Long-Term Benefits with Sirolimus-Based Therapy after Early Cyclosporine Withdrawal

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Abstract. Graft function at 6 or 12 mo is positively correlated with renal transplant survival. The 36-mo results of a study that tested whether withdrawing cyclosporine (CsA) from a sirolimus (SRL)-CsA-steroid (ST) regimen would affect renal graft survival are reported. Eligible patients ($n = 430$) who were receiving SRL-CsA-ST were randomly assigned at 3 mo to remain on SRL-CsA-ST or to have CsA withdrawn (SRL-ST group). At 36 mo, the calculated GFR was significantly better with SRL-ST (47.3 *versus* 59.4 ml/min; $P < 0.001$) as was the slope of the GFR (−3.6 *versus* 0.8 ml/min; $P < 0.001$). This was accompanied by growing trend for improved graft survival in the SRL-ST group (85.1% *versus* 91.2%, $P = 0.052$ at 36 mo; 81.4% *versus* 91.2%, $P = 0.015$ in a cumulative data analysis up to 54 mo), despite numerically more biopsy-proven acute rejections after randomization (5.6% *versus* 10.2%; $P = 0.107$). Lipid parameters were similar between groups, whereas both systolic and diastolic BP were significantly lower in the SRL-ST group. Investigator-reported hypertension, abnormal kidney function, edema, hyperuricemia, hyperkalemia, gingival hyperplasia, and *Herpes zoster* occurred significantly more often in SRL-CsA-ST patients. Abnormal liver function test results, hypokalemia, thrombocytopenia, and abnormal healing were reported significantly more often with SRL-ST. The discontinuation rate was significantly higher for SRL-CsA-ST (48% *versus* 38%; $P = 0.041$). In conclusion, withdrawing CsA from a SRL-CsA-ST regimen at 3 mo after transplantation resulted in long-term benefits for renal transplant recipients.

Multiple risk factors, including donor origin and age, acute rejection, human leukocyte antigen (HLA) mismatches, ethnic origin, and diabetes, influence long-term graft survival in renal transplantation. The calcineurin inhibitors (CNI) cyclosporine (CsA) and tacrolimus are immunosuppressive agents that have been associated with a reduced incidence of acute rejection and improved graft survival during the first year (1). CNI, however, increase BP, decrease GFR, and contribute to chronic allograft nephropathy. Studies have shown that renal function (2, 3) and BP control (4) are important predictors of graft survival. Therefore, an intervention to improve renal function and BP control by eliminating CNI-related toxicity may result in an improvement in graft survival.

The recent introduction of sirolimus (SRL; rapamycin) has provided an important evolution in transplantation therapeutics, because this immunosuppressive agent does not increase BP or reduce GFR when compared with CsA (5). A phase 2 study (6) demonstrated that progressive withdrawal of CsA beginning at month 2 after transplantation followed by SRL-steroid (ST) therapy resulted in significantly improved calculated GFR at month 12 when compared with patients who remained on SRL-CsA-ST. In contrast, another trial showed that late CsA withdrawal (>15 mo after transplantation) in stable renal transplant patients who received mycophenolate mofetil (MMF), CsA, and ST resulted in no significant differences in renal function (7). The incremental increase in biopsy-confirmed acute rejection was 3.4% for early CsA withdrawal in the SRL study and 8.2% for late CsA withdrawal in the MMF study. Both rates are lower than the 11% mean difference in acute rejection rates between the CsA withdrawal and control groups as reported in a meta-analysis of studies that tested late CsA withdrawal with azathioprine (8).

The Rapamune Maintenance Regimen study has been the largest randomized trial testing CsA withdrawal in human renal transplantation. We previously reported the 12- and 24-mo results of this phase 3 trial (9, 10), which showed a low incremental risk of acute rejection, no difference in graft survival, better renal function, and lower BP compared with

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patients who remained on CsA and SRL. This article extends the long-term reporting of the earlier results and confirms the durability of the findings at 36 mo of therapy.

Materials and Methods

Study Design

This randomized, open-label, multicenter trial was conducted in Europe, Canada, and Australia. Approval was obtained from local ethics committees, and the study was carried out according to the Declaration of Helsinki. Complete 36-mo data and cumulative data through November 7, 2002 (up to 54 mo after transplantation), for graft loss are reported. For analysis of cumulative data beyond 36 mo, it was assumed that patients without reported graft loss had functioning grafts. Investigators were to report graft losses to the sponsor immediately (within 24 h).

The study design and eligibility criteria have been previously described (9). Briefly, 525 enrolled patients received SRL 2 mg/d (tablet formulation), CsA, and ST from the time of transplantation. SRL doses were adjusted to maintain whole-blood trough levels $\geq$5 ng/ml (monoclonal immunoassay). Eligible patients ($n = 430$) were randomly assigned at month 3 $\pm$ 2 wk to one of the treatment groups (SRL-CsA-ST or SRL-ST). Exclusion criteria for random assignment included a Banff grade 3 acute rejection episode or vascular rejection within 4 wk preceding randomization, dialysis dependency, serum creatinine >400 μmol/L, or inadequate renal function to support CsA elimination.

Patients who continued on triple therapy (SRL-CsA-ST; $n = 215$) received the same SRL dose (nominally 2 mg/d, troughs >5 ng/ml), CsA, and ST. In the CsA withdrawal group (SRL-ST; $n = 215$), SRL doses were increased to attain trough concentrations of 20 to 30 ng/ml (monoclonal immunoassay) through month 12, and 15 to 25 ng/ml thereafter. By chromatographic assay, these ranges corresponded to 16 to 24 ng/ml and 12 to 20 ng/ml, respectively. The recommended CsA target whole-blood trough levels in the SRL-CsA-ST group were 75 to 200 ng/ml (monoclonal immunoassay) from randomization through 24 mo and 50 to 150 ng/ml thereafter. On the basis of concurrent medical conditions (*e.g.*, renal function), however, the investigator could adjust CsA trough levels outside these recommended ranges.

Graft loss was defined as physical loss (nephrectomy), functional loss (necessitating maintenance dialysis for >8 wk), retransplantation, death with a functioning graft, or loss to follow-up. Graft function, as determined by serum creatinine and calculated GFR (11), was a secondary end point. On the basis of a protocol amendment, serum creatinine and urea levels were collected retrospectively for discontinued patients at 12 mo after transplantation and then prospectively at yearly intervals thereafter. Local pathologists confirmed acute rejection by renal biopsy using the 1993 Banff criteria (12).

Statistical Analyses

The primary efficacy end point was noninferiority of graft survival in patients who received SRL-ST compared with SRL-CsA-ST at 12 mo; graft survival at 36 mo was a secondary end point. Noninferiority of graft survival would be established at 36 mo if the 95% confidence interval (CI) of the difference (rate for SRL-CsA-ST minus rate for SRL-ST) crossed zero and if the upper limit of the CI was no more than 10%. Noninferiority of SRL-CsA-ST compared with SRL-ST would be established if the lower limit of the CI was more than -10%. Time to graft loss was analyzed using a log-rank test. All primary analyses were based on treatment-emergent events after randomization. Graft loss, death, acute rejection, and malignancy occurring after discontinuation from the study were included in the analyses.

Calculated GFR values were analyzed in patients who continued on therapy at 12, 24, and 36 mo and also by including values from discontinued patients (intention-to-treat [ITT] analysis). A quartile analysis was performed in which patients were divided into four equal groups according to their baseline calculated GFR (last value before randomization). Random coefficients regression analyses of calculated GFR *versus* time (slope analyses) were performed using on-therapy data over 6 to 36 mo and by using all values over the same period (ITT analysis). For slope analyses, calculated GFR was set to zero at the time of physical or functional graft loss, and subsequent values were censored.

A Fisher exact test was used for comparison of adverse events and other categorical variables. Vital signs and laboratory data including renal function were analyzed by analysis of covariance, using baseline (last value before randomization) as the covariate. On-therapy (per protocol), last-observation-carried-forward (LOCF), and completers analyses were performed for these parameters. A completer was defined as a patient who was still receiving assigned therapy 1050 d after the first dose of SRL (*i.e.*, the first day of the 36-mo time slot). All data from patients who discontinued before this time were censored for the completers analyses. Using this definition, the SRL-CsA-ST and SRL-ST groups had 123 and 136 completers, respectively. The number of antihypertensive drugs used was compared between groups using a Cochran-Mantel-Haenszel test.

Results

Patient Characteristics and Drug Dosages

A total of 525 primary (90%) or secondary (10%) renal allograft recipients with cadaveric (89%) or living (11%) donors were enrolled, and 430 eligible patients were randomly assigned to SRL-CsA-ST ($n = 215$) or SRL-ST ($n = 215$). There were no significant differences in recipient or donor demographics between the two randomized treatment groups. A total of 94.5% of recipients were white (9), reflecting the ethnic composition of patients awaiting renal transplantation in the countries in which this study was performed. CsA withdrawal was successful in 93.0% of patients who were assigned to SRL-ST.

There was excellent protocol adherence in obtaining target trough levels for both SRL and CsA. Median SRL whole-blood trough levels as measured by monoclonal immunoassay (median daily doses) in the SRL-CsA-ST group were 10.4 ng/ml (2.0 mg) and 10.8 ng/ml (2.0 mg) at months 24 and 36, respectively. In the SRL-ST group, these parameters were 21.8 ng/ml (6.0 mg) and 17.8 ng/ml (5.0 mg) at months 24 and 36, respectively. Median CsA whole-blood trough levels as measured by monoclonal immunoassay (median daily doses) in the SRL-CsA-ST group were 110 ng/ml (200 mg) and 95 ng/ml (175 mg) at months 24 and 36, respectively. There was no significant difference between groups in the daily ST doses (9, 10).

Graft Survival, Acute Rejection, and Patient Survival

Differences between groups in graft survival increased over time in favor of the SRL-ST regimen (Table 1, Figure 1). To date, the cumulative data up to 54 mo indicate that the difference in graft survival continued to increase beyond 36 mo and

Table 1. Graft survival (%)[a]

Time	SRL-CsA-ST (n = 215)	SRL-ST (n = 215)	Difference (95% CI)
36 mo[b]	85.1	91.2	−6.0 (−12.1 to 0.0)
graft loss	7.0	3.3	
death with functioning graft	4.7	3.7	
lost to follow-up	3.3	1.9	
Cumulative[b,c] (up to 54 mo)	81.4	89.8	−8.4 (−15.0 to −1.8)
graft loss	7.9	3.3	
death with functioning graft	6.5	4.2	
lost to follow-up	4.2	2.8	

[a] SRL, sirolimus; CsA, cyclosporine; ST, steroids; CI, confidence interval.
[b] Patients lost to follow-up were counted as events.
[c] Cumulative data analysis assumes that patients without reported graft loss had functioning grafts.

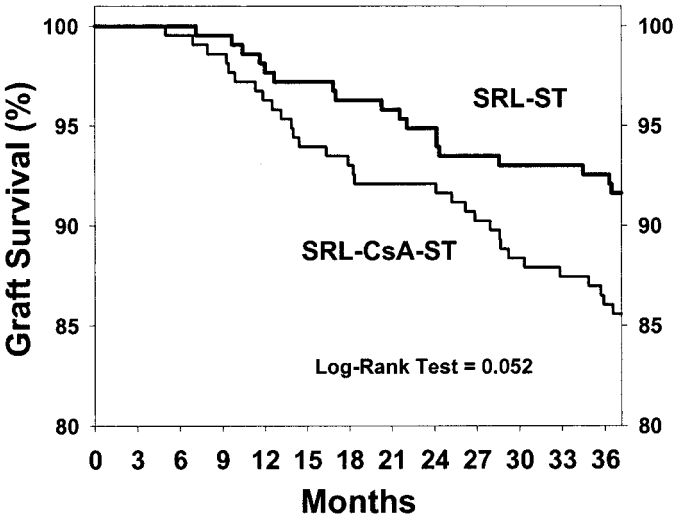

Figure 1. Time to observed graft loss at 36 mo.

subsequently became statistically significant (Table 1). The log-rank P value for the cumulative data analysis was 0.015.

The incidence of first biopsy-proven acute rejection between randomization and month 36 was 4.7% higher with SRL-ST (5.6% *versus* 10.2%), but this difference was not statistically significant (P = 0.107). Between months 12 and 36, three primary biopsy-proven acute rejections occurred in the SRL-CsA-ST group (posttransplantation days 650, 690, and 928, respectively), and 1 primary biopsy-proven acute rejection occurred after month 12 in the SRL-ST group (posttransplantation day 798).

In patients who received donor kidneys with fewer than three HLA mismatches, there was no significant difference in the incidence of postrandomization rejection between groups (6.8% *versus* 7.7%, SRL-CsA-ST *versus* SRL-ST, respectively; P = 0.823). The rate of acute rejection, however, was significantly higher with SRL-ST treatment in the presence of four or more HLA mismatches (4.5% *versus* 15.3%; P = 0.047). In both groups and regardless of HLA mismatch, all rejections after randomization were mild or moderate.

At 36 mo, patient survival excluding lost to follow-up (94.4% *versus* 96.3%, SRL-CsA-ST *versus* SRL-ST; P = 0.493) was not significantly different between groups. The primary causes of death were cardiovascular, including sudden death of unknown origin (2.3% *versus* 1.4%), infection (2.3% *versus* 2.3%), and diabetic complications (0.9% *versus* 0%) in SRL-CsA-ST *versus* SRL-ST, respectively.

Renal Function

Serum creatinine levels at 36 mo were significantly better in the SRL-ST group, either when measured in patients who remained on therapy (163 *versus* 127 μmol/L; $P < 0.001$) or when including values from discontinued patients in the ITT analysis (168 *versus* 145 μmol/L; P = 0.002). The same was true of calculated GFR (Table 2). Serum creatinine values were available for 96.4%, 94.7%, and 90.5% of patients with a functioning graft regardless of treatment status at 12, 24, and 36 mo, respectively. Figure 2 illustrates calculated GFR over time in the cohort of patients who remained on assigned therapy through month 36; values in discontinued patients were censored. Overall, the completers analysis represents the cohort of patients who tolerated and/or responded best to assigned therapy. It is interesting that this graph shows that renal function in the SRL-ST group continued to improve even well beyond month 12. In contrast, renal function in the SRL-CsA-ST group was characterized by a steady decline from the time of randomization. Overall, from month 6 through month

Table 2. Calculated GFR (Nankivell method, mL/min): Intention-to-treat analysis[a]

Time	SRL-CsA-ST	SRL-ST	ANCOVA P Value
Month 12	53.17 ± 1.46[b] (208)[c]	59.25 ± 1.46 (203)	<0.001
Month 24	48.38 ± 1.67 (203)	58.35 ± 1.60 (201)	<0.001
Month 36	47.26 ± 1.83 (194)	59.38 ± 1.82 (194)	<0.001

[a] ANCOVA, analysis of covariance.
[b] Mean ± SEM.
[c] Number of observations used to calculate the mean.

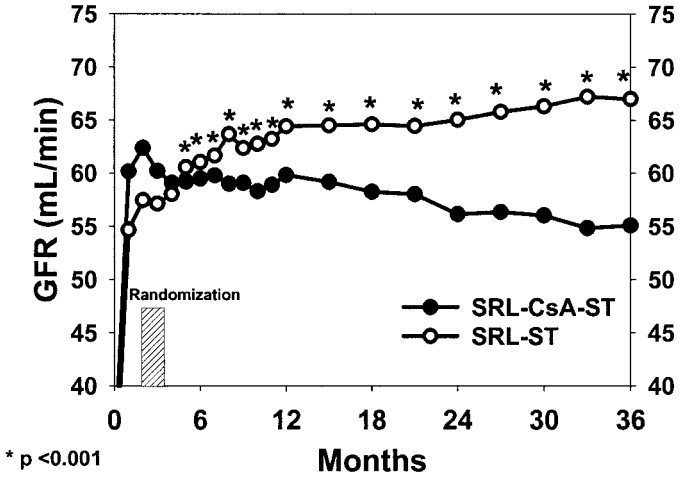

Figure 2. Calculated GFR in patients who completed 36 mo of therapy.

36, mean GFR values in patients who received SRL-ST were significantly higher than in those who received SRL-CsA-ST.

Table 3 shows the impact of acute rejection on calculated GFR, indicating poorer renal function in patients who experienced an acute rejection. Patients with an acute rejection before random assignment to SRL-ST, however, had significantly better renal function at 36 mo than those who remained on CsA. The same trend was observed for patients with acute rejections that occurred after randomization, although the difference was not statistically significant. When the occurrence of any posttransplantation rejection was included, the difference between groups (13.5 ml/min) was statistically significant (31.9 *versus* 45.4 ml/min; $P = 0.017$), favoring CsA withdrawal. Similarly, an ITT analysis of all data showed that patients who had four or more HLA mismatches and underwent CsA withdrawal also had better calculated GFR at 36 mo (47.0 *versus* 59.2 ml/min; $P < 0.001$).

To ascertain which patients responded better to CsA withdrawal over time, we divided patients into four equal groups, or quartiles, according to the last calculated GFR value before randomization (baseline). The first quartile consisted of patients with the lowest GFR values (≤45 ml/min) at baseline; the fourth quartile included patients with baseline GFR values >67 ml/min. Figure 3 shows the treatment effect on change from baseline to 36 mo in each of the quartiles (ITT analysis). For all four quartiles, the difference favored SRL-ST therapy, and these differences were statistically significant for the three quartiles with the lowest calculated GFR at baseline.

Trends in renal function were also estimated through slope analyses of calculated GFR over 6 to 36 mo (see Table 4). For both ITT and on-therapy data, the slopes were significantly negative for SRL-CsA-ST therapy, indicating a loss of renal function. The slopes were positive for the SRL-ST regimen, indicating an improvement in renal function, although this parameter was only significantly different from zero for the on-therapy analysis. The differences in slope (SRL-CsA-ST minus SRL-ST) were statistically significant (−5.4 ml/min per year [$P < 0.001$] and −3.9 ml/min per year [$P < 0.001$]) for the on-therapy and ITT analyses, respectively.

Safety

By 36 mo, significantly more patients had discontinued from assigned therapy in the SRL-CsA-ST group than in the SRL-ST group (47.9% *versus* 37.7%, respectively; $P = 0.041$), although these rates had been similar at 24 mo (34% *versus* 33%). The primary reason for discontinuation was an adverse event (66.0% *versus* 66.7% of discontinuations, SRL-CsA-ST *versus* SRL-ST, respectively). More patients who received SRL-CsA-ST discontinued because of CsA toxicity (8 *versus* 0; $P = 0.007$), nervous system disorders (5 *versus* 0; $P = 0.061$), and abnormal kidney function (11 *versus* 3; $P = 0.053$). In the SRL-ST group, more patients discontinued because of abnormal liver function test results (0 *versus* 3; $P = 0.248$) and hypertriglyceridemia (4 *versus* 7; $P = 0.543$). Discontinuation for lack of efficacy was similar between groups (7.0% *versus* 5.1%, SRL-CsA-ST *versus* SRL-ST, respectively).

The frequency of significantly different treatment-emergent adverse events is depicted in Table 5. These events are cumulative from the time of randomization and are similar to those reported at 12 mo (9). New events that were significant at 36

Table 3. Calculated GFR (Nankivell method, mL/min) at month 36 in patients with or without a primary biopsy-proven acute rejection (includes values after discontinuation)

Time of Rejection	Rejection	SRL-CsA-ST ($n = 215$)	SRL-ST ($n = 215$)	ANCOVA P Value
Prerandomization	No	48.4 ± 1.9[a] (176)[b]	60.3 ± 1.9 (174)	<0.001
	Yes	36.6 ± 5.8 (18)	51.6 ± 5.7 (20)	0.028
Postrandomization[c]	No	48.7 ± 1.9 (183)	61.3 ± 1.9 (177)	<0.001
	Yes	23.2 ± 6.6 (11)	39.5 ± 5.9 (17)	0.060
Posttransplantation[d]	No	49.9 ± 1.9 (166)	62.6 ± 1.9 (158)	<0.001
	Yes	31.9 ± 4.6 (28)	45.4 ± 4.2 (36)	0.017

[a] Mean ± SEM.
[b] Number of observations used to calculate the mean. GFR set to zero for graft loss.
[c] Includes both on-therapy period after randomization and follow-up after discontinuation periods.
[d] Any primary rejection from the time of transplantation.

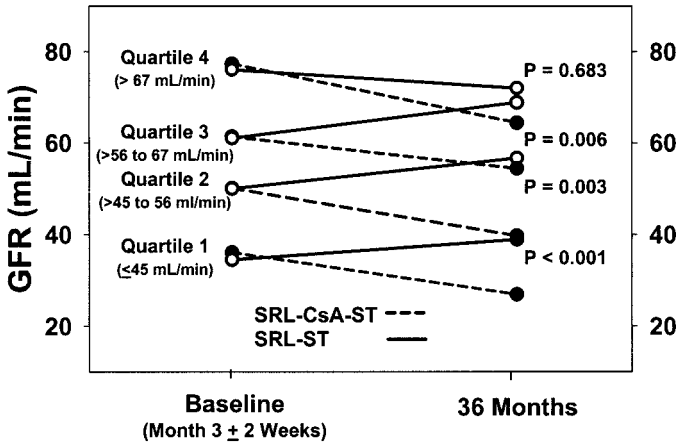

Figure 3. Quartile analysis of change from baseline to month 36 of calculated GFR (Nankivell method), including values after discontinuation. The symbols represent the means at baseline and 36 mo.

mo but not at 12 mo in the SRL-CsA-ST group included increased creatinine, abnormal renal function, toxic nephropathy, edema, gingival hyperplasia, and hyperkalemia, all of which are characteristic of CsA toxicity. In the SRL-ST group, only abnormal healing, ileus, and rectal disorder were significant events at 36 mo but not at 12 mo. Rectal disorder was generally associated with hemorrhoids.

BP remained lower with SRL-ST therapy from the time of randomization (Figure 4). At 36 mo, the differences in systolic (140.1 *versus* 131.3 mmHg; $P = 0.002$) and diastolic BP (81.2 *versus* 76.3 mmHg; $P = 0.006$) were both statistically and clinically significant (SRL-CsA-ST *versus* SRL-ST, respectively). Moreover, this difference was observed despite significantly ($P = 0.001$) lower use of antihypertensive medication in the SRL-ST group. Average weight gain between randomization and month 36 was similar between groups (3.27 kg for SRL-CsA-ST and 3.25 kg for SRL-ST).

Mean serum cholesterol values peaked at month 2, then decreased through month 9 and stabilized thereafter. Fasting total cholesterol (5.9 *versus* 6.3 mmol/L; $P = 0.059$) tended to be higher in SRL-ST patients at 36 mo, whereas HDL cholesterol (1.5 *versus* 1.6 mmol/L), LDL cholesterol (3.5 *versus* 3.6 mmol/L), and triglycerides (2.3 *versus* 2.4 mmol/L) were similar for the SRL-CsA-ST *versus* SRL-ST groups, respectively. Cumulative use of statins (75% *versus* 78%) and fibrates (25% *versus* 26%) to control serum lipids was comparable between SRL-CsA-ST and SRL-ST, respectively. From randomization through 36 mo, four SRL-CsA-ST–treated patients and seven SRL-ST–treated patients discontinued because of hypertriglyceridemia.

At 36 mo, mean hemoglobin values (126.4 *versus* 132.4 g/L; $P < 0.001$) were significantly higher in patients who received SRL-ST. One patient in the SRL-CsA-ST group (posttransplantation day 378) and two in the SRL-ST group (days 113 and 217) discontinued because of anemia. White blood cell counts (7.81 *versus* 7.45 $\times 10^9$/L, SRL-CsA-ST *versus* SRL-ST) and platelet counts (228 *versus* 227 $\times 10^9$/L) were not significantly different between treatment groups at month 36. One SRL-CsA-ST patient discontinued because of leukopenia (posttransplantation day 395), and one discontinued because of thrombocytopenia (posttransplantation day 792); no patients in the SRL-ST discontinued for either leukopenia or thrombocytopenia.

Infections were observed at a similar incidence in the two groups with the exception of *Herpes zoster*, which was significantly more frequent in the SRL-CsA-ST group (Table 5). Pneumonia, defined as any event reported by the investigator that included this term as well as other terms such as pulmonary interstitial infiltration, was significantly more frequent with SRL-ST therapy at 24 mo (13.0% *versus* 6.0%; $P = 0.021$), but the difference was no longer significant at 36 mo (14.4% *versus* 9.3%; $P = 0.135$). Most cases of pneumonia resolved with antibiotic treatment, and the patients remained in the study.

The overall incidence of malignancies at 36 mo was lower with SRL-ST (5.6% *versus* 11.2%; $P = 0.054$), which confirms the trend observed at earlier time points (Table 6). Rates of skin cancer, posttransplant lymphoproliferative disease/lymphoma, and other malignancies occurred less frequently in the SRL-ST group.

Discussion

The 36-mo results of this study showed that CsA can be eliminated successfully in *de novo* renal transplant patients who receive SRL-based therapy. By 36 mo, there was a growing trend for improved graft survival (91.2% *versus* 85.1%; $P = 0.052$) in SRL-ST patients, despite numerically more biopsy-proven acute rejections. Importantly, data available to date beyond 36 mo indicate that the advantage in graft survival continues to increase for patients who receive SRL-ST immunotherapy compared with those who remain on SRL-CsA-ST. As the cumulative data analysis assumes that patients without reported graft loss had functioning grafts, further confirmation is required when all patients have attained the future protocol-defined end points for graft survival at 48 and 60 mo.

Graft survival including loss to follow-up was the primary analysis. More patients were lost to follow-up in the SRL-CsA-ST group. Nevertheless, the conclusions would be the same for both the 36-mo and cumulative data if patients who were lost to follow-up were excluded. Notably, the advantage in the difference in graft loss censored for death in the SRL-ST group also increased from 3.7% at 36 mo to 4.7% with 41 to 54 mo of follow-up.

The incidence of first biopsy-proven acute rejection between randomization and 36 mo was 4.7% higher after CsA withdrawal. This difference was not significant, however, and could be explained by the higher incidence of acute rejection with SRL-ST therapy through month 12 in the presence of four or more HLA mismatches. All rejections after randomization were mild or moderate in severity. Moreover, patients who received SRL-ST had better renal function at 36 mo regardless of whether they had a biopsy-confirmed acute rejection or four or more HLA mismatches or both. Patients with a Banff grade 3 or vascular rejection within 1 mo before randomization (3 mo

Table 4. Slope of calculated GFR versus time (ml/min per year)[a]

Period	SRL-CsA-ST	SRL-ST	Difference
On-therapy, 6 to 36 mo			
slope (mean ± SEM)	−3.631 ± 0.444 (204)[b]	1.725 ± 0.455 (194)	−5.356 ± 0.636
95% CI	−4.505, −2.757	0.829, 2.622	−6.608, −4.104
P value[c]	<0.001	<0.001	<0.001
ITT analysis, 6 to 36 mo[d]			
slope (mean ± SEM)	−3.037 ± 0.453 (213)	0.827 ± 0.449 (212)	−3.864 ± 0.638
95% CI	−3.929, −2.146	−0.056, 1.709	−5.118, −2.610
P value	<0.001	0.066	<0.001

[a] ITT, intention-to-treat.
[b] Number of patients used to calculate the mean.
[c] Random coefficients regression model.
[d] Including values after discontinuation.

Table 5. Significantly different treatment-emergent adverse events and infections at 36 months[a]

Adverse Event	SRL-CsA-ST (n = 215)	SRL-ST (n = 215)	*P* Value[b]
Higher in SRL-CsA-ST group			
creatinine increased	33.0%	19.1%	0.001
hypertension	24.2%	10.2%	<0.001
hyperuricemia	16.7%	7.4%	0.005
abnormal kidney function	17.7%	7.4%	0.002
CsA nephrotoxicity	10.2%	2.8%	0.003
edema	10.2%	4.2%	0.024
toxic nephropathy	6.0%	0.9%	0.006
gingival hyperplasia	7.0%	2.3%	0.037
hyperkalemia	2.8%	0%	0.030
Herpes zoster	6.5%	0.9%	0.004
Higher in SRL-ST group			
thrombocytopenia	5.6%	12.1%	0.026
ALT increased	5.1%	16.3%	<0.001
AST increased	3.7%	12.6%	0.001
hypokalemia	3.3%	10.2%	0.006
healing abnormal	1.4%	5.6%	0.032
rectal disorder	0.9%	4.7%	0.036
ileus	0.0%	2.8%	0.030

[a] ALT, alanine transaminase; AST, aspartate transaminase.
[b] Fisher exact test, SRL-CsA-ST *versus* SRL-ST in randomly assigned patients.

± 2 wk) were excluded from randomization. Three of the nine patients with Banff grade 3 rejections during the first 2 mo of the prerandomization period were randomly assigned; therefore, there is insufficient information to determine the benefit of CsA withdrawal in a patient who experiences an early grade 3 rejection.

The advantage of improved renal function with CsA withdrawal increased steadily between 12 and 36 mo. This improvement was observed both in patients on therapy and in the ITT analysis. Importantly, a random-coefficients regression analysis of calculated GFR *versus* time (slope analysis) demonstrated that overall renal function declined in patients who remained on CsA, whereas it tended to improve or remain stable in SRL-ST–treated patients. Furthermore, a quartile analysis of the change in renal function between randomization and 36 mo showed that all patients benefited from early CsA withdrawal, regardless of their initial GFR levels. These differences were statistically significant for the three quartiles with lowest baseline renal function (calculated GFR <67 ml/min), indicating that patients with the poorest GFR levels at baseline benefited the most from early CsA withdrawal.

Cardiovascular disease is a major cause of morbidity and mortality in the renal transplant population. Renal impairment is an independent risk factor for cardiovascular death in renal transplantation (13), which underscores the clinical importance of improved renal function as observed in SRL-ST–treated

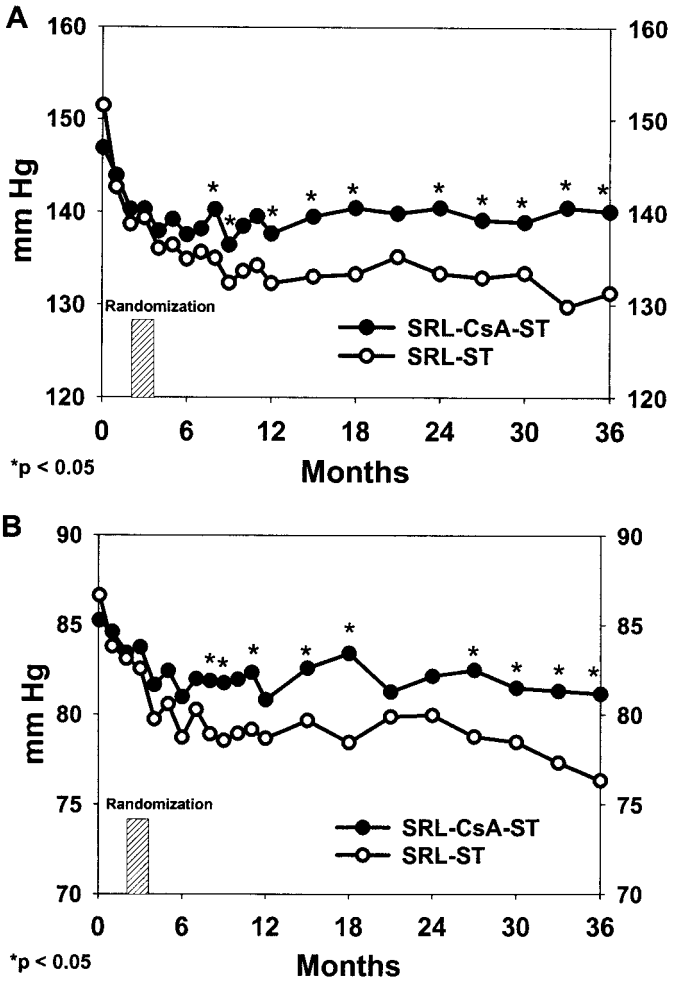

Figure 4. Mean systolic (A) and diastolic (B) BP values in patients who completed 36 mo of therapy.

Table 6. Malignancies at 36 months[a]

	SRL-CsA-ST (n = 215)	SRL-ST (n = 215)	P Value[b]
Total	11.2%	5.6%	0.054
Skin cancer	6.5%	3.7%	0.274
PTLD/lymphoma	1.4%	0.5%	0.623
Other	3.3%	1.4%	0.338

[a] PTLD, posttransplant lymphoproliferative disease.
[b] Fisher exact test for pairwise comparison, SRL-CsA-ST *versus* SRL-ST.

patients. Furthermore, CsA withdrawal resulted in a statistically significant and sustained improvement in BP, another independent and clinically meaningful cardiovascular risk factor. These results were obtained despite the use of significantly fewer antihypertensive medications. In contrast to these benefits to cardiovascular risk, SRL has been shown to cause increases in serum lipids. Nonetheless, despite the nearly twofold higher SRL trough concentrations in the SRL-ST group, unfavorable differences were not observed in laboratory measurements of these parameters at 36 mo. In both groups, serum lipids were controlled by the use of lipid-lowering agents.

The significantly lower incidence of *Herpes zoster* reported at 12 mo in the SRL-ST group was maintained through 36 mo. There was a numerically higher incidence of pneumonia in the SRL-ST group. Available evidence suggests that this increased incidence is due to an infectious rather than an atypical cause (pneumonitis). A potentially important finding was the lower incidence of malignancy at 36 mo in the group randomly assigned to SRL-ST. Furthermore, the myelosuppression observed in this study was modest and seemed to be similar between groups.

Both SRL and another mammalian inhibitor of rapamycin (mTOR) inhibitor, everolimus (currently under clinical investigation), enhance the nephrotoxicity of CsA (14–16). The present trial and other studies (5, 17) comparing SRL directly with CsA have shown a renal function benefit with SRL-based, CsA-free maintenance therapy. A similar enhancement of CNI nephrotoxicity has been reported when SRL is combined with tacrolimus (18). Minimizing CNI when combined with mTOR inhibitors is an area of intense interest, but it is unknown whether these strategies will produce a benefit in renal function as good as early CNI withdrawal or totally CNI-free therapy. The median CsA whole-blood trough levels, as measured by monoclonal immunoassay, are approximately 40% lower (unpublished data, Wyeth Research) than those used in phase 3 trials in which CsA was administered with 2- or 5-mg fixed doses of SRL (14, 15), yet despite these minimized levels of CsA, decreased renal function was still observed in patients who received SRL-CsA-ST therapy. Thus, early CsA withdrawal may be superior to CsA minimization when combining an mTOR inhibitor with CsA.

Could immunotherapy without CNI improve renal function further? An early pilot study suggests that a combination of SRL and MMF may offer advantages over CsA-MMF (19) in kidney transplantation. This approach was further refined in a subsequent pilot study adding induction therapy with basiliximab (17). Although acute rejection rates were similar at 12 mo (6.4% for SRL-MMF, 16.6% for CsA-MMF), calculated GFR was significantly higher in the SRL-MMF group (81.1 ml/min) than in the CsA-MMF group (61.1 ml/min). Large multicenter trials are warranted to test further the concept of immunotherapy completely free of CNI and to evaluate whether late conversion from CNI to SRL has the same benefits as early CsA withdrawal that the present study demonstrated. Finally, because nephrotoxicity is also problematic in nonrenal transplantation, conversion to CNI-free regimens also merits testing in selected patients who receive these types of organ transplants.

In conclusion, the 36-mo data from the Rapamune Maintenance Regimen trial provide further confirmation that early, progressive, and complete withdrawal of CsA from a combination of SRL, CsA, and ST is safe and effective in patients with mild to moderate immunologic risk. This CNI-free therapy results in long-term improvements in both renal function and BP without an increased risk of late acute rejection. These benefits have resulted in a growing advantage in graft survival.

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