ORIGINAL ARTICLE

5-Year outcomes of the prospective and randomized CISTCERT study comparing steroid withdrawal to replacement of cyclosporine with everolimus in de novo kidney transplant patients

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SUMMARY

Withdrawal of either steroids or calcineurin inhibitors are two strategies to reduce treatment-related side effects and improve long-term outcomes of kidney transplantation. The CISTCERT study compared the efficacy and safety of these two strategies. In this multicenter, randomized controlled trial, 151 incident kidney transplant recipients received cyclosporine (CsA), mycophenolic acid (MPA), and steroids during three months, followed by either steroid withdrawal (CsA/MPA) or replacement of cyclosporine with everolimus (EVL) (EVL/MPA/steroids). 5-year patient survival (89% vs. 86%; P = NS) and death-censored graft survival (95% vs. 96%; P = NS) were comparable in the CsA/MPA and EVL/MPA/steroids arm, respectively. ^{51}CrEDTA clearance was comparable in the intention-to-treat analysis, but in the on-treatment population, the EVL/MPA/steroids arm exhibited a superior ^{51}CrEDTA clearance at 1 and 5 years after transplantation (61.6 vs. 52.4, P = 0.05 and 59.1 vs. 46.2ml/min/1.73 m^2, P = 0.042). Numerically more and more severe rejections were observed in the EVL/MPA/steroids arm, which also experienced a higher incidence of posttransplant diabetes (26% vs. 6%, P = 0.0016) and infections. No significant differences were observed in cardiovascular outcomes and malignancy. Both regimens provide an excellent long-term patient survival and graft survival. Regarding graft function, EVL/MPA/steroids is an attractive strategy for patients with good tolerability who remain free of rejection. (ClinicalTrials.gov number: NCT00903188; EudraCT Number 2007-005844-26).

Transplant International 2021; 34: 313–326

Key words
calcineurin inhibitor, cyclosporine, everolimus, immunosuppression, kidney transplantation, mTOR inhibitors

Received: 17 September 2020; Revision requested: 13 October 2020; Accepted: 1 December 2020; Published online: 31 December 2020

doi:10.1111/tri.13798

Introduction

Nephrotoxic, cardiovascular, and metabolic adverse effects of immunosuppressive drugs are increasingly recognized to impair long-term outcomes of kidney transplantation. Calcineurin inhibitors (CNIs) cause nephrotoxicity, characterized by a functional decrease in renal blood flow and nonreversible histological lesions [1–3]. CNIs are associated with hypertension, hyperlipidemia, and posttransplant diabetes (PTDM) [4]. Steroids cause dyslipidemia and hypertension in kidney transplant recipients [4–7] and by inducing insulin resistance increase the risk for PTDM, which is associated with a reduced patient survival and graft survival [4–6,8–10].

One strategy to reduce the negative impact of immunosuppressants consists in steroid withdrawal in patients maintained on CNIs and mycophenolic acid (MPA) to avoid side effects of steroids and prevent PTDM [11,12]. An alternative strategy replaces CNIs with mTOR inhibitors (mTORi) to avoid chronic nephrotoxicity and reduce the long-term risk of cancer [13]. This is based on findings in preclinical models showing everolimus-induced inhibition of renal interstitial fibrosis and tubular atrophy [14,15]. Further support comes from reduced incidence of cardiac allograft vasculopathy in heart transplant patients treated with sirolimus and everolimus and from the lower incidence of posttransplant malignancies with mTORi [16–20]. Another potential advantage of mTORi is its anti-viral action against CMV and BK-polyomavirus infections [21].

Each strategy has been the subject of several trials and is currently used in clinical practice. However, they have not yet been evaluated in a comparative analysis of major clinical endpoints—that is, long-term renal function, rejection episodes, patient survival, and graft survival. The randomized controlled, multicenter CISTCERT study (CNI versus STeroid CEssation in Renal Transplantation) with 5-year follow-up was designed to address this question. In this trial, 151 patients were randomized, to convert at posttransplant month three to either steroid withdrawal or replacement of cyclosporine by everolimus. The primary endpoint was glomerular filtration rate at 1 year after transplantation.

Materials and methods

Study design and conduct

CISTCERT is a 5-year, prospective, multicenter, open-label, randomized controlled trial (RCT), conducted at 5 Belgian kidney transplant centers during the period October 2008 (FirstPatientFirstVisit) to September 2016 (LastPatientLastVisit), in which patients were randomized to discontinue either steroids or replace cyclosporine with everolimus at 3 months after transplantation. The study intended to compare maintenance immunosuppression regimens without steroids or CNI in terms of graft function, graft survival, graft histology, and surrogate markers for cardiovascular outcomes during the first five years after transplantation. The CISTCERT trial was conducted in compliance with the 2000 Declaration of Helsinki, the declaration of Istanbul 2008, and with the principles of Good Clinical Practice. The study was approved by ethics committees in all participating centers (Institutional Review Board approval number OG 085; protocol number CRAD 001ABE06T). The trial was registered in both ClinicalTrials.gov (NCT00903188) and EudraCT (2007-005844-26) registries.

Study population

Between October 2008 and September 2011, 155 adult recipients of a de novo renal allograft from a living or deceased donor were recruited. 151 patients were included in the study and randomly assigned at center level to one of the two treatment groups within 24 h prior to transplantation. After providing written informed consent, patients were randomized by opening numerically consecutive sealed envelopes containing treatment allocation generated by a validated automated procedure. Main exclusion criteria were highly immunized recipients and cytopenia. An overview of exclusion criteria is provided in Table S1. All patients had a complete 5-year follow-up, except those patients with any of following conditions: graft loss, death, withdrawal of consent, and lost to follow-up.

Immunosuppression

During the first three months of the study, all patients received the same immunosuppressive regimen consisting in anti-IL2 receptor mAbs (Simulect®), enteric-coated MPA (Myfortic®), methylprednisolone, and cyclosporine (CsA) (Neoral®) (Fig. 1). At transplantation, 76 patients were randomized to withdraw steroids at three months after transplantation while continuing on CsA and MPA, and 75 patients were randomized to replace CsA by EVL in combination with MPA and steroids (Fig. 1). Exclusion criteria for discontinuation of steroids or conversion from CsA to EVL included any episode of treated acute rejection, dialysis-

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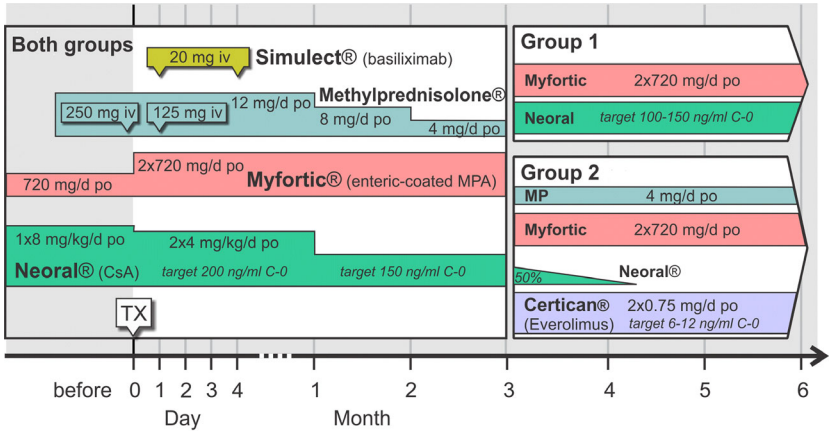

Figure 1 Overview of study medication. Target levels CsA during the first month after transplantation: 200 ng/ml (range 150–250 ng/ml) for C-0h and 1000 ng/ml (range 900–1100 ng/ml) for C-2h; during the second and third month 150 ng/ml (range 100–200 ng/ml) for C-0h and 900 ng/ml (range 800–1000 ng/ml) for C-2h. Group 1 (CsA/MPA arm): Target levels for CsA after 90 days: 100–150 ng/ml for C-0h and 750 ng/ml for C-2h. Discontinuation of steroids on day 90. Group 2 (EVL/MPA/steroids arm): everolimus started at three months after transplantation with simultaneous decrease of CsA-dose by 50% and discontinuation when EVL trough levels were within the therapeutic range. CsA—cyclosporine; MPA—enteric-coated mycophenolic acid; MP—methylprednisolone.

dependency, and any other medical condition precluding discontinuation of steroids or conversion to EVL in the opinion of the investigators. Patients randomized to the CsA/MPA arm who did not discontinue steroids, and patients randomized to the EVL/MPA/steroids arm who were not converted to everolimus, remained in the study and were analyzed on intention-to-treat (ITT) basis. Their treatment was left at the discretion of the local investigator. Per protocol all patients who received at least one day of the allocated regimen were analyzed as a modified intention-to-treat (mITT) population. Patient survival and graft survival and measured and estimated GFR are also reported for the full ITT population of all included patients.

Treatment of acute rejection

Treatment of acute rejection consisted in an IV bolus of methylprednisolone (500 or 1000 mg) on three consecutive days. In case of steroid-resistant acute rejection or vascular rejection, treatment with anti-thymocyte globulin (ATG) was given. Plasmapheresis plus intravenous immunoglobulin therapy was administered for the treatment of antibody-mediated rejection, at the discretion of the local investigator.

Concomitant therapies

Pneumocystis jirovecii prophylaxis was administered for the first three months after transplantation. CMV-prophylaxis or preemptive treatment was provided during the first three months according to center practice. The use of antihypertensive and/or lipid-lowering drugs was left at the discretion of the local investigator.

Renal biopsies

Renal biopsies at baseline and one year after transplantation were mandatory per study protocol. An additional protocol biopsy could be performed at 3 months at the discretion of the local investigator. Indication biopsies had to be performed in all suspected episodes of acute rejection. All graft biopsies were initially evaluated by a local pathologist, and then reviewed centrally by a dedicated nephropathologist (C.G.), who was blinded to the randomization and to the initial diagnosis at the local center.

Study endpoints

The primary endpoint of the CISTCERT study was the glomerular filtration rate (GFR) measured by ^{51}CrEDTA clearance at one year after transplantation. Graft function at one year after transplantation correlates with long-term graft function, long-term graft survival and patient survival [22–25]. A difference of 10 ml/min/1.73 m^2 in GFR is considered a clinically significant and a realistic target based on outcomes of other RCT [26–28].

Secondary endpoints were GFR estimated by the MDRD formula, patient survival and graft survival, rejection episodes, diabetes, malignancies, infections,

cardiovascular endpoints, and proteinuria. A detailed description of the secondary endpoints is provided in Table S2.

Statistical analysis

The sample size calculation for the primary endpoint predicted that a total population of 128 patients (64 patients per group) would provide an 80% power and two-sided significance level of 5% to detect a difference in GFR of 10 ml/min. Taking into account a dropout at the time of the conversion at 3 months of 15% of the initially included patients, 152 patients (76 patients per study group) needed to be included in the study. Analysis was performed on an intention-to-treat basis. The intention-to-treat (ITT) population was defined as the subset of all subjects who have been randomized and not censored at the moment of analysis. A modified intention-to-treat (mITT) population was defined as the set of all subjects, who have been randomized and treated with the allocated regimen for at least one day. All statistical analyses were performed in Medcalc version 18.11. Comparison between the two treatment arms was performed by means of an independent samples T test. In case of unequal distribution or low number of participants, statistical analysis was performed by the Mann-Whitney test. Patient survival and graft survival were estimated by a Kaplan–Meier survival analysis. Safety data were analyzed descriptively and were compared statistically by Chi-square and Fisher exact tests for comparison of 2 proportions. The protein/creatinine in urine was categorized ($\leq$0.5, 0.5–1.0, $\geq$1.0) and comparison between both groups was performed by Chi-square and Fisher exact tests for comparison of 2 proportions. All statistical tests were interpreted at the two-sided 5% significance level.

Results

Overview of patients and immunosuppressive regimens

At baseline, donor and recipient characteristics were well balanced between treatment arms except for a higher dialysis vintage, a trend for higher recipient age, and less NHBD in the CsA/MPA arm compared with the EVL/MPA/steroids arm (Table 1). 70 of the 76 patients who were allocated to the CsA/MPA and 54 of the 75 patients allocated to the EVL/MPA/steroids arm have been treated at least one day with the allocated treatment. 25 patients in the CsA/MPA arm (36%) and 20 patients in the EVL/MPA/steroids arm (37%) remained on the assigned treatment during the entire 5-year follow-up (Fig. 2). An overview of the reasons for discontinuation of study medication and main types of adverse events leading to discontinuation is provided in Tables S3 and S4. 53 patients in the CsA/MPA arm (76%) and 43 patients in the EVL/MPA/steroids arm (80%) completed the 5-year follow-up.

In both groups, blood concentrations of cyclosporine and everolimus were within the target limits of the study before and after randomization. Mean MPA doses were lower than intended in the protocol. MPA doses were significantly lower in the EVL arm throughout the entire follow-up (Table 2).

Table 1. Baseline donor and recipient characteristics (mITT analysis).

	CsA + Mycophenolate	Everolimus + Mycophenolate + Steroids	*P*
Number of recipients	70	54	
Recipient age (years)	55 ± 12	51 ± 11	0.09
Gender (M/F)	50/20	37/17	0.73
Ethnicity (Caucasian) (%)	94	94	1.0
Dialysis vintage (months)	30 ± 18	22 ± 17	0.0115
Panel reactive antibodies (%)	0.8 ± 3.5	0.5 ± 2.6	0.65
Number of HLA mismatches	2.7 ± 1.3	2.5 ± 1.2	0.56
Cold ischemia time (hours)	13 ± 5	14 ± 6	0.68
2nd warm ischemia time (min)	28 ± 10	30 ± 7	0.33
Donor age (years)	45 ± 13	46 ± 12	0.56
Type of donor (%)			
Brain dead	61	40	0.0337
Living	6	6	
Nonheart beating	3	8	

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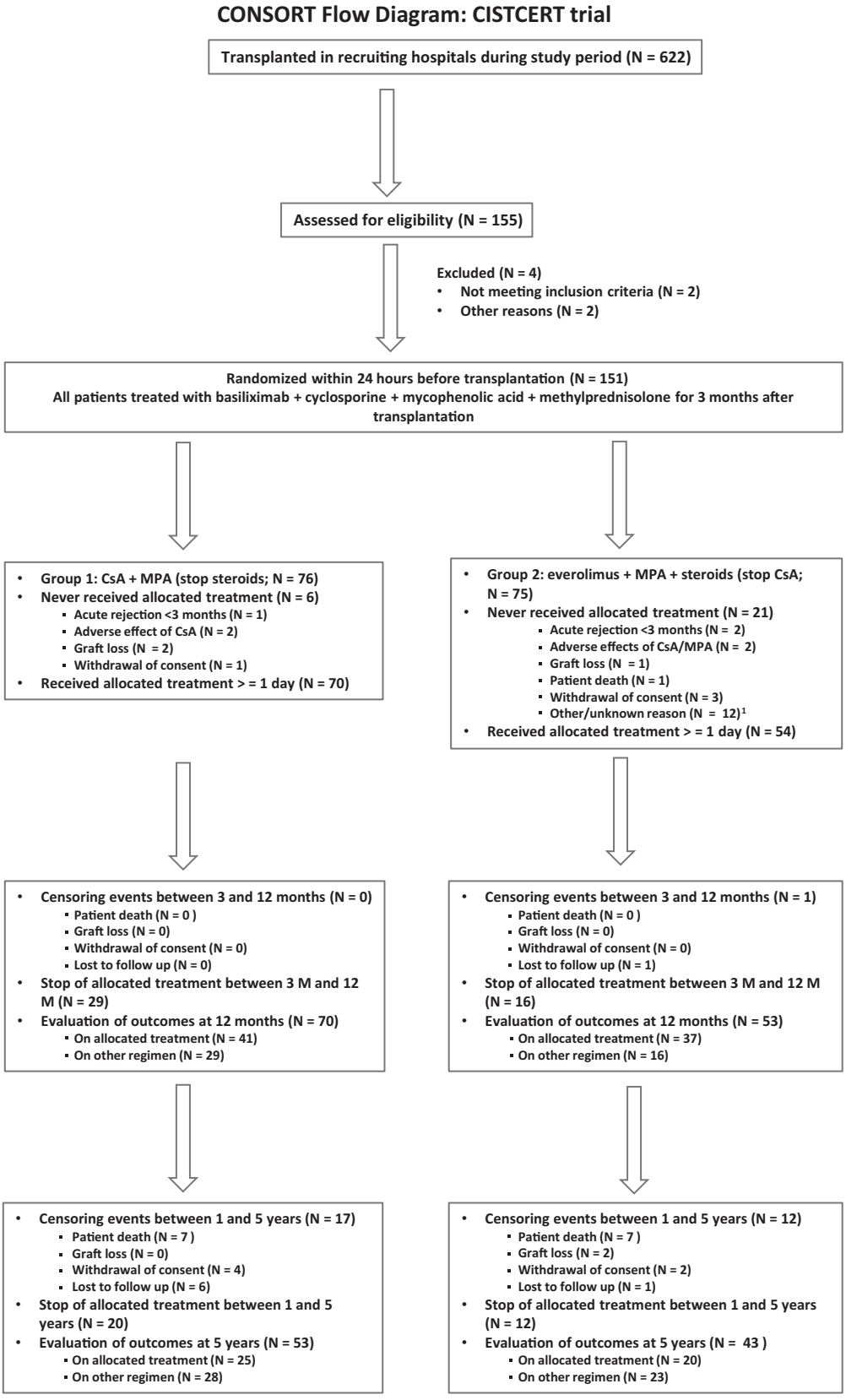

Figure 2 Study flow chart. All primary and secondary outcomes were evaluated/analyzed on the modified intention-to-treat population (mITT) defined as all patients who received at least one day of the allocated regimen. CsA, cyclosporine; MPA, mycophenolic acid. [1]deteriorating graft function: *N* = 2; prolonged delayed graft function : *N* = 2; wound healing problems: *N* = 3; administrative problems/unknown reasons: *N* = 5.

Table 2. Therapeutic drug monitoring data of cyclosporine (CsA) and everolimus (EVL)*,‡ (mITT analysis).

	W1	M1	M3	M6	Y1	Y2	Y3	Y4	Y5
CsA/MPA *n* = 70									
C0 (ng/ml)	234	247	209	187	157	164	133	145	163
C2 (ng/ml)	1085	1108	802	776	692	696	696	660	684
MPA† (mg/day)	1466	1466	1327	1270	1299	1253	1194	1199	1150
EVL/MPA/steroids *n* = 54									
C0 (ng/ml)	227	225	193						
C2 (ng/ml)	995	1088	945						
MPA† (mg/day)	1454	1480	1387	1219	1117	1038	1008	977	941
EVL (ng/ml)				7.8	8.7	8.0	6.7	6.9	7.7

*Results are expressed as arithmetic mean of available values

†Daily dose of mycophenolic acid (MPA)

‡Each time point included values from at least 75% of patients.

Graft function

As expected, the ^{51}CrEDTA clearance was similar in both treatment arms at 3 months. There was no significant difference in ^{51}CrEDTA clearance in the mITT and in the ITT analysis neither at 1y (primary endpoint) nor at 5y. We observed a significant difference of 9.2 ml/min/1.73 m^2 in the on-treatment (OT) analysis in favor of the group EVL/MPA/steroids at 1 year and of 12.9 ml/min/1.73 m^2 at 5 years (Table 3). MDRD clearance was comparable in both groups in both the ITT and the mITT analyses (Table 3).

Patient survival and graft survival

Survival outcomes were comparable in both groups, both in the mITT analysis (Fig. 3) and in the ITT analysis (Table S5). In the CsA/MPA arm and the EVL/MPA/steroids arm, respectively, 5-year death-censored graft survival was 95% and 96%, overall graft survival 85% and 83% (Fig. 3), and patient survival 89% and 86%.

Acute rejection

During the first three months after transplantation, 8 acute rejection episodes had occurred in 151 patients. After discontinuation of steroids or conversion to everolimus, respectively 5 biopsy-proven acute cellular rejections (BPAR) > borderline were diagnosed in 70 patients in the CsA/MPA arm and 8 BPAR > borderline in 54 patients in the EVL /MPA/steroids arm ($P = 0.238$). The number of borderline rejections was 4/70 in the CsA/MPA group compared with 1/54 in the EVL/MPA/steroids arm ($P = 0.27$). Two antibody-mediated rejections occurred in the CsA/MPA group and one in the EVL/MPA/steroids group. Overall, the incidence of severe acute rejections (≥ Banff 1B or ABMR) was 4/70 patients and 5/54 patients, respectively (Table 4, $P = 0.5$).

Proteinuria

Both mITT (Table S6) and OT analyses (data not shown) showed no significant differences in proteinuria between both groups, although there was a numerically higher proportion of patients with Urine Prot/Creat ≥ 1.0 g/g creatinine in the EVL/MPA/steroids group (16.6%) compared with the CsA/MPA group (8.6%). We also observed a trend to a higher proportion of patients treated with ACEI/ARB in the EVL/MPA/steroids group (70% vs. 53%; $P = 0.06$). In 3/54 patients, proteinuria was reported as a reason for discontinuation of everolimus (Table S4).

Cardiovascular endpoints

The incidence of major adverse cardiac events (MACEs) was 7/70 (10%) in the CsA/MPA group compared with 4/54 (7.4%) in the EVL/MPA/steroid group during the 5-year follow-up ($P = 0.61$). There were no significant differences between both groups in the mITT analysis for intima media thickness or left ventricular mass at baseline and at 5 years (results not shown). Blood pressure was well controlled during the 5-year follow-up without significant differences between both treatment arms. However, a trend for a lower blood pressure in the everolimus group could be observed at year 4 and 5 (Table S7).

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Table 3. ^{51}CrEDTA and MDRD clearance*.

	mITT			ITT			OT		
^{51}CrEDTA†	CsA /MPA $n = 70$	EVL/MPA/steroids $n = 54$	P	CsA /MPA $n = 76$	EVL/MPA/steroids $n = 75$	P	CsA /MPA	EVL/MPA/steroids	P
M 3‡	55.7 ± 18 (62)	55.6 ± 14 (51)	0.98	54.6± 18 (65)	53.5 ± 14 (61)	0.69	54.6± 18 (65)	53.5 ± 14 (61)	0.69
Y 1	51.8 ± 18 (53)	57.6 ± 18 (43)	0.11	51.5 ± 18 (55)	55.8± 17 (51)	0.21	52.4 ± 20 (33)	61.6 ± 17 (32)	0.050
Y 5	46.3 ± 17 (40)	54.3 ± 21 (30)	0.09	46.5 ± 17 (41)	52.5 ± 21 (37)	0.17	46.2 ± 16 (20)	59.1 ± 19 (13)	0.042

	mITT			ITT			OT		
MDRD†	CsA /MPA	EVL/MPA/steroids	P	CsA /MPA	EVL/MPA/steroids	P	CsA /MPA	EVL/MPA/steroids	P
M 3‡	51.0 ± 13 (68)	51.0 ± 12 (53)	0.99	50.0 ± 14 (71)	49.0 ± 12 (70)	0.66	50.0 ± 14 (71)	49.0 ± 12 (70)	0.66
Y 1	49.7 ± 11 (65)	54.1 ± 15 (49)	0.08	48.5 ± 12 (68)	51.7 ± 15 (63)	0.18	51.6±10 (38)	56.5 ± 12 (35)	0.07
Y 5	49.8 ± 17 (51)	54.2 ± 21 (42)	0.29	49.4 ± 17 (54)	52.6 ± 21 (52)	0.39	51.3±18 (23)	60.6 ± 22 (21)	0.13

M3—3 months after transplantation, Y1—1 year after transplantation, Y5—5 years after transplantation, MITT—modified intention-to-treat, ITT—intention-to-treat, OT—on-treatment

*Mean ± standard deviation

†Results are expressed as ml/min/1.73 m^2

‡At three months after transplantation, the OT population does not differ from the ITT population. At this moment, patients in both treatment arms still received standard triple therapy with CsA/MPA/steroids.

Posttransplant diabetes mellitus

The incidence of PTDM during the 5 years follow-up was significantly lower in the CsA/MPA arm than in the EVL/MPA/steroid arm (4/70 (6%) vs. 14/54 (26%), $P = 0.0016$).

Infections and malignancies

The incidence of infections and the incidence of serious adverse events (SAEs) associated with an infection was significantly higher in the everolimus treatment arm ($P = 0.04$ and $P = 0.015$, respectively). More urinary tract ($P = 0.0005$) and less pulmonary ($P = 0.025$) infections were observed in the CsA/MPA arm than in the EVL/MPA/steroids arm (Table 5). There was a trend toward a higher incidence of malignancy (solid organ and skin tumors) in the EV/MPA/steroids arm compared with the CsA/MPA arm during the 5-year follow-up in the mITT analysis ($P = 0.06$). The majority of skin tumors were basocellular and squamous cell carcinoma and were diagnosed during the last 2 years of follow-up. The proportion of patients developing at least one malignancy was comparable in both groups ($P = 0.1$) (Table 5).

Kidney biopsies

At baseline, the proportion of normal implantation biopsies was comparable between both groups: 66% in the CsA/MPA arm and 63% in the EVL/MPA/steroids arm (Table S8). At 1 year, protocol biopsies were available in 24 patients of the CsA/MPA group and 18 patients of the EVL/MPA/steroids group with no significant difference in chronic histological damage between the two groups (Table S9).

Discussion

Primary endpoint

Notwithstanding some rare exceptions [29], the majority of previous clinical trials [13,27,30–33], and a meta-analysis [34] have reported improvements in GFR after

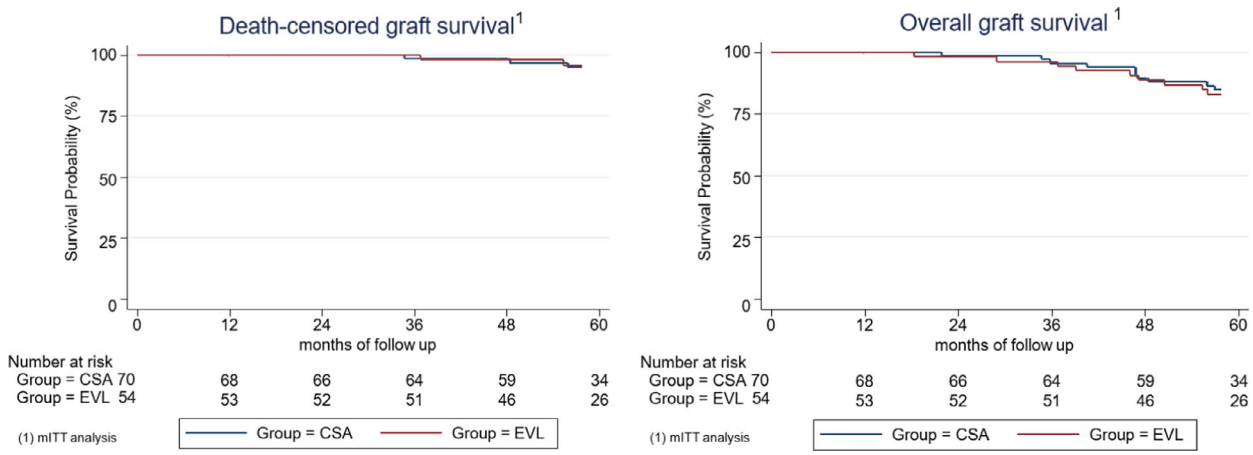

Figure 3 Death-censored graft survival and overall graft survival (mITT analysis). CsA, cyclosporine; EVL, everolimus.

Table 4. Biopsy-proven acute rejection (BPAR) episodes after 3 months and treatments administered for acute rejection*.

	CsA/MPA *n* = 70	EVL/ MPA/Steroids *n* = 54
BPAR ≥ 3months		
Total	11	10
Classification	4 Borderline	1 Borderline
	3 Banff IA	4 Banff IA
	1Banff IIA	1 Banff IIA
	1 Banff IIB	3 Banff IIB
	1ABMR GrI	1 ABMR Gr II +
	1 ABMR Gr II	Borderline changes
Treatment†	7 MP	5 MP
	1 MP + ATG	2MP + ATG + PEX + Ivlg
	2MP + PEX + Ivlg	3 MP + PEX + Ivlg
	1 MP + IV Ig	

During the first 3 months with all patients receiving CsA/MPA/steroids, 8 BPAR occurred in 151 patients.

*mITT analysis.

†MP, methylprednisolone; PEX, plasma exchange therapy; ATG, anti-thymocyte globulin; Ivlg, intravenous immunoglobulins.

replacement of a CNI by an mTORi. The beneficial effect of the conversion, however, was often limited to the on-treatment population in several studies [31–33,35].

In the CISTCERT trial, the primary endpoint was not met, since the 6 ml/min/1.73 m^2 improvement in ^{51}CrEDTA clearance of the EVL/MPA/steroids group at 1 year in the mITT analysis did not reach statistical significance. However, this lack of effect probably reflected the fact that a large proportion of patients in the ITT population in this arm received calcineurin inhibitor–based therapy. Analysis of the on-treatment data of patients who remained on EVL/MPA/steroids documented an improvement of 9 ml/min in ^{51}CrEDTA clearance that was statistically significant at one year and persisted during the 5-year follow up. Conversion toward EVL/MPA/steroids might, therefore, be a good strategy for a subset of patients, who experience good tolerability and remain free of rejection under an EVL/MPA/steroid treatment. This subset of patients was relatively small in our study (37% after 5 years) because the majority of patients had been reconverted toward another immunosuppressive regimen for a variety of reasons. Nevertheless, taking into account the excellent overall graft survival and patient survival observed in our trial, an attempt for conversion toward EVL/MPA/steroids might be justifiable in selected patients at low immunological risk.

Patient survival and graft survival

Our study shows excellent and equivalent long-term patient survival (PS) and graft survival (GS) for both treatment arms (5-year PS of 89 and 86% and death-censored GS of 95% and 96% in the CSA/MPA and EVL/MPA/steroids arm, respectively), compared with the recently published European 5-year death-censored kidney graft survival rate (84.4%) [36]. Our outcomes are similar to those of many other conversion trials [13,30–32] and a meta-analysis [34]. To our best knowledge, the CISTCERT trial is the first interventional trial in kidney transplantation with 5-year follow-up demonstrating the safety of steroid withdrawal in terms of graft survival in low-risk patients receiving CsA in combination with MPA.

Table 5. Adverse events.

	CsA/MPA $n = 70$(%)†	EVL/MPA/steroids $n = 54$ (%)†	*P*
Infections : incidence and type*			
Total	226	212	0.04
Urinary tract	90 (39%)	57 (24%)	0.0005
Pulmonary	31 (14%)	53 (22%)	0.025
Gastrointestinal	14 (6%)	25 (10%)	0. 11
Ear, nose, and throat	34 (15%)	27 (11%)	0.19
Dermatological	19 (8%)	29 (12%)	0.15
Blood/lymph	6 (3%)	5 (2%)	0.49
CMV§	18 (8%)	18 (7%)	0.68
BKV¶	10 (4%)	9 (4%)	1
Musculoskeletal	3 (1%)	5 (2%)	0.38
Wound	1 (0.4%)	1 (0.4%)	1
Other	2 (0.8%)	3 (1.2%)	0.66
SAE due to infection	49 (21.7%)	68 (28.2%)	0.0015
Incidence of malignancies and type of malignancy (number of malignancies)*			
Overall	14	19	0.06
Solid tumor	8	10	0.26
Skin	6	9	0.98
Patients developing at least one malignancy	11 (15.7)‡	15 (27.7)‡	0.1

*mITT analysis

†%: percentage of total number of infections

‡Proportion of patients

§CMV replication or disease not specified in AE reports

¶BKV replication or nephropathy not specified in AE reports

Rejection

The incidence of acute rejection of the overall cohort during the first three months was low (8/151; 5.3%), confirming the excellent efficacy of basiliximab in combination with standard immunosuppressive therapy. A significant proportion of patients in both groups developed acute rejection after either steroid withdrawal or replacement of CsA with EVL. Steroid withdrawal in combination with tacrolimus is associated with a minimal increase in the risk of acute rejection [37,38]. The present study confirms previous reports that steroid withdrawal in patients treated with cyclosporine is associated with a significant risk of acute rejection [39,40]. The higher risk of acute rejection of mTORi-based immunosuppression as compared to standard triple therapy is well documented [13,29,31,34,41–45]. The current protocol directly compared mTORi-based immunosuppression to steroid avoidance in combination with cyclosporine. The number of rejection episodes was numerically higher in the EVL/MPA/steroids arm, but the difference did not attain statistical significance. Although not powered to detect differences in acute rejection, the current study, nevertheless, allows to conclude that both strategies imply a relatively high risk of late and sometimes severe acute rejection in a population selected to be at low immunological risk.

Unfortunately, data on anti-HLA antibodies were not collected in the CISTCERT study, and in many other randomized controlled trials that were designed in the same period. However, retrospective data of conversion to CNI-free EVL–based regimens [46,47] and limited data available from randomized controlled trials [13,29,45,48] raise concerns about the development of de novo donor–specific anti-HLA antibodies (DSA) and antibody-mediated rejection. In the light of current knowledge about the detrimental effect of DSA, we would now consider pre-existing DSA as a contraindication for participation to the CISTCERT trial, and in case of occurrence of de novo DSA, we would no longer consider it safe to convert to one of the proposed minimization strategies.

Diabetes

The incidence of PTDM was significantly lower in the steroid withdrawal group remaining on cyclosporine compared with the patients that were converted to

everolimus and continued steroids. We attribute this difference mainly to the discontinuation of steroids, although a role of the known diabetogenic effect of mTORi (combined to low-dose steroids) cannot be excluded [44,49].

Several studies have shown a benefit of early steroid withdrawal and steroid avoidance on the incidence of posttransplant diabetes [50,51]. Our data are in line with those in two meta-analysis showing that both late steroid withdrawal and steroid avoidance in patients treated with CsA were associated with a 50% reduction in PTDM although at the price of a significant increase in acute rejection episodes [39,52].

Infections

We observed a significantly higher incidence of infections in the group of patients converted to everolimus. This observation is in contrast to other conversion trials [13,29,31,33] and a meta-analysis [44]. While we observed more genito-urinary infections in the cyclosporine arm and more pulmonary infections in the everolimus arm, a finding that might have been confounded by pulmonary toxicity of everolimus. Importantly, the incidence of infections reported as SAEs was significantly higher in patients who converted from cyclosporine to everolimus. This was mainly due to a higher hospitalization rate for parenteral antibiotherapy. No difference was observed in the incidence of CMV-infections between both treatment arms. The strength of these data is, however, limited due to the absence of standardized diagnostic criteria for the diagnosis of CMV-infection in the study protocol. The reported incidence of BK-polyomavirus infections was equal in both treatment arms, but the numbers were very low.

Malignancy

We observed a numerically higher incidence of overall malignancies (skin and solid organ tumors) in patients converted from cyclosporine to everolimus compared with patients remaining on cyclosporine and MPA, although for both outcomes the difference did not reach statistical significance. Interpretation remains, however, difficult due to the small sample size and the relatively large number of patients who discontinued study treatment. In fact, most patients who developed a skin tumor in the everolimus arm had previously discontinued study treatment, and had been re-converted to a CNI.

Cardiovascular endpoints

We did not detect significant differences in cardiovascular endpoints between both treatment arms, but the trial was likely underpowered to detect differences in rare events such as MACEs. Our results confirm the reports by three other RCTs that have failed to demonstrate relevant effects on cardiovascular end points after conversion from CNI to an mTORi-based CNI–free regimen [53–55].

Histology

In terms of development of allograft fibrosis, the conversion from a CNI toward an mTORi was beneficial in one previous RCT [27] but not in another [45] . We were unable to detect a lower incidence of chronic histological lesions in patients who were converted to everolimus as compared to patients remaining on cyclosporine without corticosteroids. We acknowledge that the low number of available protocol biopsies at 1 year limits a reliable comparison of the long-term histologic effects of both treatment arms.

Discontinuations and tolerability

By the end of the 5-year follow-up of our study, the proportion of patients who had discontinued study treatment was high but comparable in both treatment arms (64% in the CsA/MPA arm and 63% in the EVL/MPA/steroids arm). Historically, high rates of discontinuations have been reported in many conversion trials [27,29,31–33,35,56] and occurred predominantly in the mTORi arm as a consequence of poor tolerance or adverse events [27,29,32,56]. In our study, there was a trend for a higher number of discontinuations as a consequence of adverse events in the everolimus group, although this was not significant.

Strengths and limitations

In the EVL/MPA/steroids arm, the number of patients that had been initially randomized, but failed to be converted from CsA to EVL at three months after transplantation according to the study protocol, was higher than the 15% predicted. As a consequence, the study was slightly underpowered as to the primary efficacy endpoint. Primary and secondary outcomes were analyzed on a "modified intention-to-treat" (mITT) population, defined as the subset of patients who had taken the allocated treatment for at least one day. This

mITT population corresponds to the ITT population of the Zeus study, in which the efficacy analysis was performed on all patients who were randomized at 4.5 months after transplantation and who received at least one dose of any immunosuppressive drug [35]. In our study, the mITT population included 70 patients in the CsA/MPA arm and 54 patients in the EVL/MPA/steroids arm. The unequal number of patients (70 and 54) in both treatment arms could reflect a selection bias. However, the results of mITT and ITT (defined as all randomized patients at the time of transplantation) were comparable for both the primary endpoint and the survival analysis (Table 3 and Table S5). The sample size of our study might have been too small to discover statistically significant differences for secondary endpoints such as long-term graft function, incidence of rejection, and malignancies. The frequent crossover between both treatment strategies, with re-introduction of a CNI in the EVL/MPA/steroids arm, and re-introduction of steroids in the CsA/MPA arm represents an important limitation when outcome of these strategies is under evaluation. However, frequent adaptation of medication according to signals of over- and under-immunosuppression and drug tolerance reflects current clinical practice where tailored immunosuppression and precision medicine are increasingly recommended [57–59].

Although our study shows that replacement of CsA with EVL results in significantly improved graft function up to 5 years in those patients tolerating EVL without treatment failure, it was not powered to identify predictors of treatment failure. However, the excellent graft survival and patient survival overall in the EVL/MMF/steroids arm suggest that conversion can be attempted in selected patients with acceptable risk.

Another limitation of our study is the lack of a CNI-based triple therapy control group. The present study was indeed designed to compare two interventions aiming at either improving metabolic side effects or renal function after renal transplantation. The important question whether each of these two interventions improves outcomes as compared to standard of care therapy had been previously investigated by several large-scale intervention trials [13,39,60].

To our knowledge, this is the first study in kidney transplantation that prospectively compares the long-term outcomes of steroid withdrawal to CNI withdrawal. The 5-year follow-up, multicenter, and randomized controlled design reinforces the validity and credibility of the results.

Conclusions

In the CISTCERT trial, an advantage in graft function after conversion toward EVL/MPA/steroids could only be observed in a select group of patients, that is, those who were able to remain on treatment. Patient survival and graft survival were excellent for both immunosuppressive strategies. A relatively high number of rejections occurred in both treatment arms, with numerically more and more severe rejections in the EVL/MPA/steroids arm. Dual therapy with CsA and MPA was associated with fewer serious infections as compared to the EVL/MPA/steroids regimen. Steroid cessation in the CsA/MPA arm was associated with a significantly lower incidence of PTDM. The CISTCERT trial did not show a benefit of conversion to an mTORi in terms of malignancy, cardiovascular outcomes or graft fibrosis, but the trial was not powered to detect these differences. We conclude that the CISTCERT trial provides evidence for the feasibility of conversion to any of the investigated immunosuppressive regimens based on the individual recipient's needs and risk profile. Regarding graft function, EVL/MPA/steroids is an attractive strategy for patients with good tolerability who remain free of rejection.

Part of this work was presented at:

1. The Annual Meeting of the Belgian Transplantation Society 2017 Brussels
2. The Annual ERA-EDTA Congress 2017 Barcelona

Authorship

LP: performed the analysis of the data and the writing of the paper. DA: participated in the performance of the study and the writing of the paper. NB: participated in the performance of the study. AL: participated in the performance of the study. PP: participated in the performance of the study. SVL: participated in the performance of the study and the writing of the paper. LEW: participated in the performance of the study. JS: participated in the performance of the study and the writing of the paper. KMW: participated in the performance of the study, data analysis, and writing of the paper. CG: participated in the performance of the study. J-LB: principal investigator, participated in study design, acquisition and analysis of data, the performance of the study, and the writing of the paper.

Funding

Novartis S.A. provided financial support for this trial. The sponsor had no part in data collection, data

management, data analysis, and in drafting of the manuscript.

Conflict of interest

The authors have no conflict of interest to declare.

Acknowledgements

The authors thank Nadia Fenners for her excellent secretarial assistance, and Dirk Deweerdt for the design of Fig. 1.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 Exclusion criteria.

Table S2 Secondary endpoints.

Table S3 Reasons for discontinuation of randomized treatment (mITT analysis).

Table S4 Most frequent type of adverse event (A E > n = 1) leading to discontinuation of study medication (mITT analysis).

Table S5 5-years survival Analysis ITT POPULATION.

Table S6 Proteinuria : number (proportion) of patients according to highest value of urine Prot/Creat ratio on a spot urine sample during 5 years follow-up (mITT analysis).

Table S7 Systolic (SBP) and diastolic (DBP) blood pressure (mmHg) (mean ± SD) (m ITT analysis).

Table S8 Histologic characteristics of baseline biopsies (mITT analysis).

Table S9 Histologic characteristics of protocol biopsies at 1Y (m ITT analysis).

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