Nankivell formula [30]) at month 12. Secondary efficacy endpoints at month 12 included eGFR according to the Cockcroft-Gault [31] and MDRD-7 [32] formulae; the change in eGFR between randomization and month 12; biopsy-proven acute rejection (BPAR); graft loss; death; and treatment failure (a composite endpoint of BPAR, graft loss, death, loss to follow-up, discontinuation due to lack of efficacy or toxicity or conversion to another regimen).

Statistical analysis

Efficacy analyses were performed for the intent-to-treat (ITT) population. In the current analysis, this comprised all recipients of a graft from a living donor who were randomized at 4.5 months after transplantation, received at least one dose of any immunosuppressive drug and had at least one postbaseline assessment of the primary efficacy variable, that is eGFR (Nankivell). The per-protocol population was defined as all living-donor ITT patients who did not show major deviations from protocol. The safety population comprised all living-donor recipients who received CsA or everolimus.

The primary efficacy endpoint, adjusted eGFR (Nankivell) at month 12, was assessed by analysis of covariance (ANCOVA) with treatment and centre as factors, and eGFR at month 4.5 as a covariate, using the last observation carried forward (LOCF) method for missing values. In a *post hoc* calculation, it was estimated that a sample size of 38 in each treatment group would have 99% power to detect a difference in mean adjusted eGFR (Nankivell) of 10 ml/min/1.73 m^2, assuming that the common standard deviation was 10 ml/min/1.73 m^2. This estimate was based on a two-group *t*-test with a 0.05 two-sided significance level (NQuery, 7.0).

Results

Patient population

Figure 1 shows the patient disposition according to donor type. In total, 80 living-donor recipients and 220 deceased-donor kidney transplant recipients were randomized. In the living-donor cohort, 42 patients were randomized to everolimus, and 38 were randomized to CsA (safety population), but one CsA patient was given everolimus due to a

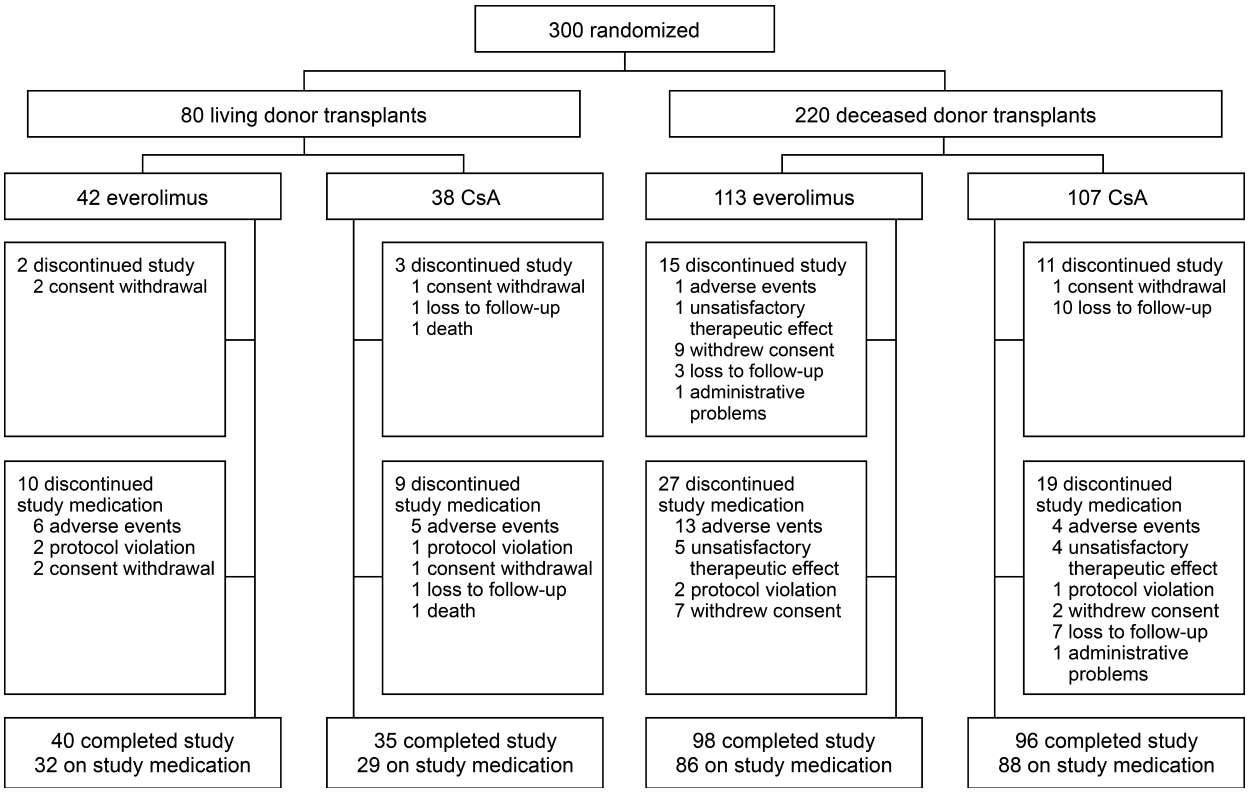

Figure 1 Patient disposition according to donor type. CsA, cyclosporine.

randomization error such that the ITT living-donor population comprised 41 everolimus patients and 39 CsA patients. The 12-month study was completed by 75 living-donor patients (93.8%), with 61 patients (76.3%) remaining on their randomized study medication. There were no marked differences in the proportion of patients discontinuing the study or study medication between the everolimus and CsA arms, the most frequent reason being adverse events in both treatment groups (Fig. 1).

Demographic and baseline characteristics of the living- and deceased-donor cohorts are shown in Table 1. In the living-donor cohort, the everolimus and CsA treatment groups were similar other than minor disparities in the incidence of pretransplant dialysis and the distribution of HLA DR mismatches (Table 1).

Immunosuppression

Table 2 shows the immunosuppression data of the living- and deceased-donor cohorts, revealing no significant differences between the two cohorts. All patients in both cohorts received induction therapy. In the living-donor group, the mean everolimus C_0 level was towards the lower end of the target range (6–10 ng/ml) at month 6 (6.6 ± 2.2 ng/ml) and month 12 (7.1 ± 2.1 ng/ml). At randomization, mean CsA C_0 levels had declined to the lower threshold of the target range for months 1 to 4.5 (150–200 ng/ml) in both treatment groups. By month 12, mean CsA C_0 had decreased to approximately 120 ng/ml. The mean dose of EC-MPS was similar in the everolimus and CsA treatment groups among living-donor recipients at randomization, becoming slightly lower in everolimus-treated patients by month 12 (Table 2).

Renal function

Table 3 and Fig. 2 show the renal function data of the living- and deceased-donor cohorts, revealing no significant differences between the two cohorts. Among living-donor recipients randomized to everolimus, unadjusted mean eGFR (Nankivell) was 66.0 ± 11.7 ml/min/1.73 m^2 at randomization, increasing to 75.0 ± 13.3 ml/min/

Table 1. Demographics and baseline characteristics according to donor type (safety population).

	Living donor		Deceased donor	
	Everolimus (*n* = 42)	CsA (*n* = 38)	Everolimus (*n* = 113)	CsA (*n* = 107)
Age (years), mean ± SD	45.1 ± 13.5	40.9 ± 12.1	47.5 ± 10.9	48.7 ± 11.1
Male, *n* (%)	30 (71.4)	25 (65.8)	72 (63.7)	61 (57.0)
White race, *n* (%)	41 (97.6)	38 (100.0)	111 (98.2)	101 (94.4)
Body mass index (kg/m^2), mean ± SD	26.3 ± 3.9	24.3 ± 4.2	25.4 ± 4.0	24.4 ± 3.9
End-stage disease leading to transplantation, *n* (%)				
Glomerulonephritis/glomerular disease*	13 (31.0)	12 (31.6)	29 (25.7)	35 (32.7)
Polycystic kidney disease	12 (28.6)	5 (13.2)	15 (13.3)	19 (17.8)
IgA nephropathy	4 (9.5)	4 (10.5)	15 (13.3)	13 (12.1)
Hypertension or nephrosclerosis	5 (11.9)	4 (10.5)	6 (5.3)	5 (4.7)
Pyelonephritis/interstitial nephritis	2 (4.8)	1 (2.6)	9 (8.0)	3 (2.8)
Diabetes mellitus	0 (0.0)	2 (5.3)	3 (2.7)	3 (2.8)
Other	2 (4.8)	6 (15.8)	22 (19.5)	18 (16.8)
Unknown	2 (4.8)	3 (7.9)	4 (3.5)	3 (2.8)
Pretransplant dialysis, *n* (%)	30 (71.4)	31 (81.6)	111 (98.3)	106 (99.1)
Previous renal transplant, *n* (%)	2 (4.8)	2 (5.3)	19 (16.8)	21 (19.6)
Number of HLA DR mismatches, *n* (%)				
0	12 (28.6)	5 (13.2)	47 (41.6)	42 (39.3)
1	16 (38.1)	24 (63.2)	52 (46.0)	49 (45.8)
2	14 (33.3)	9 (23.7)	14 (12.4)	16 (15.0)
Cold ischaemia time (h), mean ± SD	2.2 ± 0.9	2.3 ± 0.9	14.0 ± 5.7	14.6 ± 4.9
Diabetes at randomization, *n* (%)	3 (7.1)	3 (7.9)	2 (1.8)	6 (5.6)
Donor				
Age (years), mean ± SD	49.4 ± 8.6	51.5 ± 12.0	46.8 ± 13.5	46.2 ± 13.2
Male gender, *n* (%)	11 (26.2)	15 (39.5)	67 (59.3)	57 (53.3)
Living related, *n* (%)	27 (64.3)	30 (78.9)	–	–
Living unrelated, *n* (%)	15 (35.7)	8 (21.1)	–	–
eGFR at randomization, Nankivell (ml/min/1.73 m^2), mean ± SD	66.0 ± 11.7	63.1 ± 14.8	63.2 ± 19.5	62.6 ± 15.8

CsA, cyclosporine; eGFR, estimated glomerular filtration rate; HLA, human leucocyte antigen; IgA, immunoglobulin A; SD, standard deviation.
*Excluding IgA nephropathy and diabetic glomerular sclerosis.

Table 2. Immunosuppression according to donor type (safety population).

	Living donor		Deceased donor	
	Everolimus (n = 42)	CsA (n = 38)	Everolimus (n = 113)	CsA (n = 107)
Everolimus C_0, mean ± SD (ng/ml)				
Month 6	6.6 ± 2.2	–	6.3 ± 2.2	–
Month 12	7.1 ± 2.1	–	6.4 ± 2.1	–
CsA C_0, mean ± SD (ng/ml)				
Randomization	158 ± 44	154 ± 50	150 ± 53	146 ± 48
Month 12	–	117 ± 32	–	121 ± 39
EC-MPS dose, mean ± SD (mg/day)				
Randomization	1360 ± 218	1317 ± 281	1278 ± 333	1244 ± 328
Month 12	1213 ± 347	1316 ± 277	1165 ± 365	1200 ± 361
Corticosteroid therapy, n (%)				
Randomization	42 (100)	38 (100)	113 (100)	105 (98.1)
Month 12	39 (92.9)	34 (89.5)	101 (94.4)	112 (99.1)

CsA, cyclosporine; C_0, trough concentration; EC-MPS, enteric-coated mycophenolate sodium; SD, standard deviation.

1.73 m^2 at month 12. For the CsA cohort, mean values were 63.1 ± 14.8 ml/min/1.73 m^2 at baseline and remained almost unchanged at month 12 (62.2 ± 15.7 ml/min/1.73 m^2). The between-group difference at month 12 was 10.0 ml/min/1.73 m^2 in favour of everolimus ($P < 0.001$). The primary endpoint, adjusted eGFR (Nankivell) at month 12, was superior in the everolimus group: 74.3 (95% CI [70.7, 77.9]) ml/min/1.73 m^2 vs. 63.8 (95% CI [60.0, 67.7]) ml/min/1.73 m^2, a difference of 10.5 ml/min/1.73 m^2 in favour of everolimus ($P < 0.001$) (Table 3, Fig. 2a). Comparable results were obtained using the Cockcroft-Gault and MDRD formulae (Table 3). During the period from randomization to month 12, adjusted eGFR increased by a mean of 9.8 (95% CI [6.2, 13.4]) ml/min/1.73 m^2 in the everolimus group, an increase that was not seen in the CsA cohort (mean −0.7, 95% CI [−4.6, 3.1] ml/min/1.73 m^2) ($P < 0.001$). When the primary analysis was repeated based on the per-protocol population, the between-group difference (11.5, 95% CI [17.2, 5.7] ml/min/1.73 m^2, $P < 0.001$) was similar to that seen in the ITT population (Table 3).

The direction and extent of the improvement in adjusted eGFR at month 12 and the change from randomization to month 12 did not differ markedly between the deceased-donor cohort and the living-donor patients (Table 3, Fig. 2a and b). The between-group difference in adjusted eGFR (Nankivell) at month 12 in recipients of a deceased-donor graft was 9.1 ml/min/1.73 m^2 (95% CI [11.8, 6.4] ml/min/1.73 m^2; $P < 0.001$).

Mean urine protein at month 12 was significantly higher in the everolimus-treated living-donor patients (352 ± 228 mg/day) versus those receiving CsA (152 ± 103 mg/day) ($P = 0.002$). Proteinuria ≥0.5 g/day was observed at month 12 in five everolimus-treated living-donor recipients (one of whom also experienced Banff grade IA rejection) and no CsA-treated patients ($P = 0.094$).

Efficacy

From 4.5 months to month 12, there were no significant differences in any efficacy parameter between the everolimus and CsA groups in the living-donor population, including the composite treatment failure endpoint (Table 4). However, six everolimus-treated patients experienced one episode of BPAR, of which five were Banff grade I and one was grade II, whereas only one CsA-treated patient experienced one BPAR which was grade I ($P = 0.109$). The total number of biopsies carried out in living-donor recipients between month 4.5 and month 12 was 37 in the everolimus group compared to 18 in the CsA group. One living-donor recipient in the CsA group died from myocardial infarction, which was not suspected to be related to study drug, and there were no death-censored graft losses (Table 3).

Safety

All living-donor recipients experienced one or more adverse event by month 12. Thrombocytopenia, aphthous stomatitis, diarrhoea, hypercholesterolaemia and diabetes were more frequent with everolimus, while leukopenia and urinary tract infection were more frequent with CsA (Table 5). Proteinuria was reported as an adverse event in nine everolimus patients and five CsA patients (21.4% and 13.2%, respectively); all cases were graded mild. Adverse events led to hospitalization or prolongation of hospitalization in 15 everolimus-treated patients (35.7%) and 20

Table 3. Renal function at month 12 according to donor type.

	Living donor						Deceased donor					
	Everolimus	*n*	CsA	*n*	Difference [95% CI]	*P* value	Everolimus	*n*	CsA	*n*	Difference [95% CI]	*P* value
ITT population												
eGFR, Nankivell (ml/min/1.73 m^2)												
Unadjusted	75.0 ± 13.3	38	62.2 ± 15.7	39	−10.0	<0.001*	70.4 ± 20.0	109	60.8 ± 17.2	104	−8.9	<0.001*
Adjusted	74.3 (70.7, 77.9)		63.8 (60.0, 67.7)		−10.5 [−14.9, −6.1]	<0.001†	68.7 (66.0, 71.4)		59.6 (56.8, 62.5)		−9.1 [−11.8, −6.4]	<0.001†
Adjusted change from randomization	9.8 (6.2, 13.4)		−0.7 (−4.6, 3.1)			<0.001†	5.8 (3.1, 8.5)		−3.3 (−6.2, −0.4)			<0.001†
eGFR, Cockcroft-Gault (ml/min)												
Unadjusted	76.7 ± 23.4	38	61.5 ± 18.2	39	−10.9	<0.001*	68.0 ± 24.4	110	56.0 ± 20.3	104	−12.1	<0.001*
Adjusted	75.5 (71.3, 79.6)		64.6 (60.5, 68.8)		−10.8 [−15.9, −5.7]	<0.001†	64.8 (61.9, 67.6)		55.7 (52.7, 58.8)		−9.0 [−11.9, −6.1]	<0.001†
eGFR, MDRD (ml/min)												
Unadjusted	58.0 ± 17.4	32	49.0 ± 15.9	28	−9.1	0.016*	56.4 ± 21.2	88	48.0 ± 16.9	85	−8.5	0.008*
Adjusted	57.8 (53.5, 62.1)		44.7 (40.1, 49.3)		−13.1 [−18.7, −7.5]	<0.001†	54.9 (51.4, 58.4)		45.5 (42.0, 49.0)		−9.4 [−12.7, −6.1]	<0.001†
PP population‡												
eGFR, Nankivell (ml/min/1.73 m^2)												
Unadjusted	75.8 ± 14.0	29	63.0 ± 15.1	26	−11.6	<0.001*	71.2 ± 20.8	77	63.5 ± 17.3	76	−7.7	0.013*
Adjusted	75.9 (71.6, 80.2)		64.4 (59.4, 69.4)		−11.5 [−17.2, −5.7]	<0.001†	72.1 (68.7, 75.4)		63.7 (60.1, 67.2)		−8.4 [−11.7, −5.1]	<0.001†
Adjusted change from randomization	10.6 (6.3, 14.9)		−0.9 (−5.9, 4.1)			<0.001†	8.1 (4.7, 11.4)		−0.3 (−3.9, 3.2)			<0.001†
Safety population												
Serum creatinine (μmol/l)	122 ± 32	39	147 ± 45	37	–	0.028*	130 ± 46	106	142 ± 4	99	–	0.101*
Urine protein (mg/day)	352 ± 228	24	152 ± 103	16	–	0.002*	533 ± 832	48	264 ± 368	49	–	0.001*
Urine protein, *n* (%) (g/day)												
<0.5	18 (75.9)	24	16 (100.0)	16	–	0.094§	34 (70.8)	48	47 (95.9)	49	–	<0.001§
0.5–1.0	5 (20.8)		0				11 (22.9)		0			
>1.0	1 (4.2)		0				3 (6.3)		2 (4.1)			

ANCOVA, analysis of covariance; CI, confidence interval; CsA, cyclosporine; eGFR, estimated glomerular filtration rate; MDRD, modification of diet in renal disease.
Unadjusted values are shown as mean ± SD. Adjusted data are shown according to ANCOVA analysis and are presented as least square mean values [95% CI].
*Wilcoxon rank-sum test.
†ANCOVA.
‡The per-protocol population included all patients who were treated according to protocol from transplantation to month 12.
§Mantel-Haenszel chi-square.

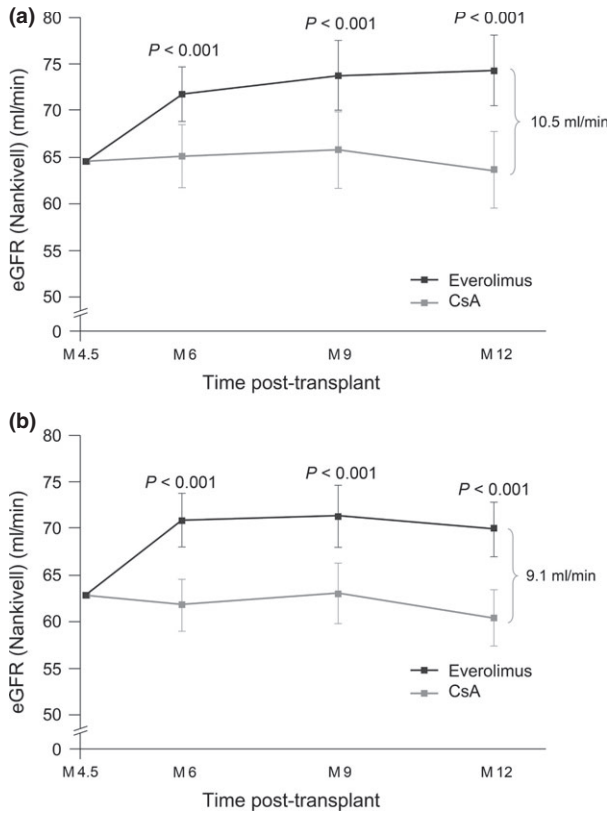

Figure 2 Estimated GFR (Nankivell) following (a) living-donor and (b) deceased-donor kidney transplantation (ITT population). Data are shown as adjusted values (least squares mean from ANCOVA model) with 95% CI. ANCOVA, analysis of covariance; CsA, cyclosporine; eGFR, estimated glomerular filtration rate; ITT, intent-to-treat.

CsA-treated patients (52.6%) ($P = 0.18$). The incidence of infections and serious infections in the living-donor cohort were similar in the everolimus and CsA groups (Table 5). Urinary tract infections were reported in 31.0% of everolimus-treated patients and 42.1% of CsA-treated patients. Rates of BK virus and cytomegalovirus infection were similar between groups. Hospitalization, or prolongation of hospitalization, due to infection occurred in 13 everolimus patients (31.0%) and 11 CsA patients (28.9%). Between randomization and month 12, discontinuation due to adverse events in the living-donor population occurred in three everolimus-treated patients (7.1%), due to allograft rejection, hypertension and pneumonia, and in five CsA-treated patients (13.2%), due to allograft rejection, leukopenia, chronic bronchitis, cough, herpes zoster infection, human polyomavirus infection and pneumonia.

Discussion

These findings suggest that initiation of everolimus with early elimination of CNI therapy in kidney transplant patients is associated with a significant benefit for renal function at 12 months post-transplant which is observed in both living-donor and deceased-donor recipients. Compared to a standard CNI-based regimen, everolimus-treated patients showed a clinically relevant [10.5 ml/min/1.73 m^2 (Nankivell)] improvement in adjusted eGFR. Efficacy and safety outcomes were similar in the two treatment groups, other than a numerical increase in mild BPAR and proteinuria with everolimus.

A previous open-label trial in which 60 living-donor kidney transplant patients were randomized to remain on CNI therapy or switch to sirolimus at 2 months post-transplant reported significantly higher eGFR in the group converted to the CNI-free regimen at 6 months [27]. In our population, a parallel analysis of the deceased-donor subpopulation suggested that the improvement in eGFR from

Table 4. Efficacy events between randomization (month 4.5) and month 12 (ITT population), *n* (%).

	Living donor			Deceased donor		
	Everolimus ($n = 41$)	CsA ($n = 39$)	*P* value†	Everolimus ($n = 113$)	CsA ($n = 107$)	*P* value†
Treatment failure*	8 (19.5)	7 (18.0)	1.00	21 (18.6)	16 (15.0)	0.589
BPAR‡						
Any	6 (14.6)	1 (2.6)	0.109	9 (8.0)	4 (3.7)	0.255
Banff I	5 (12.2)	1 (2.6)		9 (8.0)	3 (2.8)	
Banff II	1 (2.4)	0		0	1 (0.9)	
Graft loss	0	0	–	0	0	–
Death	0	1 (2.6)	0.488	0	0	–

BPAR, biopsy-proven acute rejection; CsA, cyclosporine.

*A composite endpoint of BPAR, graft loss, death, loss to follow-up, discontinuation due to lack of efficacy or toxicity or conversion to another regimen.

†Fisher's exact test.

‡On clinically indicated biopsies.

Table 5. Incidence of adverse events from time of transplant to month 12 and laboratory values at month 12 (safety population).

	Living donors			Deceased donors		
	Everolimus (n = 42)	CsA (n = 38)	P value*	Everolimus (n = 113)	CsA (n = 107)	P value*
Adverse event or infection	42 (100.0)	38 (100.0)	–	113 (100.0)	107 (100.0)	–
Serious adverse event or infection†	23 (54.8)	23 (60.5)	0.66	72 (63.7)	63 (58.9)	0.49
Infection	32 (76.2)	33 (86.8)	0.26	103 (91.2)	86 (80.4)	0.032
Serious infection	13 (31.0)	11 (28.9)	1.00	34 (30.1)	29 (27.1)	0.66
Infection						
BK virus	1 (2.4)	1 (2.6)		4 (3.5)	1 (0.9)	
Cytomegalovirus	10 (23.8)	7 (18.4)		18 (15.9)	19 (17.8)	
Nasopharyngitis	13 (31.0)	15 (39.5)		30 (26.5)	23 (21.5)	
Pneumonia	4 (9.5)	1 (2.6)		6 (5.3)	11 (10.3)	
Urinary tract infection	13 (31.0)	16 (42.1)		76 (67.3)	61 (57.0)	
Blood & lymphatic disorders						
Anaemia	10 (23.8)	9 (23.7)		32 (28.3)	25 (23.4)	
Leukopenia	6 (14.3)	8 (21.1)		18 (15.9)	17 (15.9)	
Thrombocytopenia	4 (9.5)	0		13 (11.5)	5 (4.7)	
Gastrointestinal disorders						
Aphthous stomatitis	8 (19.0)	1 (2.6)		13 (11.5)	2 (1.9)	
Diarrhoea	16 (38.1)	4 (10.5)		40 (35.4)	35 (32.7)	
Metabolism & nutritional disorders						
Hypercholesterolaemia	12 (28.6)	6 (15.8)		33 (29.2)	34 (31.8)	
Hyperlipidemia	6 (14.3)	4 (10.5)		16 (14.2)	11 (10.3)	
Hypertriglyceridemia	2 (4.8)	2 (5.3)		8 (7.1)	3 (2.8)	
Diabetes mellitus	7 (16.7)	3 (7.9)		12 (10.6)	8 (7.5)	
Proteinuria	9 (21.4)	5 (13.2)		15 (13.3)	19 (17.8)	
Urea (mmol/l), mean ± SD	7.4 ± 2.8	9.2 ± 4.0	0.019	8.2 ± 4.2	10.4 ± 4.3	<0.001
Haemoglobin						
Mean ± SD (g/dl)	12.6 ± 1.5	12.8 ± 1.6	0.494	12.4 ± 1.5	13.1 ± 1.8	0.002
<11 g/dl, n/N (%)	8/80 (10.0)	18/78 (23.1)	0.032	32/218 (14.7)	24/212 (11.3)	0.319
Blood glucose						
Mean ± SD (mmol/l)	6.0 ± 2.7	5.5 ± 2.2	0.372	5.7 ± 2.2	5.7 ± 2.3	0.929
>7.0 mmol/l, n/N (%)	0/80	0/78	–	0/214	0/210	–
White blood cells						
Mean ± SD (10^9/l)	7.5 ± 2.1	8.5 ± 3.2	0.151	7.1 ± 2.2	8.0 ± 2.4	0.002
Leucocytes <4.5 × 10^9/l, n/N (%)	0/80	8/78 (10.3)	0.003	14/220 (6.4)	14/212 (6.6)	1.00
Platelets						
Mean ± SD (10^9/l)	253 ± 74	275 ± 72	0.108	239 ± 85	256 ± 63	0.026
Platelets <100 × 10^9/l, n/N (%)	0/80	0/78	–	1/110 (0.9)	0/106	1.00
Total blood cholesterol (mmol/l), mean ± SD	6.4 ± 1.7	5.6 ± 1.2	0.012	6.6 ± 1.7	6.0 ± 1.2	0.005
LDL-cholesterol (mmol/l), mean ± SD	3.8 ± 1.2	3.3 ± 1.1	0.090	3.8 ± 1.0	3.6 ± 1.1	0.154
HDL-cholesterol (mmol/l), mean ± SD	1.4 ± 0.3	1.4 ± 0.4	0.892	1.5 ± 0.4	1.6 ± 0.5	0.246
Triglycerides (mmol/l), mean ± SD	4.3 ± 5.2	2.4 ± 1.5	0.002	3.6 ± 2.8	2.6 ± 1.3	0.004
Aspartate aminotransferase (U/l), mean ± SD	28.4 ± 9.5	22.0 ± 6.2	0.003	30.4 ± 14.8	23.4 ± 13.2	<0.001
Alanine aminotransferase (U/l), mean ± SD	33.1 ± 17.4	26.1 ± 14.5	0.042	37.5 ± 30.5	23.5 ± 12.4	<0.001

CsA, cyclosporine; HDL, high density lipoprotein; LDL, low density lipoprotein; SD, standard deviation.

*Fisher's test.

†Defined as fatal or life-threatening, resulting in persistent or significant disability/incapacity, requiring inpatient hospitalization or prolongation of existing hospitalization, medically significant, or a graft loss.

randomization to month 12 was at least as good in the living-donor cohort as in deceased-donor recipients, confirmed by three different formulae. Higher renal function at 1 year might be expected to translate to improved long-term graft survival in living-donor recipients [11]. It should be noted, however, that late conversion (more than 6 months post-transplant) from CNI therapy to an mTOR inhibitor only appears to offer a renal benefit in patients with good remaining kidney function at time of conversion [23,33].

Strategies to preserve the good baseline function in grafts from living donors are likely to be as relevant as in deceased-donor transplants. Even in kidney grafts from living donors which are functioning well in patients without any rejection episodes, histological damage is almost universal, with one study reporting chronic tubulointerstitial fibrosis in 85% of such grafts on protocol biopsy in 120 patients more than 1 year post-transplant [34]. Protocol biopsies in a series of 52 living-donor recipients showed the presence of interstitial fibrosis/tubular atrophy in 10% of patients by month 3 [35], while in a cohort of 50 paediatric patients (45 of whom received a graft from a living donor), 41.5% of protocol biopsies up to 2 years post-transplant exhibited signs of CNI-related nephrotoxicity [36]. Moreover, chronic histological changes on protocol biopsies at one and 5 years in 300 kidney transplant patients were found to be similar in grafts from living or deceased donors [37].

Efficacy endpoints did not differ between the everolimus and CsA groups to month 12. The mildest category of BPAR (grade I) occurred in 10% more everolimus-treated patients than CsA-treated patients, a finding which might have been influenced by the fact that twice as many biopsies were carried out in the everolimus group compared to the CsA group. It would seem likely that the greater propensity to undertake biopsies may have been due to a relative lack of familiarity with the CNI-free regimen, leading to investigators taking a more cautious approach, but this is speculative. Both regimens were associated with excellent graft survival, with no graft failures during months 4.5–12 and only one death in the CsA-treated group. In other studies which have detected an increased rate of acute rejection after early conversion of kidney transplant patients to an mTOR inhibitor, the excess rejection episodes have generally been mild, as observed here, and not associated with inferior graft survival [19,38]. It has been reported that mild acute rejection has only a negligible impact on subsequent graft survival if renal function recovers [39,40]. In our cohort, there was no apparent relationship between acute rejection and development of proteinuria, and as discussed above, graft function was superior in the everolimus-treated patients.

The safety profile of everolimus in living-donor recipients showed no clinically meaningful differences from the deceased-donor cohort. As in the total study population [28], total cholesterol and triglyceride levels were higher with everolimus versus CsA, and adverse events typically associated with mTOR inhibition, such as aphthous stomatitis and hypercholesterolaemia, were more frequent. The higher rate of diarrhoea in the everolimus cohort was unexpected, has not been observed elsewhere and was not seen in the deceased-donor group. A higher rate of proteinuria reported as an adverse event in everolimus-treated patients was only seen in the living-donor group, but the harder endpoint of urinary protein showed a similar between-group difference for both living-donor and deceased-donor recipients. Consistent with the literature, urinary protein of 0.5 g/dl or higher at month 12 was only observed in everolimus-treated patients, not CsA-treated patients, in both the living- and deceased-donor cohorts, although no patient discontinued everolimus due to proteinuria. The rate of adverse events or infections which required hospitalization, and the number of patients discontinuing study drug due to adverse events, was similar with everolimus or CsA in the living-donor cohort.

The ZEUS trial used a robust prospective, randomized and multicentre study design. The current analysis was undertaken *post hoc*, which must be taken into account when considering the study findings. Notably, however, enrolled patients were stratified according to donor type prior to randomization, so as to ensure balanced populations in the everolimus and CsA cohorts within the living-donor and deceased-donor subpopulations. This avoids the risk of selection bias inherent in some *post hoc* subanalyses. The primary and secondary endpoints and statistical methodology that were prespecified for the overall study population were applied here, with no new endpoints introduced. It should be kept in mind that patients who experienced rejection greater than Banff grade I prior to randomization, who required an immunosuppressive change for immunological reasons or who exhibited poor renal function (serum creatinine $\geq$265 μmol/l) were not randomized. Thus, the current findings are not necessarily applicable to individuals at high immunological risk or in whom renal function has already deteriorated substantially. It should be noted that the conversion to everolimus took place over a four-week period, as opposed to an abrupt conversion such as that used in some previous trials [18,38]; the relative merits of each approach have not yet been established. Lastly, at the time the study protocol was developed, the clinical relevance of donor specific antibodies (DSA) was not widely recognized, and regretfully, data on DSA and *de novo* DSA were not captured throughout the study. A protocol amendment requested that DSA should be recorded at the five-year study visit of the follow-up study, but information was only captured for a minority of patients (n = 53). In this subgroup, DSA was present in 6 of 28 everolimus patients (21.4%) and 5 of 25 CsA patients (20.0%) [41], although this finding should be regarded cautiously due to low numbers. Other data relating to an association between mTOR inhibitor therapy and risk of DSA are conflicting [42–46] and include one retrospective, single-centre analysis of 126 patients (including some from the ZEUS study) which observed a significant relationship between everolimus therapy and risk of DSA (42). Future studies in this area should include regular monitoring of DSA.

In conclusion, conversion from CNI therapy to everolimus with mycophenolic acid and steroids improves renal function at 1 year, an effect that is at least as great in living-donor recipients as deceased-donor recipients. Conversion at 4.5 months avoids the period of highest rejection risk and is not associated with loss of efficacy when everolimus is given in combination with mycophenolate sodium and steroids, but may avoid extensive irreversible CNI-related tubulointerstitial damage [47]. Both living-donor and deceased-donor kidney transplant patients who are not at high immunological risk and have acceptable renal function appear to be suitable candidates for conversion to everolimus-based immunosuppression.

Authorship

FL, KB, MZ, RPW, PR, UE, AM, WA, RS, KH, OW, HHW, BS, HUK, MS, IAH, SN and CS: were involved in the study conduct, collection, and review of study data. MP and E-MP: reviewed the study data. CM: provided biostatistical support.

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