TRANSPLANT INTERNATIONAL

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Minimization of maintenance immunosuppressive therapy after renal transplantation comparing cyclosporine A/azathioprine or cyclosporine A/mycophenolate mofetil bitherapy to cyclosporine A monotherapy: a 10-year postrandomization follow-up study

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Keywords
cyclosporine A, kidney transplantation, minimization, steroid-free maintenance immunosuppression.

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Conflict of interest
The authors have declared no conflicts of interest.

Received: 19 March 2015
Revision requested: 13 April 2015
Accepted: 15 June 2015

doi:10.1111/tri.12627

Summary

Long-term outcomes in renal transplant recipients withdrawn from steroid and submitted to further minimization of immunosuppressive regimen after 1 year are lacking. In this multicenter study, 204 low immunological risk kidney transplant recipients were randomized 14.2 ± 3.7 months post-transplantation to receive either cyclosporine A (CsA) + azathioprine (AZA; $n = 53$), CsA + mycophenolate mofetil (MMF; $n = 53$), or CsA monotherapy ($n = 98$). At 3 years postrandomization, the occurrence of biopsy for graft dysfunction was similar in bitherapy and monotherapy groups (21/106 vs. 26/98; $P = 0.25$). At 10 years postrandomization, patients' survival was 100%, 94.2%, and 95.8% ($P = 0.25$), and death-censored graft survival was 94.9%, 94.7%, and 95.2% ($P = 0.34$) in AZA, MMF, and CsA groups, respectively. Mean estimated glomerular filtration rate was 70.4 ± 31.1, 60.1 ± 22.2, and 60.1 ± 19.0 ml/min/1.73 m^2, respectively ($P = 0.16$). The incidence of biopsy-proven acute rejection was 1.4%/year in the whole cohort. None of the patients developed polyomavirus-associated nephropathy. The main cause of graft loss ($n = 12$) was chronic antibody-mediated rejection ($n = 6$). *De novo* donor-specific antibodies were detected in 13% of AZA-, 21% of MMF-, and 14% of CsA-treated patients ($P = 0.29$). CsA monotherapy after 1 year is safe and associated with prolonged graft survival in well-selected renal transplant recipient (ClinicalTrials.gov number: 980654).

Introduction

Minimization of immunosuppressive drugs in stable kidney transplant patients is required to reduce the consequences of long-term over-immunosuppression, particularly cancer, infection, and cardiovascular complications. Calcineurin inhibitors (CNI) minimization protocols have been widely used over the past 15 years to reduce the suspected chronic nephrotoxicity of these drugs [1,2]. Corticosteroid minimization or withdrawal is also commonly prescribed to

prevent cardiovascular and skeletal complications [3,4]. Minimization of antimetabolites is less common although patients are usually tapered to 1 g/day of mycophenolate mofetil (MMF) after few weeks or months post-transplant. However, long-term follow-up studies are missing to establish the benefit of such minimization strategies.

On the other hand, the concept of minimization is debated, as cyclosporine A (CsA) nephrotoxicity is no longer considered as a main cause of graft loss [5,6]. Second, recent advances in the detection of anti-HLA antibodies and classification of histological lesions have pointed out chronic humoral rejection, potentially favored by insufficient immunosuppressive regimen as a major cause of kidney allograft dysfunction and loss [7–9].

We have previously defined clinical predictors of successful minimization in low-risk patients [10,11]. Applying these criteria, we designed 18 years ago an open-label randomized multicenter study comparing three maintenance immunosuppressive strategies [CsA-azathioprine (AZA), CsA-MMF, and CsA monotherapy] in kidney transplant recipients selected for low risk of graft dysfunction. We herein present long-term results of minimization in this cohort of transplant recipients, who, after completion of the 36-month study, underwent yearly follow-up assessments until 10 years postrandomization.

Patients and methods

This is a multicenter, academic, prospective, randomized study conducted at five renal transplantation centers in France from September 1999 to June 2012. The study was initially designed as a prospective study with primary end point analyzed at 3 years postrandomization. After the 36-month study visit, patients were enrolled in a follow-up study on the basis of an annual observational visit until 10 years postrandomization. Written informed consent was obtained from all patients before enrollment for the whole study duration. The study, conducted in compliance with Good Clinical Practice and approved by the coordinating center ethic committee, is registered at www.clinicaltrials.gov (NCT 980654).

Study population

Inclusion criteria were as follows: first renal transplantation from deceased donor in an adult Caucasian recipient, low immunological risk recipients with historical or pretransplant peak panel-reactive antibodies (PRA) detected by lymphocytotoxicity (IgG anti-T) ≤25%, and donor age ≤45 years. Criteria for randomization between 12 and 24 months post-transplant were as follows: up to one steroid-sensitive cellular acute rejection episode during the first year, maintenance therapy with CsA and MMF, steroid withdrawal for at least 3 months and stable serum creatinine level ≤125 μmol/l, and/or a estimated glomerular filtration rate (eGFR) ≥50 ml/min/1.73 m^2.

Drug regimens

All patients received induction therapy with antithymocyte/lymphocyte antibodies (Thymoglobulin®, Lymphoglobulin®; InstitutMérieux, Lyon, France). The initial immunosuppressive regimen comprised CsA microemulsion (Neoral®; Novartis Pharma AG, Basel, Switzerland) dosed according to prespecified target ranges for through concentration (C_0) between 100 and 200 ng/ml, MMF: 2 g/day (Cellcept®; Roche Pharma AG, Basel, Switzerland) and corticosteroid withdrawal at 3–6 months post-transplant according to previously described criteria [10].

At 11–24 months post-transplant, eligible patients were randomized into three groups CsA–AZA, CsA–MMF, and CsA monotherapy into a 1:1:2 ratio, using a centralized validated system. From randomization, the CsA C_0 target was 75–125 ng/ml whatever the group. In the AZA group, MMF was abruptly switched to AZA (1–2 mg/kg/day) over 1 day. MMF dose was tapered biweekly or monthly by 500 mg, until 1 g/day in the MMF group, or withdrawal over 2–4 months in the CsA group. After randomization, patients had clinical assessment every 4 weeks until 2 years post-transplant, every 6 weeks until 4 years and then every 8 weeks. CsA trough level was measured at each visit to assess compliance. After the 36-month study visit, the assigned immunosuppressive regimen was to be maintained, but changes were permitted at the discretion of the investigator.

Study end points

The primary end point was the occurrence of biopsies performed for graft dysfunction and/or proteinuria ≥1 g/day at month 36 after randomization. Graft dysfunction was defined as an increase in mean serum creatinine level (calculated on three consecutive measures from the same laboratory) ≥20% from baseline value at the inclusion, excluding dysfunction secondary to urinary tract obstruction or graft artery stenosis. eGFR was calculated using the Cockcroft–Gault formula adjusted for body surface area [12]. Two pathologists centrally reviewed all allograft biopsies. Lesions were classified according to the 1997-revised Banff classification [13]. CNI nephrotoxicity was diagnosed if striped interstitial fibrosis and tubular atrophy, medial arteriolar hyalinosis, and/or tubular/interstitial microcalcifications were observed [14]. Efficacy analyses were performed in the intent-to-treat (ITT) population. The ITT population comprised all patients who were randomized between 11 and 24 months post-transplant, had received at

least one dose of any immunosuppressive drug, and at least an available postbaseline assessment of the primary efficacy variable. The per-protocol population was defined as all ITT patients who remained on the assigned treatment.

Secondary end points were the incidence of biopsy-proven acute rejection (BPAR) and CsA nephrotoxicity episode, patient and graft survival, and renal function evaluated by calculated creatinine clearance (Cockcroft and Gault formula).

After the initial 36-month study, data collection at each annual follow-up visit for patients that entered the 10-year follow-up study comprised the following: patient and graft survival status; routine hematology and biochemistry assessment; type and dosage of immunosuppressive drugs, CsA C_0 concentrations, BPAR, infection, diabetes, cancer, and cardiovascular events. Post-transplantation diabetes mellitus was defined as fasting plasma glucose ≥126 mg/dl or 2-h plasma glucose after oral glucose ≥200 mg/dl. Severe cardiac events were defined as acute coronary syndrome, symptomatic severe arterial stenosis, and sudden cardiac death/acute circulatory failure.

De novo donor-specific HLA antibodies (dnDSA) were determined using LABScreen Single Antigen beads (One Lambda, Canoga Park, CA, USA), retrospectively on frozen serum samples at randomization, and prospectively at the end of the follow-up period.

Statistical analysis

This study was initially designed to test the hypothesis that in a well-selected population, a minimized immunosuppressive regimen with CsA–AZA or CsA–MMF bitherapy was superior to CsA monotherapy at 36 months.

With a two-side alpha of 5%, 100 patients per arm were needed to achieve 80% power and to detect a difference of 10% at 3 years on the number of patients with biopsied graft dysfunction episode.

In the long-term follow-up study, each study group (AZA, MMF, and CsA) was compared separately.

Quantitative variables are expressed with means ± standard deviation or median [interquartile range (IQR) = quartile3–quartile1], and qualitative variables are given as number and percentage of patients. Comparison was performed using Students *t*-test for normally distributed variables, Mann–Whitney *U*-test for nonparametric variables, and chi-square or Fisher exact test for qualitative variables.

Death, graft lost, and BPAR-free survival curves were generated with actuarial method. Death with functional graft was considered for estimating graft lost-free survival curves as censored data. Survival curves were compared using the log-rank test. *P*-values <0.05 were considered to be significant. All analyses were performed using the SAS version 9.3 software package (SAS Inc, Cary, NC, USA).

Results

Baseline characteristics

Between July 1998 and January 2004, 207 eligible patients were selected. Three patients were excluded from further analysis (exclusion criteria: $n = 2$, withdrawal of consent: $n = 1$). Thus, the ITT population included 204 patients: 106 received bitherapy (AZA group: $n = 53$, MMF group: $n = 53$) and 98 patients received monotherapy (CsA group) (Fig. 1).

Baseline recipient characteristics including donor and recipient age, HLA incompatibilities, CMV status, mean time on dialysis, and cause of end-stage renal disease were similar in AZA, MMF, and CsA groups (Table 1). Steroid withdrawal was effective 5.9 ± 2.4 months post-transplant in the whole population (5.5 ± 1.9, 6.1 ± 2.7, and 6.0 ± 2.5 months in AZA, MMF, and CsA groups, respectively), all patients being free of steroids at randomization. The occurrence of BPAR episodes during the first year was similar: 8%, 9%, and 5% in AZA, MMF, and CsA groups, respectively, with a mean interval of 1.2 ± 0.7, 2.9 ± 4.3, and 2.8 ± 2.3 months post-transplant.

Randomization was performed at 14.5 ± 5.3, 13.8 ± 4.5, and 14.4 ± 4.2 months post-transplantation in AZA, MMF, and CsA groups, respectively (Table 2).

Results at 36 months

As designed in the protocol, the primary end point was analyzed at 36 months in the ITT population. Of note, at 3 years postrandomization, 91 of 104 (88%) patients under bitherapy and 77 of 96 (80%) patients in the CsA monotherapy group were still receiving the initially allocated study drug. The number of graft biopsied for graft dysfunction (≥20% increase in mean serum creatinine level and/or proteinuria ≥1 g/day) was 21 of 106 (20%) patients and 26 of 98 (27%) in bitherapy and monotherapy, respectively, without difference between the two strategies ($P = 0.25$). Secondary end points did not differ among the groups at 36 months, including BPAR [8 (8%) and 11 (11%), $P = 0.36$] and CsA toxicity [10 (9%) and 14 (14%), $P = 0.28$] in bitherapy and monotherapy groups, respectively (Table 3). Results were similar when we compared the three groups of treatment (data not shown). Death-censored graft survival at month 36 was 100% in bitherapy and monotherapy groups. eGFR was not significantly different: 69.8 ± 18.6 in patients under bitherapy and 69.4 ± 21.9 ml/min/1.73 m^2 on monotherapy ($P = 0.68$).

10-year follow-up study

Immunosuppression

The randomly assigned regimen was maintained in 70%, 66%, and 61% of patients at 10 years. At the end of follow-up, of

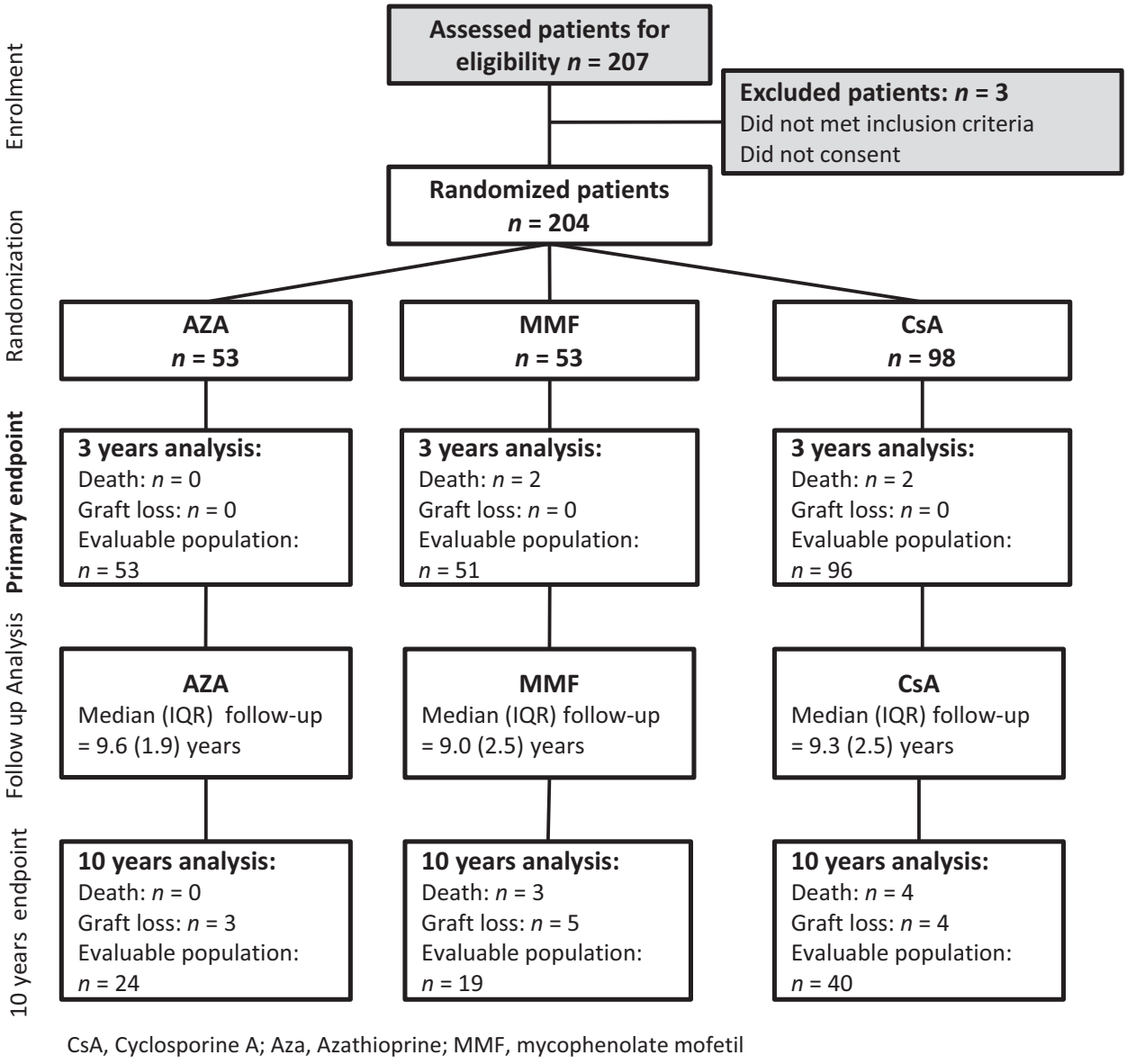

Figure 1 Patient disposition. AZA, azathioprine; CsA, cyclosporine A; MMF, mycophenolate mofetil.

204 patients, only 21 (10%) received steroids: 4%, 10%, and 15% in AZA, MMF, and CsA groups, respectively. There was no significant difference in CsA dose ($P = 0.08$) and CsA C_0 ($P = 0.41$) between the three groups (Table 4 and Fig. 2a and b).

Patient survival

Median (IQR) follow-up duration was as follows: 9.6 (1.9), 9.0 (2.2), and 9.3 years (2.5) in AZA, MMF, and CsA groups, respectively ($P = 0.26$). Patient survival was 100%, 94.2%, and 95.8% at 10 years postrandomization in AZA-, MMF-, and CsA-treated groups ($P = 0.25$) (Fig. 3a). Seven patients (three in MMF group and four in CsA group) died, all with a functioning graft. Causes of death were suicide ($n = 1$), lung cancer ($n = 1$), pancreas cancer ($n = 1$) in MMF-treated group, and acute coronary syndrome ($n = 1$), trauma ($n = 1$), lung cancers ($n = 2$) in CsA-treated group.

Graft survival

At 10 years postrandomization, death-censored graft survival was similar: 94.9% (AZA group), 94.7% (MMF group), and 95.2% (CsA group) (Fig. 3b; $P = 0.34$) as BPAR-free graft survival (Fig. 3c; $P = 0.38$). Except the seven patients who died with a functional graft, 12 patients lost their graft: 3, 5, and 4 in AZA, MMF, and CsA groups, respectively. Causes of graft lost were chronic anti-

Table 1. Demographic and background characteristics of patient at transplantation.

	Group **AZA** (n = 53)	Group **MMF** (n = 53)	Group **CsA** (n = 98)	P value**
Recipient characteristics				
Age (years)	45 ± 12	48 ± 13	47 ± 12	0.68
Gender (male), *n* (%)	32 (60)	44 (83)	68 (69)	0.71
Initial nephropathy, *n* (%)				
Glomerular	22 (42)	22 (42)	32 (33)	**0.04**
ADPKD	12 (23)	16 (30)	22 (22)	
Interstitial	7 (13)	7 (13)	17 (17)	
Vascular	4 (8)	2 (4)	11 (11)	
Diabetes	5 (9)	2 (4)	1 (1)	
Other	3 (6)	4 (8)	15 (15)	
Donor characteristics				
Age (years)	28 ± 9	28 ± 8	30 ± 9	0.07
Gender (male), *n* (%)	42 (79)	38 (72)	73 (74)	0.87
Transplant characteristics				
Panel-reactive antibodies >0%, *n* (%)	0	0	2 (2%)	0.22
Total HLA mismatch	3.6 ± 1.1	3.6 ± 1.3	3.4 ± 1.2	0.17
Postoperative days with SCr ≥200 μmol/l	6.9 ± 9.0	5.2 ± 4.4	5.9 ± 8.5	0.85
Delayed graft function, *n* (%)*	6 (11)	6 (11)	6 (6)	0.11
Cytomegalovirus status, *n* (%)				
D−/R−	23 (43)	20 (38)	33 (34)	**0.02**
D−/R+	15 (28)	12 (23)	29 (30)	
D+/R−	10 (19)	19 (36)	17 (17)	
D+/R+	5 (9)	2 (4)	19 (19)	

Quantitative variables are expressed as mean ± standard error.

ADPKD, autosomal dominant polycystic kidney disease; AZA, azathioprine; CsA, cyclosporine A; MMF, mycophenolate mofetil; *n*, number; SCr, serum creatinine concentration.

*Delayed graft function is defined by dialysis requirement during the first week after transplantation.

***P*-value for comparison between AZA + MMF group versus CsA group.

body-mediated rejection (ABMR) in six cases (50%; MMF group: n = 4, CsA group: n = 2), biopsy-proven recurrent disease in two cases (17%; AZA group: n = 1, CsA group: n = 2), and chronic allograft nephropathy in two cases (17%; AZA group: n = 1, CsA group: n = 1). The origin was unknown in two cases. Of note, of these 12 patients who lost their graft, four had been diagnosed with graft dysfunction related to CsA nephrotoxicity before month 36.

Renal function

The 10-year eGFR of the functioning grafts was 70.4 ± 31.1, 60.1 ± 22.2, and 60.1 ± 19.0 ml/min/1.73 m^2 in AZA-, MMF-, and CsA-treated groups, respectively (P = 0.16; Fig. 4). The 10-year median proteinuria was 0.17 (0.13), 0.16 (0.33), and 0.19 (0.23) g/day in AZA, MMF, and CsA groups, respectively (P = 0.72). Similar results were obtained when considering eGFR (P = 0.18) and proteinuria (P = 0.57) in per-protocol analysis patients who had remained on their randomized drug study at 10 years post-transplant.

Biopsies for allograft dysfunction

During the follow-up study (from year 3 to 10), 10 patients (19%) in AZA-, 15 patients (28%) in MMF-, and 24 patients (24%) in CsA-treated groups underwent a biopsy for allograft dysfunction. The occurrence of BPAR was similar: 1/53 (2%), 3/53 (6%), and 6/98 (5%) in AZA, MMF, and CsA groups, respectively. From randomization to the end of follow-up, the incidence of BPAR was only 1.4%/year for the whole cohort and not associated with graft loss.

Chronic CsA nephrotoxicity was diagnosed in 5/53 (9%), 4/53 (7%), and 8/98 (8%) of patients in AZA, MMF, and CsA groups, respectively. Chronic allograft nephropathy was observed in 2/53 (4%), 5/53 (9%), and 4/98 (4%) and recurrence of the initial nephropathy in 2/53 (4%), 1/53 (2%), and 1/98 (1%) of patients from each group.

Donor-specific antibodies and antibody-mediated rejection

Among 193 patients with available data at the end of the follow-up, dnDSA were detected in 6/51 AZA- (13%), 12/47 MMF- (21%), and 13/95 CsA-treated patients (14%), respectively (P = 0.29). Death-censored graft survival was lower for dnDSA-positive patients compared with dnDSA-negative patients (89.5% vs. 95.2%, respectively; P = 0.04). Biopsy-proven chronic ABMR occurred in three MMF- and four CsA-treated patients, followed by graft failure in six cases.

Table 2. Baseline characteristics of patients at randomization.

	Group AZA (n = 53)	Group MMF (n = 53)	Group CsA (n = 98)	P value*
Time between transplantation and randomization (months)	14.5 (5.3)	13.8 (4.5)	14.4 (4.2)	0.54
Rejection episode before randomization, n (%)	4 (8)	5 (9)	5 (5)	0.33
Serum creatinine (μmol/l)	109.7 ± 27.9	114.3 ± 22.7	110 ± 24.5	0.57
eGFR (ml/min/1.73 m^2)	73.8 ± 18.6	73.1 ± 17.4	75.5 ± 19.1	0.43
Proteinuria (g/day)	0.05 (0.14)	0.06 (0.17)	0.05 (0.14)	0.61
CsA dose (mg/kg/day)	3.6 ± 1	3.5 ± 1.2	3.4 ± 0.9	0.26
CsA C_0 (ng/ml)	130.6 ± 38.6	130.3 ± 32.8	127.3 ± 31.2	0.51
MMF dose (g/day)	1.9 ± 0.3	1.9 ± 0.3	1.8 ± 0.4	0.37

Quantitative variables are expressed as mean ± standard error or median (inter quartile range).
AZA, azathioprine; CsA, cyclosporine A; CsA C_0, cyclosporine concentration 12 h postdose; eGFR, estimated glomerular filtration rate using Cockcroft–Gault equation; MMF, mycophenolate mofetil.
**P*-value for comparison between AZA + MMF group versus CsA group.

Table 3. Histological findings in kidney allograft recipients at primary end point (36 months postrandomization).

	Group AZA (n = 53)	Group MMF (n = 53)	Group CsA (n = 98)	P-value*
Number of biopsied patients (%)	11 (21)	10 (19)	26 (27)	0.25
Number of biopsies	23	28	56	
Patients with acute rejection (%)†	4 (8)	4 (8)	11 (11)	0.36
Borderline	0	1	1	
Grade I	4	3	10	
Grade II	0	0	0	
Grade III	0	0	0	
Patients with CsA nephrotoxicity (%)	5 (9)	5 (9)	14 (14)	0.28
Patients with other lesions‡	2	3	5	

Values are given as number or number (percentage).
AZA, azathioprine; CsA, cyclosporine A; MMF, mycophenolate mofetil.
**P*-value for comparison between AZA + MMF group versus CsA group.
†According to Banff 97.
‡Others lesions consisted of one acute tubular nephritis and one grade I interstitial fibrosis/tubular atrophy in CsA group; one acute tubular nephritis, one recurrent glomerulonephritis, and one chronic rejection in MMF group; one acute tubular nephritis, two grade I interstitial fibrosis/tubular atrophy and two T chronic-mediated rejection in CsA group.

Safety

The proportion of patients who experienced at least one bacterial infection episode during months 12–136 was similar: 27/53 (51%) in AZA-, 28/53 (53%) in MMF-, and 64/98 (69%) in CsA-treated groups (P = 0.15). Twenty-three patients were hospitalized for bacterial infections: two in AZA group, seven in MMF group, and 14 in CsA group. The most common cause was urinary tract infection (AZA group: n = 1, MMF group: n = 3, CsA group: n = 10). Interestingly, none of the patients developed polyomavirus-associated nephropathy.

At 10 year, no significant differences were found between the three groups in hematologic and lipid parameters or glucose level. At the end of the follow-up study, post-transplantation diabetes mellitus had occurred in 25/201 patients (12%): 4/53 (7.5%), 6/51 (12%), and 15/97 (15%) in AZA, MMF, and CsA groups, respectively (P = 0.37). Severe cardiac complications occurred in 22 patients (11%): 4/53 (8%), 9/51 (18%), and 9/97 (9%) in AZA, MMF, and CsA groups, respectively (P = 0.58).

Seventeen neoplasms and 44 skin cancers (baso-cellular: n = 39, spino-cellular epitheliomas: n = 5) were reported in the whole cohort. There was no marked difference in the occurrence of cancer between treatment groups (P = 0.29). The most common neoplasms were colorectal cancer (CsA group: n = 4), prostate cancer (AZA group: n = 1, CsA group: n = 3), and renal carcinoma (MMF group: n = 3, CsA group: n = 1).

Discussion

The present study confirms that minimization of maintenance immunosuppressive drugs is safe and associated with prolonged patient and graft survival when performed in selected kidney transplant recipients. Careful selection of recipients was illustrated by the absence of detectable PRA by lymphocytotoxicity in AZA and MMF groups and a mean 2% of PRA in the CsA group. Based on three principles, that is systematic steroid withdrawal at 3–4 months, long-term CsA minimization with low trough levels and low dose of the antimetabolite drugs (AZA or MMF), we designed a three arm-maintenance therapy randomized trial comparing CsA–AZA, CsA plus low-dose MMF to CsA monotherapy. The CsA–AZA group was designed in attempt to evaluate the potential long-term superiority of MMF on AZA. In the CsA plus low-dose MMF group, we decided a long-term MMF dose of 1 g/day although data on the safety of this strategy were missing at that date. The

Table 4. Main pharmacological data at the end of follow-up.

	Group AZA (n = 53)	Group MMF (n = 53)	Group CsA (n = 98)	P-value*
Patients receiving steroids, n (%)	2 (4)	5 (9)	14 (14)	0.06
Steroids dose, mg/day	7.5 ± 3.5	11.0 ± 5.2	9.5 ± 6.2	
Patients receiving AZA, n (%)	38 (72)	3 (6)	4 (4)	**<0.0001**
AZA dose, mg/day	86.2 ± 20.7	83.3 ± 38.2	230.0 ± 326.9	
Patients receiving MMF, n (%)	11 (21)	45 (85)	25 (26)	**0.0001**
MMF dose, g/day	1.3 ± 0.6	1.3 ± 0.5	1.8 ± 0.6	
Patients receiving CsA, n (%)	48 (91)	47 (89)	85 (87)	0.66
CsA dose, mg/kg/day	2.4 ± 0.8	2.5 ± 0.9	2.3 ± 0.7	
CsA C_0 (ng/ml)	106.4 ± 26.6	112.4 ± 28.7	101.1 ± 35.3	
Patients receiving tacrolimus, n (%)	4 (8)	5 (9)	5 (5)	0.41
Patients receiving sirolimus, n (%)	0 (0)	0 (0)	2 (2)	0.22

Quantitative variables are expressed as mean ± standard error.
AZA, azathioprine; CsA, cyclosporine A; MMF, mycophenolate mofetil; CsA C_0: cyclosporine concentration 12 h postdose.
*P-value for comparison between AZA + MMF group versus CsA group.

CsA monotherapy arm derived from our previous pilot studies [10,11]. At the time of the study design, selection of donors and recipients was particularly strict and around half of transplant recipients in the participating centers fulfilled the inclusion and randomization criteria. Although donor and recipient characteristics changed over the past 15 years, donors <45 years still represent approximately 30% of deceased donors in French centers and a significant proportion of living donors [15]. Therefore, this study still provides useful clinical information.

First, our results demonstrate the feasibility of a tailor-made strategy of reduced maintenance immunosuppression including steroid withdrawal. To our knowledge, this is the first study reporting such a prolonged follow-up period in a randomized trial comparing AZA with reduced-dose MMF with CsA monotherapy in low-risk kidney transplant recipients. Several observations deserve comments. First, 64% of patients still received CsA at the end of follow-up, with trough levels in accordance with the study recommendations (75–125 ng/ml). Secondly, at the end of follow-up, steroid-free immunosuppressive regimen was maintained in 94% of patients withdrawn from steroid during the first year, highlighting a low incidence of immunological events. The primary end point of this study was the occurrence of biopsy-documented graft dysfunction at month 36. Only 20% and 27% patients experienced graft dysfunction (BPAR or CsA nephrotoxicity) at 36 months post-transplant in bitherapy and monotherapy groups, respectively. This resulted in impressive patient, and death-censored graft survival rates of more than 95% at 10 year postrandomization, similar in patients treated with bitherapy or monotherapy. Our results are in line with those from the MYSS follow-up study, which compared the outcomes of 157 patients with competing steroid withdrawal and randomized to receive either MMF or AZA [16,17]. At month 72 following randomization, patient mortality (AZA: 2.5%; MMF: 2.6%) and graft loss (AZA: 3.8%; MMF: 2.8%) were similar. In contrast, our results appear better than those reported by Montagnino *et al.* In a study of 354 patients assigned to receive CsA monotherapy or CsA + steroids or CsA + AZA + steroids, the 9-year patient and graft survival of the CsA monotherapy arm were 94.0% and 73.3%, respectively [18]. However, patients in this study were older and CsA monotherapy regimen was initiated earlier after transplantation. The present results validate the predictors of long-term success of steroid withdrawal and antimetabolite withdrawal that we previously suggested in a homogenous population with low risk of graft dysfunction [10,11]. Moreover, our minimization strategy in low-risk patients may represent a valuable approach to reduce the occurrence of severe complications that strongly impact long-term allograft outcome, including BK virus nephropathy and new-onset diabetes mellitus [19,20]. In a recent study, Naesens *et al.* [6] reported that polyomavirus-associated nephropathy accounts for 8.9% of all causes of biopsy-documented graft failure occurring between 1 and 5 year post-transplantation. Interestingly, none of the patients in our trial was diagnosed with polyomavirus-associated nephropathy, a condition that has been associated with tacrolimus [21], high MMF exposure [22], and steroid maintenance regimen [23]. Similarly, the prevalence of post-transplantation diabetes mellitus reported in our series was only 12% at 10 year, compared to 17% at 10 weeks in a large recent study [24]. Several factors probably explained this finding, including young recipient age (46 years), early steroid withdrawal, and the use of CsA rather than tacrolimus [25,26]. Similarly, the respective incidence of cardiovascular events was lower than previously reported in patients under CsA-based immunosuppression. In a cohort of 2071 patients, 53% of patients had developed cardiovascular

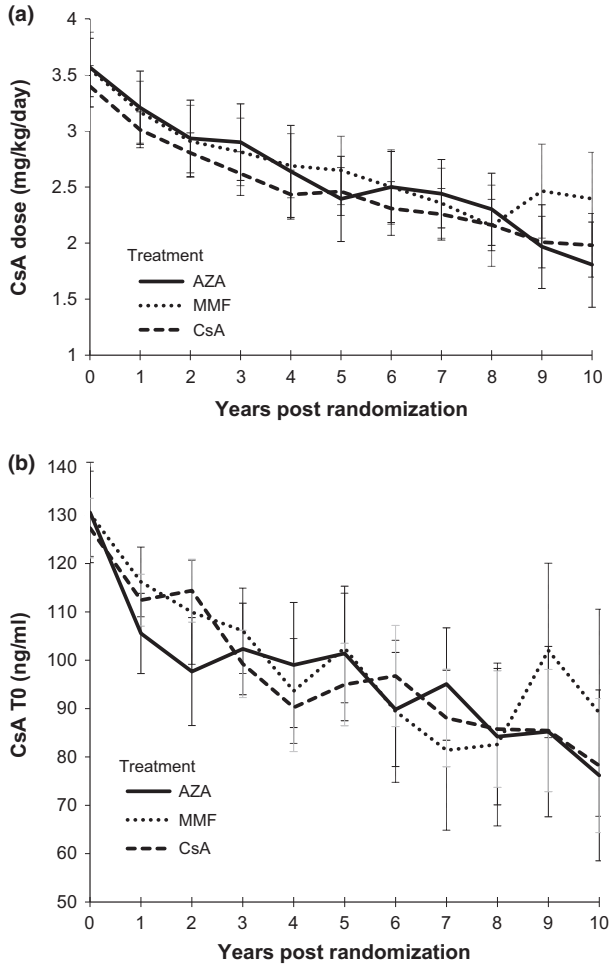

Figure 2 Mean of cyclosporine A (a) dosage (in milligrams per kilogram per day) (b) trough level (in nanograms per milliliter) according to treatment arm. Errors bars indicate standard deviation. AZA, azathioprine; CsA, cyclosporine A; MMF, mycophenolate mofetil.

complications after 15 years [27]. Finally, a similar malignancy incidence of 10% at 10 years was reported by Rizzari *et al.* [3] in a series of 1241 patients after rapid discontinuation of prednisone.

Another important finding is that long-term minimization protocols are not associated with an increased frequency of immunologic events, thanks to careful selection of patients using rigorous criteria: first renal transplantation and historical or pretransplant peak PRAs <25%. The low occurrence of BPAR (4.9%) in the follow-up study is in accordance with the study published by Etienne *et al.* in which a minimization based on CsA low-dose exposure is associated with an incidence of BPAR of 6% at 24 months [28]. However, in our study, six grafts were lost over 10-year follow-up because of chronic ABMR. These findings are in harmony with the recently established crucial role of ABMR in kidney transplant failure in the era of modern immunosuppressive regimens [7,8]. For instance, in the series of 315 recipients reported by Sellarés *et al.* [9], ABMR or mixed rejection accounted for 64% of kidney failure in their biopsy-for-cause cohort. DSA exert a crucial role in the mediation of chronic allograft destruction and constitute the first step in the natural history of ABMR [29]. The emergence of dnDSA has been ascribed to insufficient maintenance immunosuppressive regimen, nonadherence [9], or conversion to mTOR inhibitors [30]. Because DSA are associated with shorter kidney graft survival [29], their detection was particularly relevant in our follow-up study. To this aim, a protocol amendment was performed to introduce systematic DSA screening by the sensitive single antigen flow beads method at the end of follow-up period. Interestingly, despite minimization, dnDSA were detected at the end of the follow-up in only 16% of patients in the whole cohort, without significant differences between those treated with bitherapy and CsA monotherapy. By comparison, in the ZEUS study that used a CNI minimization strategy based on conversion to everolimus, DSA were detected at 5 years in 21.4% and 20.0% of patients treated with everolimus or CsA patients, respectively [31]. Our study has several limitations, particularly the absence of protocol biopsy and DSA screening leading to diagnose the subclinical ABMR. Nevertheless, our results suggest that the clinical and biological criteria used in the present study to select low-risk recipients were reliable and allowed successful minimization without major immunological events.

Chronic CNI nephrotoxicity was considered until recently as a main factor of chronic allograft dysfunction, which risk progresses with duration of treatment [14,32]. The occurrence of chronic renal failure after transplantation of a nonrenal organ was 16.5% in a cohort study of 11 426 patients [33]. In our study, renal function was stable in the long term with a median decrease in eGFR between 0.1 and 2 ml/min/year, close to the physiological reduction in GFR observed in subjects after 40 years. These results suggest that CNI nephrotoxicity can be managed by maintaining moderate CsA level target. However, donor characteristics, with a mean age of 29 $\pm$ 9 years, probably had a strong impact on our results. Older age of cadaveric donors increase is an important risk of graft lost and a major predictor of CNI-induced long-term graft dysfunction [34]. In nonrenal organ transplantation, the risk of chronic renal failure correlates also with increasing recipient age [33].

Finally, the present study may help to refine our minimization strategies. Although the study was designed to show a superiority of bitherapy with CsA–AZA or CsA–MMF to monotherapy with CsA, the three groups were identical at 3 years for the primary end point with similar patient and graft survival or renal function at 10 years in the follow-up study. These results compared with those of Montagnino

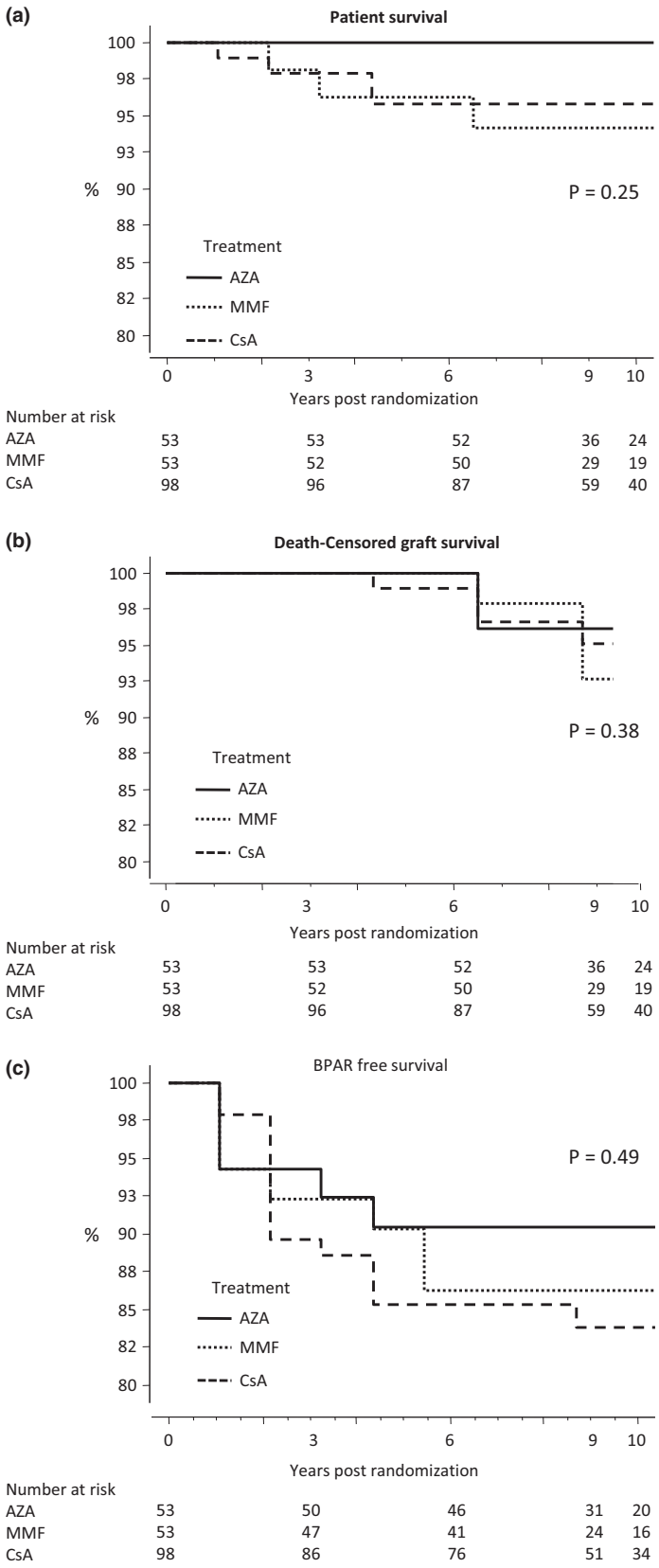
(a)
Patient survival
100
98
95
93
% 90
88
85
82
80
P = 0.25
Treatment
AZA
MMF
CsA
0 3 6 9 10
Years post randomization
Number at risk
AZA 53 53 52 36 24
MMF 53 52 50 29 19
CsA 98 96 87 59 40
(b)
Death-Censored graft survival
100
98
95
93
% 90
88
85
82
80
P = 0.38
Treatment
AZA
MMF
CsA
0 3 6 9 10
Years post randomization
Number at risk
AZA 53 53 52 36 24
MMF 53 52 50 29 19
CsA 98 96 87 59 40
(c)
BPAR free survival
100
98
95
93
% 90
88
85
82
80
P = 0.49
Treatment
AZA
MMF
CsA
0 3 6 9 10
Years post randomization
Number at risk
AZA 53 50 46 31 20
MMF 53 47 41 24 16
CsA 98 86 76 51 34

Figure 3 (a) Patient survival. There was no significant difference in patient survival between the three groups ($P = 0.25$). (b) Death-censored graft survival. There was no significant difference in patient survival between the three groups ($P = 0.38$). (c) BPAR-free graft survival. There was no significant difference in patient survival between the three groups ($P = 0.49$). *P* values were calculated using the log-rank test. AZA, azathioprine; CsA, cyclosporine A; MMF, mycophenolate mofetil; BPAR, Biopsy-proven acute rejection.

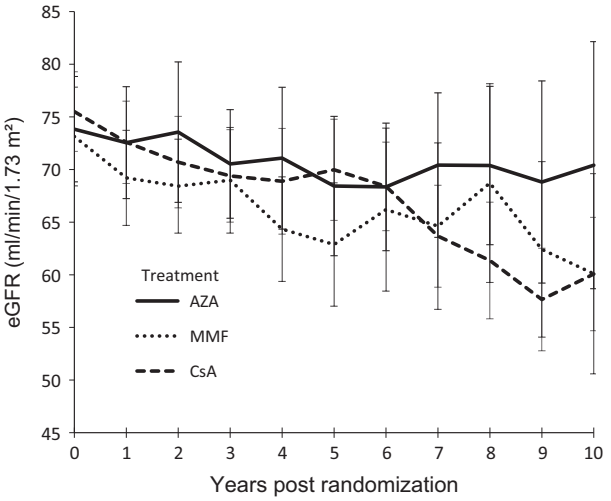

Figure 4 Renal function. Development of renal function (estimated glomerular filtration rate/Cockcroft–Gault formula) over time according to treatment arm ($P = 0.16$). *P* value was calculated using the log-rank test. AZA, azathioprine; CsA, cyclosporine A; MMF, mycophenolate mofetil.

et al., in which patients assigned to receive CsA monotherapy were not affected in terms of patient and graft survival [18]. Whereas MMF has been shown to reduce acute rejection compared to AZA during the first year [35], its long-term benefit in low immunological risk recipients is debated. Indeed, the MYSS study has challenged the belief that MMF improves outcomes in kidney transplantation compared to AZA, when used in addition to CsA and steroid without induction antibody [16,17]. Moreover, in patients withdrawn from steroids, a clinical diagnosis of acute rejection was made in 16% of MMF- and 12% of AZA-treated patients with a BPAR rate of 7% in both groups. At 5 years after transplantation, eGFR was not statistically different suggesting that the long-term benefit/risk profile of MMF and AZA therapy is similar [17]. Here, we confirm that maintenance therapy with AZA or MMF is not different. As the costs of MMF exceed largely those of AZA (×10–15), including with generic drugs, we suggest that AZA is an alternative to MMF for maintenance immunosuppressive regimen, after 1 year in low-risk recipients.

In conclusion, our data suggest a favorable long-term outcome obtained with tailoring immunosuppressive therapy, based on low-dose antimetabolite exposure or CsA monotherapy. This clinician's chosen minimized immunosuppressive strategy, in well-selected patients for low risk of graft dysfunction is not associated with graft loss increase by chronic ABMR.

Authorship

AT, RAA, YL, IE, CC, CL, VV, BHL, JPR, NB, FB, ME, and GT: performed study. AT, YL, IE, CC, VJ, EG, JCA, and GT: analyzed data. LE: collected and analyzed data. AT and YL: wrote the manuscript. GT: designed and wrote the manuscript.

Funding

This work was supported by grants from Programme Hospitalier de Recherche Clinique régional (1997) CHU Poitiers and Association pour la Recherche et l'Enseignement en Néphrologie en Poitou-Charentes (AREN-PC).

Acknowledgements

We thank all the patients, nurses, clinicians (Sylvie Lavaud, Olivier Toupance and Claude Leroux-Robert), and Benedicte Ayrault, Fabien Duthé, Chantal Buet, who collected data from medical charts. The five centers participating in this study are members of the René Spiesser Group. Preliminary results of this study were reported at the 2005 American Transplant Congress in Seattle and at the 2008 American Transplant Congress in Toronto.

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