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Sirolimus Conversion Regimen Versus Continued Calcineurin Inhibitors in Liver Allograft Recipients: A Randomized Trial

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A large prospective, open-label, randomized trial evaluated conversion from calcineurin inhibitor (CNI)- to sirolimus (SRL)-based immunosuppression for preservation of renal function in liver transplantation patients. Eligible patients received liver allografts 6–144 months previously and maintenance immunosuppression with CNI (cyclosporine or tacrolimus) since early posttransplantation. In total, 607 patients were randomized (2:1) to abrupt conversion (<24 h) from CNI to SRL (n = 393) or CNI continuation for up to 6 years (n = 214). Between-group changes in baseline-adjusted mean Cockcroft–Gault GFR at month 12 (primary efficacy end point) were not significant. The primary safety end point, noninferiority of cumulative rate of graft loss or death at 12 months, was not met (6.6% vs. 5.6% in the SRL and CNI groups, respectively). Rates of death at 12 months were not significantly different, and no true graft losses (e.g. liver transplantation) were observed during the 12-month period. At 52 weeks, SRL conversion was associated with higher rates of biopsy-confirmed acute rejection (p = 0.02) and discontinuations (p < 0.001), primarily for adverse events. Adverse events were consistent with known safety profiles. In conclusion, liver transplantation patients showed no demonstrable benefit 1 year after conversion from CNI- to SRL-based immunosuppression.

Key words: Calcineurin inhibitor, liver transplantation, maintenance therapy, nephrotoxicity, sirolimus

Abbreviations: ACR, acute cellular rejection; ANCOVA, analysis of covariance; AZA, azathioprine; BCAR, biopsy-confirmed acute rejection; CI, confidence interval; CL_{cr}, creatinine clearance; CNI, calcineurin inhibitor; CORE, computerized randomization/enrollment; CRF, chronic renal failure; DSMB, Data Safety and Monitoring Board; ESRD, end-stage renal disease; GFR, glomerular filtration rate; HCV, hepatitis C virus; ITT, intent-to-treat; LT, liver transplantation; MMF, mycophenolate mofetil; SCr, serum creatinine; SRL, sirolimus; TEAE, treatment-emergent adverse event.

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Introduction

Calcineurin inhibitors (CNIs) are associated with improved patient survival and decreased acute cellular rejection (ACR) rates in solid-organ transplant recipients (1). Nephrotoxicity, the most common side effect of CNIs, occurs as acute or chronic renal failure (CRF) (2,3), with >50% of patients with acute or CRF experiencing end-stage renal disease (ESRD) requiring hemodialysis or kidney transplantation (4). In a 13-year follow-up of liver transplantation (LT) patients, 10.3% developed CRF, with 5.4% progressing to ESRD. Of those with ESRD, 73% were diagnosed with CNI toxicity (5).

When renal function is impaired, improvement and preservation of renal function, without increasing the risk of

ACR, becomes paramount. Common strategies comprise reducing or eliminating CNI administration (6). Sirolimus (SRL), an inhibitor of mammalian target of rapamycin, is an immunosuppressive drug for solid-organ transplantation management (7). For renal allograft recipients, maintenance therapy conversion from CNI to SRL was safe and effective in patients with CNI-induced nephrotoxicity, resulting in excellent patient and graft survival with no difference in ACR episodes after 24-month follow-up (8). Superior renal function was observed in patients remaining on SRL through 12 to 24 months, particularly those with baseline glomerular filtration rates (GFR) >40 mL/min. For LT recipients, several small studies suggested that introducing SRL with concomitant CNI reduction or elimination in maintenance therapy can improve renal function in patients experiencing renal impairment (7,9–14).

Retrospective studies observed no improvement in kidney function (15) and increased proteinuria risk in SRL-treated LT patients (15,16). One randomized, single-center trial observed improvements in creatinine clearance at 3 months following CNI withdrawal and conversion to SRL, but between-group differences were not significant at 12 months (17). A second single-center trial evaluating 27 patients ≥6 months post-LT showed that patients switched to SRL had improvement in GFR after 3 ($p = 0.001$) and 12 months ($p = 0.02$) compared with those continuing CNI-based immunosuppression (18).

We report the results of the first large scale, randomized clinical trial prospectively evaluating the safety, tolerability and efficacy of a SRL-conversion regimen versus continuation of CNI-based immunosuppression in stable LT recipients at least 6 months posttransplantation.

Materials and Methods

Study design

The Sirolimus Liver Conversion Trial was a large international, randomized, open-label, parallel-group clinical trial. Eligible patients were randomly assigned (2:1 [SRL:CNI]) to undergo abrupt conversion (within 24 h) from CNI to SRL or to continue CNI therapy. Treatment was determined via a computerized randomization/enrollment system, with patients stratified by hepatitis C virus (HCV) status and whether or not they received antimetabolite therapy within 30 days prerandomization. Randomization was sequential, utilizing blocks of numbers assigned by the study sponsor for each transplantation center. Coprimary end points, assessed at 12 months (cut-off date for analyses was set for day 450 from study start), were baseline adjusted, calculated Cockcroft–Gault GFR (19) and the cumulative rate of graft loss or death.

Enrollment began in October 2002. After enrolling 49 patients, study enrollment was placed on voluntary hold (March 2003) at the request of the independent external Data Safety and Monitoring Board (DSMB) owing to safety concerns related to two deaths among SRL-conversion patients who experienced ACR with complications. Several protocol changes were approved by the DSMB and local ethics committees, and enrollment resumed (November 2003). Changes included limiting inclusion to patients with GFRs of 40–90 mL/min, extending the posttransplantation period for inclusion to 10 years (from 5), and establishing target trough levels of 8–16 mg/mL for patients receiving SRL.

This study was conducted according to the Good Clinical Practice guidelines and the Declaration of Helsinki. The study protocol and amendments were approved by the Institutional Review Board or independent ethics committee of each participating center. All patients provided written informed consent before enrollment. Those who remained on-study beyond 24 months provided a second informed consent.

Eligibility criteria

Eligible patients were aged ≥13 years, underwent orthotopic LT within 6 months to 12 years, and must have been on a stable immunosuppressive regimen of the same CNI (cyclosporine or tacrolimus) or a combination of CNI with corticosteroids and/or antimetabolite therapy for ≥4 weeks prior to randomization. Use of SRL or any of its derivatives prior to or at the time of the study was not permitted. Patients were required to have baseline Cockcroft–Gault GFR values of 40–90 mL/min. Doppler ultrasonography must have shown no evidence of thrombosis or clinically significant stenosis of the hepatic artery, hepatic vein, or portal vein. Patients were excluded if treated for biopsy-confirmed or clinically diagnosed ACR within 3 months pre-enrollment, or if they had a Banff 1997 grade III rejection within 12 months pre-enrollment.

Treatment

Patients randomized to CNI-continuation remained on cyclosporine or tacrolimus administered to attain trough concentrations of 50–250 ng/mL or 3–10 ng/mL (by standard monoclonal assays), respectively. Switching CNIs was permitted. Patients assigned to SRL-conversion received divided loading doses of SRL (10–15 mg). First dose was given after SRL trough level collection ≥4 h after the last CNI dose; second doses were given 12 h later. On study days 2 through 6, doses of 3–5 mg/day were administered. Thereafter, appropriate daily SRL doses were initially concentration-controlled to 6–16 ng/mL (chromatographic) and subsequently (following amendment at the request of the study's Data Safety and Monitoring Board) to 8–16 ng/mL (chromatographic) or 10–20 ng/mL (immunoassay).

Antimetabolite therapy with mycophenolate mofetil (MMF) or azathioprine (AZA) was allowed in either group. Those receiving daily antimetabolite therapy for ≥4 weeks prerandomization were permitted to continue. Those not on antimetabolite therapy at baseline could have MMF or AZA initiated at the time of ACR or for toxicity requiring CNI or SRL reduction. Antimetabolite therapy could be stopped, restarted or switched at investigator's discretion. Patients receiving corticosteroids at baseline were required to continue for at least the first 90 days of treatment. Corticosteroids could be started for an ACR episode and continued or discontinued at investigator's discretion.

Patients discontinuing the CNI or SRL treatment phase of the study could remain on-study for a follow-up phase. The time wherein a patient received originally assigned treatment was defined as the on-therapy period; time on-study after discontinuation of assigned treatment was deemed the follow-up period. Upon entering the follow-up phase, patients could receive whichever primary immunosuppressant (SRL, CNI) the investigator chose, even if it differed from the immunosuppressant to which the patient was initially randomized. Adverse events (AEs) and other study data, however, were analyzed as belonging to the original treatment group—regardless of immunosuppressant administered during follow-up. For example, a patient randomized to remain on CNI therapy who later discontinued the treatment phase of the study could enter the follow-up phase on SRL therapy, with any AEs or accrued data in the follow-up phase regarded as CNI-group data.

A 24-month treatment phase with up to 48 months of follow-up was amended in March 2007 to include a total treatment phase of up to 6 years.

End points

The primary efficacy end point was baseline-adjusted Cockcroft–Gault GFR in the intent-to-treat (ITT) population 12 months postrandomization. The primary safety end point was a composite of cumulative rates of graft loss or death at 12 months postrandomization; patients with missing graft and survival data at 12 month were counted as graft losses. Secondary end points included mean GFR values at 28 days, 2, 3 and 6 months, and yearly thereafter, incidence and severity of biopsy-confirmed acute rejection (BCAR), hypertension and incidence of malignancy/clinically important infection. Treatment failure was defined as the first occurrence of BCAR or premature treatment discontinuation.

Post hoc analyses were performed, stratifying for HCV status and concomitant antimetabolite therapy, to determine the percentage of patients showing clinically meaningful benefit (i.e. improvement from baseline GFR of ≥5.0 mL/min) from SRL-conversion or CNI-continuation.

Statistical analyses

The graft survival portion of the primary safety end point was used to determine sample size. Based on sample sizes of 400 and 200 patients in the SRL-conversion and CNI-continuation groups, respectively, the study had 88% power to reject the null hypothesis that graft survival rates in both groups were not equivalent. For the primary efficacy end point (renal function) with this sample size, a two-sided *t*-test at the 0.05 level of significance would have 89% power to detect a difference in 12-month mean baseline-adjusted GFR between both groups of 5 mL/min, assuming a standard deviation of 15 mL/min in both groups.

Analysis of covariance (ANCOVA) methods were used for the primary efficacy end point, with GFR at month 12 as the dependent variable, treatment and center as fixed effects, and baseline GFR as covariate. Differences in BCAR rates were assessed by 95% confidence intervals (CIs) and Fisher's exact test; rejection severity was assessed by a row-mean score-rank test. Kaplan–Meier methodology and log-rank test were used to evaluate patient and graft survival. Breslow–Day tests were used to assess significant interactions between treatment and strata.

Results

Patients

In total, 607 patients participated at 82 transplantation centers worldwide, comprising the ITT population (SRL-conversion, n = 393; CNI-continuation, n = 214). Of these, 599 (SRL-conversion, n = 389; CNI-continuation, n = 210) received at least one dose, comprising the safety population. Patients were aged 21–76 years, with no significant between-group differences in any baseline characteristic (Table 1). Over 90% of patients received livers from deceased donors. Over 50% of patients in each group were at least 3 years posttransplantation at the time of study entry (Figure 1).

Among CNI-continuation patients, 71 (34%) received cyclosporine and 139 (66%) received tacrolimus at study start. During the first 12 months, mean daily doses ranged from 147.4 (month 12) to 184.1 mg (day 7) for cyclosporine, and 3.6 (month 12) to 4.6 mg (day 7) for tacrolimus. For SRL-conversion patients, the mean±SD of the loading dose at the start of SRL therapy was 10.3 mg±2.1 (range, 2–20); thereafter, mean daily SRL doses during the first 12 months ranged from 3.44 (month 12) to 3.84 mg (month 2). Antihypertensive medications in the CNI and SRL groups were taken by 210 (54%) and 121 (58%) patients, respectively; lipid-lowering medications were taken by 28 (13%) and 49 (13%) patients, respectively. Concomitant use of anticholinesterases and angiotensin-receptor blockers were not significantly different between groups. All patients in both groups received nonstudy immunosuppressive medications.

Table 1: Baseline characteristics for all enrolled patients

Parameter	SRL conversion, (n = 393)	CNI continuation, (n = 214)
Mean age, y (SD)	55.4 (9.5)	54.7 (9.7)
Sex, % men	69	70
Ethnicity, n (%)		
White	313 (80)	173 (81)
Asian	43 (11)	20 (9)
Black	20 (5)	6 (3)
Hispanic	8 (2)	9 (4)
Other	9 (2)	6 (3)
HCV positive, n (%)	47 (12)	27 (13)
Receiving antimetabolite therapy, n (%)	149 (38)	86 (40)
Mean baseline Cockcroft–Gault GFR, mL/min (SD)	65.5 (18.7)	65.7 (19.6)
Time from transplant to enrollment, years, mean±SD (25th, 75th percentile)	4.0 ± 2.9	
(1.6, 5.8)	3.8 ± 2.7	
(1.7, 2.7)		

CNI = calcineurin inhibitor; GFR = glomerular filtration rate; HCV = hepatitis C virus; SRL = sirolimus.

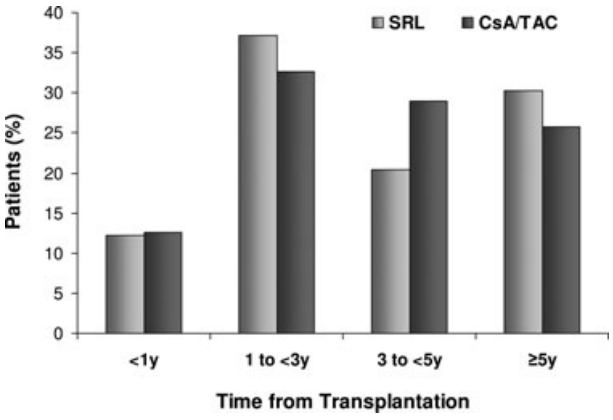


Figure 1: Proportions of patients based on time from transplantation to study inclusion.

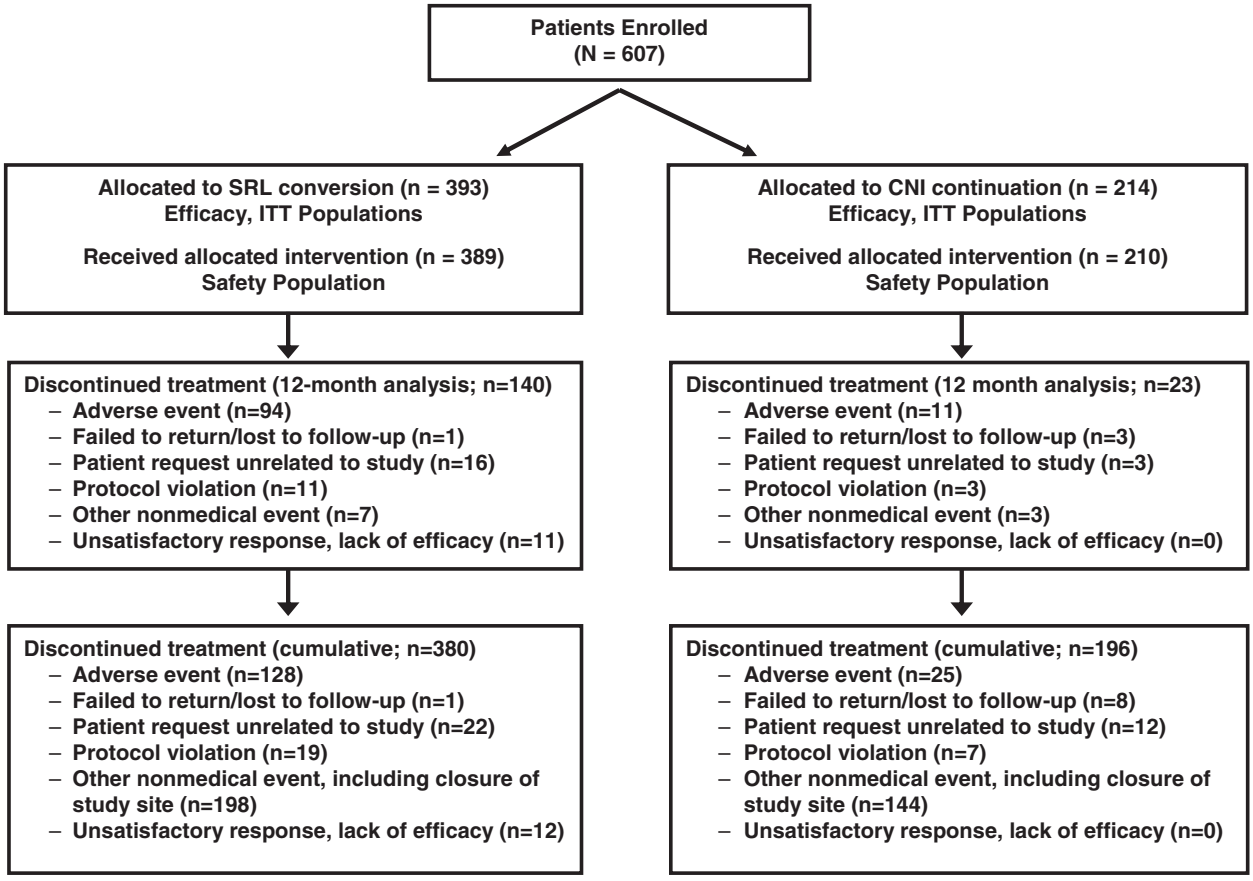


Figure 2: Patient disposition.

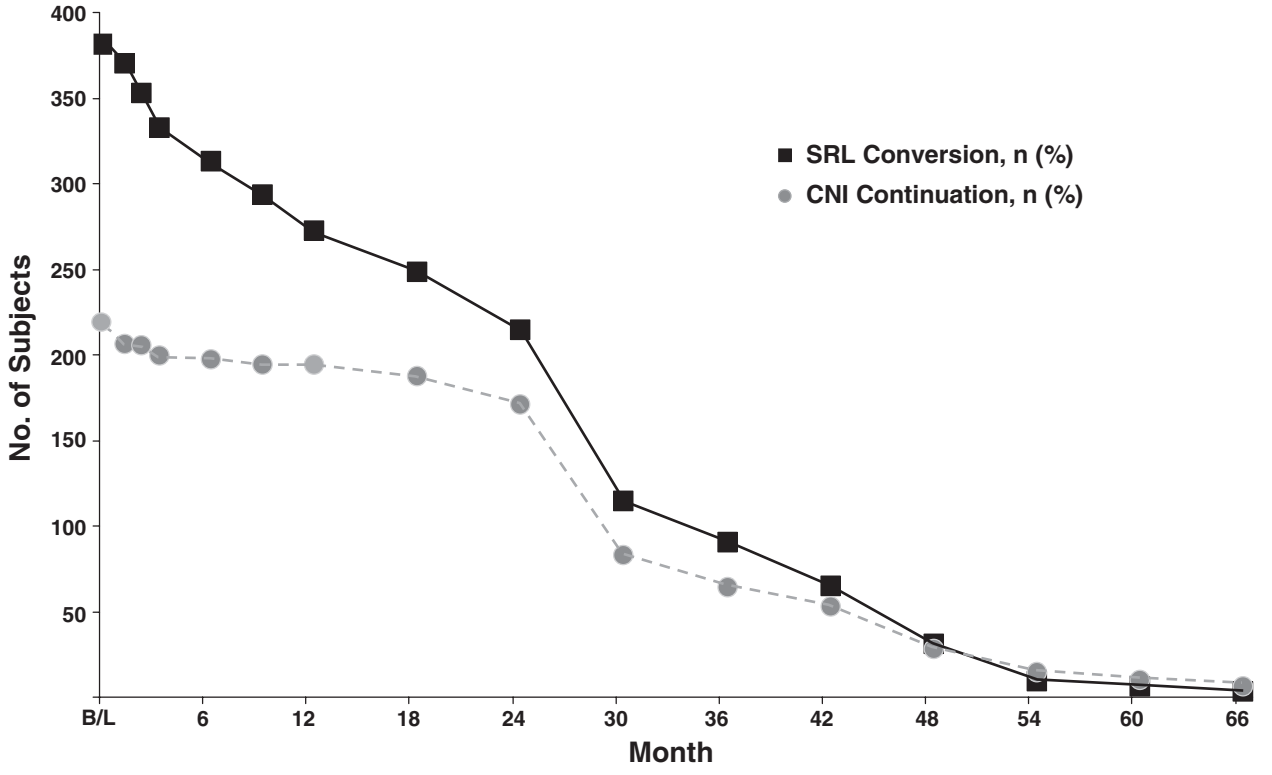
Patient disposition is delineated in Figure 2. Rates of discontinuation from the treatment phase of the study during the first 12 months were significantly greater in the SRL-conversion group (36% vs. 11%, $p < 0.001$), largely owing to AEs or unsatisfactory response. Cumulatively, 380 SRL-conversion patients and 196 CNI-continuation patients discontinued the treatment phase, although the majority (198 in SRL, 144 in CNI) did so owing to nonmedical events. Many patients were discontinued because of early study termination once it was evident that the study failed to meet both primary end points (Figure 3).

Among patients randomized to SRL, 194 (49.9%) switched back to a CNI upon discontinuation from the treatment phase. Thus, many patients in the SRL group received a CNI, often for a longer period (follow-up phase) than they received SRL during the treatment phase. In contrast, among patients randomized to CNI continuation, only 12 (5.7%) were switched to SRL during the follow-up phase.

Graft and patient survival

The primary safety end point was defined as the absence of graft loss due to retransplantation or death with a functioning graft within the first 12 months post-treatment. No graft losses due to transplantation occurred during the 12-month period (Table 2). Twelve-month survival rates for the composite end point were 93.4% with SRL conversion and 94.4% with CNI continuation. The weighted difference in rates was −1.2%; 95% confidence interval [CI], −5.2, 2.8. As the criterion for declaring noninferiority was prespecified as the lower boundary of the 95% CI for the difference in 12-month rates of graft loss being not less than 5%, the study did not meet its primary safety end point of noninferiority in graft loss rates.

All cumulative graft loss events in the SRL-conversion group and all but 1 in the CNI-continuation group (resulting in retransplantation) were attributable to death. Between-group differences in graft loss events through month 72 were not significant (Figure 4A). Cumulative data did not assign missing data as events; patients missing their last visit were censored at time of last follow-up. Differences in rates of death in the SRL-conversion and CNI-continuation groups in time-to-event analyses were not significant at 12 months (13 and 3, respectively) or cumulatively through 72 months (19 and 7, respectively). Most deaths occurred during the first 2 years.



SRL, n (%)	389 (100)	313 (80.5)	272 (69.9)	248 (63.8)	214 (55.0)	114 (29.3)	90 (23.1)	65 (16.7)	31 (8.0)	9 (2.3)	6 (1.5)	3 (0.8)
CNI, n (%)	210 (100)	197 (93.8)	194 (92.4)	187 (89.0)	171 (81.4)	83 (39.5)	64 (30.5)	53 (25.2)	28 (13.3)	14 (6.6)	10 (4.8)	6 (2.9)

Figure 3: Number of patients on study medication over time.

Biopsy-confirmed acute rejection

The BCAR rate at month 12 was significantly higher in the SRL-conversion group ($p = 0.02$) (Table 2). Any patient whose clinical rejection page was missing was scored as an ACR event. When excluding events for missing patients, rates were 6.4% versus 1.9% in the SRL and CNI groups, respectively. At 12 months, all moderate and most mild BCARs occurred in the SRL-conversion group. Cumulative BCAR rates through month 72 (Figure 4B) were numerically higher in the SRL group, but frequency was low and the difference was not significant. As with the cumulative graft loss/death analysis, patients with missing data were censored at time of last follow-up. Most BCARs in the SRL-conversion group (23/25) occurred early (within 3–6 months). In the CNI-continuation group, 3/7 events occurred in the first 10 months.

Treatment failure

Cumulative treatment failure rates (defined as occurrence of ACR or premature discontinuation of assigned treatment) as of study end are shown in Figure 4C. Overall, treatment failure occurred in almost twice as many SRL-conversion patients as in CNI-continuation patients (48.3% and 26.7%, respectively; $p < 0.001$). Among patients with treatment failure, 6.4% and 3.3% ($p = 0.104$) in the SRL-conversion and CNI-continuation groups, respectively, had ACR. Rates of dose discontinuation without acute rejection were 41.9% and 23.3%, respectively. Most treatment failures in the SRL-conversion cohort occurred in the first 12 months (126/188). In contrast, in the CNI-continuation cohort, 20/56 treatment failures occurred in the first 12 months.

Efficacy end points

Table 3 shows the intent-to-treat analysis of the change in baseline-adjusted mean Cockcroft–Gault GFR at 12 months. Mean changes were not significantly different between groups. Nonparametric analysis by rank ANCOVA confirmed the absence of significant differences in adjusted GFRs between groups. Therefore, the study's primary efficacy objective was not met.

A separate treatment comparison was made between mean baseline-adjusted GFRs for all patients with a GFR value available for each study visit. This comparison included patients still receiving their randomized, assigned therapy at that particular time point, with no values imputed for death, graft loss, or missing data. Mean observed GFR values and changes from baseline were significantly greater in the SRL-conversion group between day 28 and

Table 2: Primary analysis of safety end points at 12 months after randomization (ITT population)

End point	SRL conversion (n = 393)	CNI continuation (n = 214)
Patient and graft survival		
Overall graft survival, n (%)	367 (93.4)	202 (94.4)
Event rate, n (%)[1]	26 (6.6)	12 (5.6)
Weighted difference (95% CI) in event rates (CNI–SRL)	−1.2 (−5.2, 2.8)	
Components of overall graft survival, n (%)		
Graft loss	0	0
Death	13 (3.3)	3 (1.4)
Missing data counted as event	13 (3.3)	9 (4.2)
Biopsy-confirmed acute rejection		
Overall event rate, n (%)[2]	46 (11.7)	13 (6.1)
Weighted difference (95% CI) in event rates (CNI–SRL)	−6.0 (−10.6, 1.4)	
Missing data counted as BCAR	21 (5.3)	9 (4.2)
Acute rejection	25 (6.4)	4 (1.9)
Mild	14 (3.6)	4 (1.9)
Moderate	11 (2.8)	0 (0)

[1]Event rate is graft loss and death; Cochran–Mantel–Haenszel p = 0.56 (p values < 0.05 are significant).
[2]Cochran–Mantel–Haenszel p = 0.02 (p values < 0.05 are significant); log-rank p = 0.02.
Breslow–Day tests for significant interactions between treatment and strata); $p = 0.826$.
CI = confidence interval; CNI = calcineurin inhibitor; ITT = intent-to-treat; SRL = sirolimus.

month 3 ($p \leq 0.001$), but not thereafter. Following this early increase, mean GFR gradually returned to levels approximating those at baseline (Figure 5).

In a *post hoc* analysis of patients achieving an increase from baseline GFR of ≥5.0 mL/min, 30.5% and 29.9% of patients in the SRL-conversion and CNI-continuation groups, respectively, achieved this binary efficacy end point. Similar to the prespecified primary end point analysis, this analysis included values imputed to be zero, i.e. values missing because of death, graft loss or loss to follow-up before month 12. This analysis was also performed for the four concomitant antimetabolite therapy/HCV status strata (receiving antimetabolites/HCV+, no antimetabolites/HCV+, receiving antimetabolites/HCV−, no antimetabolites/HCV−); no significant differences were observed.

Mean serum creatinine (SCr) levels for on-therapy patients were similar at baseline, but at day 28 and months 2 and 3, mean values were significantly lower in the SRL-conversion group ($p < 0.001$). Thereafter, mean values were numerically but not significantly different through 5 years. After an

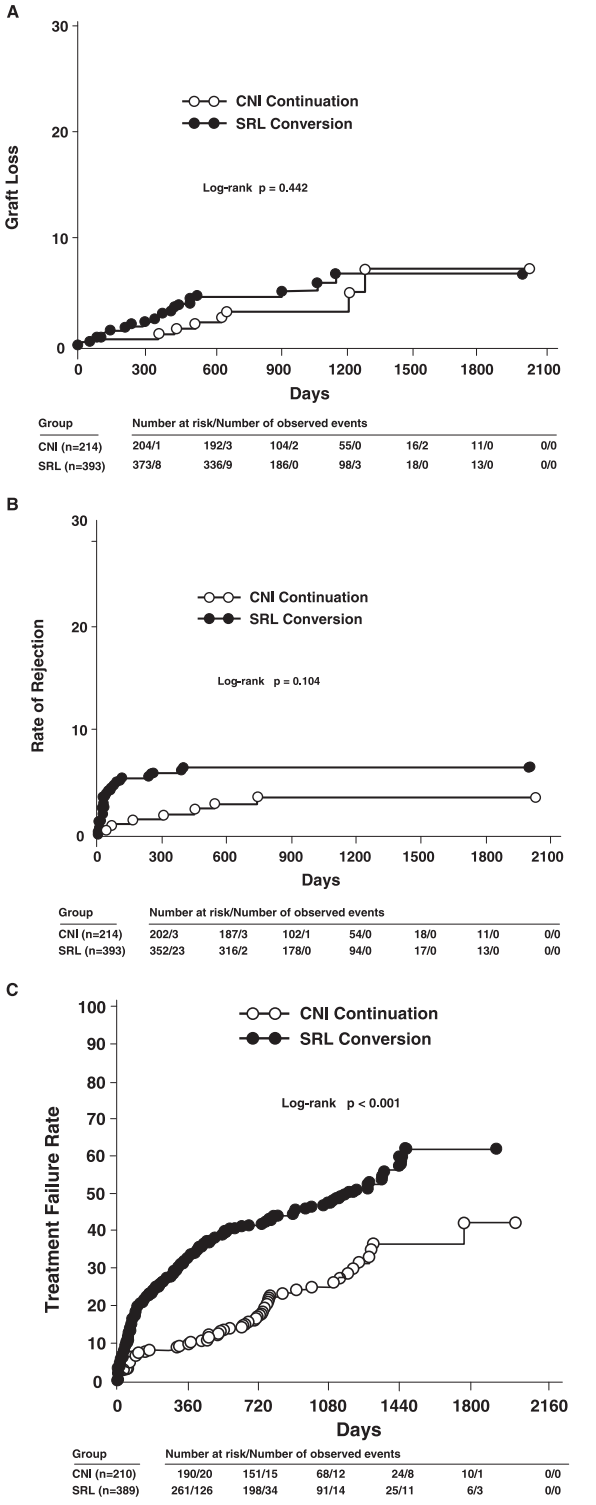


Figure 4: Cumulative analysis of safety end points (ITT population). Incidence of (A) graft loss or death, (B) biopsy-confirmed acute rejection and (C) treatment failure through month 72. Cumulative analyses (A and B) did not assign missing data as events; patients who missed their last visit were censored at the time of last follow-up.

Table 3: Primary efficacy end point: change from baseline in Cockcroft–Gault GFR at 12 months (ITT population)[1]

	Treatment		
Cockcroft–Gault GFR, mL/min	SRL conversion (n = 393)	CNI continuation (n = 214)	Rank ANCOVA p-Value
Change from baseline of the adjusted mean ± SE	−4.45 ± 1.12	−3.07 ± 1.36[1]	0.34
Maximum	49.67	45.85	—
75th percentile	6.75	6.00	—
50th percentile	−0.92	0.24	—
25th percentile	−9.98	−7.72	—

[1]Mean value of calculated Cockcroft–Gault GFR for all values where there was a valid baseline. Missing data counted as GFR = 0. Adjusted for baseline value; antimetabolite therapy status and hepatitis C status entered as fixed effects.
CNI = calcineurin inhibitor; GFR = glomerular filtration rate; ITT = intent-to-treat; SE = standard error; SRL = sirolimus.

initial decrease, the mean SCr in the SRL-conversion group gradually increased.

Treatment-emergent adverse events

Treatment-emergent adverse events (TEAEs) are reported for patients in both treatment groups, irrespective of whether they crossed over to receive the other treatment. Any TEAEs occurring at rates of ≥10% or differing significantly between groups at 12 months or cumulatively are listed in Table 4. At month 66, only four patients remained on therapy. Overall, significantly more SRL-conversion patients experienced ≥1 TEAE during the study compared with CNI-continuation patients (386 [99.2%] vs. 201 [95.7%]), p = 0.005). A significantly higher percentage of patients had infection-related TEAEs in the SRL conversion group compared with the CNI continuation group at 12 months (p = 0.001*)*, but differences in the cumulative data were not significant. Overall, 20 and 7 deaths occurred in the SRL-conversion and CNI-continuation arms, respectively (Table 5). Although the percentage of patients with malignancy-related TEAEs was not significantly different in the first 12 months, 6/15 deaths in the SRL-conversion arm and 2/3 deaths in the CNI-continuation arm at 12 months were malignancy related. Skin carcinoma occurred in a significantly lower percentage of patients in the cumulative data in the SRL-conversion group (p = 0.008), but no difference was seen at 12 months (p = 0.17).

Discussion

The use of CNIs as maintenance immunosuppression in LT patients is associated with long-term nephrotoxicity (20). The present study examined whether abrupt conversion from CNI to SRL in maintenance LT recipients would prevent further CNI nephrotoxicity, thus preserving renal function. Mean GFR at baseline in both treatment groups was 66.5 mL/min, indicating the study population had moderate renal insufficiency prior to study entry. However, the study did not meet its predefined primary efficacy end point of difference in baseline-adjusted Cockcroft–Gault GFR.

In the absence of significant deterioration in the CNI-continuation patients, no "benefit" (i.e. absence of nephrotoxicity) could be demonstrated in the SRL-conversion group. Of note, a retrospective analysis of measured GFRs conducted to predict renal failure after LT showed a slow but steady decline in GFR, and the lower the initial post-transplant GFR, the sooner renal failure developed (21). Although based on a small number of patients, those with GFR <60 mL/min/1.73 m^2 at month 3 had higher risk of renal failure, whereas those who did not develop renal failure seemed to maintain renal function long term. These observations may, in part, explain the lack of CNI-induced reduction in renal function, as mean baseline GFR was 66 mL/min. While 12 months may have been insufficient to detect significant deterioration in the CNI-continuation group, continued follow-up beyond 12 months, albeit with diminishing numbers of patients remaining on assigned treatment, revealed little if any change over time. It is also important to note the prolonged time from transplantation to enrollment in this study. While inclusion criteria allowed for patients to enroll as early as 6 months posttransplantation, mean time from transplantation to randomization was 4.0 and 3.8 years (median 3.1 and 3.4) for the SRL-conversion and CNI-continuation groups,

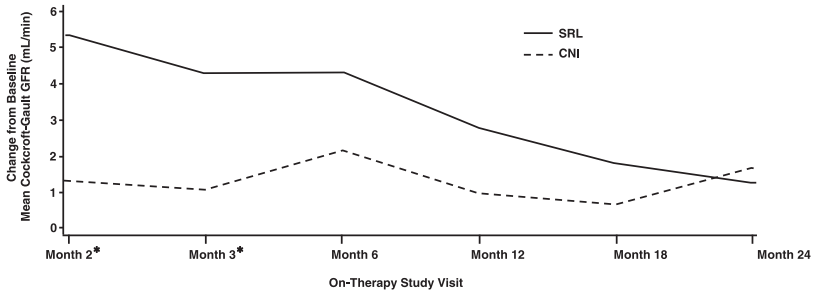


Figure 5: Observed change from baseline in Cockcroft–Gault calculated GFR. ∗p < 0.05 by ANCOVA with treatment as factor and baseline as covariate; all other timepoints not significant. Note: A spurious value was deleted from the CNI group at month 6.

Table 4: Between-arm comparison of treatment-emergent adverse events[1] occurring in at least 10% or having significant differences in the safety population[2]

	Month 12			Cumulative through 72 months		
Adverse event	SRL conversion (n = 389)	CNI continuation (n = 210)	p-Value[2]	SRL conversion (n = 389)	CNI continuation (n = 210)	p-Value[3]
Infections, n (%)						
Any	232 (60)	94 (45)	<0.001	269 (69)	130 (62)	0.08
Herpes simplex	29 (8)	1 (0.5)	<0.001	33 (9)	3 (1)	<0.001
Pneumonia	12 (3)	1 (0.5)	0.04	30 (8)	9 (4)	0.12
Hepatitis	7 (2)	0 (0)	0.10	9 (2)	0 (0)	0.03
Malignancies, n (%)						
Any	13 (3)	13 (6)	0.14	47 (12)	36 (17)	0.11
Skin carcinoma	7 (2)	8 (4)	0.17	14 (4)	19 (9)	0.008
Other, n (%)						
Any	385 (99)	178 (85)	<0.001	386 (99)	201 (96)	0.005
Hyperlipidemia	133 (34)	15 (7)	<0.001	159 (41)	20 (10)	<0.001
Diarrhea	109 (28)	22 (10)	<0.001	136 (35)	38 (18)	<0.001
Peripheral edema	100 (26)	19 (9)	<0.001	127 (33)	29 (14)	<0.001
Rash	105 (27)	11 (5)	<0.001	111 (29)	16 (8)	<0.001
Hypercholesterolemia	98 (25)	7 (3)	<0.001	110 (28)	9 (4)	<0.001
Stomatitis	94 (24)	2 (1)	<0.001	103 (27)	4 (2)	<0.001
Anemia	77 (20)	7 (3)	<0.001	94 (24)	19 (9)	<0.001
Headache	72 (19)	12 (6)	<0.001	84 (22)	23 (11)	<0.001
Fever	64 (17)	15 (7)	0.001	79 (20)	26 (12)	0.02
Asthenia	61 (16)	17 (8)	0.008	79 (20)	28 (13)	0.03
Pain	72 (19)	12 (6)	<0.001	71 (18)	39 (19)	0.91
LFTs abnormal	51 (13)	16 (8)	0.04	59 (15)	25 (12)	0.32
Thrombocytopenia	51 (13)	5 (2)	<0.001	58 (15)	8(4)	<0.001
Leukopenia	49 (13)	8 (4)	<0.001	55 (14)	10 (5)	<0.001
Acne	51 (13)	2 (1)	<0.001	52 (13)	4 (2)	<0.001
Pruritus	46 (12)	9 (4)	0.002	50 (13)	14 (7)	0.02
Mouth ulceration	40 (10)	1 (0.5)	<0.001	44 (11)	2 (1)	<0.001
SGPT increased	33 (9)	8 (4)	0.04	40 (10)	9 (4)	0.01
Creatinine increased	10 (3)	11 (5)	0.11	24 (6)	26 (12)	0.01

[1]Treatment-emergent adverse events were investigator-reported, and all, where applicable, may not have been proven diagnostically.
[2]Not all patients received their randomized assigned therapy for the entire follow-up period; 49.9% of patients who started on SRL were switched back to CNI when they discontinued the treatment phase, and 5.7% in the CNI group received SRL during follow-up.
[3]Fisher's exact test p value (two-tail).
CNI = calcineurin inhibitor; LFTs = liver function tests; SGPT = serum glutamic pyruvic transaminase; SRL = sirolimus.

respectively. As a result, a substantial proportion of patients had extended CNI exposure and may have incurred irreversible nephrotoxic effects prior to switching to SRL.

For the primary safety end point, no significant between-group difference was noted in graft survival rates. The majority of graft loss events (85%) in the SRL-conversion group occurred within 20 months; in the CNI-continuation group, half occurred after 20 months. With the exception of one retransplantation in the CNI-continuation group, all graft loss events were deaths (although no deaths were due to graft loss). Similarly, patient survival rates were not significantly different between groups. Again, the majority of deaths in the SRL-conversion group occurred within 20 months. The numerically lower patient survival rate with SRL treatment was partly attributable to the deaths of four patients who, in violation of the protocol, had either clinical documentation of preexisting malignancy at enrollment, or in whom a malignancy was strongly suspected to have existed pre-enrollment, based on its early presentation after randomization.

The significantly higher BCAR rate in the SRL-conversion cohort within the first 12 months (23/25) was primarily attributable to the abrupt change in immunosuppression, because most of these events occurred within the first 3 months. In current clinical practice, immunosuppression conversions are more often gradual, with weaning of the prior agent while exposure to the new agent is progressively increased. In the CNI-continuation groups, BCARs were evenly distributed for the study's duration. BCAR event rates at 12 months in both groups were due in part to the strict requirements for reporting, i.e. assigning missing clinical rejection data as BCAR events. It is important to bear this in mind, as after eliminating these missing data events, BCAR rates in the SRL and CNI-continuation groups were lowered by 46% and 69%,

Table 5: Patients who died and causes of death—cumulative data

Cause of death	Time of event (study day)	Treatment emergent? (Y/N)	Related to study drug?
Sirolimus conversion			
Infection related			
Sepsis	58	Y	Probably
Pneumonia	82	Y	Probably
Shock	335	Y	Definitely not
Sepsis	411	Y	Probably not
Septic shock	444	Y	Probably
Malignancy related			
Skin carcinoma	27	N[1]	Probably not
Carcinoma	49	Y[2]	Definitely not
Lymphoma	105	N	Definitely not
Carcinoma	190	N	Definitely not
Carcinoma	500	N	Probably not
Gastrointestinal carcinoma	297	N[3]	Definitely not
Carcinoma of lung	332	N[4]	Not related
Hepatic neoplasia	833	Y	Possibly
Skin melanoma	865	Y	Possibly
Gastrointestinal carcinoma	1020	Y	Probably not
Cardiovascular related			
Cerebrovascular accident	196	N	Definitely not
Myocardial infarct	529	Y	Possibly
Other			
Accidental injury	236	Y	Definitely not
Death	383	Y[5]	Definitely not
Liver damage	428	Y	Probably not
Gastrointestinal hemorrhage	504	Y	Probably not
CNI continuation			
Malignancy related			
Carcinoma of lung	524	Y	Probably not
Skin melanoma	356	Y	Probably
Gastrointestinal carcinoma	362	Y	Possibly
Gastrointestinal carcinoma	492	Y	Probably not
Carcinoma of larynx	1291	N	Definitely not
Other			
Aplastic anemia	43	Y	Definitely not
Pulmonary embolus	639	Y	Definitely not

[1]On immunosuppressive regimen other than SRL alone (e.g. CNI, CNI/MMF, CNI/SRL) at time of death.
[2]Protocol violation (previous cancer) but remained in study.
[3]Protocol violation (previous cancer) and was withdrawn from study treatment on day 29.
[4]Terminated participation in study before death; death is not included in death-related analyses and is included here for completeness.
[5]Case report form was missing at time of data lock, but identified subsequently; death is not included in death-related analyses and is included here for completeness.

respectively (Table 2). Ascertainment bias, due to a higher threshold for conducting biopsies in the conversion group versus standard of care, may have also contributed to reporting frequency of the presence of ACR. It is important to note that reporting bias of the presence/absence of rejection may have occurred as well, potentially owing to selection bias of biopsy performance in those patients with and without hepatitis C who exhibited abnormal liver enzymes.

The majority of treatment failures throughout the study out to 66 months (67%) in the SRL-conversion group occurred within the first 12 months, versus 36% in the CNI-continuation group. The largest contribution to the between-group difference resulted from discontinuation owing to TEAEs. The open-label nature of the study may have skewed some discontinuations because of perceived limited efficacy or AEs reflexively attributed to SRL by patients aware they were taking a new medication. Although this was an open-label, randomized study, change was introduced in only the SRL-conversion group, and results should be viewed with that consideration.

Over the entire study, significantly more patients in the SRL-conversion group experienced $\geq$1 TEAE (excluding infections and malignancies). The individual TEAEs that were significantly more frequent in the SRL-conversion group, excluding asthenia, were consistent with the known AE profile of SRL. Of note, among the 607 patients in this study, only one case of hepatic artery thrombosis was reported (SRL-conversion group), which did not result in graft loss.

The overall incidence of infection-related TEAEs was significantly different at 12 months but similar between treatment groups in the cumulative analysis. Significantly more patients in the SRL-conversion group had TEAEs of

hepatitis and presumptive (i.e. investigator reported but not necessarily confirmed by culture, stain or other definitive means) herpes simplex in the cumulative analysis. The clinical significance of the imbalance in investigator-reported hepatitis is unclear, as mean HCV RNA titers in all HCV-positive patients revealed significantly lower values in the SRL-conversion group at month 3 and numerically lower values otherwise.

Additionally, previous studies in renal transplant patients reported lower malignancy rates for those converted to SRL-based, CNI-free maintenance therapy (8). Although no difference in overall malignancy incidence was observed, it is possible that longer follow-up with more patients was needed to achieve statistical significance.

This trial was complicated by enrollment difficulties, amendments resulting in population changes, high discontinuation rates and treatment crossovers in the follow-up period, and less deterioration in renal function in the CNI-continuation arm than expected based on historical data. Study limitations include the potential effects of confounding variables, such as age and the wide range in time from transplantation at time of enrollment, both of which are associated with declining renal function as they increase. It is unknown whether conversion to SRL based on baseline creatinine clearance (CL_{cr}) could have potentially altered the study results. The primary end point, however, was adjusted for baseline GFR; thus, this does not appear to be a significant confounding factor. The accuracy of measured GFR versus calculated CL_{cr} in liver transplant patients, as described by Gonwa et al. (22), should also be considered. Given the nature of the study (multicenter, multinational), calculated GFR was deemed the most reasonable measure of renal clearance across the study sites, given the impractical and cumbersome nature of other, more accurate means in the context and time of this clinical trial. Other variables potentially influencing efficacy include time to initiation of concomitant antimetabolite therapy and/or time on concomitant therapy. In addition, patients switched early after transplantation may have an increased ACR risk, whereas those switching later may have already experienced additional detrimental effects on kidney function. We also acknowledge the large loading dose of sirolimus mandated by the study protocol due to the abrupt withdrawal of calcineurin inhibitor immunosuppression (10–15 mg; mean loading dose 10.3 $\pm$ 2.1 mg [range, 2–20]), which may have impacted certain aspects of the results (e.g. TEAEs, discontinuations, etc).

Based on these results, conversion from CNI-based to SRL-based immunosuppression did not result in renal function preservation compared with CNI continuation in maintenance LT recipients. However, given the confounding variables and limitations encountered during this trial, this strategy may still warrant further investigation.

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Conflicts of interest

At the time of this study, Dr. Maller, Dr. Scarola and Mr. Goldberg-Alberts were employees and stockholders of Wyeth Research. The remaining authors declare that they have no conflicts of interest to disclose as described by the *American Journal of Transplantation.*

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