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REVIEW

mTOR inhibition: the learning curve in kidney transplantation

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Summary

All immunosuppressive medications require a learning curve that enables clinicians to improve the therapeutic index of agents. Mammalian target of rapamycin (mTOR) inhibitors are potentially a less nephrotoxic form of immunosuppression than calcineurin inhibitors (CNIs) that has been used in kidney transplant recipients for more than two decades. This drug class has a novel immunosuppressive action, probably mediated in part through inhibition of growth receptor signaling mechanisms. In addition, it has a unique drug toxicity, which is partially dose-related. This medication class also possesses antiproliferative activity, which may be useful in-post-transplant patients with increased atherosclerotic and malignancy risks. mTOR inhibitors have been utilized for *de novo* immunosuppression with CNIs, corticosteroids, and antimetabolites. mTOR inhibitors also have been used as CNI-sparing agents both early and late post-transplant. Much debate remains over how to best utilize mTOR inhibition in kidney transplantation.

Mammalian target of rapamycin (mTOR) inhibition is potentially a less nephrotoxic form of immunosuppression. Compared with calcineurin inhibitors (CNIs), mTOR inhibition has been extensively studied for more than two decades in recipients of kidney transplants [1,2]. Optimal use of the two mTOR inhibitors, sirolimus (SRL) and everolimus (EVL), needs to be better defined in kidney transplantation.

Both of the currently marketed CNIs, cyclosporin A (CsA) and tacrolimus (Tac), display nephrotoxicity that not only acutely results in deterioration of glomerular filtration rate (GFR) but also contributes to long-term anatomical injuries of tubular atrophy and interstitial fibrosis [3–6]. Given that mTOR inhibitors are less nephrotoxic, especially in the absence of CNIs, much attention has focused on their use as a substitute for CNIs or as a strategy to diminish CNI exposure [7–10]. mTOR inhibitors also have a unique mechanism of action that may be beneficial to retard progressive atherosclerotic injury [11,12] and are associated with a decreased incidence of malignancies [13,14]. Given that cardiovascular disease [15] and malignancy [16] are important clinical concerns following transplantation, mTOR inhibition may be an important long-term therapeutic consideration for renal transplant recipients.

Mammalian target of rapamycin inhibitors also possess unique side effects that require understanding [1,2,7,8,10,17]. Most of these are dose-related, requiring monitoring of drug levels, with some side effects potentially requiring treatment cessation. This review provides

an important perspective on the use of mTOR inhibition as an immunosuppressive strategy in renal transplant recipients and a balanced perspective on its use either alone, or in conjunction with other medications, to provide an improved therapeutic index for maintaining adequate immunosuppression and reducing the likelihood for kidney graft deterioration, and possibly atherosclerosis and malignancy.

Basic science and pharmacology of mTOR inhibitors

Sirolimus is a macrolide antibiotic produced by *Streptomyces hygroscopicus*. It was formerly known as rapamycin, as it was first isolated from Rapa Nui (Easter Island) [18]. SRL was initially identified as an antifungal agent in the 1970s [12,19], but was identified as an immunosuppressant in 1988 [18] and was approved by the Food and Drug Administration (FDA) for transplantation in 1999 [20].

The search for mechanisms of action of SRL led to the identification of the TOR in 1991 [21]. Subsequently, mTOR was found to play a critical role in cellular responses to nutritional and energy signals from the environment as well as growth factors, and to direct cell growth, differentiation, and proliferation [12]. mTOR is the downstream target of several different signaling pathways, and in turn, mTOR mediates signals leading to the translation of proteins that allow cells to progress through the cell cycle and proliferate, and impair programmed cell death. Some of these pathways are summarized in Fig. 1, and many of these signaling molecules play additional roles in pathways not shown in the figure.

Some of the most important clinical effects of mTOR inhibitors are reflected in the three FDA-approved agents that work by this mechanism: (i) SRL, for prevention of kidney transplant rejection [20]; (ii) SRL-eluting stents, for prevention of coronary artery restenosis [22]; and (iii) temsirolimus, for treating advanced renal cell carcinoma [23]. T and B lymphocytes proliferate in response to interleukin 2 (IL-2) binding to the IL-2 receptor and subsequent downstream signaling through pathways shown in Fig. 1. Therefore, the immunosuppressive action of SRL arises out of mTOR inhibition causing impaired activation and proliferation of lymphocytes [24]. SRL also impairs the proliferation of smooth muscle and endothelial cells, thus preventing intimal hyperplasia in human coronary artery disease [25] and animal transplant models of graft vascular disease [26]. Finally, the growth of many different tumor cells can be inhibited by mTOR blockade. For example, phosphatase and tensin homologue deleted on chromosome 10 (PTEN) is a tumor suppressor the abnormal expression of which is associated with many malignancies, and mTOR inhibition can potentially block the resultant abnormal signaling (Fig. 1) [27]. In addition, animal [28] and human data [29] demonstrate that SRL can simultaneously prevent graft rejection and cause regression of tumors.

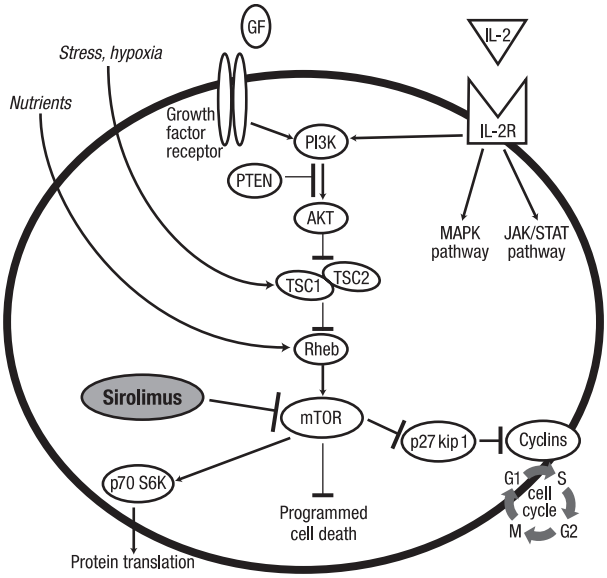

Figure 1 Engagement of the IL-2 receptor or other growth factor receptors activates PI3K (phosphatidylinositol 3-kinase), which results in the activation of Akt. Akt inhibits the TSC1/TSC2 (tuberous sclerosis complex), which in turn inhibits Rheb (Ras homologue enriched in brain). The net effect of this inhibition of the inhibitor TSC1/TSC2 is positive signaling through Rheb and mTOR, which leads to cell proliferation by several mechanisms. PTEN inhibits this cascade upstream of Akt, whereas stress and hypoxia stimulate TSC1/TSC2, also resulting in inhibition of mTOR signaling. In contrast, nutrients increase signaling through Rheb and mTOR. mTOR phosphorylates p70 S6K, leading to the translation of proteins that increase cell growth and proliferation. mTOR also inhibits programmed cell death and blocks the p27 kip1 (kinase inhibitory protein 1) inhibition of cyclins, which has the net effect of increasing progression from the G1 to S phase of the cell cycle. By blocking mTOR, SRL prevents all 3 mechanisms from enhancing cell proliferation.

Everolimus, an SRL derivative, also inhibits mTOR, and is also being studied in preventing graft rejection, drug-eluting stents, and the treatment of cancer. Although less extensively studied than SRL, available data suggest that EVL has similar effects on intracellular signaling pathways [30].

Experimental evidence suggests that SRL may promote tolerance by increasing numbers of regulatory T cells, blocking costimulatory signals, and promoting apoptosis and other mechanisms, whereas CNIs impair these tolerogenic mechanisms [31].

Pharmacology of mTOR inhibitors

Sirolimus is available as either an oil-based solution or a tablet. Bioavailability is low, from ~14% for the solution to ~27% higher for the tablets [20]. Time to peak blood concentration is 0.5–3 h [32]. Administration of SRL with a fatty meal increases area under the curve (AUC) by 35%, so it should be taken consistently either with or without a meal [33]. SRL has a large volume of distribution, ~12 l/kg, and is present in high levels in red blood cells and tissues compared with plasma [20,34]. SRL is metabolized to some extent in the small intestine and extensively in the liver by cytochrome P450 3A4 (CYP3A4). In addition, transport across intestinal and liver cells by P-glycoprotein affects drug absorption and disposition [20,35]. Clearance of SRL and its metabolites is 91% fecal and 2% urinary [20]. The elimination half-life is approximately 62 h [20].

Because of large variations between individuals in absorption and metabolism, drug dose correlates poorly with blood levels, and monitoring of levels is essential. Trough measurements correlate well ($r^2 = 0.85$) with AUC, so monitoring of trough levels is sufficient for accurate therapeutic drug monitoring [32]. Despite differences between the pharmacokinetics of the solution and tablet, trough levels strongly predict drug exposure, and the two preparations have been demonstrated to be therapeutically equivalent [36].

Exposure to SRL may be substantially altered by drugs that are substrates, inhibitors, or inducers of CYP3A4 or P-glycoprotein [20]. For example, nondihydropyridine calcium channel blockers and azole antifungals may increase SRL levels, whereas phenytoin may reduce SRL levels. SRL interacts with CsA by pharmacokinetic and probably pharmacodynamic mechanisms. This interaction between the two drugs can increase their toxicities [37].

Everolimus has similar pharmacology to SRL, in that peak concentrations are reached 1–2 h after oral administration, and the metabolism of both is mostly by liver P450 enzymes, with minimal renal excretion [38]. However, the elimination half-life of EVL is shorter, at approximately 28 h [39]. EVL is not metabolized to SRL; that is, it is not a prodrug [40]. As with SRL, EVL has the expected interactions with drugs affecting the CYP3A4 enzyme [41].

Pivotal randomized controlled trials

The clinical development of mTOR inhibitors has proceeded on two bases: as adjunctive therapy to a CNI or as base therapy, either following conversion from a *de novo* CNI regimen or as the foundation of *de novo* or chronic treatment. The former strategy is based on the synergistic pharmacodynamic and pharmacokinetic interactions between CsA and mTOR inhibitors. The majority of early clinical trial data was derived from experience with SRL. Following a single-center, randomized, double-blind, phase I dose escalation study in stable renal transplant patients treated with CsA plus prednisone (Pred) [42], and a single-center, phase II ascending SRL dose protocol in recipients of living related kidneys treated with full exposure to CsA and attenuated courses of steroids [43], a multicenter trial evaluated the outcomes of renal transplants among subjects treated with full versus reduced CNI doses [44] (Table 1). Among non-African American patients, reduced CsA doses yielded outcomes similar to full doses, with improved renal function. However, reduction in CsA doses among African Americans, who are known to be strong immune responders, resulted in enhanced rates of acute rejection (AR) episodes [44]. The phase III, pivotal, randomized, double-blind trial compared the outcomes of cohorts treated with SRL versus those receiving azathioprine (Aza; United States) or placebo (global) and followed for 2 years [47]. The rate of AR episodes was significantly lower within the SRL arm. The apparent requirement for greater SRL doses among African Americans in the phase III US trial was addressed in a further study of high-risk recipients, which also included retransplants and patients displaying >80% panel reactive antibody [49]. These subjects showed >90% graft survival with good renal function at 1 year, using regimens based on modestly reduced exposures to CsA or Tac in combination with SRL and Pred, usually after antibody induction treatment.

Although the renal function was improved using SRL versus CsA in two trials, namely in combination with Aza + Pred [45] or mycophenolate mofetil (MMF) + Pred [46], the AR rates of ~35% were unacceptable. Unfortunately, a randomized clinical trial among a variegated US population had to be prematurely discontinued because of a higher than anticipated 1-year incidence of AR episodes (30%) among subjects treated with daclizumab induction accompanied by SRL/MMF/Pred versus a CNI/MMF/Pred regimen [20,50]. *Post hoc* analysis revealed that the majority (56%) of recipients with AR in the CNI-free arm had subtherapeutic C_0 levels the first 6 months [50].Thus, clinical studies have suggested that a CNI-free regimen based on SRL with antibody IL-2 receptor antagonist induction may be useful only among immunologically low-risk, primary renal transplantations, with careful attention to therapeutic drug level monitoring.

Seeking to exploit the relatively nonnephrotoxic properties of SRL, a phase III trial randomized patients at approximately 3 months who had not experienced a rejection episode within 4 weeks before randomization to either a CNI-free regimen of SRL + Pred or continued

Table 1. Pivotal clinical trials of sirolimus in renal transplantation.

Phase	Setting	Regimens	Study	Efficacy				Discontinuation rate (%)		
					GFR (ml/min)	BCAR				
I	Stable patients	Add to CsA + Pred: SRL versus PBO	Murgia 1996 [42]	SRL	53.5[a] 51.9[c]	–		SRL[b]: 3.33; 13.33		
				PBO	57.4[a] 51.1[c]	–		PBO[b]: 30; 20		
II	*De novo* LRD	Ascending-dose SRL	Kahan 1998 [43]		–	18 months				
				SRL + CsA + Pred		7.5%[d]		SRL: 25		
				CsA + Pred	–	32%		–		
	De novo	Full versus reduced CsA + SRL + Pred	Kahan 1999 [44]	Full		6 months	12 months	SRL: 25 PBO: 4		
				SRL + CsA	–	8.5%[e]	10.6%[f]			
				PBO + CsA	–	32%	32%			
				Reduced						
				SRL + CsA	–	19.5%[g]	–			
				PBO + CsA	–	32%	32%			
	De novo	SRL versus CsA with Aza + Pred	Groth 1999 [45]	SRL	–	41%			6 months	12 months
								SRL	46.3	58.5
				CsA	–	38%		CsA	40.4	45.2
	De novo	SRL versus CsA with MMF + Pred	Kreis 2000 [46]	SRL	–	27.5%[h]			6 months	12 months
								SRL	25	43
				CsA	–	18.4%		CsA	13	26
III	*De novo*	SRL or Aza/PBO with CsA + Pred	Kahan 2003 [47]			24 months; SRL			Global	US
						Global, SRL versus PBO	US, SRL versus Aza	SRL (2 mg/day)	56.4	55.3
				SRL (2 mg/day)	–	29.5[i]	23.6[h]	SRL (5 mg/day)	70.3	61.7
				SRL (5 mg/day)	–	26.0[j]	17.5[k]	PBO	57.7	–
				Aza	–	–	32.3	Aza (2–3 mg/day)	–	58.4
				PBO	–	43.1	–			
	Withdrawal	Discontinued CsA at 3 months versus continued CsA, all with SRL + Pred	Johnson 2001 [48]	SRL + CsA + ST	57[k]	13.5%[l]		SRL + CsA + ST:18[m]		
				SRL + ST	63	20.2%		SRL + ST: 27		
	High-risk *de novo*	CsA versus Tac + SRL + Pred	Gaber 2008 [49]		12 months	6 months	12 months	SRL + Tac	43.3	
				SRL + Tac	54.5[n]	12.5%[o]	13.8%[p]	SRL + CsA	40.2	
				SRL + CsA	52.6	14.7%	17.4%			
	De novo	CsA versus SRL + MMF + Pred	Flechner 2007 [50]	SRL + Tac elimination versus Tac + MMF	–	$P = 0.0998$		–		

Table 1. continued

Phase	Setting	Regimens	Study	Efficacy			Discontinuation rate (%)			
					GFR (ml/min)	BCAR				
				SRL + MMF versus Tac + MMF	–	$P < 0.0001$	–			
	Late conversion	Continued CNI versus SRL conversion + MMF + Pred	Schena 2009 [51]		>12 months				12 months	24 months
				SRL	24.6[q] 59.0[r]		SRL		15.7[s]	25.8[t]
				CNI	27.2[q] 57.7[r]		CNI		9.5	20.0
			Mulay 2006 [52]		–	–	SRL RCT: 28			
							SRL non-RCT: 17			
			Patel 2007 [53]	SRL	–[u]	–	–			
				CNI	–	–				
				Tac	–	–				

Aza, azathioprine; BCAR, biopsy-confirmed acute rejection; CNI, calcineurin inhibitor; CsA, cyclosporin A; GFR, glomerular filtration rate; LRD, living related donor transplants; MMF, mycophenolate mofetil; NS, non-significant; PBO, placebo; Pred, prednisone; RCT, randomized controlled trial; SRL, sirolimus; ST, steroid; Tac, tacrolimus.
[a]At baseline; in mg/dl; [b]Two discontinuation rates cited; [c]At study end; in mg/dl; [d]$P = 0.003$; [e]$P = 0.018$; [f]$P < 0.05$; [g]$P = 0.27$; [h]NS difference; [i]$P < 0.01$; [j]$P < 0.05$; [k]$P < 0.001$; [l]$P = 0.093$; [m]$P = 0.028$; [n]$P = 0.466$; [o]$P = 0.582$; [p]$P = 0.362$; [q]Baseline GFR = 20–40 ml/min; $P = 0.575$; [r]Baseline GFR >40 ml/min; $P = 0.278$; [s]$P = 0.013$; [t]$P = 0.070$; [u]Percentage change in GFR 12 months post-transplant: SRL = 28.8, CNI = 8.2, and Tac = 4.7.

treatment with CsA + SRL + Pred [48]. As expected, patients who were continuously exposed to CNI showed impaired function relative to those in whom the nephrotoxic agent was withdrawn. The conversion from a chronic CNI-based regimen to only SRL showed potential benefit among patients with a baseline GFR >40 ml/min [51–53].

De novo use of mTOR inhibitors

To obtain superior renal function coupled with low rates of AR, a regimen using an induction antibody (basiliximab) followed by SRL, MMF, and steroids was conducted. In primary deceased and live donor recipients, this regimen produced significantly better 1-year renal function with AR rates of ≤10%, compared with a CsA-based regimen [54]. Longitudinal reports from this trial at 2 and 5 years demonstrated superior renal histology and preservation of renal function in the SRL-based, CNI-free group [9,17]. At 5 years, the estimated GFR (eGFR; by the abbreviated Modified Diet in Renal Disease formula) was 67 versus 51 ml/min ($P = 0.008$) for the CNI-free patients [17]. A similar regimen in live donor recipients reported at 2 and 5 years demonstrated improved eGFR by >15% and produced low initial AR rates of ∼12% [55, 56]. Subsequent randomized controlled trials (RCTs), both single center and multicenter, reported comparable findings [57–60], although some groups felt that the use of a depleting antibody was beneficial to the outcomes. Additional large reports from South America expanded this experience [61]. An important feature of these regimens, highlighted by the investigators, was the need for therapeutic drug monitoring of SRL, keeping C_0 levels at least 10–15 ng/ml in the first 6 months [9,17,54,55,57–62]. This was confirmed in a meta-analysis of RCTs of CNI-free, SRL-based protocols [2]. It should also be noted that these RCTs excluded ischemically damaged and older-aged donor kidneys, as well as patients with greater immunologic risk for rejection.

The use of a *de novo* SRL-based, CNI-free regimen has a distinct learning curve and requires some appreciation of side effects, patient education, and attention to therapeutic drug level monitoring. For example, when patients are switched from *de novo* SRL to a CNI due to intolerance to the mTOR drug, the renal function usually deteriorates [7,63]. In a large, primarily European multicenter trial, subtherapeutic dosing targets of SRL at 4–8 ng/ml were employed, resulting in high rates of AR, which abrogated any renal function advantages for the CNI-free group [10]. The target range of 10–20 ng/ml during the first 6 months had been confirmed in a meta-analysis of randomized trials [2].

Two single-center retrospective series reported an increased rate of delayed graft function and slower recovery from delayed graft function with *de novo* use of SRL [64, 65]. However, this has never been substantiated in RCTs compared with *de novo* use of CNI drugs [10,54,66,67].

The early development of EVL was closely wedded to the continued use of CNI drugs. The initial trials of *de novo* use compared EVL (in divided doses of either 1.5 or 3 mg) with MMF, and either full- or 50% reduced-dose CsA and steroids [68,69].

In both studies, the 12-month incidence rates of biopsy-proven AR (BPAR) were similar for the EVL- and MMF-treated groups: 19.7% vs. 24.0% [68] and 22.2% vs. 24.0%, respectively ($P = 0.51$) [69]. Further analysis showed that EVL patients with C_0 levels ≥3 ng/ml had significantly reduced BPAR [68,70]. Patients receiving EVL had lower eGFR than those receiving MMF (49.3 vs. 56.9 ml/min) [68]. Refinements to the protocol, such as lowering CsA target trough levels to 50–75 ng/ml after 12 months, decreased mean serum creatinine levels slightly. Further modifications, including use of the non-depleting antibody IL-2 receptor antagonist basiliximab for induction, and employing C_2 rather than C_0 monitoring of CsA (1200 ng/ml during week 1 tapered to 400 ng/ml after week 16), diminished BPAR rates to 14.3%, with an eGFR of 68 ml/min at 12 months [71]. In addition, a recent 6-month RCT using basiliximab induction with EVL (1.5 mg), low-dose Tac (C_0, 4–7 ng/ml), and steroids demonstrated a BPAR rate of 14% and eGFR of 75 ml/min [72]. There have not been many complete and peer-reviewed trials using *de novo* EVL in a CNI avoidance regimen. One novel *de novo* regimen for 52 recipients at high risk for delayed graft function employed EVL with the sphingosine analog, fingolimod (FTY720) and steroids [73]. Whereas the combination was well tolerated, BPAR rates of 50% led to abandonment of the regimen. However, these results may be related to the failure to achieve target EVL C_0 values. Lastly, rapid (7-day) steroid elimination was reported among 68 patients in an RCT of basiliximab induction, EVL (3 mg), and CsA (C_0, 150–350 ng/ml during month 1) [74]. The 12-month BPAR rate was 32% off steroids versus 18% on steroids ($P = 0.059$). However, graft survival and eGFR were not different at any time point from 12 to 36 months.

In summary, the use of a *de novo* CNI avoidance regimen, including an induction antibody followed by SRL, MMF, and steroids, has been used worldwide (>1000 patients), consistently demonstrating improved renal function at 1, 2, and now 5 years. This combination has a somewhat different side effect profile, and wider experience has revealed that the use of *de novo* SRL requires careful therapeutic drug level monitoring, maintaining C_0 levels of SRL at 10–15 ng/ml the first 6 months to keep AR rates at 10–15%. In addition, SRL should be withheld in patients at high risk for early mTOR-related problems, particularly the obese, those with years of prior steroid use, those who had extensive pelvic surgery or radiation, or those with grafts with early severe oliguria. For such recipients, as well as those at greater immunologic risk, the initial use of a CNI drug for two to possibly 9–12 months may be preferred. The introduction of EVL has focused on CNI minimization protocols; however, its role in CNI avoidance and conversion strategies is highly likely to emerge in the near future.

Conversion to SRL: optimal timing for mTOR inhibition-based therapy

Optimal strategies for employing mTOR inhibition post-transplant have been clarified by recent clinical trials evaluating the timing, safety, and efficacy of substitution of an mTOR inhibitor for CNIs [51,75,76]. Whereas there are numerous pros and cons concerning *de novo* mTOR inhibition, with or without a CNI, it is evident from the literature that conversion from CNIs to mTOR inhibitors after transplantation and before the development of renal injury may be an important strategic use of this therapy.

Three prospective RCTs have evaluated conversion from CNI to SRL at varying periods after transplantation [51,75,76]. The SRL CONVERT Trial study group randomized 830 patients, between 6 and 120 months after transplantation, to either continue CNI ($n = 275$) or be converted to SRL therapy ($n = 555$) [51]. Patients also received center-specific regimens, including induction therapy, MMF, Aza, and corticosteroids [51]. Patients were monitored for change in eGFR using the Nankivell formula, as well as the composite rate of BPAR, graft loss, and death at 1 year. Enrollment in the 20- to 40-ml/min stratum was halted prematurely because of a higher incidence of safety end points in the SRL conversion arm. Investigators noted, however, that patients with a baseline GFR >40 ml/min who remained on therapy had a significantly ($P = 0.009$) higher GFR after SRL conversion through 24 months [62.6 ml/min ($n = 370$) vs. 59.9 ml/min ($n = 201$)]. On the other hand, patients with GFR <40 ml/min did not fare as well and had an increased rate of death compared with those not converted (P = NS), and had no significant difference in graft outcome. At 24 months, the rates of AR episodes were low and similar in each group: 7.8% in the conversion group versus 6.5% for those who stayed on CNI [51]. Graft and patient survivals were nearly identical. Median urinary protein/creatinine ratios were similar in each group at baseline, but increased significantly 6–24 months after

conversion in the SRL group. The overall rates of adverse events were similar at 24 months [51].

Lebranchu *et al.* [75] conducted a multicenter, prospective, open-label trial that randomized 235 *de novo* renal transplant recipients at week 12 to switch from CsA to SRL or to continue CsA. All patients received induction therapy with daclizumab, MMF, and corticosteroids. The objective was to evaluate eGFR as determined by the Cockcroft-Gault formula at 1 year postconversion. Steroids were withdrawn in both groups at 8 months [75]. GFR improved from a baseline of 60 ml/min to 69 ml/min in the conversion group, compared with 64 ml/min for those who remained on CsA ($P = 0.02$) [75]. Biopsy-proven rejection episodes were more common in the conversion group (17%) than in the group that remained on CsA (8%; $P = 0.07$) [75].

In the Spare the Nephron (STN) trial, 305 first-kidney transplant patients were randomized to be converted from center-specific use of CNIs (81% Tac, 19% CsA) to SRL between 1 and 6 months post-transplant or to remain on CNIs [76]. Patients also received center-specific use of induction treatment, MMF (2 g/day), and corticosteroids. The mean conversion time post-transplant was 117 days. The primary outcome measure was change in measured GFR (cold iothalamate). Secondary outcomes included the incidence of BPAR and graft loss at 1 year after conversion. Mean percentage improvement in measured GFR was 26% in the conversion group; whereas in the CNI maintenance group, it only improved by 11%. The incidence of BPAR was low (~7%) in each group; there was no difference in graft loss or patient survival. Approximately 80% of patients who were converted remained on therapy at 1 year.

These three studies, which evaluated conversion in low- to moderate-risk patients, suggested that conversion from CNI to mTOR inhibition can be safely accomplished in patients with GFR >40 ml/min with a low rate of BPAR and graft loss that was not different from remaining on CNI therapy [51,75,76]. None of the studies utilized protocol biopsies at the time of conversion. More than 75% of patients tolerated the conversion at 1 year; there was improvement in either estimated or measured GFR compared with those remaining on CNI therapy. Of note is that all patients in these studies received MMF with or without corticosteroids. A variety of induction regimens were used in some patients; whereas in others, there was no induction. These studies also suggest that there may be a modest, but not statistically significant increase in urinary protein excretion among patients who were converted.

The therapeutic index for later conversion post-transplant once kidney function has diminished (e.g. eGFR <40 ml/min) or at greater levels of urine protein excretion (e.g. ≥800 mg/day) is yet to be established. In the CONVERT study [51], patients with eGFR <40 ml/min did not fare as well as those with greater degrees of eGFR. Likewise, Diekmann *et al.* [77] noted that patients with >800 mg of protein in urine per day sustained marked increases in urinary protein excretion and renal function deterioration in response to conversion. Wali *et al.* [78] reported analysis of an ongoing, single-center, retrospective experience in 136 patients with biopsy-proven allograft nephropathy who were converted from CNI to SRL at variable periods within the first 2 years after transplantation. Goal SRL trough levels of 8–10 ng/ml were used. They noted that eGFR improved in 74% of patients after conversion. However, they also noted that conversion was ineffective in patients with creatinine >3.8 mg/dl or eGFR <18.4 ml/min. They recommended that earlier conversion (preferably <6 months post-transplant) was associated with greater improvement in renal function; once substantial renal injury had occurred, later conversion would be less beneficial. These studies suggested that more information is required about later conversion strategies, especially in patients with lower GFR or greater degrees of clinical proteinuria. On the other hand, these studies showed that the majority of patients tolerate conversion and that it is associated with a low rate of rejection (~10%).

In summary, the available studies indicate that conversion from CNI to mTOR inhibitors can be accomplished safely and effectively in the majority of low- to moderate-risk renal transplant recipients within the first 6 months after transplantation. One- and 2-year follow-up data indicate improvements in estimated and measured GFR. The conversion strategy is tolerable in >75% of patients and is associated with a low risk of rejection that is comparable to remaining on CNI. More information will be required to evaluate conversion strategies in patients with greater risk, as well as those patients with lower GFR (<40 ml/min) and greater degrees of proteinuria. In addition, studies with EVL will need to be conducted to evaluate its utility for CNI conversion.

Malignancies

Another potential opportunity for mTOR inhibition is the possible reduction of risk for post-transplant malignancy. The prevalence of post-transplant malignancies increases with time after engraftment. With advances in long-term graft survival, malignancies and cardiovascular disease have become the most common causes of death after renal transplantation in most countries, especially in Australia and New Zealand [79]. Furthermore, the survival after diagnosis of almost any type of cancer in transplant cohorts is dramatically lower compared with age- and disease stage-matched general populations [80].

The strongest predictors for the development of post-transplant malignancies are age, race, and time after engraftment [81,82]. It has been commonly acknowledged that a major contributor to post-transplant malignancies is the mandatory immunosuppressive therapy after transplantation, specifically, the use of depleting antibodies for induction and treatment of rejections, as well as Aza- and CNI-based immunosuppressive regimens [83,84].

With the introduction of mTOR inhibitors as immunosuppressive agents in clinical transplantation approximately 20 years ago, there are now some long-term data available to assess whether this group of drugs can actually reduce the incidence of post-transplant malignancies.

Three years after the first experimental studies described a slower growth of inoculated tumors and longer survival of immunoincompetent mice in 2002, the first analyses of large registries and a meta-analysis showed that the use of mTOR inhibitor-based immunosuppression was associated with a reduced risk of developing a post-transplant malignancy, particularly cutaneous malignancy [2,85–87].

In the Rapamune Maintenance Regimen study, the incidence of any non-skin cancer was significantly lower at 5 years in the intent-to-treat study arm that received high-dose SRL and steroids compared with those patients who were treated with low-dose SRL, CsA, and steroids [88]. The relative risk for the development of skin cancer was reduced by 65%. This study has been criticized because both study groups received SRL. Studies on the other available mTOR inhibitor that included at least one study arm without EVL had only short-term follow-up, and thus, few malignancies [69,89,90].

The other large, randomized, controlled, *de novo* study that used SRL only in one study arm reported malignancies in 4–9 patients in the various study groups at 1 year after transplantation [91]. In a pooled analysis of 2-year data of two pivotal phase III SRL trials and two phase II studies, Mathew *et al.* [13] summarized that SRL immunotherapy may be beneficial in protecting renal transplant patients from cancer. The other SRL *de novo* trials were either too small or do not yet have a sufficiently lengthy follow-up to evaluate the effect on the development of malignancies [17,59,76,89,92]. Even in the large SYMPHONY study, investigators did not find a difference in the rate of malignancies between the CNI and mTOR inhibitor arms at the 1-year follow-up [10].

The strongest evidence that mTOR inhibitors truly may reduce the incidence of post-transplant malignancies comes from the largest conversion study so far [51]. In the CONVERT trial, 11.0% of the 273 patients on CsA developed a malignancy within 2 years after randomization, compared with only 3.8% of the 551 SRL-converted subjects ($P < 0.001$). The reduction in incidence of malignancies with the mTOR inhibitor was mainly as a result of the reduction in incidence of skin tumors (7.7% vs. 2.2%, $P < 0.01$) [51]. At inclusion in the CONVERT study, patients had been transplanted slightly more than 3 years on average.

An evaluation of the incidence of EVL-associated post-transplant malignancies is not possible because EVL was always dosed with CsA. In the 3-year analysis of the B201 and B251 trials, Lorber *et al.* [69] and Vítko *et al.* [89] found a malignancy rate of ~5%. The combined analysis of the 1-year data from the 2306 and 2307 trials showed a malignancy incidence of ~2%, which also was not differently distributed between groups [71]. In summary, the impact of EVL on the development of post-transplant malignancies remains unclear because there are no head-to-head comparisons of CsA with EVL.

Another solid piece of evidence that mTOR inhibitors may reduce tumor growth comes from nontransplanted patients with renal cell cancer. Temsirolimus, a water-soluble derivative of SRL, is the first drug ever to have been shown to prolong survival of patients with metastatic renal cell carcinoma [93].

Adverse effects of mTOR inhibitors

A substantial but varied percentage of discontinuations for adverse events have been observed among mTOR inhibitor-treated patients in nonrandomized (17%) [52] and randomized studies with either *de novo* use: 15.5% [57], 38% [7], and 7.8% [10]; or after conversion: 28% [52] and 12% (CONCEPT) [75]. The main adverse events reported were wound-healing complications [7], gastrointestinal [10,51] and mucocutaneous side effects [51,57], bone marrow suppression [17], disorders of the blood [52] and lymphatic systems, infection [10], hyperlipidemia [17], and proteinuria [10,52] (Table 2). Unexplained interstitial pneumonitis leading to discontinuations or dose reduction has been described [95]. A significant increase in cholesterol and triglyceride levels and percent-

Table 2. Selected frequent adverse events during the 12-month follow-up of randomized studies.

Adverse events	Rate of occurrence (%)
Surgical complications [10,57,89,94]	9–15
Acne, folliculitis [51,57,75,94]	16–25
Mouth ulcers/aphthous stomatitis [51,57,75]	8–46
Diarrhea [10,51,57,75,89]	24–39
Hypokalemia [57]	23
Peripheral edema [51,75,89]	22–32
Bronchopulmonary complications [51,57,94]	6–16

age of patients receiving lipid-lowering agents has been reported in most controlled studies [2,7,17,52,57,96]. Other adverse events have been recognized recently, including new-onset diabetes [97,98] and reduced male fertility [99]. Significant risk factors of surgical complications have been identified (delayed graft function and body mass index >30 kg/m^2) [100], and experience is critical to limit their occurrence. From studies with EVL, as well as the few studies that compared the side effects of SRL with those of EVL, there is no evidence of a significant difference in the incidence and severity of these adverse events [101,102].

Comparisons of low-dose and high-dose mTOR inhibition confirmed that disturbances of hematologic and lipid indices were dose dependent [2]. Therefore, increased experience has prompted dose reduction and loading dose avoidance [103]. The synergistic effects of mTOR inhibitors with mycophenolic acid (MPA) on bone marrow suppression and gastrointestinal disorders have led to dose reduction of MPA [104] because MPA exposure is increased in patients receiving SRL rather than CsA [105–107]. Furthermore, the highly variable oral bioavailability could result partly from genetic polymorphism of the *CYP3A5* gene because patients with the genotype of non-expressor 3*/3* have a decreased clearance of SRL and an increased AUC/dose, suggesting that the determination of this polymorphism could be useful for a dose adjustment [108]. Similar observations may be seen with EVL.

Some trials have indicated that mTOR inhibitors may be associated with increased proteinuria. Data on proteinuria in randomized *de novo* trials comparing CNI-containing regimens with SRL-based regimens show inconclusive results. Flechner *et al.* [9,17] found no difference in proteinuria at 1 and 5 years, whereas Büchler *et al.* [57] found a higher amount of proteinuria in the SRL group (0.64 ± 0.8 vs. 0.18 ± 0.3 g/day).

Two randomized early conversion studies also examined protein/creatinine ratios or 24-h proteinuria at 1 year. In STN, a slight, however, statistically significant difference was found (CNI = 0.14 vs. SRL = 0.21 g/g creatinine) [109]. In the CONCEPT trial, no significant difference was found [75].

In the analysis of the largest multicenter trial of late conversion (i.e. >6 months post-transplant), a quantitative but not statistically significant difference in proteinuria was observed compared with the CNI control arm [51].

In patients who were converted from a CNI-containing regimen to SRL for chronically deteriorating kidney function, a considerable increase of proteinuria has been observed. In a retrospective analysis of 149 patients, Ruiz *et al.* [110] observed an increase in mean proteinuria from 6 months after conversion. In this study, an increase of >500 mg/day was associated with a higher serum creatinine compared with those patients who had no or a moderate increase. In this patient series, the *de novo* incidence of nephrotic-range proteinuria was 15/149 patients. The majority of these patients had some degree of proteinuria before conversion. However, 3% of patients with declining renal function without proteinuria <300 mg/day before conversion experienced an increase to nephrotic-range proteinuria.

Letavernier *et al.* [111] observed nephrotic syndrome and focal segmental glomerulosclerosis in three patients who received SRL *de novo* and five patients who were converted to SRL. All patients developed classic focal segmental glomerulosclerosis lesions, but advanced sclerotic lesions were only exhibited in switched patients. In general, it is not clear to what extent reconversion can lead to regression of proteinuria; however, Letavernier *et al.* [112] published data of a series of patients in whom a significant regression of proteinuria was observed after switching back to a CNI.

In summary, several randomized, multicenter trials demonstrated a quantitative increase in proteinuria. General risk factors for increased proteinuria, such as poor organ quality, are influential factors in SRL-treated patients. Whether those changes in proteinuria are meaningful in terms of negatively influencing graft outcome or just reflect the absence of the antiproteinuric action of CNIs remains unknown. In patients with proteinuria >800 mg/day, conversion to mTOR inhibitors is not advisable, as renal function is more likely to worsen. The etiology of the increase in proteinuria remains unknown.

Conclusions: optimal clinical use

In summary, there are risks and benefits in every form of immunosuppression. With mTOR inhibitors, there are unique aspects of adverse events that need to be carefully considered and monitored. mTOR inhibition is a novel therapeutic approach with a difficult learning curve for post-transplant immunosuppression. The first trials with mTOR inhibitors were started more than two decades ago. Consequently, there is insufficient evidence of the impact of this therapy on graft and patient survival. The main advantage of these drugs is that they are less nephrotoxic than CNIs and have antiproliferative properties, which may be important for retarding atherosclerosis or malignancy development. There is still some debate about which patients are most likely to benefit from this form of immunosuppression. In some patients, particularly those who are overweight, or those who receive kidneys from deceased donors with longer cold storage time, the use of mTOR inhibitors in the immediate perioperative and post-transplant period is not ideal given the risk of

impaired wound healing and worsening of delayed graft function. On the other hand, conversion from CNI to mTOR inhibition within the first year post-transplant before CNI-mediated chronic renal injury occurs may be helpful for improving 1-year GFR. Longer-term studies will need to demonstrate the durability and clinical relevance of this benefit. Later conversion from CNI to mTOR inhibition, once there is loss of GFR, especially if there is clinical proteinuria, may be risky, as there is less evidence of benefit regarding eGFR change over time and a greater proclivity for increasing proteinuria. Thus, later conversion decisions should be carefully individualized. Tolerability of mTOR inhibitors, as with all immunosuppression drugs, is important. There are unique adverse events associated with mTOR inhibition that may respond to dose reduction or may require drug discontinuation. Future clinical trials, both in single center and multicenter, will assist clinicians to define better the role of mTOR inhibition as a long-term therapy in immunosuppression protocols.

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