Long-term Results of Conversion From Calcineurin Inhibitors to Sirolimus in 150 Maintenance Kidney Transplant Patients

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Abstract

Objectives: **This retrospective single-center study evaluated long-term renal function after conversion from calcineurin inhibitors to sirolimus-based immunosuppression in kidney transplant recipients.**
Materials and Methods: **From 2001 to 2009, one hundred fifty kidney transplant recipients were converted from calcineurin inhibitors to sirolimus at least 3 months after transplant.**
Results: **After a mean follow-up of 171 weeks, 56.7% of converted patients remained on sirolimus. The 5-year survival rate of the patients (including intent-to-treat) and grafts was 85.5% and 83.6%. Patients on sirolimus showed significant improvement in renal function with a creatinine clearance of 50.9 ± 20.7 and 52.9 ± 20.8 mL/minute at month 0 and month 24. Independent predictive factors associated with a stable estimated glomerular filtration rate at the last follow-up of sirolimus patients were (1) having a living donor, (2) absence of anti-HLA alloantibodies at month 0, and (3) cyclosporine versus tacrolimus used before conversion.**

Adverse effects were reported in 134 patients (89.3%). They included (1) hospitalization for infection (n=52), (2) de novo proteinuria (n=40), and (3) eight patients with biopsy-proven acute rejection. Sirolimus was stopped and replaced by calcineurin inhibitors in 37 patients after a mean of 16 months treatment. After stopping sirolimus, renal-allograft function remained stable at 2 years.
Conclusions: **Conversion of calcineurin inhibitors to sirolimus in kidney transplant recipients was associated with improved renal function. The reintroduction of calcineurin inhibitors was safe in patients who were withdrawn from sirolimus owing to adverse effects.**

Key words: *Cyclosporin, Tacrolimus, mTOR, Renal transplant, Adverse effects*

Introduction

The use of calcineurin inhibitors (CNIs), cyclosporine and tacrolimus, has increased the half-life of kidney transplants compared with patients not taking CNIs, that is, receiving azathioprine-based immunosuppressive therapy.[1] However, long-term kidney transplant patients can develop interstitial fibrosis and tubular atrophy (IF/TA), in which the leading causative factor may be the long-term use of CNIs, as well as de novo cancers.[2, 3]

Sirolimus, a mammalian target of the rapamycin inhibitor, was launched in 2000 as an immunosuppressive therapy for kidney transplant patients because of its promising results in the phase 3 Rapamune Maintenance Regimen trial.[4] The use of this drug in de novo kidney transplant patients has been associated, in the mid-term, with improved allograft function compared to patients receiving cyclosporine.[5] In maintenance kidney transplant

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patients presenting with interstitial fibrosis and tubular atrophy (IF/TA), conversion from CNIs to sirolimus-based immunosuppression is associated with improved renal function and stabilization of kidney-allograft lesions compared with patients maintained on CNIs.[6, 7] Furthermore, de novo and maintenance kidney transplant patients receiving sirolimus-based immunosuppressive therapy have a significantly lower incidence of cancer than patients receiving CNIs.[8, 9]

In 2001, the Department of Nephrology, Dialysis and Organ Transplantation, at Toulouse University Hospital (France) began converting kidney transplant patients who developed IF/TA or de novo cancer from CNIs to sirolimus. By 2009, a total of 150 patients qualified for inclusion in this retrospective study, which assessed the long-term efficacy, safety, and tolerability of this strategy.

Materials and Methods

This single-center retrospective study included 150 (9.7%) of the 1553 patients in our kidney transplant cohort, who had been converted from CNIs (tacrolimus or cyclosporine) to a sirolimus-based immunosuppressive treatment between January 1, 2001 and May 1, 2009. All protocols were approved by the local ethics committee of the institution before the study began, and they conformed to the ethical guidelines of the 1975 Helsinki Declaration. Written informed consent was obtained from patients or their guardians. Patients who had received sirolimus as part of their immunosuppression therapy within the first 3 months after transplant or less than 12 months after sirolimus follow-up were excluded.

Conversion was abrupt: CNIs were stopped after an evening dose and 5 to 15 mg of sirolimus was given as a loading dose the following morning. Three milligrams per day was given thereafter. The first assessment of sirolimus trough levels was performed on day 10 after conversion, with the goal of obtaining a level between 8 and 12 ng/mL. Patients receiving concomitant mycophenolic acid therapy had their daily doses reduced by 25%. After conversion to sirolimus, prophylaxis for *Pneumocystis jiroveci* was implemented for 6 months at the discretion of the referring transplant physician.

Clinical and biological data were collected at the following intervals: (1) 12, 6, and 3 months before conversion, (2) on the day of conversion (M0), and (3) at 3, 6, 12, 18, and 24 months after conversion, and then yearly until the last follow-up. Clinical data collected included blood pressure, type of immunosuppressive regimen, the use of antihypertensive drugs, lipid-lowering agents, and other standard medical information.

The tolerability of sirolimus was addressed by recording any potential adverse effects, for example, cutaneous lesions, gastrointestinal symptoms, pneumopathy. Biological data, such as hemoglobin levels, white blood-cell counts, lymphocyte and platelet counts, serum-creatinine levels, eGFR (as assessed by the modification of diet in renal disease and Cockcroft-Gault formulae), sirolimus trough levels, natremia, calcemia, kalemia, serum bicarbonate, phosphorus, alkaline phosphatase, parathormone level, aspartate and alanine aminotransferases, gamma glutamyl transpeptidase, and proteinuria were recorded, as well as the occurrence of any acute rejections after conversion. A kidney biopsy was performed in 108 patients (72%) before conversion and classified according the Banff 2007 classification[10] to rule-out subclinical acute rejection and to determine the presence and/or severity of IF/TA lesions.

In cases where sirolimus therapy was stopped, we continued to monitor the same clinical and biological parameters as listed above at the following time points: the day sirolimus was stopped, and the patient was converted back to a CNI (T0), at 3 months (T3), 6 months (T6), 12 months (T12), and at 24 months (T24).

Statistical Analyses

Data are presented as means ± SDs, or medians (ranges) for quantitative variables, and the numbers of observations and frequencies for qualitative variables. The paired *t* test was applied to compare the differences in means between each time point and the baseline visit. For cases where the difference was not normally distributed, the Wilcoxon signed rank test was applied, and normality of distributions was tested by the Kolmogorov-Smirnov test. Patient and graft survival rates were assessed using Kaplan-Meier survival analysis. Predictive factors were calculated using univariate logistic regression. Parameters with a significance level of $P \leq .25$ were included in a multivariate model using a stepwise regression method. Odds ratios (ORs) are reported with 95% confidence intervals. All tests were 2 sided, and a P value of $< .05$ was considered statistically significant.

Statistical analyses were performed using SAS software (SAS Institute, Inc., version 9.1.3, Cary, NC, USA).

Results

Table 1 summarizes the baseline demographic data at the time of conversion to sirolimus for the 150 patients included in this study. There were 107 men and 43 women (mean age, 51.1 ± 14.7 y). All were receiving a CNI-based immunosuppressive therapy (cyclosporine, 64.7%; tacrolimus, 35.3%). Of the total, 100 were receiving long-term steroid therapy, and almost all were receiving antiproliferative drugs (mycophenolic acid, n=118; or azathioprine, n=11). The major reason for converting from CNI to sirolimus was the presence of IF/TA (58.7%) or the occurrence of de novo posttransplant cancer (29.3%), of which the majority (25/44) were skin cancers. When a kidney biopsy was performed before conversion (n=108), none displayed subclinical acute rejection, and the mean IF/AT score was 1.4 ± 0.8.

Table 1. Demographic data from kidney-transplant patients converted from calcineurin inhibitors to sirolimus.

Factor	Data
Sex: M/F (%)	107/43 (71.3/28.7)
Age at conversion (y)	51.1 ± 14.8
Time between transplant and conversion (mo)	51 (3-301)
Rank of transplant (1 vs > 1)	131/19
PRA level at transplant (%)	0 (0-96)
Patients with PRA > 0 (%)	12.9
HLA A/B/DR mismatches	3.2 ± 1.3
History of BPAR (yes, %)	24.8
Immunosuppression at conversion:	
Tac/CsA	53/97
MPA (Y/N)	118/32
Daily MPA dosage (mg/d)	1506 ± 464
AZA (Y/N)	11/139
Steroids (Y/N)	130/20
CAN score	1.4 ± 0.8
0, n	13
1, n	53
2, n	32
3, n	10
Reason for conversion to sirolimus:	
CAN (%)	88 (58.7)
De novo cancer (%)	44 (29.3)
Other (%)	18 (12)

Abbreviations: AZA, azathioprine; BPAR, biopsy-proven acute rejection; CAN, chronic allograft nephropathy; CsA, cyclosporine; F, female; HLA, human leukocyte antigen; M, male; MPA, mycophenolate acid; PRA, panel-reactive alloantibodies; SRL, sirolimus; Tac, tacrolimus

Patient and graft survival rates

The median follow-up after conversion (including intent-to-treat) from CNIs to sirolimus was 147 weeks (range, 3 to 373), that is, 2.8 years, and the median follow-up, during which the patients received sirolimus (per protocol), was 103.6 weeks (range, 1 to 360), that is, 2 years. Patient survival after conversion was 85.5% at 5 years. During follow-up, 13 converted patients died (8.6%) after receiving sirolimus for 16 months (range, 1 to 43). Among these 13, eight died while receiving sirolimus therapy for 17.25 months (1 to 43). Of these eight, 6 died owing to progression of the underlying cancer (which was the reason for conversion to sirolimus), 1 died of septic shock, and the other committed suicide. The remaining 5 patients died after they had been converted back from sirolimus to CNIs. Their causes of death included progression of bronchial adenocarcinoma (n=1), sudden death (n=2), cardiovascular disease (n=1), and hemorrhagic shock after a car accident (n=1). At the last follow-up, 14 patients (9.3%) had developed end-stage renal failure that required chronic dialysis after a median time of 30 months (range, 1 to 67 mo) of sirolimus therapy. Six of the 14 were still receiving sirolimus-based immunosuppression when they developed end-stage renal disease; hence, death-censored kidney-allograft survival at 5 years after conversion intent-to-treat was 83.6%.

Immunosuppression after conversion

The mean loading dose of sirolimus was 9.7 ± 5.4 mg. Mean daily doses of sirolimus on day 1 and month 24 were 0.05 (± 0.02) and 0.04 (± 0.03) mg/kg. The mean sirolimus trough level at 10 days after conversion was 12.1 ng/mL (± 7 ng/mL). This level had decreased significantly by month 3 (9.7 ± 4 ng/mL) and month 24 (8.2 ± 3.7 ng/mL) ($P < .001$). At the time of conversion, the daily dosage of mycophenolic acid was 1506 ± 466 mg. Dosage was decreased significantly to 1287 ± 414 mg by month 3, and to 1259 ± 373 mg by month 24 ($P < .001$). The daily dosage of prednisone was 0.1 mg/kg (range, 0.02 to 1.67 mg/kg) on conversion day; thereafter, the dosage was significantly decreased to 0.09 mg/kg (range, 0.02 to 0.53 mg/kg) by month 3, and to 0.07 mg/kg (range, 0.01 to 0.33 mg/kg) by month 24 ($P < .001$).

Outcome of renal function after conversion

Estimated glomerular filtration rate (eGFR) was significantly greater at 1 year before conversion (53.6 ± 16.8 mL/min) compared to the day of

conversion (50.2 ± 18.7 mL/min; $P = .002$) (Figure 1). After conversion, eGFR significantly increased to 52.9 ± 20.7 mL/minute at month 3 ($P < .001$), 53.3 ± 20.3 mL/minute at month 12 ($P = .05$), and 52.9 ± 20.8 mL/minute by month 24 ($P = .03$). After month 24, eGFR plateaued (Figure 1). On multivariate analyses, the predictive factors associated with improved or stable eGFR, when baseline values were compared to those at last follow-up after sirolimus conversion, were (1) having a living donor [OR = 12.66 (1.59 to 100.00); $P = .02$], (2) the absence of anti-HLA alloantibodies at M0 [OR=10.75 (2.28 to 50.00); $P = .003$], and (3) the type of CNI used before conversion, that is, cyclosporine instead of tacrolimus [OR=2.53 (1.09 to 5.87); $P = .003$] (Table 2).

Table 2. Independent predictive factors associated with stabilization or improvement of estimated GFR at last follow-up after conversion to sirolimus.

Variable	Odds ratio	*P* value
Living kidney donor	12.66 (1.59-100.00)	.02
Absence of anti HLA alloantibodies at M0	10.75 (2.28-50.00)	.03
Type of CNI before conversion (CsA instead of Tac)	2.53 (1.09-5.87)	.003

Abbreviations: CNI, calcineurin inhibitors; CsA, cyclosporine; HLA, human leucocyte antigen;Tac, tacrolimus

Proteinuria and sirolimus therapies

During the follow-up after sirolimus conversion, 50 patients (33%) presented with proteinuria > 0.5 g/d. Proteinuria was de novo in 40 cases (27% of the cohort), whereas the other 10 patients had proteinuria before conversion, of which 9 cases were mild proteinuria (range, 0.5 to 1 g/d) and 1 had proteinuria < 3 g/d. There were 5 additional patients with a history of mild proteinuria (range, 0.5 to 1 g/d) before conversion to sirolimus, but no detectable proteinuria after conversion. Proteinuria was > 0.8 g/d in 40 of the 50 cases. Also, during follow-up, 12 patients (8%) presented with proteinuria > 3 g/d. Of these, 5 had preconversion proteinuria of > 0.8 g/d (Figure 2).

Other biological parameters

There were no statistically significant changes in natremia, serum bicarbonate, parathormone, or alkaline phosphatase during follow-up. Conversely, there was a significant decrease in kalemia ($P = .004$) and calcemia ($P < .001$). Serum potassium dropped from 4.25 ± 0.54 at month 0 to 4.04 ± 0.43 mmol/L by month 24, and serum calcium dropped from 2.38 ± 0.28 on day 0 to 2.3 ± 0.15 mmol/L by month 24.

Hemoglobin levels had significantly decreased as early as 3 months after conversion, dropping from 12.9 ± 1.5 on month 0 to 12.2 ± 1.4 g/dL by month 3. There was also a significant decrease in mean

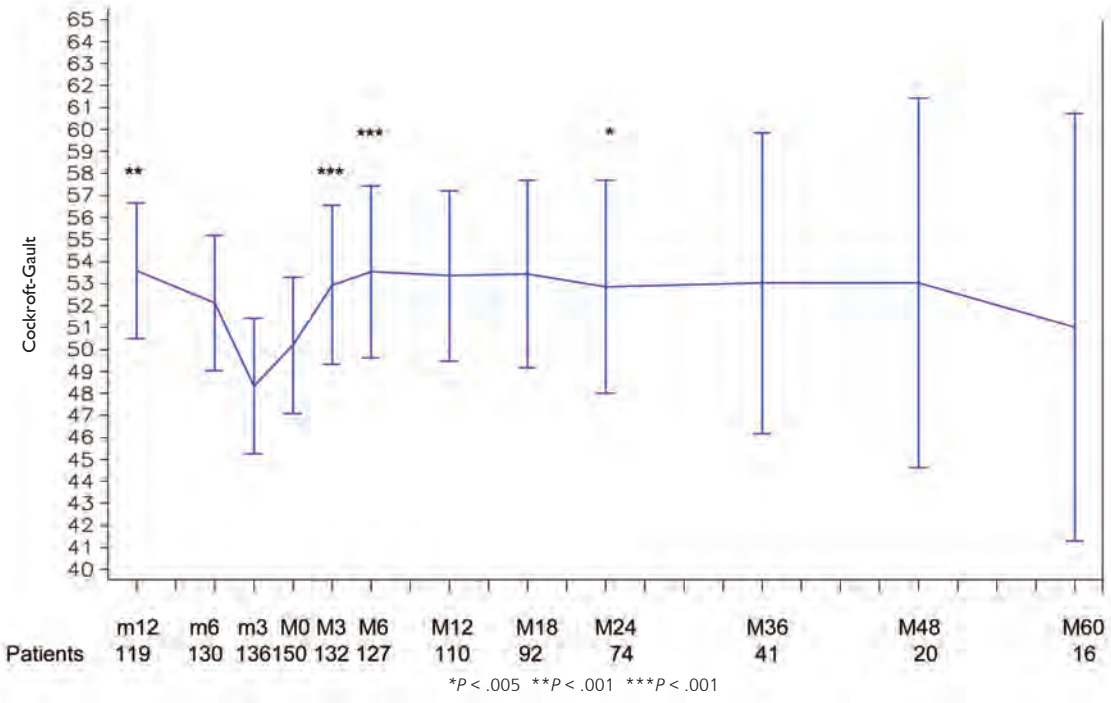

Figure 1. Outcome of creatinine clearance before and after conversion from CNIs to sirolimus therapy.
Abbreviations: CNIs, calcineurin inhibitors; m, months before conversion; M, month/s after conversion

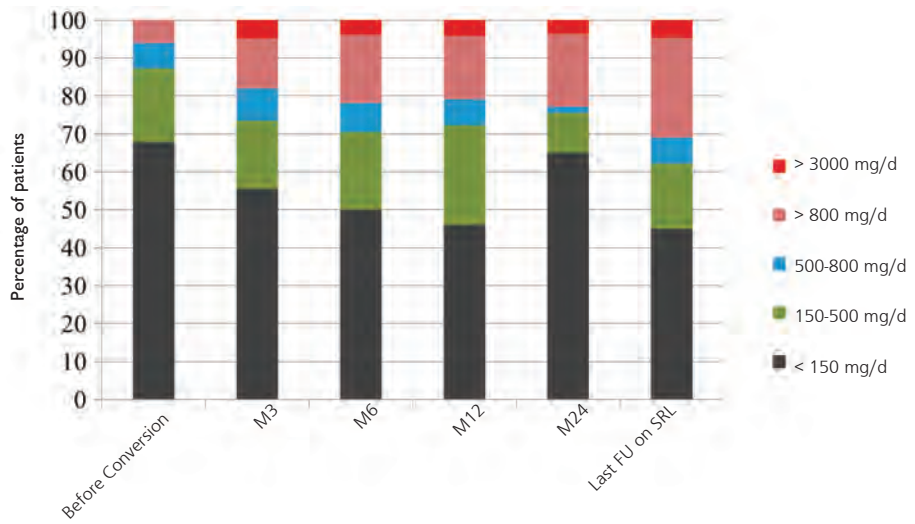

Figure 2. Outcome of proteinuria after conversion to sirolimus.
Abbreviations: FU, follow-up; M, month; SRL, sirolimus

corpuscular volume from 91 ± 5.2 on month 0 to 85.5 ± 9 fl by month 3 ($P < .001$), but no significant changes were seen in serum-iron concentrations, ferritin, or C-reactive protein levels. Because of sirolimus-induced anemia, the proportion of patients requiring recombinant erythropoietin therapy increased from 22.8% at month 0 to 34% by month 3, and stabilized thereafter. Sirolimus therapy appeared to be associated with significant decreases in total leukocyte and polymorphonuclear-cell counts ($P < .001$); however, their mean values remained within normal ranges (data not shown).

Sirolimus therapy was associated with statistically significant but clinically irrelevant increases in alanine and aspartate aminotransferases levels. However, total cholesterol, LDL cholesterol, and total triglycerides were significantly increased after conversion to sirolimus (Table 3), resulting in an increased number of patients requiring lipid-lowering agents ($P < .0001$). Independent predictive factors for the occurrence of dyslipidemia that required statin therapy after conversion were (1) needing conversion for a cause other than neoplasia [OR=4.96 (1.07 to 22.97); $P = .04$] and (2) the patient's age at conversion [OR=0.95 (0.92 to 0.98); $P = .01$].

Adverse effects after conversion to sirolimus

At least 1 adverse effect was reported by 134 patients (89.3%). Fifty-two patients developed infectious complications that required hospitalization. Of these, 22 were pulmonary infections, including opportunistic infections: 6 cases of pneumocystosis occurred at 3 months (range, 1 to 22 mo) after conversion, 2 cases of pulmonary aspergillosis occurred at 20 and 21 months after conversion, 1 case of nocardiosis at 1 month after conversion, and 1 case of herpetic pneumopathy at 3 months after conversion. Other infections included febrile urinary infections (n=15), sigmoiditis (3 episodes in a single patient), erysipelas (n=1), herpes zoster (n=1), and bacteremia (n=1). Independent predictive factors that required hospitalization at after conversion were being female [OR=3.12 (1.3 to 48); $P = .01$] and length of time on dialysis [OR=1.009 (1.001 to 1.016); $P = .02$].

Forty-seven patients (31.3%) presented with a total of 72 manifestations of cutaneous adverse effects after a median time of 1.25 months (range, 1 to 75 mo) after conversion. These included maculopapular skin eruptions (n=23), mouth ulcerations (n=23), peripheral edema (n=8), folliculitis

Table 3. Outcome of lipid parameters after conversion to sirolimus.

	M0 (n=150)	M3 (n=132)	M12 (n=110)	M24 (n=74)	M36 (n=41)	Last follow-up	*P* value
Triglycerides (mmol/L)	1.8 ± 1.08	2.28 ± 1.2	2.11 ± 1.43	1.9 ± 0.89	2.23 ± 1.34	2.46 ± 1.66	< .001
Total cholesterol (mmol/L)	4.83 ± 1.02	5.66 ± 1.25	5.37 ± 1.14	5.07 ± 1.08	5.2 ± 1.08	5.33 ± 1.55	.005
HDL cholesterol (mmol/L)	1.42 ± 0.54	1.55 ± 0.63	1.49 ± 0.48	1.47 ± 0.35	1.45 ± 0.4	1.4 ± 0.23	NS
LDL cholesterol (mmol/L)	2.64 ± 0.79	3.27 ± 1.05*	3 ± 0.95*	2.93 ± 0.86	2.93 ± 0.88	2.85 ± 0.96	NS
Statins (% of patients)	55	59.1	67.9	85.1	85.7	72.1	< .0005

Abbreviations: HDL, high density lipoproteins; LDL, low-density lipoproteins; M, month; NS, not significant
*denotes *P* value < .001 as compared to baseline.

(n=7), acne requiring treatment (n=6), and others (n=5). The independent predictive factor for cutaneous adverse effects after conversion to sirolimus was increased age [OR=1.03 (1.002 to 1.06); $P = .04$].

Eight patients (5.3%) presented with an episode of biopsy-proven acute rejection after conversion to sirolimus. In 4 cases, this was a steroid-sensitive acute cellular rejection that occurred at month 2 (n=1), month 3 (n=1), and at month 6 (n=2) after conversion; however, sirolimus was replaced by tacrolimus in only 1 patient after a biopsy-proven acute rejection. The fifth patient developed acute humoral rejection at month 2. Although he was treated with plasmapheresis and rituximab therapy, he lost his allograft 1 month later. The sixth and the seventh patients presented with late acute humoral rejection at month 18 and month 56. The sixth patient was treated with plasmapheresis, rituximab, and was converted from sirolimus to tacrolimus; however, he lost his allograft 31 months later. The seventh patient was treated with renal transplant and was given an increased sirolimus dosage, which allowed stabilization of renal function. The eighth patient presented with transplant glomerulopathy at month 36 after conversion. He was treated with renal transplant, which allowed stabilization of his renal function. The latter 4 patients with humoral rejection developed donor-specific alloantibodies at the time of rejection.

Discontinuation of sirolimus treatment

At the last follow-up, 65 patients had stopped sirolimus therapy owing to adverse effects (n=57) or death (n=8). The most frequent causes leading to sirolimus withdrawal were cutaneous adverse effects (n=15), heavy proteinuria (n=14), early degradation of renal function, or acute renal failure (n=6) (ie, sirolimus and/or gemcitabine-related microangiopathy [n=1], acute humoral rejection [n=1], chronic humoral rejection [n=1], and after conversion sirolimus-related acute renal failure [n=3]), progression to end-stage renal failure (n=6), gastrointestinal adverse effects (n=6), sirolimus-related pneumopathy (n=4), and other causes (n=10).

Outcome of patients who were converted back from sirolimus to calcineurin inhibitor-based immunosuppression

For 37 patients, sirolimus was stopped and replaced by either tacrolimus (n=30) or cyclosporine (n=7). This occurred at a median of 16 months (range, 1 to 32 mo) after conversion with sirolimus. After stopping sirolimus (T0), renal-allograft function remained stable: eGFR was 46.2 ± 17.9 mL/min at T0 and 46.9 ± 22.6 mL/min at 2 years after switching back to CNIs. Of the 37 patients, 5 developed end-stage renal disease after 27 months (range, 4 to 31 mo). One year after withdrawal of sirolimus-based therapy, a significant decrease was seen in triglyceride ($P < .004$) and total cholesterol levels ($P = .006$). Significant increases also were seen in hemoglobin level ($P < .01$) and mean corpuscular volume ($P = .001$) (data not shown).

Of the 37 patients who were converted back from sirolimus to CNIs, 13 had proteinuria > 0.8 g/d. Of these, proteinuria remained at > 0.5 g/d in 8 cases. Of the 12 patients that presented with nephrotic-range proteinuria after conversion to sirolimus, only 3 were converted back to CNIs, resulting in resolution of proteinuria in 1, a decrease to 1.5 g/d in the second, and no change in the third. Of the remaining patients, 2 were switched from sirolimus to an immunosuppressive regimen based on mycophenolic acid and steroids. Proteinuria disappeared in the first patient and decreased to 1.2 g/d in the second. Three patients progressed to terminal chronic insufficiency. One patient died from progression of ovarian adenocarcinoma with nephrotic syndrome, but had stable renal function. The last 2 patients' proteinuria decreased to < 1 g/d with rennin-angiotensin blockers and their renal function remained stable.

Occurrence of de novo cancer after conversion from calcineurin inhibitors to sirolimus

Seventeen patients (11.3%) presented with at least 1 episode of de novo cancer that occurred by 24 months (range, 2 to 67 mo) after conversion to sirolimus (Table 4). Noncutaneous cancers developed in 7 patients by 33 months (range, 2 to 67 mo) after conversion to sirolimus. In the other 10 patients, 17 de novo skin cancers had developed by 23 months (range, 5 to 34 mo) after conversion to sirolimus.

Discussion

This study included 150 patients, 60% of whom remained on sirolimus-based immunosuppression 2 years after conversion. In most cases, the reason for conversion from CNIs to sirolimus was IF/TA (58.7%) or de novo posttransplant malignancy (29.3%). Although eGFR steadily declined within the year

Table 4. Occurrence of de novo cancer after conversion from calcineurin inhibitors to sirolimus.

Patient No.	Conversion to sirolimus for de novo cancer (Y/N)	Time after conversion (mo)	Type of cancer
1	N	2	Renal adenocarcinoma
2	Y	24	Esophageal epidermoid cancer
3	Y	25	SCC
4	Y	13	SCC
5	Y	49	Prostate adenocarcinoma
6	N	13	Colon adenocarcinoma
7	N	13 (n=3)	BCC
8	N	27	SCC
9	Y	29 and 53	SCC then BCC
10	N	34	BCC
11	N	23	SCC
12	Y	15 and 45	BCCs
13	N	33	Tonsillar epidermoid cancer
14	Y	19	BCC
15	Y	5, 8, 10 (n=3), 15	BCCs
16	N	60	Anal epidermoid cancer
17	N	67	Duodenal large-cell lymphoma

Abbreviations: BCC, basal skin-cell carcinoma; N, no; SCC, squamous skin-cell carcinoma; Y, yes

before conversion to sirolimus, after conversion, there was significant and sustained improvement in eGFR, with a net gain of 3 to 4 mL/minutes for at least 3 years. Such a gain in eGFR after conversion from CNI- to sirolimus-based immunosuppression has been previously observed in 2 randomized studies.[6, 8]

In the CONVERT trial, 830 CNI-based kidney transplant patients, at 6 to 120 months after transplant, were randomly assigned to continue CNIs, or were converted from CNIs to sirolimus. At 12 and 24 months, on-therapy analysis showed significantly higher eGFR levels in sirolimus patients whose baseline eGFR was > 40 mL/minute. Post hoc analyses identified a subgroup with a baseline eGFR of > 40 mL/minute and a urinary protein/creatinine ratio of ≤ 0.11, who had a more-favorable outcome after conversion.[8] Stallone and associates studied 84 CNI-treated kidney transplant patients at 12 to 36 months after transplant. These patients had stable serum-creatinine levels, but their kidney-allograft biopsies showed IF/TA lesions. By 2 years after randomization, the allograft survival rate was significantly higher in patients converted to sirolimus (group 2) than in patients who had a CNI-dose reduction (group 1) (P = .03). Furthermore, for those who had repeat biopsies, the IF/TA lesions were significantly increased in group 1 but remained stable in group 2. After 24 months, all group 1 kidney biopsies showed increased alpha-smooth-muscle actin expression at interstitial and muscular levels. In contrast, alpha-smooth-muscle actin expression was dramatically reduced in group 2 patients.[6]

Other uncontrolled trials have examined conversion from CNIs to sirolimus in maintenance kidney transplant patients, but only 1 study had more than 85 patients.[11-14] Cardinal and associates studied 193 kidney transplant patients; however, their follow-up was limited to 2 years, and the authors were unable to demonstrate any significant change in eGFR. There was a small but significant increase in dipstick proteinuria at 1 year. From multivariate analyses, proteinuria ≥ 1 g/L at the time of conversion was the only predictor for deteriorating GFR at 1 year.[15]

Ours is the second largest uncontrolled study and the only one to have a median follow-up of 2.8 years after conversion from CNIs to sirolimus. We found that conversion was associated with significant improvement in eGFR. Among the independent predictive variables associated with improved eGFR, at the last follow-up after conversion, was preconversion treatment with cyclosporine-based immunosuppression rather than tacrolimus. This finding agrees with that of Witzke and associates who examined 78 kidney transplant patients who were converted from CNIs to sirolimus because of IF/TA.[14] They observed that the overall slope of creatinine clearance significantly improved only in those who were converted from cyclosporine, but not in patients receiving tacrolimus. This benefit, conferred by conversion to sirolimus, may be because cyclosporine induces more nephrotoxic renal lesions than tacrolimus, or because replacing cyclosporine with sirolimus may result in more potent immuno-suppression.

The magnitude of proteinuria at conversion has been associated with the renal outcome during after conversion to sirolimus. Dieckmann and associates demonstrated that patients with preconversion proteinuria of > 0.8 g/d had no renal benefit.[16] Witzke and associates found that low proteinuria at conversion was associated with improved renal outcome.[14] Our study did not confirm these findings, possibly because, at inclusion, only 10 of our 150 patients had proteinuria of 0.5 to 1 g/d, except for 1 case.

Other independent predictive factors for improved eGFR at last follow-up were having a kidney from a live donor and having no anti-HLA alloantibodies before conversion. These factors have

not been significant predictors for positive outcomes in previous conversion studies, possibly because the majority of patients studied have been recipients of a deceased- rather than a living-donor graft, and because anti-HLA alloantibodies were not routinely searched for before conversion to sirolimus.

In our study, de novo proteinuria occurred in 40 patients: 27% of the cohort. The occurrence of new-onset proteinuria during sirolimus therapy has been well-described in de novo and maintenance kidney transplant patients. Stephany and associates have shown that de novo kidney transplant patients treated with sirolimus had higher rates of proteinuria (≥ 1 g/d) at 12 months than those treated with CNIs.[17] The independent predictors for proteinuria at 12 months were GFR at 1 month (OR 0.62 per 10 mL/min/1.73; $P < .001$), delayed graft function (OR 11.5; $P = .02$), and receiving a sirolimus-based regimen (OR 4.18; $P = .002$). Dieckmann and associates have reported that CNI-free protocols in kidney transplant patients with advanced-age donors, and sirolimus-based treatment compared to mycophenolate mofetil, are associated with increased proteinuria in intent-to-treat cohort analysis.[18] In 68 maintenance kidney transplant patients converted from CNIs to sirolimus, Letavernier and associates demonstrated that baseline proteinuria increased from 0.39 ± 0.69 to 1.44 ± 1.90 g/d at 3 months ($P < .001$), and remained elevated at 6, 12, and 24 months. Moreover, when sirolimus was withdrawn from 19 patients, their proteinuria decreased from 1.95 ± 2.06 to 0.9 ± 1.4 g/d ($P < .05$).[19] Marx and associates have shown that the independent predictors for development of proteinuria after conversion from CNIs to sirolimus were preexisting proteinuria (> 0.15 g/d) and reduced glomerular filtration rate.[20]

After kidney transplant, the occurrence of de novo proteinuria may be related to alloimmune processes, such as transplant glomerulopathy, which is associated with donor-specific alloantibodies.[21] In our study, 11 patients presented with donor-specific alloantibodies, but in those presenting with increased proteinuria during sirolimus therapy, only 8 had donor-specific alloantibodies. Therefore, other mechanisms may be involved in causing either de novo or worsening of proteinuria during sirolimus therapy. Indeed, podocyte injury and focal-segmental glomerulosclerosis have been related to rapamycin inhibition in some patients, but the pathways underlying these lesions remain hypothetical.[22-24] Recent studies have provided evidence that sirolimus-induced proteinuria may be a dose-dependent effect of the drug on key podocyte structures, such as slit diaphragm-associated protein expressions.[25, 26]

At last follow-up, 43.3% of our converted patients had to interrupt sirolimus therapy for several reasons. This rate of withdrawal is average. Across previously published conversion studies, the main causes of sirolimus discontinuation include mouth ulcers, proteinuria, cutaneous rashes, and sirolimus-related pneumopathy.[8, 27, 28] Conversely, in our study the most common cause of sirolimus withdrawal was related to the allograft: 14 cases developed increased proteinuria and 12 developed worse renal function. Sirolimus withdrawal for cutaneous adverse effects occurred in only 15 patients.

We conclude that conversion from CNIs to sirolimus in maintenance kidney-transplant recipients is associated with improved renal function. Although 43.3% of patients had interrupted sirolimus therapy because of various adverse effects by the last follow-up, the reintroduction of CNIs was safe and renal-allograft function remained stable.

References

1. Hariharan S, Johnson CP, Bresnahan BA, Taranto SE, McIntosh MJ, Stablein D. Improved graft survival after renal transplantation in the United States, 1988 to 1996. *N Engl J Med*. 2000;342(9):605-612.
2. Nankivell BJ, Borrows RJ, Fung CL, O'Connell PJ, Allen RD, Chapman JR. The natural history of chronic allograft nephropathy. *N Engl J Med*. 2003;349(24):2326-2333.
3. Kauffman HM, Cherikh WS, Cheng Y, Hanto DW, Kahan BD. Maintenance immunosuppression with target-of-rapamycin inhibitors is associated with a reduced incidence of de novo malignancies. *Transplantation*. 2005;80(7):883-889.
4. Oberbauer R, Segoloni G, Campistol JM, et al. Early cyclosporine withdrawal from a sirolimus-based regimen results in better renal allograft survival and renal function at 48 months after transplantation. *Transpl Int*. 2005;18(1):22-28.
5. Ekberg H, Bernasconi C, Tedesco-Silva H, et al. Calcineurin inhibitor minimization in the Symphony study: observational results 3 years after transplantation. *Am J Transplant*. 2009;9(8):1876-1885.
6. Stallone G, Infante B, Schena A, et al. Rapamycin for treatment of chronic allograft nephropathy in renal transplant patients. *J Am Soc Nephrol*. 2005;16(12):3755-3762.
7. Pontrelli P, Rossini M, Infante B, et al. Rapamycin inhibits PAI-1 expression and reduces interstitial fibrosis and glomerulosclerosis in chronic allograft nephropathy. *Transplantation*. 2008;85(1):125-134.
8. Schena FP, Pascoe MD, Alberu J, et al. Conversion from calcineurin inhibitors to sirolimus maintenance therapy in renal allograft recipients: 24-month efficacy and safety results from the CONVERT trial. *Transplantation*. 2009;87(2):233-242.
9. Campistol JM, Eris J, Oberbauer R, et al. Sirolimus therapy after early cyclosporine withdrawal reduces the risk for cancer in adult renal transplantation. *J Am Soc Nephrol*. 2006;17(2):581-589.

10. Solez K, Colvin RB, Racusen LC, et al. Banff 07 classification of renal allograft pathology: updates and future directions. *Am J Transplant*. 2008;8(4):753-760.
11. Sundaram V, Abraham G, Fathima N, et al. Management of chronic allograft dysfunction by switch over to rapamycin. *Saudi J Kidney Dis Transpl.* 2010;21(1):37-42.
12. Gutiérrez MJ, González E, Andrés A, Morales JM. Clinical implications of proteinuria in renal transplant recipients switching to rapamycin for chronic allograft dysfunction. *Transplant Proc*. 2009;41(6):2348-2350.
13. Sola E, Lopez V, Gutierrez C, et al. Evaluation of the efficacy and safety of conversion to sirolimus in 85 renal transplant recipients. *Transplant Proc*. 2009;41(6):2137-2138.
14. Witzke O, Viklicky O, Türk TR, et al. Conversion to sirolimus of patients with chronic allograft nephropathy–a retrospective analysis of outcome and influencing factors. *Langenbecks Arch Surg*. 2009;394(6):1073-1078.
15. Cardinal H, Froidure A, Dandavino R, et al. Conversion from calcineurin inhibitors to sirolimus in kidney transplant recipients: a retrospective cohort study. *Transplant Proc*. 2009;41(8):3308-3310.
16. Diekmann F, Budde K, Slowinski T, et al. Conversion to sirolimus for chronic allograft dysfunction: long-term results confirm predictive value of proteinuria. *Transpl Int*. 2008;21(2):152-155.
17. Stephany BR, Augustine JJ, Krishnamurthi V, et al. Differences in proteinuria and graft function in de novo sirolimus-based vs. calcineurin inhibitor-based immunosuppression in live donor kidney transplantation. *Transplantation*. 2006;82(3):368-374.
18. Diekmann F, Gutiérrez-Dalmau A, López S, et al. Influence of sirolimus on proteinuria in de novo kidney transplantation with expanded criteria donors: comparison of two CNI-free protocols. *Nephrol Dial Transplant*. 2007;22(8):2316-2321.
19. Letavernier E, Pe'raldi MN, Pariente A, Morelon E, Legendre C. Proteinuria following a switch from calcineurin inhibitors to sirolimus. *Transplantation*. 2005;80(9):1198-1203.
20. Marx C, Busch M, Ott U, Gerth J, Wolf G. Proteinuria after conversion to sirolimus in kidney transplant recipients: impact of pre-existing proteinuria, graft function, and angiotensin-converting enzyme inhibitors/angiotensin-receptor antagonists. *Clin Transplant*. 2010;24(5):626-630.
21. Fotheringham J, Angel CA, McKane W. Transplant glomerulopathy: morphology, associations and mechanism. *Nephron Clin Pract*. 2009;113(1):c1-c7;discussion c7.
22. Letavernier E, Bruneval P, Mandet C, et al. High sirolimus levels may induce focal segmental glomerulosclerosis de novo. *Clin J Am Soc Nephrol*. 2007;2(2):326-333.
23. Letavernier E, Legendre C. mToR inhibitors-induced proteinuria: mechanisms, significance, and management. *Transplant Rev (Orlando)*. 2008;22(2):125-130.
24. Bumbea V, Kamar N, Ribes D, et al. Long-term results in renal transplant patients with allograft dysfunction after switching from calcineurin inhibitors to sirolimus. *Nephrol Dial Transplant*. 2005;20(11):2517-2523.
25. Stallone G, Infante B, Pontrelli P, et al. Sirolimus and proteinuria in renal transplant patients: evidence for a dose-dependent effect on slit diaphragm-associated proteins. *Transplantation*. 2011;91(9):997-1004.
26. Guan F, Villegas G, Teichman J, Mundel P, Tufro A. Autocrine VEGF-A system in podocytes regulates podocin and its interaction with CD2AP. *Am J Physiol Renal Physiol*. 2006;291(2):F422-F428.
27. Lebranchu Y, Thierry A, Toupance O, et al. Efficacy on renal function of early conversion from cyclosporine to sirolimus 3 months after renal transplantation: concept study. *Am J Transplant*. 2009;9(5):1115-1123.
28. Pearson TC, Mulgaonkar S, Patel A, et al. Efficacy and safety of mycophenolate mofetil (MMF)/sirolimus (SRL) maintenance therapy after calcineurin inhibitor (CNI) withdrawal in renal transplant recipients: final result of the Spare-the-Nephron (STN) trial. *Am J Transplant*. 2008;8 (suppl 2):213.