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ORIGINAL ARTICLE

Eight-year results of the Spiesser study, a randomized trial comparing *de novo* sirolimus and cyclosporine in renal transplantation

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Summary

We present the results at 8 years of the Spiesser study, a randomized trial comparing *de novo* sirolimus and cyclosporine in kidney transplant recipients at low immunologic risk. We assessed estimated glomerular filtration (eGFR), graft, patient, and death-censored graft survival (log-rank compared), *de novo* DSA appearance, risk of malignancy, post-transplant diabetes mellitus (PTDM), and anemia. Intent-to-treat and on-treatment analyses were performed. Graft survival was similar in both groups (sirolimus: 73.3%, cyclosporine: 77.7, $P = 0.574$). No difference was observed between treatment groups concerning patient survival ($P = 0.508$) and death-censored graft survival ($P = 0.858$). In conditional intent-to-treat analysis, mean eGFR was greater in sirolimus than in cyclosporine group (62.5 ± 27.3 ml/min vs. 47.8 ± 17.1 ml/min, $P = 0.004$), in particular because graft function was excellent in patients maintained under sirolimus (eGFR = 74.0 ml/min). Importantly, no detrimental impact was observed in patients in whom sirolimus has been withdrawn (eGFR = 49.5 ml/min). Overall, 17 patients showed *de novo* DSAs, with no difference between the two groups ($P = 0.520$). Malignancy did not differ by treatment. An initial maintenance regimen based on sirolimus provides a long-term improvement in renal function for kidney transplant patients, especially for those maintained on sirolimus.

Introduction

For about 30 years, calcineurin inhibitors (CNIs) have been considered the cornerstone of immunosuppressive strategies in renal transplantation [1,2]. Indeed, their use has considerably reduced the rate of acute rejection and improved graft survival. However, the CNIs cyclosporine and tacrolimus can promote tubular atrophy and interstitial fibrosis, although the relative contribution of this CNI-nephrotoxicity on graft loss remains debated [3,4]. Moreover, serious extra-renal effects ascribed to CNIs include increased risk of malignancy and cardiovascular events, which are frequent causes of death with kidney transplantation [5]. CNI-free immunosuppressive strategies are therefore of interest.

Maintenance regimens based on mammalian target of rapamycin (mTOR) inhibitors, a class of immunosuppressive agents with a favorable nephrologic and cardiac toxicity profile, which bind the mTOR complex and inhibit immune cell proliferation and differentiation, have been considered the most promising option to avoid CNI therapy in terms of safety and effectiveness [6]. The Spiesser study was a randomized trial that compared a *de novo* sirolimus–mycophenolate mofetil (MMF) strategy to a classical maintenance regimen based on cyclosporine–MMF for kidney transplant recipients with low immunological risk receiving Thymoglobulin induction (Genzyme, Saint-Germain en Laye, France). Importantly, steroids were planned to be withdrawn in both groups at 6 months. At 1 year, the rate of sirolimus withdrawal was high. Nevertheless, patients who retained sirolimus treatment showed excellent renal function at 1 year [7]. Most importantly, a persistent renal benefit was observed until 5 years post-transplant [8].

On the other hand, a possible increased incidence of *de novo* donor-specific antibodies (DSAs) and antibody-mediated rejection has been reported in kidney transplant recipients who converted from cyclosporine to everolimus at 3–4.5 months after transplantation [9]. This observation questioned the long-term effect of mTOR-based immunosuppressive strategy in such patients. Because we did not observe more *de novo* DSAs at 5 years in the Spiesser and Concept studies in patients receiving sirolimus [8,10], we decided to extend the Spiesser study.

Here, we report on glomerular filtration (eGFR) as the primary outcome in the intent-to-treat analysis as well as graft and patient survival and risk of *de novo* DSA appearance at 8 years after kidney transplantation with sirolimus and cyclosporine treatment.

Material and methods

Patients

This observational study was an 8-year follow-up of the Spiesser study, an open-label, randomized, comparative trial assessing the efficacy of a sirolimus–MMF-based regimen. The original study was approved by a local independent review board and complied with good clinical practice and the Declaration of Helsinki. The details of the study were previously published [7]. Briefly, 150 recipients from 18 to 65 years old were included; 145 received a graft from a heart-beating deceased donor between April 2002 and September 2003 in 13 French Transplant centers. The presence of panel-reactive antibodies >80%, determined by lymphocytotoxicity testing, was an exclusion criteria. Patients were assigned before transplantation to receive a sirolimus–MMF-based regimen (sirolimus group) or cyclosporine–MMF-based regimen (cyclosporine group). All patients received a 5-day course of antithymocyte globulins (1.5 mg/kg/day, thymoglobulin; Genzyme, Saint-Germain en Laye, France), MMF (2 g/day, Cellcept®; Roche, Neuilly-sur-Seine, France), and corticosteroids were planned to be withdrawn at the end of month 5. Sirolimus or cyclosporine was started within 48 hr after transplantation. At the end of the study (1 year), 133 of 145 patients had a functional graft.

Clinical and biological data at 2, 3 and 5 years were retrospectively recorded in a first observational study for patients who agreed to participate [8]. At 5 years, graft function was analyzed in 114 patients (sirolimus group, 55; cyclosporine group, 59). Data were missing for five patients (sirolimus group, two; cyclosporine group, three). For this second observational study, a questionnaire was send to each center for these patients to complete (n = 119).

Data studied

At 8 years after transplantation, we recorded graft failure and death (date and cause), serum creatinine level (μmol/l), proteinuria (g/24 h), immunosuppressive regimen (type and daily dose), systolic and diastolic arterial pressure, antihypertensive drugs (class, dose), hemoglobinemia level (g/dl), erythropoietin use, post-transplant diabetes mellitus (PTDM), and malignancies (type, number). No data were available for nine patients who were considered lost to follow-up.

We recorded the date and specificity of anti-human leukocyte antigen (HLA) antibodies that appeared after transplantation. Of note, anti-HLA antibodies were detected and identified by ELISA when patients were enrolled, and solid-phase assay was routinely used in all centers to monitor all patients only after 2007. Importantly, when anti-HLA antibodies were identified by single-antigen bead assay after transplantation, especially that characterized as DSA, sera harvested before transplantation were tested by solid-phase assay. DSAs were considered as *de novo* in patients without anti-HLA antibodies before transplantation.

Statistical analysis

Descriptive statistics are expressed as percentage or mean ± SD for normally distributed variables and median (range) for non-normally distributed variables. Qualitative data were compared by chi-square test. Quantitative data were compared by Mann–Whitney *U*-test when two groups were compared, and by ANOVA (followed by pairwise comparisons using PSLD Fisher test) when more than two groups were compared.

The primary outcome was eGFR, estimated by the Modification of Diet in Renal Disease (MDRD) formula 8 years after transplantation, in accordance with previous reports of the Spiesser study. We used an intent-to-treat analysis conditional on a functioning graft 8 years after transplantation (termed conditional ITT) comparing all patients with eGFR available at 8 years and on-treatment analysis that included patients maintained at 8 years on the study drug initially allocated.

Kaplan–Meier curves were used to estimate uncensored graft survival (death or end-stage renal disease [ESRD]), patient survival (ESRD was censored), and death-censored graft survival. End of follow-up was defined as death (with a functioning graft) or graft loss (i.e., dialysis or re-transplantation). For nine patients lost to follow-up, the date of the last visit was used. *De novo* anti-HLA antibody incidence, malignancy occurrence, and PTDM appearance were estimated by Kaplan–Meier methods. Log-rank test was used to compare survival curves. For these analyses, all patients randomized were analyzed.

Statistical analyses involved use of Microsoft Excel 2008 (Redmond, WA, USA). $P < 0.05$ was considered statistically significant.

Results

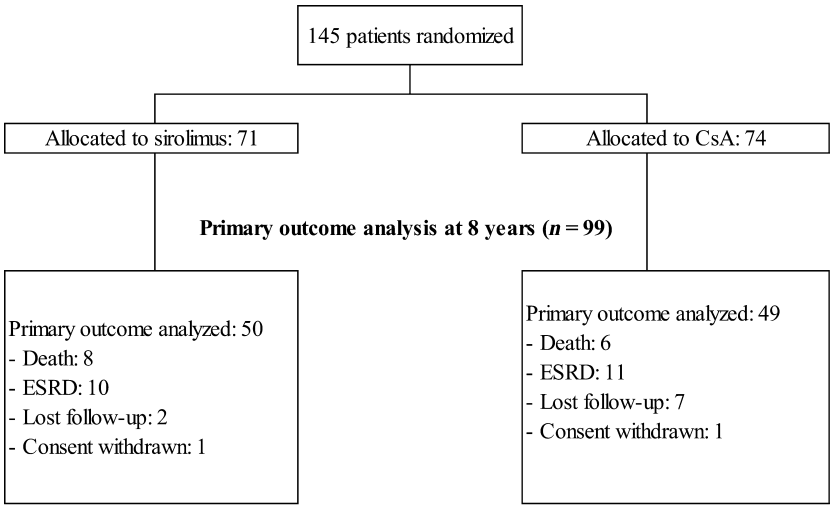
Patients

Of the 150 patients included, 145 were randomized to receive sirolimus ($n = 71$) or cyclosporine ($n = 74$), with similar baseline characteristics; 99 had a functional graft at 8 years after transplantation and eGFR was available for 50 patients in sirolimus group and 49 in cyclosporine group (Fig. 1). As shown in Table 1, baseline characteristics of patients were similar with the two treatments.

Immunosuppressive regimen

After the fifth year, the study drug was withdrawn for six and two patients in the sirolimus and cyclosporine groups, respectively. Sirolimus was still replaced by CNIs, especially tacrolimus ($n = 5$), and cyclosporine was replaced by tacrolimus ($n = 1$) or everolimus ($n = 1$). Overall, sirolimus was withdrawn in 43 patients (year 1: 20, years 2–5: 17 and years 5–8: six) and replaced by CNI in 42 patients (cyclosporine: 12, tacrolimus: 30). Cyclosporine was withdrawn in 28 patients (year 1: six, years 2–5: 20 and years 5–8: two), most often for tacrolimus ($n = 23$).

Finally, 26 of 50 (52%) patients remained on sirolimus and 36 of 49 (73%) remained on cyclosporine at 8 years. The daily dose at 8 years was slightly lower than that reported at 5 years (sirolimus, 2.3 ± 1.2 vs. 2.7 ± 1.1 mg/day, $P = 0.0003$; cyclosporine, 149 ± 47 vs. 177 ± 55 mg/day, $P = 0.014$). Almost all patients received MMF or mycophenolic sodium regardless of drug allocated, and the daily doses were similar in both groups. Importantly, about three quarters of patients did not receive any steroids at 8 years (Table 2).



ESRD, end-stage renal disease; eGFR, estimated glomerular filtration rate

Figure 1 Study flow chart.

Table 1. Baseline characteristics of all randomized patients (n = 145) and patients with primary outcome available at 8 years after transplantation (n = 99, intent-to-treat population).

	At inclusion			At 8 years posttransplantation		
	Sirolimus (n = 71)	Cyclosporine (n = 74)	P-value	Sirolimus (n = 50)	Cyclosporine (n = 49)	P-value
Recipient age (years)	45.6 ± 10.3	45.1 ± 12.4	0.913	45.8 ± 10.2	47.4 ± 12.1	0.266
Male recipient (%)	62	61	0.886	62	61	0.402
Donor age (years)	38.7 ± 14.4	41.3 ± 14.0	0.403	37.9 ± 13.4	42.5 ± 13.6	0.163
Cold ischemia time (hours)	19.3 ± 5.2	20.2 ± 5.5	0.508	20.1 ± 5.5	20.4 ± 5.4	0.744
HLA mismatches (A, B, DR)	3.5 ± 1.2	3.4 ± 1.4	0.617	3.6 ± 1.2	3.4 ± 1.2	0.534

HLA, human leukocyte antigen.

Table 2. Immunosuppressive regimen at 8 years in 98 patients with primary outcome analyzed.

	Study drug as randomized			
	Sirolimus		Cyclosporine	
	At randomization (ITT)	At 8 years (OT)	At randomization (ITT)	At 8 years (OT)
Number	49	26	49	36
Daily dose of allocated study drug (mg/day)				
Mean ± SD	2.3 ± 1.2	2.3 ± 1.2	150 ± 47	149 ± 47
Range	0.5–7	0.5–7	80–300	80–300
Patients receiving MMF or MPA (%)	94	100	96	100
Daily dose of MMF (mg/day)*	1435 ± 487	1528 ± 429	1564 ± 517	1680 ± 450
Patients receiving steroids (%)	27	12	24	17
Daily dose of steroids	7.7 ± 2.3	6.7 ± 2.4	7.7 ± 2.0	6.6 ± 2.0

*360 mg of MPA was considered equivalent at 500 mg of MMF.

Renal outcome

In the conditional ITT population (n = 99), eGFR was higher with sirolimus than cyclosporine at 1 year (54.4 ± 17.7 vs. 49.7 ± 14.6 ml/min, P = 0.051), 2 years (55.5 ± 18.1 vs. 47.7 ± 17.8, P = 0.007), 3 years (54.8 ± 19.2 vs. 47.1 ± 19.4 ml/min, P = 0.006), 5 years (57.0 ± 22.5 vs. 47.3 ± 18.8, P = 0.009), and 8 years (62.5 ± 27.3 vs. 47.8 ± 17.1, P = 0.004). The difference tends to progressively increase from 1 to 8 years (Fig. 2) and, after 1 year,

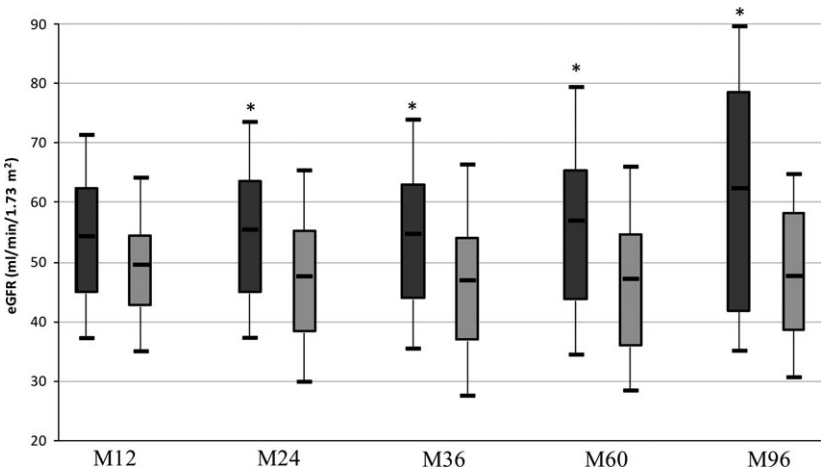


Figure 2 Estimated glomerular filtration rate estimated by Modification of Diet in Renal Disease formula in the sirolimus and cyclosporine groups at 12, 24, 36, 60, and 96 months (conditional intent-to-treat analysis). Dark and light gray bars represent sirolimus and cyclosporine groups, respectively. The mean is represented by a black bar, with the first and third quartiles. Upper and lower whiskers represent SD. *P < 0.05 (Fisher test).

eGFR was decreased ≥ 20% for 34% in cyclosporine group as compared with 16% of those who initially received sirolimus ($P = 0.037$). Importantly, eGFR in patients in whom sirolimus has been withdrawn ($n = 24$) was not lower than in cyclosporine group (49.5 ± 25.7 ml/min vs. 47.8 ± 17.1 ml/min, $P = 0.801$). Proteinuria was available for 75 patients (sirolimus group, 38; cyclosporine group, 37). The two groups did not differ in proteinuria (sirolimus, 0.7 ± 1.2 vs. cyclosporine, 0.6 ± 1.6 g/day, $P = 0.471$), while renin–angiotensin system blockers were used similarly in the two groups (63% and 65%).

For the on-treatment population ($n = 62$), eGFR was excellent in the patients remained under sirolimus ($n = 26$) in comparison with GFR in patients remained under cyclosporine ($n = 36$) or in whom sirolimus has been switched to CNI ($n = 22$). At years 1, 2, 3, 5 and 8, ANOVA showed that eGFR was different between the 3 groups at all times (Fig. 3). First, pairwise analyses indicated that GFR was higher in patients on sirolimus than in patients on cyclosporine at 1 year (59.4 ± 17.8 vs. 49.8 ± 14.0 ml/min, $P = 0.016$), 2 years (60.3 ± 18.1 ml/min vs. 47.4 ± 18.5 ml/min, $P = 0.001$), 3 years (61.7 ± 18.2 vs. 46.5 ± 19.7 ml/min, $P < 0.0001$), 5 years (67.5 ± 21.7 vs. 47.0 ± 18.8, $P < 0.0001$), and 8 years (74.0 ± 23.6 vs. 46.9 ± 16.6, $P < 0.0001$). Of note, proteinuria at 8 years was similar for patients on sirolimus or cyclosporine (0.7 ± 1.5 vs. 0.6 ± 1.8 g/day, $P = 0.710$). Second, eGFR in patients dropped out from sirolimus arm at 1 year (48.7 ± 14.6 ml/min), 2 years (50.2 ± 17.0 ml/min), 3 years (47.0 ± 17.6 ml/min), 5 years (45.1 ± 17.1 ml/min), and 8 years (53.9 ± 24.7 ml/min) was lower than in patients remained on sirolimus after third year post-transplantation but was not different to GFR of patients on cyclosporine for 8 years.

Patient and graft survivals

Intent-to-treat graft, patient, and death-censored graft survival were analyzed in all patients enrolled in the original Spiesser study and are reported in Fig. 4.

Patient survival was excellent in the two treatment groups (sirolimus, 86.3%; cyclosporine, 90.6, $P = 0.508$) (Fig. 4).

In total, 14 patients died during follow-up, eight of 71 (11%) and 6 of 74 (8%) in the sirolimus and cyclosporine groups, respectively. Among the four deaths occurring between 5 and 8 years, three occurred in the sirolimus group (two sepsis and one metastatic skin squamous-cell carcinoma) and one in the cyclosporine group (cirrhosis). Graft survival at 8 years was 75.6%, 73.3%, and 77.7% for all patients, sirolimus, and cyclosporine groups, respectively, with no difference between the treatment groups ($P = 0.574$).

Overall, 21 patients showed graft loss, 10 in sirolimus and 11 in cyclosporine groups. Among the eight graft losses that occurred after year 5, six occurred in the cyclosporine group: graft losses were due to transplant glomerulopathy ($n = 3$), chronic rejection without DSA ($n = 1$), and nonadherence ($n = 1$). No cause was recorded for the sixth patient. Chronic allograft nephropathy without DSA was admitted as cause of graft loss for two patients in sirolimus group, with nonadherence in one. We observed no acute cellular rejection between 5 and 8 years. Finally, death-censored graft survival was estimated at 85.5% and 85.8% in sirolimus and cyclosporine groups, respectively ($P = 0.858$).

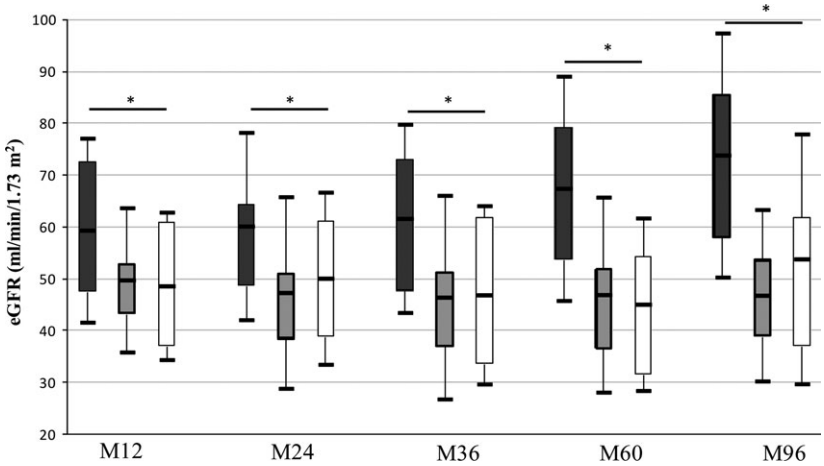


Figure 3 Estimated glomerular filtration rate estimated by Modification of Diet in Renal Disease formula in the sirolimus and cyclosporine groups at 12, 24, 36, 60, and 96 months (on-treatment analysis). Dark and light gray bars represent patients remained under sirolimus ($n = 26$) and cyclosporine ($n = 36$) groups at 8 years, respectively. White bars represent patients in whom sirolimus has been switched to a CNI inhibitor ($n = 22$). The mean is represented by a black bar, with the first and third quartiles. Upper and lower whiskers represent SD. *$P < 0.05$ (ANOVA).

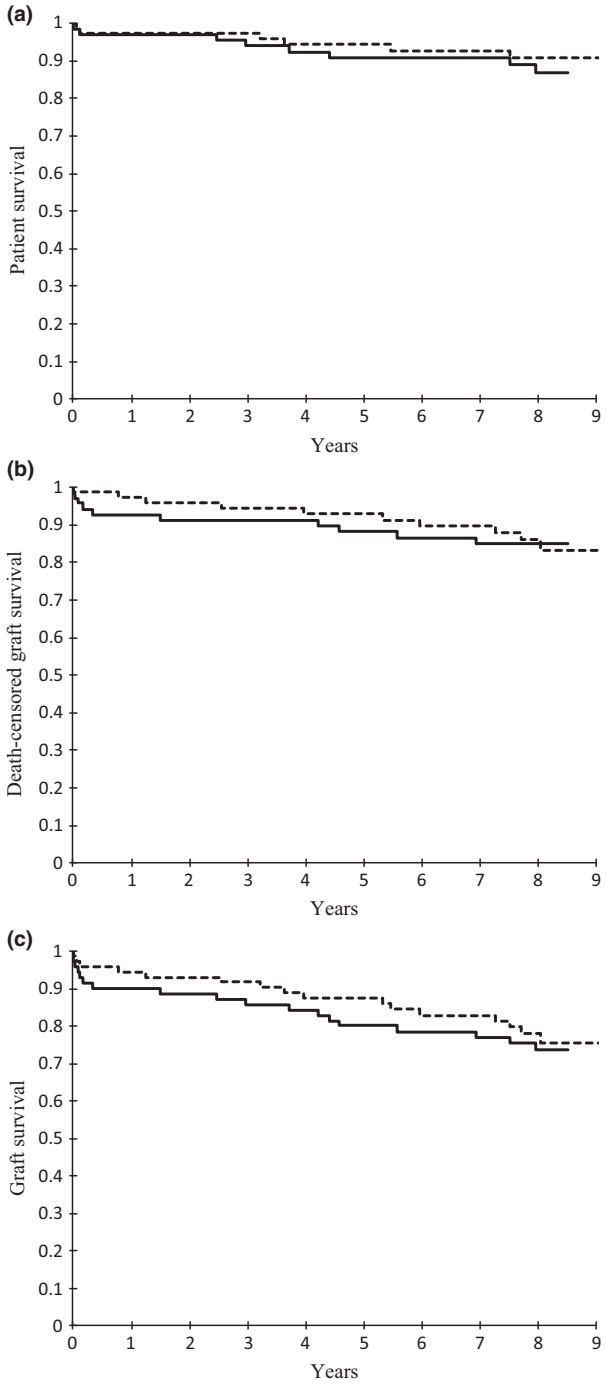


Figure 4 Patient, death-censored, and graft survival were similar in sirolimus and cyclosporine groups (ITT analysis). Estimated patient survival (a), death-censored graft survival (b), and graft survival (c) in sirolimus (bold black lines, $n = 71$) and cyclosporine groups (dashed lines, $n = 74$).

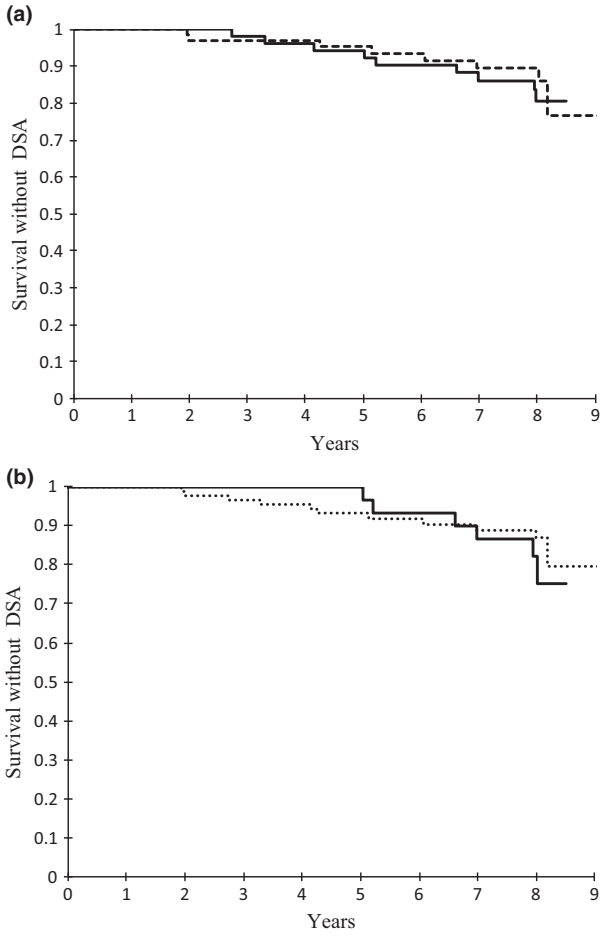


Figure 5 Risk of post-transplant DSA appearance was not increased in patients treated with sirolimus. Upper panel: intent-to-treat (ITT) analysis of estimated free donor-specific anti-human leukocyte antigen antibody (DSA) survival in patients without anti-HLA antibodies initially randomized in sirolimus (bold black lines, $n = 69$) and cyclosporine groups (dashed lines, $n = 73$). Lower panel: on-treatment (OT) analysis of estimated free DSA survival in CNI-exposed patients (dashed lines, $n = 111$) and others (bold black lines).

Anti-HLA antibody appearance

Presence of anti-HLA antibodies was reported in 30 patients after transplantation, 15 in each treatment group. With solid-phase assay, anti-HLA antibodies were identified in three of 30 patients before transplantation (sirolimus, two; cyclosporine, one). No pre-transplant anti-HLA antibodies was DSAs. Finally, anti-HLA antibodies appeared in 27 patients (sirolimus, 13; cyclosporine, 14; $P = 0.793$).

DSAs appeared in nine patients in the sirolimus group and eight patients in the cyclosporine group in an intent-to-treat analysis (Fig. 5). These DSAs were directed against class II HLA for 13 patients and class I HLA for 6 patients. Risk of DSA appearance was similar in both groups ($P = 0.520$). At the time of DSA appearance, five of 17 received sirolimus, while 12 received CNI (tacrolimus: three). To analyze carefully the risk of *de novo* DSA

according to the immunosuppressive regimen, we assessed the incidence of *de novo* DSA in patients without anti-HLA antibodies before transplantation ($n = 142$) according to immunosuppressive treatment before DSA appearance or last follow-up. Among 17 patients with *de novo* DSA, four had never been exposed to a CNI before DSA appearance. Finally, risk of *de novo* DSA was similar between 111 patients exposed to a CNI at any time (sirolimus group: $n = 38$, cyclosporine group: $n = 73$) and 31 patients exclusively treated with sirolimus ($n = 31$), as shown in Fig. 5 ($P = 0.439$).

Safety data

Malignancies

In total, 28 malignancies were diagnosed in 22 patients (15%) during the follow-up: 16 were skin cancers (3 squamous-cell carcinoma, 10 basal-cell carcinoma, and three Bowen's disease). The types of solid tumors were kidney ($n = 3$), cervical cancer ($n = 1$), oral cavity adenocarcinoma ($n = 1$), salivary gland tumor ($n = 1$), bladder cancer ($n = 1$), lung cancers ($n = 2$), and pulmonary lymphangitic carcinomatosis ($n = 1$). No post-transplant lymphoproliferative disease occurred after year 5, while two cases had been previously reported in sirolimus group. The two treatment groups did not differ in malignancy at 8 years (Fig. 6, $P = 0.757$). However, three of five patients with cancer in sirolimus group changed to tacrolimus before the diagnosis. Overall, 77% of patients in whom cancer developed had received a CNI before the diagnosis. To better assess the impact of CNI sparing on risk of malignancy, we compared patients who had never received a CNI before the occurrence of malignancy or at last follow-up ($n = 38$) and those who had received a CNI ($n = 107$) and found that the incidence of all cancers (log-rank, $P = 0.864$) or skin cancer (log-rank, $P = 0.558$) did not differ between the groups.

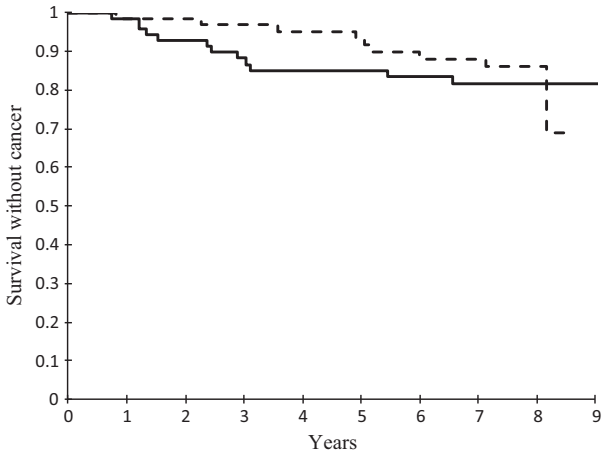


Figure 6 Risk of malignancy was similar in sirolimus and cyclosporine groups (ITT analysis). Estimated survival without post-transplant cancer in sirolimus (bold black lines, $n = 71$) and cyclosporine groups (dashed lines, $n = 74$).

PTDM, arterial pressure and anemia

For 99 patients analyzed at 8 years, only one PTDM was reported between 5 and 8 years after transplantation in each treatment group. In total, PTDM was diagnosed in 19 patients (sirolimus group, 11; cyclosporine group, eight), and the incidence was similar in two groups (log-rank $P = 0.215$). Antihypertensive treatment was required for 90% and 89% of patients receiving sirolimus and cyclosporine, respectively. The 2 treatment groups did not differ in mean systolic and diastolic pressure (138 ± 18 vs. 138 ± 15 mmHg, $P = 0.777$, and 82 ± 12 mmHg vs. 78 ± 11 mmHg, $P = 0.108$, respectively) or blood hemoglobin level (12.8 ± 1.8 vs. 12.9 ± 1.8 g/dl); 15% and 21% received erythropoietin in the sirolimus and cyclosporine groups, respectively. In patients remaining on sirolimus, hemoglobin level was 13.2 ± 1.8 g/dl.

Discussion

Our study reports the longest follow-up results for a randomized prospective clinical trial assessing the efficacy and safety of a *de novo* sirolimus-based strategy avoiding the use of CNIs. The benefit of sirolimus on renal function observed 5 years [8] after transplantation remained significant at 8 years, with a mean difference in eGFR of up to 15 ml/min as compared with cyclosporine-based treatment. More importantly, on conditional intent-to-treat analysis, eGFR was better with sirolimus than cyclosporine, although sirolimus was withdrawn in 48% of patients, more often replaced with tacrolimus. This global renal benefit in sirolimus arm was due to the very large improvement of eGFR observed in patients remaining under sirolimus at 8 years post-transplantation. However, no detrimental impact has been observed in patients in whom sirolimus has been withdrawn. Otherwise, the difference between the two treatment groups cannot be explained by a cyclosporine overexposure as daily dose decrease between the fifth and eighth post-transplant year, while mean trough cyclosporine level was 94.7 ± 23.6 ng/ml at 5 years.

Beside our trial, only 5-year results from two studies evaluating early conversion from cyclosporine to an mTOR inhibitor-based immunosuppressive regimen (CONCEPT and ZEUS trials) have been published [11,12]. Renal function was significantly better with mTOR inhibitor than CNIs in both studies in the intent-to-treat population, with eGFR (calculated by the MDRD formula) as an outcome. Indeed, eGFR was measured at 59.1 and 49.3 ml/mn in the sirolimus and cyclosporine groups in the post-CONCEPT study and at 66.2 and 60.9 ml/mn in everolimus and

cyclosporine (for 245/269 patients from the ZEUS study). This difference was greater for patients who remained under the randomized treatment at 5 years in both studies (mean difference 14.9 and 8.2 ml/min in the post-CONCEPT and ZEUS studies, respectively).

However, the better eGFR for patients under mTOR inhibitors observed between 5 and 8 years posttransplantation had no significant impact on graft and patient survival. Few data are available concerning long-term graft survival in patients at low immunological risk who received a kidney from a deceased donor and received cyclosporine, MMF, and steroids. Recently, Silva *et al.* reported a patient and graft survival of 92.5% and 85.3% at 4 years post-transplantation [13], similar to data observed in the Spiesser study (93.4% and 86.1%, respectively). Consequently, the outcome of patients in the cyclosporine group seems not to be biased. Of note, 75% of cases of graft loss that occurred after 5 years was in the cyclosporine group, and Gallagher *et al.* underlined that graft loss due to CNI-nephrotoxicity may appear as late as 15 years after transplantation [14]. These two elements argue for extending the follow-up of the Spiesser study.

De novo DSA appearance is a key event after transplantation because it increases the risk of antibody-mediated acute and/or chronic rejection, considered the first cause of graft loss [15]. The risk of DSA appearance following conversion of CNI to mTOR inhibitors remains disputed [16,17]. In our study, the risk of *de novo* DSAs was clearly similar in the 2 treatment groups, and the incidence of graft loss due to transplant glomerulopathy or chronic antibody-mediated rejection was not increased in patients with sirolimus. Liefeld *et al.* reported in renal graft recipients that early conversion from cyclosporine to everolimus was associated with increased risk of *de novo* DSAs and antibody-mediated rejection after the switch [9]. The number of patients randomized, dose of antimetabolic drug, and rate of steroids withdrawn were similar to our study, and beyond the mTOR inhibitor used and the different concerns already well described by Pascual concerning this trial [18], several important differences from our study should be pointed. First, basiliximab was used as initial immunosuppressive regimen in the ZEUS and HERAKLES trials [9], whereas thymoglobulin, which could reduce the occurrence of *de novo* DSA [19], induction was used in our study. Second, the sirolimus–MMF-based regimen was started at the time of the transplantation in the Spiesser study, whereas everolimus was introduced after 3 to 4.5 months with a cyclosporine-based immunosuppressive regimen in the ZEUS and HERAKLES studies. Our study findings are reassuring, in agreement with results of the ZEUS study at 5 years, which failed to confirm results previously reported by Liefeld *et al.* because DSAs were detected in 21.4% of patients in everolimus group and 20.0% in cyclosporine group, without increased risk of antibody-mediated rejection in patients receiving mTOR inhibitors [12]. Of note, the efficacy of the CNI-free regimen could depend on the daily dose of MMF [20]. In our study, most of patients received 1.5 g of MMF per day.

Finally, our long-term extended follow-up gives some information about the safety and efficacy of sirolimus in kidney transplant patients. Numerous data argue for a protective role of mTOR inhibitors that have antineoplasic properties [21,22] and prevent recurrence after a first skin cancer in kidney transplant patients [23,24]. In our study, we could not confirm such a protective effect. The number of patients in whom cancer developed during the 5-year follow-up tended to be higher in the cyclosporine group than in the sirolimus group [8]; results at 8 years were disappointing, even if most of the cancers developing reported in the sirolimus group occurred in patients switched to a CNI. The cumulative incidence of cancer in our study was similar to that reported in a large population of kidney transplant patients from registries [25]. Of note, the Spiesser study was not designed to evaluate the risk of cancer and results should not be over interpreted on this point. Among metabolic complications which could be considered as a side effect of mTOR inhibitors, only PTDM was available in this study. Thirteen percents of patients developed PTDM during follow-up, in agreement with several previous reports which assessed long-term incidence of PTDM [26,27]. Nevertheless, the risk of PTDM was similar in both groups, as recently reported in a recent meta-analysis [28].

The present study has several limitations. First of all, the rate of conversion from the randomized immunosuppression to other regimens is high both groups, especially in the sirolimus group. Nevertheless, the ITT analysis shows a clear renal benefit. Second, it is a retrospective analysis, but only 9 patients were lost to follow-up, mainly in the cyclosporine group. Third, concerning the identification of anti-HLA antibodies, solid-phase assay was routinely used in all centers only after 2007. Nevertheless, when DSAs were identified after transplantation, sera harvested before transplantation were usually tested by solid-phase assay to determine whether these antibodies existed before transplantation. Finally, risk of underestimating the incidence of DSA appearance after transplantation concerned mainly patients deceased or assumed to be under dialysis before 2007 regardless of treatment group. Of note, we observed only one vascular rejection during the first year with sirolimus [7].

Conclusion

This observational extended follow-up study confirmed the benefit of a CNI-free immunosuppressive strategy based on

sirolimus, which improved renal graft function at 8 years without increased risk of DSA appearance.

Authorship

PG: designed research/study, performed research/study, collected data, analyzed data, and wrote the manuscript. DB, CC, BHdL, P-FW, J-PR, AT, JS, BM, RS, JR, A-EH: collected data. BS: designed research/study and collected data. MB: analyzed data and wrote the manuscript. YL: designed research/study, analyzed data, and wrote the manuscript.

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