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Renal function three years after early conversion from a calcineurin inhibitor to everolimus: results from a randomized trial in kidney transplantation

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Conflicts of interest
LM has served as a consultant to Novartis, Astellas, and AbSorber and has received lecture fees from Astellas and Roche. SSS has served as an Advisory Board member for Bristol-Myers Squibb and AbSorber. BvzM has served as a consultant to AstraZeneca, Merck Sharp & Dome, Novartis, and Astellas. BG has served as a consultant to Genzyme and Bristol-Myers Squibb. DS is an employee of Novartis. HH has served as a consultant to Bristol-Myers Squibb, Novartis, AstraZeneca, Astellas, and Schering-Plough and has received lecture fees from Novartis and AstraZeneca, as well as having served as national co-coordinator for the SHARP study at Oxford University's Clinical Trial Service Unit. JMH has received lecture fees from GlaxoSmithKline. BJ, CB, and HA have no conflict of interests to declare.

Summary

In a 36-month, open-label, multicenter trial, 202 kidney transplant recipients were randomized at week 7 post-transplant to convert to everolimus or remain on cyclosporine: 182 were analyzed to month 36 (92 everolimus, 90 controls). Mean (SD) change in measured GFR (mGFR) from randomization to month 36 was 1.3 (14.0) ml/min with everolimus versus −1.7 (15.4) ml/min in controls ($P = 0.210$). In patients who remained on treatment, mean mGFR improved from randomization to month 36 by 7.9 (11.5) ml/min with everolimus ($n = 37$) but decreased by 1.4 (14.7) ml/min in controls ($n = 62$) ($P = 0.001$). During months 12–36, death-censored graft survival was 100%, patient survival was 98.9% and 96.7% in the everolimus and control groups, respectively, and 13.0% and 11.1% of everolimus and control patients, respectively, experienced mild biopsy-proven acute rejection (BPAR). Protocol biopsies in a limited number of on-treatment patients showed similar interstitial fibrosis progression. Donor-specific antibodies were present at month 36 in 6.3% (2/32) and 18.0% (9/50) of on-treatment everolimus and control patients with available data ($P = 0.281$). During months 12-36, adverse events were comparable, but discontinuation was more frequent with everolimus (33.7% vs. 10.0%). Conversion from cyclosporine to everolimus at 7 weeks post-transplant was associated with a significant benefit in renal function at 3 years when everolimus was continued.

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Introduction

Chronic nephrotoxicity caused by maintenance calcineurin inhibitor (CNI) therapy following kidney transplantation is a well-established phenomenon [1]. CNI avoidance appears to offer inadequate rejection prophylaxis in standard-risk kidney transplant recipients [2–6], while long-term administration of reduced-exposure CNI therapy is associated with some degree of ongoing tubulo-interstitial and glomerular injury [7]. Various immunosuppressive strategies have been explored in an attempt to avoid or minimize CNI exposure [8–10], of which CNI withdrawal before significant renal damage occurs appears to be a promising option [8]. Conversion to a CNI-free regimen based on mycophenolic acid can lead to an unacceptable rate of acute rejection [11], and attention has instead focused on switch to a mammalian target of rapamycin (mTOR) inhibitor [12]. Several randomized, controlled trials have examined efficacy and renal outcomes after conversion from CNI to an mTOR inhibitor at various time points ranging from as early as 10 days to more than 6 months after transplantation [13–21]. An overall renal benefit has been observed only when conversion takes place before month 6 post-transplant [13–15,17]. Subsequently, an improvement is restricted to patients who have good function at the time of conversion [18,19]. In some trials, however, early switch to an mTOR inhibitor increased the risk of acute rejection [14,15,17,21], raising questions over whether a renal advantage following CNI withdrawal is sustained long term.

CENTRAL (CErtican Nordic Trial in RenAL transplantation) was an open-label, multicenter trial in which *de novo* kidney transplant recipients at low to medium immunological risk were randomized at week 7 post-transplant to remain on cyclosporine (CsA) or convert to everolimus [14]. Both groups received mycophenolic acid and steroids. From baseline to month 12, mean measured glomerular filtration rate (mGFR) improved in the everolimus group by 4.9 ml/min compared with no change in the CsA group, a difference that was statistically significant. However, biopsy-proven acute rejection (BPAR) was more frequent in the everolimus group by month 12 and more everolimus-treated patients discontinued due to adverse events. Here, we describe efficacy and safety outcomes during months 12–36 post-transplant among patients who completed the first 12 months of the study, with a particular emphasis on progression of renal function as assessed by mGFR.

Methods

Study design and conduct

CENTRAL was a 36-month, open-label study in which *de novo* kidney transplant recipients were randomized at week 7 post-transplant to remain on CsA or convert to everolimus. The trial was undertaken at eight transplant centers in Sweden, Norway, and Denmark during March 2008 to April 2013, in accordance with the principles of Good Clinical Practice and the Declaration of Helsinki, following approval by the institutional review board at each center. Written informed consent was obtained from all patients.

Eligibility criteria

Adult recipients of a first or second single kidney transplant from a deceased or living donor were eligible to enter the study. Exclusion criteria at study entry were multiorgan transplantation or a previous nonrenal transplantation, receipt of a graft from an HLA-identical sibling donor, or panel reactive antibodies >30% at the most recent assessment. At randomization (week 7 post-transplant), patients had to be treated with CsA, enteric-coated mycophenolate sodium (EC-MPS, minimum 1080 mg/day), and corticosteroids (≥10 mg/day) with no previous acute rejection or treatment for acute rejection and had to have received basiliximab induction therapy. Exclusion criteria at randomization included hemoglobin <8.0 g/dl, platelets $<50 \times 10^9$/l and/or white blood cell count $\leq 2.5 \times 10^9$/l, total cholesterol ≥9 mmol/l and/or triglycerides ≥6 mmol/l despite lipid-lowering treatment, urinary protein/creatinine ratio ≥150 mg/mmol, ongoing wound healing problems or any other severe surgical complication, requirement for dialysis and/or estimated GFR (eGFR) <20 ml/min (Cockcroft–Gault formula). All

patients had a negative B-cell CDC cross-match before transplantation (a flow cross-match was not required for entry to the study).

Immunosuppression

In the everolimus arm, everolimus was initiated at week 7 post-transplant at a target C_0 concentration of 6–10 ng/ml, with a target EC-MPS dose of 1080 mg/day (minimum 720 mg/day), and CsA was withdrawn overnight. In the control group, CsA target concentration was C_0 75–200 ng/ml (C_2 700–900 ng/ml) from randomization to month 6, and C_0 50–150 ng/ml (C_2 600–800 ng/ml) after month 6 with a target EC-MPS dose of 1440 mg/day (minimum 720 mg/day). All patients received corticosteroids and dosed after weeks 10–12 according to local practice (minimum 5 mg/day) to month 12, after which they could be discontinued.

Study endpoints

The primary endpoint of the study was change in mGFR from randomization to month 12 post-transplant. Secondary efficacy endpoints after completion of the 12-month visit included progression of mGFR to month 36, eGFR using the Cockcroft–Gault, and Modification of Diet in Renal Disease Study Group (MDRD) equations, a composite treatment failure endpoint (graft loss, death, or loss to follow-up), progression of interstitial fibrosis/tubular atrophy (IF/TA), in protocol biopsies, and the incidence and severity of BPAR according to Banff classification [22]. Safety endpoints comprised the time to first diagnosed malignancy, use of lipid-lowering therapy and lipid profile, use of antihypertensive medication and blood pressure, the incidence and extent of proteinuria, adverse events, and laboratory assessments.

Assessment of mGFR was based on iohexol or ^{51}chromium-labeled ethylene-diaminetetraacetic acid (^{51}Cr-EDTA) clearance. Biopsies performed in response to suspected acute rejection were assessed locally. Protocol biopsies were performed at months 12 and 36 and were assessed centrally using Banff 97 criteria for IF/TA, by lesion scores for interstitial fibrosis [22]. For assessment of DSA, serum samples were collected at the time of transplantation and at 36 months post-transplant. All samples were tested for HLA antibodies with Luminex®-based bead assay, either quantitatively screened with LABScreen Mixed Beads (LSM12) and if positive further characterized with LABScreen Single Antigen Beads (LS1A04 or LS2A01) (both One Lambda, Canoga Park, CA, USA) or qualitatively with LABScreen Single Antigen beads only, dependent on center practice.

Statistical analysis

The change in mGFR from week 7 to month 36 was compared between groups using analysis of covariance (ANCOVA) with treatment and center as factors and baseline mGFR (i.e., mGFR at week 7) as covariate. Categorical variables were compared between treatment groups using the Fisher's exact test and continuous variables by t-tests. The occurrence of malignancy was assessed by Kaplan–Meier statistics and compared between treatment groups using the log rank test.

The safety population and the intent-to-treat (ITT) population consisted of all randomized patients who received at least one dose of study drug. *Post hoc*, a modified ITT (mITT) population was defined as all ITT patients who completed the month 12 study visit and was used for all data analyses to month 36.

Results

Study population

In total, 341 patients were included at time of transplant, of whom 204 (59.8%) met the inclusion criteria at week 7 and were randomized. 202 patients were randomized and received study medication (102 everolimus, 100 controls). Of these, 182 (90.1%; 92 everolimus, 90 controls) completed the 12-month visit and formed the mITT population for the analysis of data to month 36. The month 36 visit was completed by 176 patients (96.7%; 90 everolimus, 86 controls), of whom 111 (43 everolimus, 68 controls) remained on study drug as shown in Fig. 1, which also describes the frequency and main causes of study drug discontinuation throughout the study. The rate of study drug discontinuation varied considerably between countries (everolimus 0%, 54.8%, and 71.1% in Denmark, Norway and Sweden, respectively; controls 21.4%, 37.7%, and 30.4%).

Baseline characteristics of the mITT population (Table 1) were comparable between treatment groups and similar to those of the randomized population [13].

Immunosuppression

Immunosuppressive regimens at month 12 and month 36 are summarized in Table 2. Mean values for everolimus C_0 were toward the middle of the target range (6–10 ng/ml) at months 12 and 36, as were mean values for CsA C_0 (target range 50–150 ng/ml) (Table 2). Steroid therapy was administered to all 92 everolimus patients and 90 control patients for at least part of the extension phase, with a median dose of 5 mg/day in both groups after month 12.

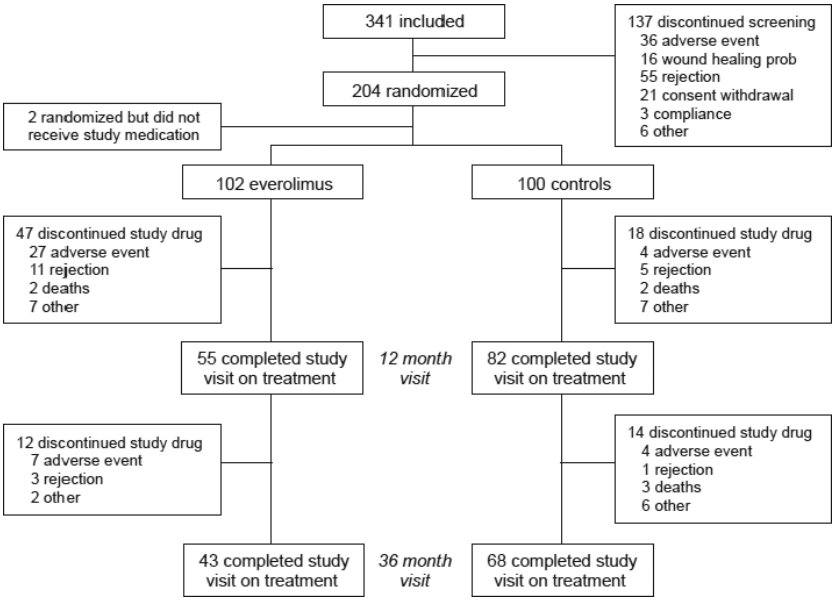

Figure 1 Patient flow: study visit completion on treatment.

Renal function

Data on mGFR were available at month 36 in 83.5% of patients (152/182), and mean (SD) values were similar between groups ($P = 0.418$) (Table 3). ANCOVA confirmed that mGFR at month 36 was not significantly different between groups: least square means (95% CI) values were 48.4 (45.3, 51.6) ml/min in the everolimus group and 45.8 (42.6, 48.9) ml/min in the control group (difference 2.6 ml/min in favor of everolimus; 95% CI −1.8, 7.1; $P = 0.241$). The mean (SD) change in mGFR from randomization to month 12 was significantly higher in the everolimus group versus controls ($P = 0.003$), but the difference became nonsignificant by month 36 ($P = 0.210$) (Table 3).

The mean (SD) change in mGFR from month 12 to month 36 was similar in both groups using all three estimation formulae (Table 3). In a prespecified analysis, mGFR was assessed in patients who remained on study drug to month 36. Of these, 99 of 107 patients (92.5%, 37 everolimus, 62 controls) provided mGFR measurements at month 36. In this on-treatment subpopulation, mean (SD) mGFR improved from randomization to month 36 by 7.9 (11.5) ml/min in the everolimus group but decreased by 1.4 (14.7) ml/min in the control arm ($P = 0.001$). ANCOVA of the on-treatment population showed least square means (95% CI) values for mGFR at month 36 to be 54.3 (50.0, 58.5) ml/min in the everolimus group and 46.1 (42.8, 49.4) ml/min in the control arm (difference 8.2 ml/min in favor of everolimus; 95% CI 2.8, 13.5; $P = 0.003$).

For the 37 patients who remained on everolimus at the month 36 visit, mean (SD) mGFR was 45.3 (13.5) ml/min at baseline and 53.1 (13.4) ml/min at month 36. Thirty-nine everolimus-treated patients were switched back to CNI therapy before month 36. In this group, mean (SD) mGFR was 48.4 (13.6) ml/min at baseline and 43.5 (14.6) ml/min at month 36 ($P < 0.001$).

In total, 28 everolimus-treated patients and 26 control patients had an mGFR value <40 ml/min at randomization. Of these, 13 and 16 patients, respectively, completed the month 36 visit on randomized treatment. In this group of 29 patients, the mean (SD) mGFR at baseline was 31.2 (7.5) ml/min and 32.8 (5.3) ml/min in the everolimus and control arms, respectively, and the change in mGFR baseline to month 36 was +14.5 (9.9) ml/min and 2.7 (14.5) ml/min ($P = 0.019$).

The increase in mean (SD) urine protein:creatinine ratio from month 12 to month 36 was similar in both treatment groups ($P = 0.534$), although data were available at month 36 in only 24 everolimus-treated patients and 21 control patients (Table 4). Proteinuria was reported as an adverse event in seven patients in the everolimus group and three control patients (7.6% and 3.3%, respectively).

Efficacy

The composite efficacy endpoint (graft loss, death, or loss to follow-up) occurred in three patients in the everolimus group and six patients in the control group during months 12–36 ($P = 0.443$) (Table 5). During months 12–36, 13.0% and 11.1% of patients in the everolimus and con-

Table 1. Baseline characteristics (mITT population).

	Everolimus (n = 92)	Controls (n = 90)	P value
Recipient characteristics			
Age, years	55.6 (11.0)	54.1 (12.1)	0.378*
Female gender, *n* (%)	32 (35.0)	24 (27.0)	0.237†
Caucasian, *n* (%)	89 (97.0)	90 (100)	0.227†
End-stage disease leading to transplantation, *n* (%)			
Glomerulonephritis/glomerular disease	34 (37.0)	22 (24.0)	0.530†
Polycystic disease	19 (21.0)	20 (22.0)	
Diabetes mellitus	11 (12.0)	9 (10.0)	
Hypertension/ nephrosclerosis	6 (7.0)	9 (10.0)	
Interstitial nephritis	1 (1.0)	5 (6.0)	
Other	13 (14.0)	13 (14.0)	
Unknown	5 (5.0)	5 (6.0)	
Donor characteristics			
Age, years	50.3 (16.3)	52.2 (14.9)	0.425*
Female gender, *n* (%)	45 (49.0)	55 (61.0)	0.099†
Deceased, *n* (%)	64 (70.0)	65 (72.0)	0.694†
Transplant characteristics			
Cold ischemia time, minutes	647 (442)	676 (455)	0.678*
Panel reactive antibodies 0, *n* (%)	90 (97.8)	87 (96.7)	0.63†
Retransplant, *n* (%)	9 (8.8)	6 (6.0)	0.651†
0 HLA mismatch			
A, *n* (%)	14/92 (15.2)	22/90 (24.4)	0.450†
B, *n* (%)	10/92 (10.9)	12/90 (13.3)	0.657†
DR, *n* (%)	24/92 (26.1)	23/90 (25.6)	0.477†

Continuous variables are shown as mean (SD).
*t-test.
†Cochrane-Mantel-Haenszel test.

Table 2. Immunosuppression (mITT population).

	Month 12		Month 36	
	Everolimus (n = 92)	Controls (n = 90)	Everolimus (n = 92)	Controls (n = 90)
Drug therapy, *n* (%)				
Everolimus	58 (63.0)	1 (1.1)	49 (53.3)	4 (4.4)
CsA	39 (42.4)	81 (90.0)	47 (51.1)	70 (77.8)
EC-MPS	84 (91.3)	85 (94.4)	86 (93.5)	80 (88.9)
Prednisolone	85 (92.4)	90 (100.0)	82 (89.1)	78 (86.7)
Tacrolimus	15 (16.3)	11 (12.2)	28 (30.4)	20 (22.2)
MMF	0	1 (1.1)	0	0
Azathioprine	0	2 (2.2)	0	1 (1.1)
Drug exposure				
Everolimus C_0, ng/ml	7.5 (1.6)	–	7.7 (4.3)	–
CsA C_0, ng/ml	–	106 (40)	–	95 (31)
EC-MPS, mg/day	1048 (383)	1096 (373)	1029 (363)	1216 (309)
Steroid dose, mg//day	5.8 (1.9)	5.5 (1.5)	5.5 (2.6)	5.0 (1.1)

Continuous variables are shown as mean (SD).
CsA, cyclosporine; EC-MPS, enteric-coated mycophenolate sodium; MMF, mycophenolate mofetil.

trol groups, respectively, experienced BPAR (P = 0.720). All BPAR episodes were Banff grade I (Table 5). Analyses of fibrosis score at month 12 and month 36, in available protocol biopsies from patients on study drug treatment, showed no marked differences between groups. The score progressed markedly during the first year post-transplant, with little change between months 12 and 36. Interstitial fibrosis grade ci0, ci1, ci2, or ci3 was observed in 19%, 81%, 0%, and 0% of everolimus-treated patients at baseline (n = 26); 11%, 46%, 27%, and 16% at month 12 (n = 37); and 15%, 41%, 24%, and 18% (n = 33) at month 36 (n = 33), respectively. In the control arm, the corresponding proportions were 29%, 63%, 4%, and 4% at baseline (n = 24); 11%, 46%, 26%, and 16% at month 12 (n = 61); and 12%, 46%, 26%, and 16% at month 36 (n = 57).

In a *post hoc* analysis, the presence of DSA was analyzed at month 36. Data were available in 60 of 92 everolimus-treated patients and 57 of 90 control patients. The proportion of patients found to be DSA positive was 15.0% in the everolimus group (9 of 60 patients) and 21.1% in the control arm (12 of 57 patients) (P = 0.600). Among patients who completed the month 36 visit on their randomized study drug (everolimus 25, controls 44), the proportion of patients who were DSA positive was 8.0% with everolimus (2 of 25 patients) and 18.2% for controls (8 of 44 patients) (P = 0.428).

Safety

The proportion of patients experiencing one or more adverse event during months 12–36 was similar between groups (everolimus 81.5%, controls 90.0%; P = 0.103). The incidence of adverse events with a suspected relation to study drug was 29.3% and 41.1% in the everolimus and control arms, respectively (P = 0.098). Serious adverse events also occurred with a similar frequency in both groups (everolimus 45.7%, controls 45.6%; P = 0.990) although the number of events was higher with everolimus versus controls (107 vs. 78; P = 0.053). The most frequent serious adverse events were urinary tract infection (everolimus 9.8%, controls 3.3%), pyelonephritis (4.3%, 2.2%), pneumonia (5.4%, 5.6%), gastroenteritis (3.3%, 2.2%), urosepsis (2.2%, 3.3%), chest pain (3.3%, 2.2%), and increased blood creatinine (4.3%, 2.2%). Throughout the whole study, the most frequent adverse events that led to everolimus discontin-

Table 3. Renal endpoints (mITT population).

	Month 12				Month 36			
	Everolimus (n = 92)		Controls (n = 90)		Everolimus (n = 92)		Controls (n = 90)	
	n		n		n		n	
mITT population								
mGFR (ml/min)								
Mean (SD)	87	51.5 (14.4)	90	47.8 (15.4)	76	48.2 (14.7)	76	46.1 (17.0)
95% CI		48.4, 54.5		44.6, 51.0		44.8, 51.5		42.2, 49.9
P value*		0.104				0.418		
Change in mGFR from randomization (ml/min)								
Mean (SD)	87	5.6 (11.5)	90	0.0 (12.9)	76	1.3 (14.0)	76	−1.7 (15.4)
95% CI		3.2, 8.1		−2.7, 2.7		−1.9, 4.5		−5.3, 1.8
P value*		0.003				0.210		
Change in mGFR from month 12 (ml/min)								
Mean (SD)	–	–	–	–	71	−3.8 (9.8)	76	−2.7 (11.6)
95% CI						−6.1, −1.5		−5.3, −0.0
P value*						0.514		
eGFR								
MDRD (ml/min/1.73 m^2)								
Mean (SD)	83	65.0 (19.9)	82	59.4 (20.1)	89	60.1 (19.7)	79	57.4 (20.2)
95% CI		60.7, 69.3		54.9, 63.8		56.0, 64.2		52.9, 61.9
P value*		0.107				0.533		
eGFR Cockcroft-Gault (ml/min)								
Mean (SD)	80	45.4 (14.6)	71	43.1 (16.1)	86	45.6 (15.4)	72	42.1 (13.1)
95% CI		42.2, 48.7		39.3, 46.9		42.3, 48.9		39.1, 45.2
P value*		0.932				0.699		
On-treatment population								
mGFR (ml/min)								
Mean (SD)	37	55.3 (15.6)	62	49.2 (15.0)	37	52.8 (13.2)	62	47.0 (17.0)
95% CI		50.1, 60.5		45.4, 53.1		48.4, 57.2		42.7, 51.3
P value*		0.069				0.178		
Change in mGFR from randomization (ml/min)								
Mean (SD)	37	10.4 (10.6)	62	0.9 (11.4)	37	7.9 (11.5)	62	−1.4 (14.7)
95% CI		6.9, 14.0		−2.0, 3.8		4.1, 11.8		−5.1, 2.4
P value*		<0.001				0.001		
Change in mGFR from month 12 (ml/min)								
Mean (SD)	–	–	–	–		−2.5 (10.0)		−2.3 (11.9)
95% CI						−5.8, 0.9		−5.3, 0.7
P value*		–				0.926		

CI, confidence interval; eGFR, estimated GFR; IQR, interquartile range; mGFR, measured GFR; MDRD, Modification of Diet in Renal Disease; mITT, modified intent-to-treat; SD, standard deviation.

**P* value for difference between groups (Student's *t*-test).

uations were infection, proteinuria, hyperlipidemia, and edema.

The incidence of infections reported as adverse events was comparable in the everolimus group (55.4%) and control group (51.1%) (P = 0.560). Urinary tract infection occurred in 27.2% of everolimus patients versus 18.9% of controls, but no other marked differences in infections were observed. There were no cases of pneumonitis.

The proportion of patients receiving lipid-lowering therapy at month 36 was 73.3% (66/90) in the everolimus group and 62.8% (54/86) in the control arm (P = 0.135) (data were not available in all patients). Mean values for total cholesterol, LDL-cholesterol, and triglycerides decreased slightly in both groups from month 12 to month 36 with no significant difference in the extent of the decrease between treatment groups (Table 4). Other laboratory values showed no significant differences between the everolimus group and controls in terms of the changes from month 12 to month 36, other than a smaller decrease in platelet count in the everolimus arm (Table 4).

Five patients in each group developed a malignancy during months 12–36. The mean time to first diagnosis of

Table 4. Laboratory values (mITT population).

	Everolimus (n = 92)		Controls (n = 90)		
	Month 12	Month 36	Month 12	Month 36	P value*
Plasma creatinine, μmol/l	119 (35)	128 (40)	131 (45)	140 (58)	0.588
Urine protein:creatinine	26.4 (25.8)	36.2 (64.3)	29.5 (43.9)	25.9 (21.7)	0.826
Total cholesterol (mmol/l)	6.1 (1.7)	5.6 (1.4)	5.3 (1.1)	4.8 (1.2)	0.724
LDL-cholesterol (mmol/l)	3.6 (1.4)	3.2 (0.9)	3.1 (1.0)	2.8 (0.8)	0.762
HDL-cholesterol (mmol/l)	1.5 (0.5)	1.5 (0.4)	1.4 (0.4)	1.5 (0.5)	0.106
Triglycerides (mmol/l)	2.5 (1.6)	2.2 (1.3)	1.9 (0.9)	1.6 (0.9)	0.952
Alanine aminotransferase (IU/l)	27.4 (15.1)	31.4 (35.9)	22.4 (14.2)	27.7 (21.5)	0.854
Aspartate aminotransaminase (IU/l)	28.2 (10.5)	34.7 (37.5)	24.7 (11.9)	28.3 (15.4)	0.781
Fasting glucose (mmol/l)	6.4 (3.1)	6.1 (1.6)	5.8 (1.4)	6.0 (1.5)	0.132
Hemoglobin (g/dl)	13.3 (1.5)	13.3 (1.8)	13.5 (1.6)	13.5 (1.6)	0.748
White blood cell count ($\times 10^9$/ml)	6.9 (2.3)	7.1 (2.4)	7.6 (2.7)	7.5 (2.3)	0.478
Platelet count ($\times 10^9$/ml)	258 (61)	245 (71)	283 (73)	242 (56)	0.030

Values are shown as mean (SD).
LDL, low-density lipoprotein; HDL, high-density lipoprotein; mITT, modified intent-to-treat.
*P value for difference in change from month 12 to month 36 between treatment groups.

Table 5. Efficacy endpoints during months 12–36, n (%) (mITT population)

	Everolimus (n = 92)	Controls (n = 90)
Composite efficacy endpoint*	3 (3.3)	6 (6.7)
Death-censored graft loss	0	0
Death	1 (1.1)	3 (3.3)
Biopsy-proven acute rejection†		
IA	10 (10.9)	6 (6.7)
IB	2 (2.2)	4 (4.4)
Total	12 (13.0)	10 (11.1)

*Graft loss, death, and loss to follow-up.
†Unscheduled biopsy results.

malignancy was 36.5 and 35.5 months in the everolimus and control arms, respectively (log rank P = 0.899).

Discussion

Three-year data from this randomized, multicenter study demonstrate that abrupt conversion from CsA to everolimus 7 weeks after kidney transplantation was associated with a significant benefit for renal function if the everolimus-based regimen was continued, but not in the population overall. For those patients who remained on everolimus at 3 years, mean mGFR was approximately 9 ml/min higher than in the control group who continued to receive CsA. Graft and patient survival were similar between groups, but BPAR was significantly more frequent in the everolimus cohort during the first twelve months, largely accounted for by mild episodes of rejection. Everolimus was less well tolerated than CsA, with a higher rate of discontinuations due to adverse events.

The renal effect of switching from CNI therapy to an mTOR inhibitor has been documented to 4 years post-transplant for the CONCEPT trial [23] and to 3 years post-transplant for the ZEUS study [24], both based on eGFR values. In CONCEPT, kidney transplant recipients switched from CsA to sirolimus at 3 months or remained on a CsA-based regimen. In the ITT and on-treatment populations, the mean between-group difference in eGFR (Cockcroft-Gault) was 4.5 ml/min (P = 0.013) and 9.9 ml/min (P = 0.002), respectively [23]. In the ZEUS trial, conversion from CsA to everolimus took place at 4.5 months, and the mean difference in eGFR (Nankivell) between groups was 7.5 ml/min/1.73 m^2 and 13.8 ml/min/1.73 m^2 for the ITT and on-treatment populations (both $P < 0.001$) [24]. Interestingly, in our population, even the subgroup of patients with poor renal function (mGFR <40 ml/min) at baseline still showed a significant renal benefit at month 36 post-transplant versus those randomized to CNI therapy. This is in contrast to the CONVERT study, which found the risk:benefit profile for conversion to sirolimus at approximately 40 months post-transplant to favor those with eGFR >40 ml/min [18], but in line with evidence from the Rapamune Maintenance Regimen Study in which conversion took place earlier (month 3 post-transplant) [25]. While cross-study comparisons must always be regarded cautiously, these findings suggest that the earlier conversion from CNI therapy (at 7 weeks post-transplant) in the current study does not confer a greater benefit for preservation of renal function than later conversion, but that delaying beyond 6 months obviates a population-wide renal benefit [18,19].

The current study offers the advantage of directly measured GFR values, instead of the most usual estimated data.

While more challenging to undertake, this overcomes the known inaccuracy of estimated values [26,27]. Indeed, the mean values of mGFR versus GFR estimated by the MDRD and Cockcroft–Gault formulae in our population differed markedly (Table 3).

It is notable that the 12-month incidence of BPAR was not significantly higher in the mTOR inhibitor treatment group in either the CONCEPT study [15] or the ZEUS study [17]. Here, following conversion at 7 weeks post-transplant, the everolimus group had a significantly higher rate of BPAR at month 12 versus the control arm in the current trial (27.5% vs. 11.0%, $P = 0.004$). Most BPAR episodes in both treatment arms occurred during the first 10 weeks after randomization [14], as would be expected, after which no further divergence in BPAR rates was observed. The incidence of BPAR after month 12 was low in the mITT population, comparable in both treatment groups, and all episodes were mild. Nevertheless, the disparity in early rejection rates suggests that slightly later switch from CNI therapy may reduce the risk of early mild rejection episodes. Saturation of interleukin-2 receptors on activated T-lymphocytes by basiliximab declines after approximately 40 days [28], that is, shortly prior to switch from CNI takes place, compounding the rejection risk. If early conversion is undertaken, a higher initial target for everolimus C_0 concentration may be appropriate, and perhaps also a higher dose of EC-MPS than was chosen in this study.

There is experimental, as well as clinical, evidence demonstrating that mTOR inhibitors have antiproliferative effects that may inhibit the development of interstitial kidney graft fibrosis [29,30]. In our protocol biopsy analysis, we found a similar rate of fibrosis progression in both treatment arms. Although the analysis was performed only in the on-treatment subpopulation, this finding should be interpreted with caution as the number of patients was low and baseline biopsies already showed a high incidence of slight fibrosis, probably reflecting the age of our donor population. Other reports have indicated that progression of fibrosis may occur in some grafts even when using CNI-free, mTOR inhibitor-based regimens [31,32].

Results showed no significant difference in the presence of DSA between the everolimus and control arms at month 36, either overall or in the subgroup of patients who remained on study drug. This contrasts with recent reports from a retrospective analysis [33] and a single-center analysis [34] that suggested a higher rate of DSA in everolimus-treated patients at a median of approximate 3 years' follow-up. In those analyses, kidney transplant patients were converted at a later time point to everolimus with a CNI-free regimen than in the current trial. An adequately powered prospective trial is required to assess the relative effect of everolimus-based or CNI-based regimens on risk of *de novo* DSA development.

Interpretation of adverse event rates during months 12–36 should take into account that only those events which first occurred after month 12 were reported, as well as the fact that a sizeable proportion of patients were no longer receiving study drug. The safety profile of both drugs was consistent with expectations, including increased use of lipid-lowering therapy and greater dyslipidemia in the everolimus arm. The incidence of malignancies did not differ between groups. Fewer adverse events with a suspected relation to study drug were reported in the everolimus group compared to controls, with a similar rate of severe adverse events, findings which appear to contradict the higher rate of study drug withdrawals due to adverse events among everolimus-treated patients. It was notable that patients randomized to everolimus who switched back to CNI therapy did not see any renal benefit, underscoring the value of managing adverse events where possible before resorting to a change in immunosuppression.

The main limitation of this trial was the high rate of study drug withdrawals, mainly due to adverse events, in patients receiving everolimus, leaving only 43% on treatment after 3 years. Other studies of conversion to mTOR inhibitors, most of which undertook conversion at later time points after grafting, have also reported similar problems with various rates of discontinuation [13,15,16,18,19], although lower than here due to shorter follow-up. This may be partly explained by simple psychology and options when the patient presents with adverse events after switch to this new study drug, as compared to adverse events in the CNI arm where the only option is to change to another type of CNI with similar side effects. It is striking how much the rate of study drug discontinuation varied between centers. Where follow-up switched rapidly from the transplant center to the patient's local nephrology department (typically in Sweden and sometimes in Norway) withdrawal rates were high, while patients who were followed up by the investigator at the transplant center throughout (notably in Denmark) had very low withdrawal rates. Apparently, either the doctor's experience or, maybe more important, his or her motivation to follow the study protocol, strongly influenced this outcome. In one way, these differences could be interpreted positively; it is possible for adverse events to be managed by an experienced and dedicated physician. The relatively low number of patients who remained on everolimus treatment and frequent switches back to CNI therapy also made the statistical analysis and conclusions more difficult. However, we have partly overcome some of these problems by keeping most of the patients who discontinued study drug in the follow-up schedule and by undertaking both ITT and *post hoc* per protocol analyses.

These results show that kidney transplant patients who remained on everolimus therapy after conversion from CNI therapy at 7 weeks post-transplant continued to experience a clinically relevant improvement in renal function to 3 years post-transplant, although there was no obvious difference in the development of graft fibrosis. In the study population overall, however, the renal benefit observed at 1 year post-transplant was lost by 3 years. In the absence of randomized trials directly comparing time points for conversion, no robust conclusions regarding optimal timing can be drawn, but switching regimen at 7 weeks post-transplant does not appear to offer a renal advantage versus conversion between 3 and 6 months, and is associated with an increased risk of mild acute rejection with no histological improvement.

Authorship

LM, SSS, BvzM, BJ, JMH, CB, HA, BG and HH: performed the study and collected data. DS: acted as medical advisor. All authors critically reviewed the manuscript and approved it for publication.

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