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REVIEW

Clinical application of sirolimus in renal transplantation: an update

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Summary

In addition to an analysis of the final results of phase I/II and phase III clinical trials of sirolimus (SRL), this review focuses on the recent results of several studies in renal transplantation, which include diverse combinations of SRL with other immunosuppressive agents. While SRL was initially introduced as an adjunctive agent to calcineurin inhibitors, it is now serving as the base for therapies that spare or avoid these nephrotoxic drugs. However, to optimize the use of SRL as base therapy, further work is necessary to determine target concentrations, requirement for concomitant steroids and/or nucleoside synthesis blockers, and countermeasure therapy to overcome the drug's adverse effects.

Introduction

Sirolimus (SRL; rapamycin, Rapamune®; Wyeth Research, Philadelphia, PA, USA), a macrocyclic lactone, is a potent new immunosuppressant with a mechanism of action that is distinct from that of either calcineurin inhibitors (CNIs) or antimetabolites. It inhibits mammalian target of rapamycin (mTOR), a kinase that acts during both co-stimulatory and cytokine-driven pathways [1]. Since the approval by the US Food and Drug Administration in 1999 and by the European Agency in 2000, SRL has provoked great interest in the field of transplantation, as evidenced by the exponential increase in clinical applications.

The mechanism of action, preclinical findings, clinical pharmacology, results of phase I through phase III clinical trials, and the general safety and toxicity of SRL, especially in combination with cyclosporine (CsA), have been extensively reviewed [1–4]. Therefore, the present manuscript only briefly covers the results of the prelaunch clinical trials of SRL, but rather focuses on recent results published up to mid-2003 that describe recent regimens that combine SRL with other immunosuppressants for renal transplantation.

Combinations of sirolimus with CsA

The initial phase I/II dose-escalation trial of SRL with limited courses of steroids and a concentration-controlled regimen of full CsA exposures in mismatched living-donor renal recipients revealed a dramatic reduction in the incidence of acute allograft rejection episodes to 7.5% over 3 years (when compared with 32% from a control cohort of CsA-steroid-treated patients) [5]. This documentation of the potent immunosuppressive activity of SRL led to a randomized-controlled, multicenter phase II trial of SRL (1 or 3 mg/m^2/day) plus steroids that showed the possibility of CsA dose reduction among nonAfrican-American (but not African-American) recipients of cadaveric grafts who displayed an 8.5% incidence of biopsy-proven acute rejection episodes within the first 6 months after transplant, a result equal to that achieved with full-dose CsA [6].

Two large-scale phase III prospective, randomized, double-blind trials including nearly 1300 renal transplant

patients compared the efficacy and safety of two dose levels of SRL versus azathioprine (Aza; USA) or placebo (Global) comparators administered with a CsA-steroid baseline regimen. At 6 months, the rate of efficacy failure (a composite of the occurrence of acute rejection, graft loss, or death) was lower among the two SRL groups (2 mg 18.7%, 5 mg 16.8% for the USA; 24.7% and 25.6%, respectively, for the Global trial) than among the Aza or placebo comparator groups (32.3% and 47.7%, respectively; all $P \leq 0.002$). The frequency of biopsy-confirmed acute rejection episodes at 6 months was also lower among the SRL groups (2 mg 16.9%, 5 mg 12.0% for the USA; 30.0 and 19.2% for the Global trial) than among their respective comparator groups (29.8% or 41.5%, all $P \leq 0.003$). Patients treated with SRL showed a delay in the time to first acute rejection episode and decreased frequency of moderate and/or severe histologic grades of rejection episodes as well as in the requirement for antilymphocyte antibody treatment. The 12-month graft and patient survival rates were similar among all groups in the two trials.

The recently published analysis of the 24-month data from the phase III USA and Global trials revealed that patients in the 5 mg SRL groups continued to show a significant delay in onset and reduction in incidence of acute rejection episodes versus comparator regimens ($P = 0.02$/$P = 0.001$). Graft and patient survival rates as well as the occurrence of transplant-related infections, post-transplant lymphoproliferative disorders (PTLD), or other malignancies were similar among all treatment arms. A *post hoc* analysis that studied the relation between outcomes and drug concentrations documented that the SRL–CsA combination displays pharmacodynamic synergy in humans [7].

Between 12 and 24 months, patients treated with 2 mg SRL displayed stable mean serum creatinine (Cr) values (about 1.8 mg/dl), which were not significantly higher than those of the comparators. In contrast, both 5 mg groups showed a significantly increased mean serum Cr values. Both SRL dose groups showed persistently elevated triglyceride levels compared with Aza-treated patients at month 24; a difference that was less pronounced in the Global trial. Furthermore, despite continued therapy, SRL-treated patients showed persistent hyperlipidemia, which required continued countermeasure therapy, whereas other adverse events tended to display progressive resolution over time.

These trials (summarized in Tables 1 and 2) suggested that the impressive immunosuppressive potency was counterbalanced by enhanced CsA-related adverse effects, including nephrotoxicity, hypertension, and new-onset post-transplant diabetes mellitus (PTDM). Companion pharmacokinetic (PK) analyses demonstrated that concomitant treatment with SRL produces increased CsA exposure per milligram of administered dose. This effect was explained by the observation that SRL and CsA share both the cytochrome P450 3A4 metabolic pathway and the p-glycoprotein countertransport mechanisms. In an animal model, the exacerbation of renal dysfunction seemed to be attributable to a PK interaction of SRL to greatly increase CsA concentrations in whole blood and, particularly, in kidney tissue. In contrast, CsA exerted pharmacodynamic effects to potentiate SRL-induced myelosuppression and hyperlipidemia independent of PK interactions [8].

The major SRL-related hematologic complication – thrombocytopenia – usually appears during the first 4 weeks of treatment. Its occurrence, but neither the severity nor the persistence, correlates significantly with SRL trough concentrations above 16 ng/ml. In 89% of patients, the first episode resolved spontaneously. Among the remaining 11%, 7% responded to SRL dose reduction and 4% to temporary drug suspension; no patient required permanent cessation of SRL therapy [9]. The leukopenia that occurred among a smaller cohort displayed similar characteristics.

One striking finding in studies of long-term SRL use is the low incidence of post-transplant malignancy. Among 1008 renal recipients treated at a single center with SRL–CsA for 1–10 years (mean follow up 60.3 months), only 30 cases of malignancy were encountered in 29 patients, a lower incidence than that observed with tacrolimus (TRL) and mycophenolate mofetil (MMF) [10]. This finding was consistent with *in vitro* and animal studies that showed SRL to inhibit the proliferation of Epstein–Barr virus (EBV)-infected B cell lines from PTLD patients as well as *in vivo* growth of cells from murine renal and other cancers [11–13].

The overall favorable experience with SRL in combination with an 80% reduced exposure of CsA has led to the routine use of the strategy for 542 immediately functioning renal grafts at The University of Texas, Health Science Center at Houston. The initial CsA target concentration at 2-h postdose (C_2) is 200–400 ng/ml: the lower end of the range is employed for low-risk, and the upper, for high-risk recipients. The SRL regimen begins with a pretransplant loading dose of 15 mg followed on day 1 with 10 mg once or twice, then 5–10 mg/day, depending on the perceived rejection risk, targeting a C_0 value of 10 ± 3 ng/ml within 5 days. Between 1 week and 3 months, CsA dose adjustments are tailored according to renal function, aiming to achieve serum Cr values <1.2 mg/ml, and Cr clearances (CrCl) above 65 ml/min. Patients displaying SRL toxicity require the reduction of the C_0 target to 5 ng/ml with an increased CsA C_2 level of about 600 ng/ml [10].

Although not targeted to as low a CsA exposure *de novo* as used in Houston, Formica *et al.* [14] administered SRL (target C_0 value, 10–15 ng/ml) combined with

Table 1. Study designs and results of representative SRL–CsA combined immunosuppression in renal transplantation.

Drug	Other IS	*n*	SRL	CsA	Follow up (months)	Graft/patient survival (%)	Study type	Reference
SRL + CsA	ST	40	0.5–7.0 mg/m^2/day	550 ± 50 for 1 month, then tapered	36	94/97	p/nr/c	[5]
CsA	ST	65	–	Same as SRL group		86/98		
SRL + full/ reduced CsA	ST	124	1 or 3 (full) 1 or 3 or 5 (reduced) mg/m^2/day	Full: trough 200–350 ng/ml for 1 month, then tapered; reduced 50% of full dosage	12	87/91 for all patients	p/r/c	[6]
Placebo + CsA	ST	25	–	Full dosage		ND		
SRL + CsA	ST	558 (284/274)	2 or 5 mg/day	Trough 200–350 ng/ml for 1 month, then tapered	12	94.2/97 (2 mg), 92.7/96 (5 mg)	p/r/c	[63]
Aza + CsA	ST	161	–			93.7/97		
SRL + CsA	ST	446 (227/219)	2 or 5 mg/day	Trough 200–350 ng/ml for 1 month, then tapered	6	93/98 (2 mg), 93/96 (5 mg)	p/r/c	[64]
Placebo + CsA	ST	130	–			88/95		
SRL + CsA	Post-ST pulse and ATG	24	10–15	550 ± 50 ng/ml for 1 month, then tapered	12	83/88	np/nr/c (rescue ARE)	[16]
MMF + CsA	ST	12	MMF dosage 1.5–3 g/day			67/92		
SRL + CsA	Bax, ST	43	10–20 ng/ml	Delayed until sCr <2.5	12	93/98	np/nr/c (DGF)	[21]
CsA	ATG, ST	18	–	Delayed for 7–14 days		78/95		
SRL + full CsA	ST	97	2 mg/day	Trough 200–400 ng/ml for 1 month, then tapered	12	93/97	p/nr/c	[39]
SRL + CsA elimination	ST	100	10–20 ng/ml	Trough 100–175 ng/ml for 1 month, tapered after 2 months if no ARE		95/96		

IS, immunosuppressant(s); ST, steroid; SRL, the concentration ranges of sirolimus or the dose or concentration ranges of the comparator in that specific group; CsA, the concentration ranges of cyclosporine or the dose or concentration ranges of the comparator in that specific group; ND, not determined/not mentioned; Bax, basiliximab; ATG, antithymocyte globulin; ARE, acute rejection episode; DGF, delayed graft function; sCr, serum creatinine.

Study type: p, prospective; np, not prospective (retrospective); r, randomized; nr, not randomized; c, comparative; nc, single treatment arm.

reduced-dose CsA (target C_0 value, 50–100 ng/ml) in 62 of 121 renal transplant recipients; the other 59 received full exposure to CsA and MMF. They observed that renal function was not adversely affected by the drug combination. However, similar to earlier clinical experiences, altered hematopoiesis and lipid metabolism, albeit readily controlled, were observed among SRL patients.

Refractory rejection

Sirolimus successfully reversed biopsy-proven acute rejection episodes that were ongoing despite repeated antilymphocyte antibody treatments [15]. Extension of this experience into a nonrandomized trial of 36 renal recipients showing Banff grade IIB or grade III ongoing rejection episodes, despite prior treatment with pulse or oral recycling of steroids, documented the greater efficacy of SRL ($n = 24$) versus MMF ($n = 12$) added to a baseline regimen of CsA-steroids. SRL rescue therapy reversed the renal dysfunction in 96% of patients, whereas MMF was effective in 67% ($P = 0.03$), despite the fact that greater fractions of patients in the SRL than the MMF group had experienced two or more episodes of acute rejection before study entry (17 of 24 vs. six of 12) and had previously displayed recurrent bouts within the first 6 months post-transplant (94% vs. 50%; $P = 0.005$). Among the patients who were reversed successfully, the rates of rebound acute rejection were similar (4% vs. 8%). The mean serum Cr values were slightly, although not significantly, lower among the SRL than MMF patients at 1, 3, 6 and 12 months. The 1-year patient and graft survival rates were similar: namely, 88% vs. 92%, and 83% vs. 67% for the SRL versus MMF groups [16].

Steroid withdrawal or sparing regimens

In phase I/II and phase II studies, steroids were successfully withdrawn from the regimens of 67–93% of renal allograft recipients within 1 week to 3 months after transplantation [5,17]. A further single-center open-labeled

Table 2. Study designs and toxicities of representative SRL–CsA combined immunosuppression in renal transplantation.

Drug	Other IS	*N*	Follow up (months)	ARE (%)	sCr (mg/dl)	Adverse events	Discontinued (%)	Reference
SRL + CsA	ST	40	36	7.5/18 months	2.3	*, †, ‡, §, ¶	25	[5]
CsA	ST	65		32/18 months	2.2	ND	ND	
SRL + full/ reduced CsA	ST	124	12	10.6/12 months (full); 10.7% for reduced CsA and non-AA	1.35–2.1	–	11	[6]
Placebo + CsA	ST	25		32/12 months	1.58	–	20	
SRL + CsA	ST	558 (284/274)	12	22/2 mg, 15/5 mg	1.78–1.9	*, †, ‡, **	32–37	[63]
Aza	ST	161		31/12 months	1.5	More efficacy failure	44	
SRL + CsA	ST	446 (227/219)	6	24.7/2 mg, 19.2/5 mg	1.8	*, †, ‡, **	35–40	[64]
Placebo + CsA	ST	130		41.5	1.7	–	45	
SRL + CsA	Post-ST pulse and ATG	24	12	96 rescue	2.8	*, †, ‡, §	ND	[16]
MMF + CsA	ST	12		67 rescue	3.2	–	ND	
SRL + CsA	Bax, ST	43	12	16	2.0	*, †, ‡, §	ND	[21]
CsA	ATG, ST	18		39	1.5	Cytokine release syndrome	ND	
SRL + full CsA	ST	97	12	18.6	1.82	Hypertension, edema, hypomagnesemia	18/6 months	[39]
SRL + CsA elimination	ST	100		22	1.38	Abnormal liver function, **, hypokalemia, ‡	18/6 months, 72 CsA elimination	

ARE, acute rejection episode; IS, immunosuppressant(s); ST, steroids; Aza, azathioprine; Bax, basiliximab; ATG, antithymocyte globulin; MMF, mycophenolate mofetil; SRL, the concentration ranges of sirolimus or the dose or concentration ranges of the comparator in that specific group; CsA, the concentration ranges of cyclosporine or the dose or concentration ranges of the comparator in that specific group; ND, not determined/ not mentioned.

*Hypercholesterolemia; †hypertriglyceridemia; ‡thrombocytopenia; §leukopenia; ¶anemia; **diarrhea.

observation of 156 recipients treated with SRL-CsA-steroid therapy examined steroid withdrawal between 1 week and more than 2 years post-transplant. With a mean follow up of 379 days, there was a 75.4% success rate of steroid withdrawal with 7.7% graft loss [18]. Success was associated with average concentration exposures of CsA and SRL C_0 that were above 200 ng/ml and 10 ng/ml, respectively.

Among 30 long-term stable renal recipients treated with a CsA-steroid regimen who requested withdrawal for a variety of steroid-induced side effects, SRL was successfully substituted for steroids in most (87%, 26 of 30) patients. The benefit of the withdrawal was evidenced by better quality of life assessments, especially improved physical activity, in all patients. There were no significant adverse effects on blood pressure, serum cholesterol, triglyceride, or serum Cr levels. SRL was targeted to 10 ng/ml, while the CsA exposure was reduced by more than 50% of the pre-enrollment levels at the time of withdrawal. Two grafts were lost at 7 and 11 months after steroid withdrawal because of chronic rejection [19].

Delayed graft function

Possible exacerbation of the ischemia-reperfusion injury, which produces delayed graft function (DGF) after cadaveric renal transplantation, has been addressed by avoidance or delayed introduction of CNIs. In a pilot series of six consecutive patients with demographic features that placed them at risk for DGF, a SRL-based strategy including chimeric anti-interleukin-2 receptor (IL-2R) monoclonal antibodies (mAb) and steroids was combined with delayed inception of CsA therapy until the serum Cr levels had recovered to below 2.5 mg/dl. During the first 2 months post-transplant, none of the six patients displayed evidence of an acute rejection episode, cytokine release syndrome, or hypersensitivity reactions. All patients recovered renal function within 8 weeks post-transplant and maintained stable allograft function [20].

An extension of these observations led to an analysis of three contemporaneous (but nonrandomized) cohorts: DGF patients (group 1; $n = 43$) were treated with the new protocol in contrast to group 2 patients who displayed

immediate function and were treated *de novo* with CsA-anti-IL-2R mAb-steroid ($n = 21$). The group 3 cohort included DGF patients induced with the previous regimen of antilymphocyte preparations steroid-CsA delayed for 7–14 days ($n = 18$). The incidence of acute rejection episodes was significantly lower among group 1 (16%) compared with groups 2 (52%, $P = 0.004$) or 3 (39%, $P = 0.05$). Among the seven rejection episodes in group 1, six occurred among African-American or retransplant recipients. A separate cluster of six was associated with SRL trough concentrations equal to or below 9 ng/ml. Furthermore, fewer patients in group 1 required additional antilymphocyte antibody treatment to reverse either steroid-resistant or Banff grades II and/or III acute rejection episodes. Patient and graft survival rates, as well as mean serum Cr values, were similar at 12 months among the three groups. However, group 1 patients displayed higher serum cholesterol and triglyceride levels, as well as lower hemoglobin, platelet, and leukocyte values compared with the other two groups, presumably because of the higher SRL exposure [21]. The protocol was modified for high-risk recipients (African-Americans or retransplants) by substitution of a 14-day course of thymoglobulin for c-IL-2R mAb, leading to a significant decrease in the incidence of acute rejection episodes from 33 to 3%; however, with the penalty of a higher incidence of infectious complications [10]. Reduction of the thymoglobulin course to 7 days obviated these complications without appreciably increasing the rate of acute rejection episodes.

Flechner *et al.* [22] compared the use of SRL plus MMF *de novo* versus CsA plus MMF in a randomized, open-label, prospective study of adult primary kidney transplant recipients. Thirty-one patients received a single 15-mg loading dose of SRL followed by 5 mg daily doses, which were adjusted to keep the SRL C_0 levels at 10–12 ng/ml for 6 months and 5–10 ng/ml thereafter. Thirty patients began CsA at 6–8 mg/kg/day with $C_0 = 200$–250 ng/ml. Mean follow up at 18.1 months (range: 12–26) revealed similar rates of 1-year patient and graft survivals as well as of biopsy-confirmed acute rejection rates between SRL-treated and CsA-treated patients (namely, 96.7%, 96.7%, and 6.4% vs. 100%, 95.4%, and 16.6%, respectively). At 6 and 12 months, the SRL patients demonstrated significantly better kidney function ($P = 0.008$ and $P = 0.004$, respectively); namely, mean serum Cr levels of 1.29 and 1.32 mg/dl, than the CsA-treated patients of 1.74 and 1.78 mg/dl. However, the benefits may have been due at least in part to increased exposure to mycophenolic acid (MPA). As previously noted by Kreis *et al.* [23], SRL-treated recipients displayed significantly higher 1-year C_0 levels of MPA (4.16 ng/ml) than CsA-treated patients (1.93 ng/ml; $P = 0.001$). These findings were confirmed in a small series of 16 renal recipients with DGF or marginal donor kidneys. Shaffer *et al.* [24] failed to observe an episode of acute rejection after administration of thymoglobulin, SRL, MMF, and steroid; all grafts survived at a mean follow up of 243 days.

However, a retrospective review of 14 consecutive kidney transplant recipients showed a less favorable outcome of DGF following treatment with MMF (1.5–3 g/day), SRL (5–15 mg loading, then 2–5 mg/day maintenance), daclizumab, and steroids. The mean time to initiation of CNIs was 21 ± 13 days [25]. Nine patients required hemodialysis after transplantation. Average serum Cr levels at the initiation of SRL and at 1 month after transplantation were 8.4 ± 2.7 and 2.1 ± 1.2 mg/dl, respectively. The two patients (14%) who experienced an acute rejection episode within the first month after transplantation showed initially undetectable SRL levels. No grafts were lost during the follow up period.

Indeed, a series of other reports suggest SRL tends to prolong DGF. A retrospective review of DGF cases including 55 SRL patients and 77 recipients treated with other regimens showed a hazard ratio of 0.48 ($P = 0.0007$), suggesting that recipients treated with SRL are twice as likely to remain on dialysis [26]. However, there was no adverse effect on graft or patient survival or on allograft function at either 3 or 12 months. Another study reported a higher incidence of acute rejection episodes among SRL-treated patients versus those treated with a regimen containing a lymphocyte-depleting antibody without SRL [27]. However, the authors failed to present convincing data that adequate SRL exposure had been obtained early after transplantation.

Smith *et al.* also observed a higher risk of developing DGF among patients receiving versus not receiving SRL on the day of transplant ($P = 0.02$). Furthermore, the development of DGF seemed to be significantly associated with increasing SRL doses (odds ratio = 1.13 per additional mg of SRL, $P = 0.004$) [28]. Stallone *et al.* also suggested that SRL prolonged DGF among recipients of suboptimal cadaveric donors (25 vs. 15 days, $P = 0.02$) when compared with patients receiving CsA-based immunosuppressants. However, after recovery, SRL-treated patients displayed better allograft renal function; namely, mean serum Cr values of 1.4 vs. 1.9 mg/dl at 1 year post-transplant ($P = 0.04$) [29]. These studies together with animal model data suggest the potential for renal dysfunction associated with SRL therapy. Whether this is a uniform finding or an idiosyncratic reaction is presently unclear.

Elimination of CsA from maintenance sirolimus–CsA regimens

To evaluate whether CsA could be safely eliminated at 3 months from a SRL-CsA-steroid induction regimen,

Johnson *et al*. conducted an open-label study. A total of 525 renal allograft recipients were enrolled to initially receive 2 mg SRL (C_0: >5 ng/ml), full exposure to CsA, and steroid. At 3 months ± 2 weeks, 430 (82%) patients eligible for CsA elimination had not experienced a Banff grade 3 or a vascular cellular acute rejection episode in the 4 weeks preceding randomization, were not dialysis-dependent, and did not display a serum Cr more than 400 μM or other evidence of inadequate renal function. This subgroup was randomized (1:1) either to remain on SRL-CsA-steroid or to undergo CsA withdrawal with continued SRL therapy (C_0: 20–30 ng/ml) in combination with steroid. Among the randomized patients, there was no difference in graft survival (95.8% vs. 97.2%) or patient survival (97.2% vs. 98.1%, respectively), for SRL-CsA-steroid versus SRL-steroid cohorts. The incidence of biopsy-confirmed primary acute rejection episodes among these patients was 13.1% during the prerandomization period. Thereafter, the acute rejection rates were 4.2% for SRL-CsA-steroid vs. 9.8% for SRL-steroid ($P = 0.035$). Graft loss was not increased at 1 year. Renal function as assessed by calculated glomerular filtration rate (GFR) was 57 vs. 63 ml/min ($P < 0.001$), respectively. Furthermore, blood pressure readings were significantly improved among patients in whom CsA had been withdrawn. In addition to hypertension and CsA nephrotoxicity, hyperuricemia, herpes zoster infections occurred significantly more often among patients remaining on CsA. In contrast, thrombocytopenia, abnormal liver function tests, and hypokalemia were more common among the SRL-steroid therapy group [30]. An analysis of the subgroup of patients who underwent protocol biopsies at transplantation and at 1 year revealed a chronicity score of progression among 64% of SRL-CsA-steroid vs. 47.4% SRL-steroid patients, a difference that was not significant [31].

Follow up at 36 months showed a significantly higher discontinuation rate for the SRL-CsA-steroid group (48% vs. 38%, $P = 0.041$) [32]. This discontinuation rate may explain the improved graft survival among the SRL-steroid versus SRL-CsA-steroid cohort (81.4% vs. 89.8% or 85.6% vs. 92.6%, if loss to follow up was excluded). Furthermore, mean renal function (GFR: 47.3 vs. 59.0 ml/min), and hypertension (including systolic, diastolic, and mean) were also mitigated [32–34]. Significantly better renal function outcomes were observed among patients with moderately impaired function or risk factors for reduced renal function [e.g. a cadaveric donor, DGF, donor age >50 years, or human leukocyte antigen (HLA) mismatch ≥4] [35].

At 3 years, there were no significant difference in the incidence of death (7.4% vs. 4.2%), biopsy-proven acute rejection (6.0% vs. 10.2%), or serum lipid levels, including total cholesterol, triglyceride, and low-density lipoprotein-associated cholesterol [33,34]. An assessment of health-related quality of life at months 12, 24, or 36 vs. month 3 suggested better appearances, less fatigue, greater vitality, and higher social functioning scores among SRL-steroid patients (all $P \geq 0.05$) [36,37]. Based on the actual GFR values, the slope of GFR (−3.02 vs. 0.77 ml/min/year, $P < 0.001$), and the graft loss rate, a predictive model estimated a 20% difference in graft survival between these two groups of patients at 10 years [38].

A preliminary open-label, controlled, randomized study compared renal function among 97 patients receiving SRL (2 mg/day, fixed dose) plus CsA (full exposure) plus steroid (group A) versus concentration-controlled SRL (10–20 ng/ml) plus CsA (reduced dose) plus steroid with subsequent elimination of CsA after 2 months (group B, 100 patients) [39]. Patients who experienced adverse events such as DGF or acute rejection episodes were excluded from the randomization (group C). Group B patients showed better renal function at 12 months, with similar rates of biopsy-confirmed acute rejection, graft survival, and patient survival. Group A included 97 patients, and in group B, 76 of 100 randomized recipients completed the CsA withdrawal. However, group B patients displayed significantly greater incidences of abnormal liver function tests, diarrhea, hypokalemia, and thrombocytopenia. A subgroup analysis of African-American recipients in group B revealed better renal function than among those in group A.

A small, randomized study that enrolled 280 patients analyzed only 172 patients for the efficacy and renal function. The patients received concentration-controlled SRL (C_0: 4–12 ng/ml) plus CsA (full exposure – C_0: 125–250 ng/ml) plus steroid for 3 months and either underwent CsA elimination ($n = 59$) or CsA minimization (C_0: 50–100 ng/ml; $n = 58$) with increased SRL maintenance concentrations (C_0: 8–16 ng/ml). Patients withdrawn from CsA demonstrated better renal function: although they showed a twofold greater rate of acute rejection episodes after randomization. The other adverse events in the two groups were similar [40].

While these studies suggest that elimination of CsA is safe and effective, it does not address the question of whether minimal CsA exposures *de novo* would only lead to better eventual renal function without an increased risk of early acute rejection episodes. This protocol might reveal better tolerability as high SRL exposures would not be necessary as is the case when CsA is totally eliminated from the regimen and since steroids could be readily withdrawn.

Sirolimus in combination with tacrolimus: results of clinical trials

Although *in vitro* immunologic studies suggested that SRL and TRL might compete for FK-binding proteins

(FKBP), producing antagonistic effects [41], the large amount of cytosolic FKBP seems to obviate this possibility. Furthermore, early pharmacodynamic studies suggested that SRL augmented the immunosuppressive effects of TRL. Peripheral blood lymphocytes from 10 stable renal transplant recipients showed significantly decreased proliferation in response to phytohemagglutinin (PHA), Con A, or anti-CD3 among patients who received both TRL plus SRL versus TRL alone. The mRNAs for the proinflammatory cytokine tumor necrosis factor (TNF)-α and for cyclins G and E (all $P < 0.05$) were decreased, while those for tumor growth factor (TGF)-β and p21 (both $P < 0.05$) were increased among patients treated with the combination of SRL and TRL. Circulating levels of interferon (IFN)-γ, IL-4, and IL-2 (all $P < 0.05$) were significantly reduced and TGF-β elevated ($P < 0.04$) [42]. Although Chen *et al.* [43] reported a beneficial interaction of the two drugs on the survival of nonhuman primate kidneys, the failure to include drug measurements obviated any discrimination of whether the interaction was additive or synergistic.

A published letter alluded to 32 recipients of liver, kidney, or pancreas transplant patients treated with a SRL and TRL combination. The low rate of biopsy-confirmed acute rejection episodes [44] was difficult to evaluate as steroid treatment was administered to additional recipients who did not undergo biopsies. Despite the initial enthusiasm for a SRL–TRL combination for primary immunosuppression, variable and frequently equivocal results have been noted in preliminary, retrospective, nonrandomized, or single-arm treatment reports including small patient numbers [44–51]. Furthermore, the different concentration ranges and use of various other immunosuppressants (detailed in Tables 3 and 4) obfuscate any firm conclusion about this regimen [52–59]. These factors make a scientific and nonbiased evaluation of the efficacy and safety of a SRL–TRL combination difficult.

A randomized, multicenter, open-label kidney transplantation trial compared SRL ($n = 185$) versus MMF ($n = 176$) in combination with TRL and steroids [54]. At 6 months follow up, the incidences of biopsy-confirmed acute rejection episodes, patient and graft survivals, as well as occurrences of PTDM were similar. However, the SRL cohort showed a greater incidence of drug discontinuation ($P = 0.008$), and inferior renal function ($P = 0.018$) compared with the MMF group. Hyperlipidemia and elevated diastolic blood pressures were significantly more prevalent among the SRL group. In contrast, the MMF group showed significantly more leukopenia and gastrointestinal adverse events. The investigators concluded that TRL was equally effective in renal transplantation when combined with either SRL or MMF [60].

A recent 6-month study evaluated the safety and efficacy of TRL in combination with three dose levels of SRL and steroid in 104 renal transplant recipients. Patients were randomized into four groups: the control group received TRL and steroid ($n = 28$) and the three other cohorts also received daily SRL doses of 0.5 mg ($n = 25$), 1 mg ($n = 25$), or 2 mg ($n = 26$). Exposure to TRL was adjusted to whole-blood trough levels, and steroid was tapered from 20 to 5 mg/day. The SRL groups underwent a second randomization to discontinue the drug at either month 3 or 5. Six-month patient survivals of 100%, 100%, 96.0%, and 100% and graft survival rates of 96.4%, 84.0%, 88.0%, and 84.6%, respectively, were not significantly different. At 3 months, the safety profile, including the incidences of infections, also was similar in all groups. The 3-month incidences of hypercholesteremia (cholesterol >240 mg/dl or low-density lipoprotein cholesterol >160 mg/dl) were significantly higher among the SRL groups (21.4%, 36.0%, 48.0%, and 50.0%; $P = 0.019$). After withdrawal of SRL, lipid levels improved. The 3-month incidences of biopsy-proven acute rejection were significantly higher in the control group versus the SRL cohorts (28.6%, 8.0%, 8.0%, and 3.8%; $P = 0.014$).

Liver transplantation

The initial experience suggested that SRL combined with either CsA or TRL was associated with an increased incidence of hepatic artery thrombosis in liver transplant patients. However, among a cohort of patients treated with SRL but no CNI, Trotter showed the incidence of hepatic artery thrombosis to be two of 104 (2%), compared with 8% in a historic control group [61]. Data from an open-label, single-center experience with SRL and TRL also reported a low incidence of hepatic artery thrombosis (2%) [62]. A series of 56 liver transplant recipients administered a combination of SRL and TRL (target trough levels, 7 and 5 ng/ml, respectively) showed survival of 52 patients (93%) and 51 grafts (91%) at 23 months (range: 6–35) with one episode (1.8%) of hepatic artery thrombosis. The absence of an increased risk of thrombotic episodes also was confirmed by a retrospective comparison of CsA-treated renal transplant recipients without versus with SRL.

A diminished incidence of cytomegalovirus (CMV) disease has been reported among renal and liver transplant recipients administered SRL alone, with CsA, or with TRL. Trotter reported an incidence of <2% among their cohort of SRL patients [61] without a significant change in CMV prophylaxis or a reduction in the proportion of mismatched (CMV IgG) recipients. McAlister *et al.* reported CMV disease in four of 56 (8%) of their

Table 3. Study designs and results of representative SRL–TRL combined immunosuppression in renal transplantation.

Drug	Other IS	*n*	SRL	TRL	Follow up (months)	Graft/patient survival (%)	Study type	Reference
SRL + TRL	ST	185	4–12	5–15 (8.5)	6	93/97.3	p/r/c	[39]
MMF + TRL	ST	176	1.5 g/day MMF	(8.7)		95.5/97.7		[54]
SRL + TRL	Bax/ATG + ST	25	5 mg/day	3–5	9	96/92	p/nr/c	[48]
MMF + CsA	ST	38	2 g/day MMF	CsA_{0-4} *AUC* 4400–5500		100/97.3		
SRL + TRL	ST	48	5–15	5–15	12	93.8/97.9	np/nr/c	[51]
Aza + CsA	ST	103	Aza 2–3 mg/kg/day	CsA-ND		89.3/99		
SRL + TRL	ST	24	8–12 then 5–10	3–6	12	ND	p/nr/c	[47]
MMF + CsA	ST	75	MMF 2 g/day	C_2 CsA 1700–2100, then 800–1000				
SRL + TRL (A)	Dac/ST	50	8 (b.i.d.)	10/<3 months, 6–8/3–12 months, 6/>12 months	12	96/ND	p/r/c	[52]
MMF + TRL (B)	Dac/ST	50	MMF 2 g/day	10		95/ND		
SRL + CsA (C)	Dac/ST	50	8 (b.i.d.)	C_0 CsA 200–250/<3 months, 175–225/3–12 months, 150–200 >12 months		92/ND		
SRL + TRL	ATG/ST	41	10.9	4.4	12	85/98	p/r/c	[53]
SRL + MMF	ATG/ST	27	14.2	MMF 2 g/day		93/100		[57]
Low TRL + SRL	ST	184	9.5	3–7 (5.9)	6	94.6/96.2	p/r/c	[58]
High TRL + SRL	ST	177	8.2*	8–12 (9.2*)		96.6/98.3		[59]
SRL + TRL (tapered)	ST	42	8–16	3–8/<3 months, then taper	6	ND	p/r/c	[55]
SRL + high TRL	ST	44	4–8	8–12/<3 months, 5–10/>3 months				
TRL + SRL	Bax/ST (2 days taper)	20	10	10–15	12	95/95	p/r/c	[56]
TRL + MMF	Bax/ST (2 days taper)	29	MMF 2 g/day	10–15		95/100		
SRL + TRL	Bax/ST (5 days taper)	66	8–15	6–9/<1 month, 4–8/>1 month	6	100/100	p/nr/nc	[50]
SRL + TRL	ST	11	6–8	5–7	13.8	100/100	np/nr/nc	[46]
SRL + TRL	ST	30	6–10/<3 months, 5–7/>3 months	8–10/<3 months, 5–7/>3 months	7.7	93/97	np/nr/nc	[44]
SRL + TRL	ST, 65% Bax/Dac	74	13.9/<1 month, 7.5/>1 month	10/<1 month, 5–10/>1 month	19	100/ND	np/nr/nc	[49]
SRL + TRL	Bax ST	20	10–15	10–15/<2 months, 5–10/>2 months	13	100/100	np/nr/nc	[45]

IS, immunosuppressant(s); SRL, the concentration ranges of sirolimus or the dose or concentration ranges of the comparator in that specific group; TRL, the concentration ranges of tacrolimus or the dose or concentration ranges of the comparator in that specific group; Bax, basiliximab; ATG, antithymocyte globulin; Dac, daclizumab; ND, not determined/not mentioned; MMF, mycophenolate mofetil; ST, steroids.

Study type: p, prospective; np, not prospective (retrospective); r, randomized; nr, not randomized; c, comparative; nc, single treatment arm; sCr, serum creatinine.

*Statistically significant difference when compared between the study group and its comparator.

patients administered SRL and TRL [62]. The incidence of either systemic CMV or tissue-invasive CMV in renal transplant recipients receiving CsA and steroid with either SRL or Aza was 23 of 550 (4.2%) compared wtih 11 of 159 (6.9%) receiving Aza (P = NS) [63]. MacDonald reported no significant difference in the incidence of CMV disease in a cohort of SRL renal transplant recipients compared wtih those receiving placebo at 6 months [64].

Experience with combination regimens of SRL and TRL in pediatric liver transplantation is limited. Markiewicz *et al.* [65] administered SRL as rescue therapy to nine children over 2 years: in three due to chronic rejection and six due to impaired renal function. SRL was initiated at 2 months to 2.5 years after transplantation. Target trough levels for TRL for patients with chronic rejection was 8–10 ng/ml and SRL 10–12 ng/ml; for patients with impaired renal function, 4–6 ng/ml and

Table 4. Study designs and toxicities of representative SRL–TRL combined immunosuppression in renal transplantation.

Drug	Other IS	*n*	Follow up (months)	ARE (%)	sCr (mg/dl)	Adverse events	Discontinued (%)	Reference
SRL + TRL	ST	185	6	13	1.77	HyperCHO, LDL	21.1	[39]
MMF + TRL	ST	176	6	11.4	1.44*	↑MMF dose changes	10.8*	[54]
SRL + TRL	Bax/ATG/ST	25	9	16	1.4	ND	ND	[48]
MMF + CsA	ST	38		8.9	1.54	ND	ND	
SRL + TRL	ST	48	12	8.3	ND	3/48 lymphocele 2/48 pneumonia	3/48	[51]
Aza + CsA	ST	103		38.8*		ND	ND	
SRL + TRL	ST	24	12	16.7	GFR = 75.9 ml/min	ND	ND	[47]
MMF + CsA	ST	75		32	GFR = 73.8 ml/min	ND	ND	
SRL + TRL (A)	Dac/ST	50	12	ND	ND	↑SRL dose to	ND	[52]
MMF + TRL (B)	Dac/ST	50		ND	ND	C_0 of 8 than (C)	ND	
SRL + CsA (C)	Dac/ST	50		ND	ND	More hyperlipidemia	ND	
SRL + TRL	ATG/ST	41	12	Protocol biopsy at 3 months 10	GFR = 68	1/41 HUS, ↑ PTDM, wound complications	ND	[53]
SRL + MMF	ATG/ST	27		19 (3/5 SCAR)	GFR = 81*	ND	ND	[57]
Low TRL + SRL	ST	184	6	14.9	1.38, GFR = 70.2	Anemia, hyperlipidemia	29.3	[58]
High TRL + SRL		177		10.1	1.65*, GFR = 58.9*	Anemia, hyperlipidemia, diarrhea	29.9	[59]
SRL + TRL (tapered)	ST	42	6	11.1 (22.7)	1.3	Off TRL in 70%, ↑ CHO* 27% low plt	ND	[55]
SRL + high TRL		44		10.7 (9.5)	1.5*	4% low plt*		
TRL + SRL	Bax/ST (2 days taper)	20	12	5, 15 SCAR, 20 CAN	1.8	Protocol biopsy, no PTDM	ND	[56]
TRL + MMF	Bax/ST (2 days taper)	29		14, 14 SCAR, 25 CAN	1.7		ND	
SRL + TRL	Bax/ST (5 days taper)	66	6	6	1.38	80% off ST better BP control, renal function better over time	12/66	[50]
SRL + TRL	ST	11	13.8	0	1.6	Hyperlipidemia	ND	[46]
SRL + TRL	ST	30	7.7	16, 12 (SCAR)	1.8	10% PTDM, protocol biopsy	30	[44]
SRL + TRL	ST, 65% Bax/Dac	74	19	13.5/1 year	ND	8% PTDM	4.1	[49]
SRL + TRL	Bax/ST	20	13	5	1.2	Pediatric patients, 15% lymphocele, 5% PTLD	ND	[45]

IS, immunosuppressant(s); SRL, the concentration ranges of sirolimus or the dose or concentration ranges of the comparator in that specific group; TRL, the concentration ranges of tacrolimus or the dose or concentration ranges of the comparator in that specific group; ARE, incidence of acute rejection episodes; SCAR, subclinical acute rejection; Bax, basiliximab; ATG, antithymocyte globulin; Dac, daclizumab; CAN, chronic allograft nephropathy; ND, not determined/not mentioned; MMF, mycophenolate mofetil; CHO, cholesterol; LDL, low-density lipoprotein; sCr, serum creatinine; PTDM, post-transplant diabetes mellitus; CsA, cyclosporine; ST, steroids; plt, platelets.
*Statistically significant difference when compared between the study group and its comparator.

8–10 ng/ml, respectively. For patients administered only SRL and steroid, the target level was 12–20 ng/ml. SRL patients were evaluated from 3 to 21 months for liver function, renal function, and side effects. All patients were alive at the conclusion of the study. In three patients displaying chronic rejection, follow up biopsies showed no signs of chronic rejection. Follow up GFR in five patients showed significant improvement overall. All patients showed elevated serum cholesterol values. SRL was discontinued in three patients due to elevated liver enzymes ($n = 1$), persistently high serum cholesterol ($n = 1$), and repeated bouts of opportunistic infection ($n = 1$). The authors concluded that addition of SRL with reduced doses of TRL or switching to SRL alone significantly improves renal function in pediatric liver transplant patients. These observations are presently being extended in a multicenter open-label trial among adult liver transplant recipients who display impaired renal function.

Pharmacologic considerations

The tablet formulation of SRL offers more convenience than the original liquid formulation, and shows a similar area under the concentration–time curve (*AUC*) and C_0 at 2, 4, and 8 weeks after a milliliter-to-milligram conversion, without any episode of acute rejection or with changes in other laboratory values. The only significant difference was the lower dose-corrected maximal concentration (C_{max}) values of the tablets ($P < 0.05$). *AUC* values of CsA were not appreciably different [66].

The pharmacologic interactions between SRL and TRL are less well understood than those between SRL and CsA, the combination used in 1250 patients in the phase III trials [67] and 500 recipients in reported single-center studies [68], as reviewed extensively elsewhere [2,4,67].

In contrast to the observation that simultaneous administration of SRL and CsA increases SRL exposure [69], the PK profiles of neither SRL nor those of TRL seemed to be altered in a preliminary study of 25 liver or kidney-pancreas transplant recipients treated with a combination of SRL (C_0: 6–12 ng/ml) and low-dose TRL (C_0: 3–7 ng/ml). However, both drug measurements were performed using nonselective techniques that detect metabolites as well as parent compounds, namely, the IMx assay (Abbott Diagnostics, Mississauga, Ontario, Canada) for SRL and a TDx assay (Abbott Diagnostics) for TRL. C_0 levels seemed to correlate with *AUC* (TRL: $r^2 = 0.82$; SRL: $r^2 = 0.83$), suggesting that trough level monitoring is useful to control therapy for both drugs. These findings raise the question of whether SRL improves the PK behavior of TRL as this TRL formulation was reported to only show an $r^2 = 0.58$ [70].

However, there are few data comparing simultaneous versus spaced co-administration of TRL and SRL. Undre [71] reported that co-administration of the two drugs, while having no effect on exposure to SRL at doses of at least 2 mg/day, reduced TRL exposure, suggesting that TRL concentrations should be monitored. Another report focusing on recipients of a low-dose SRL regimen with standard exposures to TRL concluded that SRL dose increments were required over time to maintain constant drug exposure [72]. Interestingly, Sindhi *et al.* [73] showed that in 85 pediatric patients TRL exposure was not affected significantly after addition of SRL. Other important PK interactions of SRL with TRL in pediatric patients included a shorter SRL half-life (13–19 h), which suggests the necessity of twice daily dosing. Furthermore, recipients of liver and small intestinal grafts seem to require larger doses to achieve target drug exposure [73,74].

The combination of SRL and TRL has been associated with serious side effects. Three fatal cases of bronchial anastomotic dehiscence at two centers were reported among lung transplant recipients [75]. Furthermore, two living-donor kidney recipients experienced severe acute oliguric renal failure after receiving full doses of SRL and TRL (both drugs targeted at 5–15 ng/ml), requiring temporary dialysis therapy and cessation of these agents [76]. Another consideration is that exposing patients simultaneously to two highly potent immunosuppressants, while appearing to produce a low incidence of short-term acute rejection episodes, as shown in Table 4, might easily lead to over-immunosuppression and unwanted long-term adverse effects as seen in the liver transplant experience [76]. Thus, long-term follow up for PTLD and polyoma virus infection is urgently needed before the safety of a regimen that combines SRL with TRL can be assumed.

Sirolimus-based therapy *de novo* and for maintenance

Totally CNI-free sirolimus-based studies

Two early phase II studies [23,77] explored the use of SRL as the cornerstone of an immunosuppressant regimen. These two randomized, open-label, concentration-controlled European studies in low immunologic risk patients compared SRL to CsA in triple-drug regimens with either Aza-steroid or 2 g/day MMF-steroid. At 12 months, graft survival, patient survival, and the incidence of biopsy-proven acute rejection episodes were similar between both arms of each trial. The last metric showed 41% SRL vs. 38% CsA with Aza [77], and 27.5% SRL vs. 18.4% CsA with MMF [23]. The incidence of antibody (ATG or OKT3) treatment for acute rejection episodes was 17% vs. 12% in Groth *et al.* [77] and 7.5% vs. 5.3% in Kreis *et al.* [23]. In both studies, there was a trend toward better renal function among SRL-treated patients.

The most frequently reported side effects of SRL at higher exposures were thrombocytopenia (37–45%), leukopenia (39%), hypertriglyceridemia (51%), hypercholesterolemia (44%), and diarrhea (38%). Other conditions significantly more often associated with SRL included higher incidences of herpes simplex (24%) and pneumonia (17%), increased liver enzymes, and hypokalemia. These abnormalities improved 2 months after transplantation when the SRL target C_0 level was lowered from 30 to 15 ng/ml.

The pooled 2-year renal function parameters from these two studies showed that from week 10 through year 2, the calculated GFR was significantly higher among SRL- than CsA-treated patients (69.3 vs. 56.8 ml/min, at 2 years, $P = 0.004$). Serum uric acid was significantly higher and magnesium significantly lower among CsA- than SRL-treated patients. Indeed, these parameters were

more likely to be within normal limits in the SRL group. Mean serum potassium and phosphorus were lower in SRL-treated patients [78].

The overall results of these studies suggest that base therapy with concentration-controlled SRL, like CsA, is associated with only a moderate degree of prophylaxis of acute rejection episodes among renal transplant recipients, namely, rates below 10%. Indeed, it is not clear that the lesser degree of renal dysfunction associated with high exposures to SRL compared with a conventional CsA regimen proffers a significantly great advantage to offset the more significant toxicities associated with the proliferation signal inhibitor.

Conversion from CNI to SRL for maintenance immunosuppression

A pilot study reported the results of conversion to SRL (5 mg/day) immunosuppression at the time of marked reduction or elimination of the CNI. In 20 patients at 0–204 months post-transplant, TRL was either discontinued or substantially reduced in dosage. The indications for study entry were chronic CNI nephrotoxicity ($n = 12$), acute CNI toxicity ($n = 3$), severe facial dysmorphism ($n = 2$), PTLD in remission ($n = 2$), or hepatotoxicity ($n = 1$). During a 7–24 months follow up, the 12 patients switched because of chronic nephrotoxicity showed a significant decrease in mean serum Cr (2.6–2.3 mg/dl; $P < 0.05$). Facial dysmorphism allegedly improved in both patients. No relapse of PTLD was observed. SRL was discontinued in four of 20 patients because of adverse effects. However, there were significant adverse reactions: five patients developed pneumonitis and two, a picture resembling bronchiolitis obliterans. Although there were no deaths, the authors concluded that SRL conversion (in the fashion that they performed it), was associated with excessive initial immunosuppression requiring careful therapeutic drug monitoring [79].

Wyzgal *et al.* [80] converted 13 renal transplant recipients with biopsy-proven CNI nephrotoxicity to SRL therapy, targeting SRL C_0 levels of 12–20 ng/ml. Although the renal function (including serum Cr and GFR) significantly improved at 6 months, the severity of proteinuria continued to increase ($P = 0.04$). One patient experienced an episode of acute rejection after conversion, and SRL was discontinued in two patients because of pneumonia.

Diekmann *et al.* converted initially 20 [81], further increased to 59 [82], renal transplant recipients with biopsy findings suggestive of CNI nephrotoxicity to SRL. They targeted SRL C_0 levels at 8–12 ng/ml. After 1-year follow up, graft survival was 90%. Although 55% of patients showed improved or stable graft function (mean serum Cr from 2.76 to 2.22 mg/dl; $P < 0.01$), the other patients' function continued to significantly deteriorate with increased severity of proteinuria (mean serum Cr from 3.23 to 4.43 mg/dl, $P < 0.01$). Important adverse effects in this series included anemia, necessitating erythropoietin therapy in 65% of patients, and dyslipidemia. SRL was discontinued in 14% of patients because of side effects or graft failure. The investigators identified patients with mild proteinuria or a serum Cr below 3 mg/dl as more likely to benefit from SRL conversion [81,82].

An anecdotal experience of converting 107 renal recipients with biopsy-proven chronic allograft nephropathy revealed an improvement in CrCl among 70% of patients. Interestingly, the most significant benefit was observed among the group with lower baseline CrCl values before conversion (28.4 ± 19.4 ml/min) [83].

Other reports of conversion to SRL of patients with moderate renal insufficiency or chronic allograft nephropathy revealed similar results: only a low risk of acute rejection episodes (3.3–7%) or graft loss, and a trend toward improved or stable renal function. However, a fraction of patients (7–30%) discontinued the regimen because of the adverse effects of SRL [58,84–86].

The present strategy for chronic maintenance therapy at The University of Texas, Health Science Center at Houston utilizes the SRL–CsA combination with prompt steroid elimination. The relative exposures to CsA or SRL are individualized based on the severity of adverse effects during chronic therapy. Generally, CsA exposure is gradually reduced over time. Virtually all patients receive ≤50 mg CsA microemulsion twice daily by 6 months, thereafter tapered to 50 mg once daily by 2 years (Fig. 1). The 140 patients treated in this fashion showed a significantly reduced incidence of chronic allograft nephropathy. When the serum Cr was ≥2.0 mg/dl, CsA is further reduced to alternate or every third day dosing with the SRL C_0 level maintained at 10 ng/ml, the level that displays the lowest incidence of chronic allograft nephropathy, as found by a receiver operating characteristic analysis [68]. Patients whose serum Cr fails to improve with this drastic CsA reduction are withdrawn from the CNI to SRL monotherapy with the exception of a few high-risk patients in whom CsA is substituted with MMF as a two-drug regimen [10].

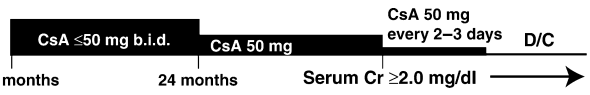

Figure 1 The current strategy for renal transplant maintenance therapy at The University of Texas Health Science Center at Houston uses an individualized combination of sirolimus–cyclosporine (CsA) with prompt steroid withdrawal. CsA therapy is gradually reduced over time and adjusted according to serum creatinine values.

Chronic sirolimus monotherapy

Swanson *et al.* [87] reported the results of 12 patients who received aggressive T-cell depletion with high-dose rabbit antithymocyte globulin (RATG; 8–10 days) combined with SRL monotherapy (C_0 target = 10–15 ng/ml). Only three doses of 125–500 mg methylprednisolone were given as a premedication for RATG. This approach was well-tolerated: all patients achieved good renal function at 12 months, and most of them (10 of 12) did not require chronic steroid or CNI treatment. Protocol biopsies revealed three rejection episodes (one Banff 1A, one Banff 1B, one subclinical 1A); their occurrence correlated with low SRL concentrations, indicating the need for therapeutic drug monitoring. In comparison with the authors' standard regimen, mRNA transcriptional analysis of the protocol biopsies were reported to show less intragraft inflammation (CD3, CD28, CD154, IL-2, IL-12) in the RATG-SRL-treated grafts at 1 month post-transplant and at the time of an acute rejection episode [88]. Adverse events included eight hospital admissions in six patients, including reactivation of varicella zoster ($n = 1$) that resolved with acyclovir; bacterial urinary tract infections ($n = 2$) with infected polycystic kidneys and a pretransplantation neurogenic bladder; diabetic foot cellulitis 15 months post-transplant ($n = 1$); hernia ($n = 2$); lymphocele requiring transperitoneal drainage ($n = 1$); and postpolio motor neuropathy 1 year after transplantation ($n = 1$) that responded to intravenous immunoglobulin.

Donati *et al.* [89] described a protocol of lymphocyte depletion using thymoglobulin (7 mg/kg, cumulatively) from days 0 to 6, followed by SRL maintenance therapy (C_0: 10–15 ng/ml during the first 3 months, then 5–10 ng/ml) with short-term MMF therapy (for 5 months) and steroid (for 3 months), but without CNI. Graft and patient survivals were both 96% in 23 patients enrolled during a follow up of 80–350 days. Two grafts were lost. The mean serum Cr level in the remaining 21 grafts was 1.27 mg/dl, but thrombocytopenia, leukopenia, bacterial and fungal infections, hematoma, lymphocele, and delayed wound healing were of serious concern with this approach. Clearly, these experiences in small series need to be expanded to randomized trials versus conventional regimens to discern benefit-risk ratios.

Application of sirolimus in special patient populations

African-Americans

Recipients of African-American ethnicity have long been known as a high-risk patient group. The 2-year outcomes of African-American renal transplant recipients treated with either a CsA-steroid ($n = 90$) or a SRL-CsA-steroid ($n = 47$) regimen were compared with those of 120 Caucasian patients treated with SRL-CsA-steroid. Addition of SRL to the CsA-steroid regimen reduced the incidence of acute rejection episodes among African-Americans from 43.3 to 19.2% ($P = 0.004$), a value similar to that among Caucasian patients. The 97.9% 2-year graft survival rate among 47 African-American patients treated with SRL-CsA-steroid was significantly higher than the 85.6% rate among 90 CsA-steroid-treated African-American transplant recipients ($P = 0.0479$) and similar to that in Caucasians. The 95.7% patient survival rate among the African-American SRL-CsA-steroid group was similar to the 97.8% rate in the African-American CsA-steroid cohort [90]. An extended cohort recruiting more African-American renal recipients ($n = 122$) treated with SRL-CsA-steroid for at least 3 years also showed a decreased cumulative incidence of acute rejection episodes from 60 to 22%, with similar graft and patient survival rates, despite CsA doses reduced by more than 50% compared with the CsA-steroid cohort. Interestingly, African-Americans treated with SRL-CsA-steroid experienced significantly fewer SRL-related side effects than the Caucasians treated with the same regimen [91].

Hricik *et al.* [92] reported a 2-year study comparing 56 African-Americans treated with steroids, SRL (target C_0 at 10–20 ng/ml), and low-dose TRL (target C_0 at 5–8 ng/ml), without the use of induction antibody therapy versus 65 Caucasian renal recipients treated with steroids, MMF, and high-dose TRL (target C_0 at 8–12 ng/ml). The incidence of acute rejection episodes in the first 3 post-transplant months was 7.1% among African-Americans and 16.9% among Caucasians (P = NS). PTDM was a serious problem; namely, incidences of 36% in African-Americans vs. 15% in Caucasians ($P = 0.024$) [92]. An amendment to the protocol attempted to withdraw steroids after 3 months in 30 African-Americans treated with steroid, SRL, and low-dose TRL seeking to reduce the incidence of PTDM. The incidence of acute rejection episodes was 13%, and graft as well as patient survivals was 97% and 100%, respectively. About 80% of the 30 recipients completed steroid withdrawal, but there was a significant deterioration in long-term graft renal function [mean serum Cr increased from 1.4 mg/dl before tapering steroid, to 1.65 among those without rejection, or 2.2 mg/dl among all recipients (both $P < 0.05$)] [93].

In a study of 70 kidney recipients of African-American ethnicity randomized after day 7 to medium (target C_0 at 8–12 ng/ml; $n = 34$) or high (target C_0 at 15–20 ng/ml; $n = 36$) SRL levels, combined with reduced exposure to CsA (C_0 at 1 month = 170; at 6 months = 70 ng/ml) and steroid, the incidences of biopsy-proven acute rejection episodes at 6 months were low in both groups (11.7% and 8.3%, respectively). Only three graft losses occurred among

70 patients. Except for the lower hemoglobin levels in the high SRL group, renal function, lipid profiles, and episodes of other adverse events were similar in both groups [94].

Pediatric recipients

The experience in applying SRL in pediatric transplant recipients is limited, mainly because the initial clinical trials did not include patients under the age of 13 years. In a subgroup analysis of 12 patients, 13–18 years of age in the USA phase III clinical trial of 719 patients, six received SRL at 2 mg/day, three received SRL at 5 mg/day, and three received the comparator Aza. Two acute rejection episodes (22%) occurred in the nine patients receiving SRL, whereas no rejection occurred in the Aza group. Both acute rejection episodes occurred among patients receiving 2 mg SRL, but both the 5 mg SRL and Aza groups displayed an increased incidence of infection. Except for three patients in the 2 mg SRL group, the other nine recipients were withdrawn from the trials for both medical and nonmedical reasons. Diarrhea occurred in three of the nine recipients treated with SRL, and hyperlipidemia in two of the 5 mg SRL group and two in the Aza groups. No episode of hematologic abnormalities occurred in either SRL group. The significance of these findings is difficult to assess because of the small number of patients [95].

Twenty pediatric renal recipients aged 3–18 years treated with basiliximab induction were maintained on SRL (target: 10–15 ng/ml), TRL (target: 10–15 ng/ml for 2 months, then 5–10 ng/ml), and steroid. There was only one episode of acute rejection at month 13 because of noncompliance; graft and patient survivals at 1 year were both 100%. Three patients (15%) experienced lymphoceles that required surgical drainage, and one patient developed PTLD [45].

Sindhi *et al.* [73] treated a mixed population of 85 pediatric nonrenal organ transplant recipients (liver, $n = 47$; liver-intestine, $n = 15$; intestine, $n = 7$; thoracic, $n = 14$; bone marrow, $n = 2$) with SRL and TRL for a variety of indications (primary immunotherapy, $n = 22$; acute rejection, $n = 24$; acute rejection and PTLD, $n = 11$; nephrotoxicity, $n = 19$; and other, $n = 9$). Acute rejection episodes were prevented in 17 of 22 primary patients, rescued in 17 of 24 patients, and in nine of 11 children who experienced acute rejection with PTLD. SRL was discontinued in 10 of the 85 children (12%) due to serious adverse effects.

Conclusions

Sirolimus, originally designed to be an adjunctive immunosuppressant to CNI therapy, has developed over the past 10 years into a baseline agent in organ transplantation. Some drug combinations have shown excellent long-term outcomes in renal transplantation; whereas others that seemed feasible based upon early reports, await the results of longer follow up of adverse effects before being regarded as practical alternatives for routine applications. SRL monotherapy is becoming a cornerstone of immunosuppression in renal transplantation because of its high immunosuppressive potency. However, the optimal target concentrations for long-term therapy are not yet certain; in view of the pleiotropic array of side effects, immunosuppression must be meticulously tailored to the needs of the individual patient. Overall, SRL is a powerful agent when judiciously used in combination with other immunosuppressants, achieving excellent outcomes with few adverse effects.

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