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Use of Sirolimus in Solid Organ Transplantation

Joshua J. Augustine, Kenneth A. Bodziak and *Donald E. Hricik*

The Department of Medicine and the Transplantation Service, Case Western Reserve University, and University Hospitals of Cleveland, Cleveland, Ohio, USA

Contents

Abstract

Sirolimus is a mammalian target of rapamycin (mTOR) inhibitor that inhibits cell cycle progression and has proven to be a potent immunosuppressive agent for use in solid organ transplant recipients. The drug was initially studied as an adjunct to ciclosporin (cyclosporine) to prevent acute rejection in kidney transplant recipients. Subsequent studies have shown efficacy when combined with a variety of other immunosuppressive agents. The most common adverse effects of

sirolimus are hyperlipidaemia and myelosuppression. The drug has unique antiatherogenic and antineoplastic properties, and may promote immunological tolerance and reduce the incidence of chronic allograft nephropathy. Although sirolimus is relatively non-nephrotoxic when administered as monotherapy, it pharmacodynamically enhances the toxicity of calcineurin inhibitors. Ironically, the drug has been used to facilitate calcineurin inhibitor-free protocols designed to preserve renal function after solid organ transplantation. Whether sirolimus can be used safely over the long term with low doses of calcineurin inhibitors requires further study. The use of sirolimus as a corticosteroid-sparing agent also remains to be proven in controlled trials. Postmarketing studies have revealed a number of unforeseen adverse effects including impaired wound healing and possibly proteinuria, oedema, pneumonitis and thrombotic microangiopathy. Overall, sirolimus is a powerful agent when used judiciously with other available immunosuppressants. As is true for all immunosuppressive drugs available for treatment of solid organ transplant recipients, the efficacy of the drug must be balanced against its considerable adverse effects.

Sirolimus (rapamycin; Rapammune®)[1] is a macrocyclic lactone antibacterial isolated from the fungus *Streptomyces hygroscopius*, first discovered in soil samples from Easter Island. Structurally similar to the calcineurin inhibitor (CNI) tacrolimus (figure 1), it proved to have potent immunosuppressive properties with a novel mechanism of action that was first delineated by Sehgal[1] and later applied in animal transplant models by Calne et al.[2] and Morris and Meiser.[3] Initial clinical trials focused on the use of sirolimus as adjunctive therapy with ciclosporin (cyclosporine) and corticosteroids in kidney transplant recipients, often substituting for the antimetabolite azathioprine. Results of phase III clinical trials demonstrated that sirolimus in combination with ciclosporin and corticosteroids reduces the incidence of acute rejection episodes, leading to its approval by the US FDA in 1999.

Since the initial FDA approval, there has been considerable off-label clinical experience combining sirolimus with other immunosuppressants in both kidney and extrarenal organ transplant recipients. Postmarketing experiences have revealed a number of toxicities, only some of which can be explained by the main mechanism of action of sirolimus, which is inhibiting a cytosolic molecule, now known as the mammalian target of rapamycin (mTOR). Use of sirolimus in combination with CNIs has been associated quite regularly with impaired renal function worse than that observed when the CNIs are used with other immunosuppressants. However, the relative lack of intrinsic nephrotoxicity of sirolimus in humans ironically has provided its potential to supplant the nephrotoxic CNIs in maintenance immunosuppression. Sirolimus was registered by the European Agency in 2000 and by the FDA in 2003 as an alternative for maintenance therapy after discontinuation of ciclosporin at 3 months post-transplant in immunologically low-risk kidney transplant recipients. This article reviews the mechanisms of action, clinical efficacy and adverse effects of sirolimus in solid organ transplantation, focusing on its approved and off-label uses in kidney transplantation.

1. Mechanism of Action

Sirolimus is a proliferation signal inhibitor that blocks growth factor-induced transduction signals that mediate cellular division in response to alloantigens. After entry into the cell, sirolimus binds to the abundant immunophilin, FKBP-12, that also serves as a cytosolic receptor for the CNI

1 The use of trade names is for product identification purposes only and does not imply endorsement.

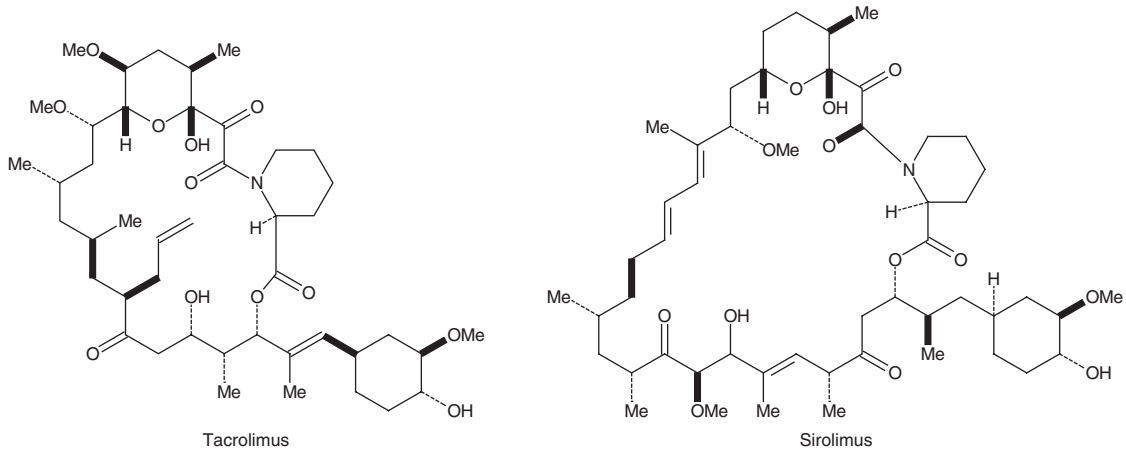

Fig. 1. Molecular structures of tacrolimus and sirolimus.

tacrolimus. The tacrolimus-FKBP-12 complex binds to the calcium-dependent serine-threonine phosphatase calcineurin, and thus blocks the pathway of T-cell activation that leads to G_0 to G_1 transition and transcription of key growth factors and cytokines.[4] In contrast, the sirolimus-FKBP-12 complex binds to mTOR (figure 2). mTOR is known to have two major functions: activation of $p70^{S6}$ kinase, which plays a key role in signal transduction leading to DNA synthesis, and activation of the eukaryotic initiation factor 4E (eIF-4E)-phosphorylatable heat stable protein I (PHAS-I) pathway that is more involved in protein synthesis.[4] By binding to and inhibiting mTOR, sirolimus inhibits activation of $p70^{S6}$ kinase, leading to arrest of the cell cycle at the G_1 to S phase. Both interleukin (IL)-2 receptor-dependent and CD28-dependent signalling pathways are inhibited by these effects on mTOR. In contrast to the CNIs, the mTOR inhibitors block growth factor-driven proliferation of both haematopoietic and nonhaematopoietic cells, including vascular and bronchial smooth muscle cells.[5,6]

Indirectly, the mTOR inhibitors block effector functions of CD4+ T helper cells and CD8+ cytotoxic T cells, activation of monocytes, and proliferation and differentiation of B cells.[7-9] Because apoptosis is independent of mTOR activity, the mTOR inhibitors, unlike the CNIs, do not interfere with apoptosis of T cells. This phenomenon forms the basis for animal experiments demonstrating that the combination of costimulatory blockade with mTOR inhibition can lead to long-term graft acceptance without subsequent maintenance immunosuppression.[10-12]

Clinical studies suggest that the immunosuppressive effects of sirolimus and ciclosporin are pharmacodynamically synergistic[13] (section 3.1.1). Although *in vitro* studies suggested that sirolimus and tacrolimus might compete for FKBP-12 and act as antagonists,[14] the effect is obviated, at least at pharmacological concentrations of the two drugs, by the large amount of FKBP-12 in the cell cytoplasm. In fact, both preclinical[15] and clinical studies[16] subsequently confirm that sirolimus augments the immunosuppressive activity of tacrolimus.

2. Pharmacology

2.1 Absorption and Distribution

Sirolimus is absorbed rapidly, with mean peak whole-blood concentrations (C_{max}) occurring 1–2 hours after administration.[17] Systemic availability is approximately 15%,[18] and is reduced by intestinal and first-pass hepatic metabolism by the cytochrome P450 (CYP) 3A enzyme and p-glycoprotein intestinal countertransport pump.[19] Time to C_{max} can be reduced by ingestion with a fatty meal[20] and with use of liquid formulations.[21] While total expo-

sure, estimated by the pharmacokinetic area under the concentration-time curve (AUC), is increased with fatty food, there is no increase in the AUC with the liquid versus tablet. Thus, the two formulations are thought to be interchangeable. However, it is recommended that sirolimus be administered consistently with or without food. Hepatic dysfunction may also increase AUC by 60%, although sex, age and ethnicity do not appear to affect exposure.[22]

2.2 Metabolism

Sirolimus undergoes oxidative metabolism and is converted to multiple metabolites. The immunosuppressive activity of sirolimus metabolites is thought to be low, although not all metabolites have been clinically defined.[23] Ninety percent of the parent compound is excreted in the faeces, with very little urinary excretion.[19] Mean terminal half-life to elimination is approximately 60 hours in patients with stable renal allograft function.[17] The long half-life has allowed for once-daily administration of sirolimus, while a loading dose of approximately three times the maintenance dose has been recommended because of the gradual accumulation of the drug.[17]

Although early phase III studies with sirolimus were dose-based,[24] therapeutic drug monitoring is recommended.[25,26] Clinical data suggest that efficacy and toxicity are related to sirolimus concentrations,[12] and there is high interpatient variability with drug exposure.[25] There is good correlation between AUC and 24-hour trough concentrations,[27,28] leading to ease of therapeutic monitoring. Because of the long half-life, drug monitoring should occur at approximately 5–7 days from the time of initiation or dose change. Whole-blood concentrations should be measured, as 95% of the drug is sequestered in red blood cells.[29] Measurements of drug concentration are generally made using high performance liquid chromatography with tandem

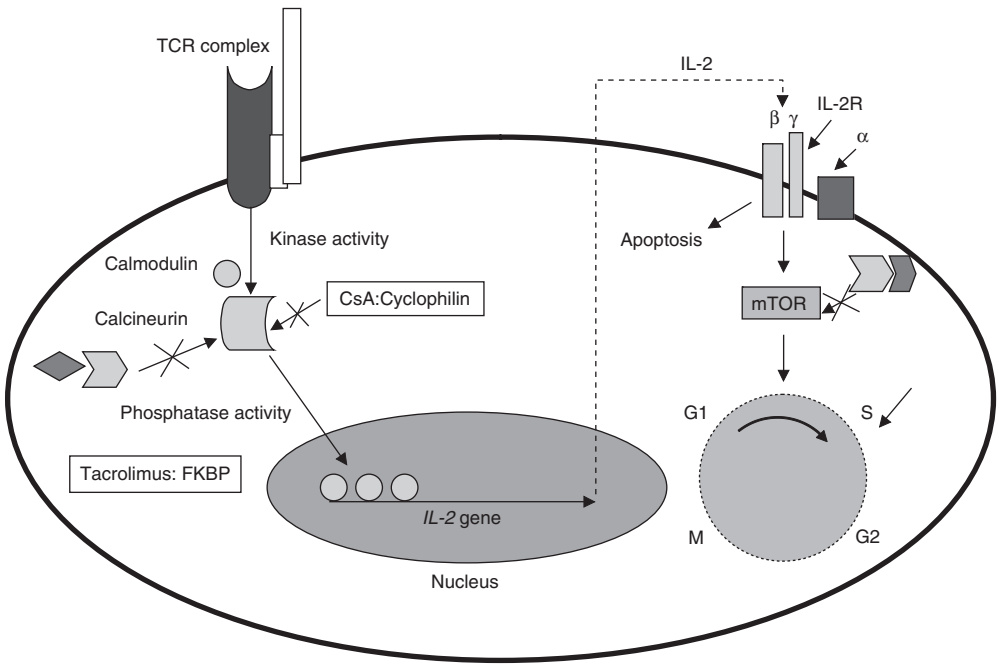

Fig. 2. Mechanism of action of sirolimus within the T cell. Ciclosporin (CsA) and tacrolimus bind to intracellular immunophilins (cyclophilin and FK binding protein [FKBP], respectively) and then inhibit the phosphatase activity of calcineurin, thus inhibiting T-cell activation by altering signal transduction to the nucleus and inhibiting generation of interleukin (IL)-2. In contrast, the sirolimus/FKBP combination has no effect on calcineurin and instead inhibits the mammalian target of rapamycin (mTOR), leading to inhibition of IL-2-mediated cell cycle progression. **TCR** = T-cell receptor.

mass spectroscopy, or by the less cumbersome liquid chromatography with ultraviolet detection. Both methods have shown good sensitivity with a broad linear range and do not measure inactive sirolimus metabolites.[30,31] In contrast, a recently approved immunoassay measures both parent compound and metabolites,[32] resulting in values that are approximately 23% higher than those obtained with liquid chromatography and mass spectroscopy.

2.3 Drug Interactions

Because sirolimus is a substrate for the CYP3A-metabolising enzyme, other drugs that induce or inhibit this enzyme have been shown to interact significantly with the drug. Inhibitors such as non-dihydropyridine calcium channel antagonists (e.g. diltiazem, verapamil), azole antifungals (e.g. voriconazole, itraconazole, ketoconazole, fluconazole), clarithromycin and erythromycin are particularly likely to increase sirolimus concentrations and should be used only with careful drug monitoring. In addition, grapefruit juice can inhibit CYP3A and should be avoided. Enzyme inducers such as rifampicin, phenytoin and carbamazepine can markedly reduce serum sirolimus concentrations. Ciclosporin microemulsion formulations administered with sirolimus led to increased exposure of both drugs when given in combination,[24] leading to a recommendation that the drugs be administered 4 hours apart. This may result from competition for the intestinal p-glycoprotein countertransporter between ciclosporin and sirolimus or by competitive suppression of CYP3A.[33] Because tacrolimus is administered in dosages 100-fold lower than ciclosporin, the drug does not share this specific interaction, and sirolimus concentrations are similar either with simultaneous administration with tacrolimus or with dose separation.[34]

3. Therapeutic Efficacy

3.1 Kidney Transplantation

3.1.1 Use with Ciclosporin (Cyclosporine)

An initial phase I/II dose-escalation trial limited to HLA-mismatched living donor kidney transplant recipients showed that use of sirolimus with corticosteroids and concentration-controlled administration of ciclosporin resulted in a 7.5% cumulative incidence of acute rejection over 3 years, compared with a 32% incidence in a control cohort maintained on ciclosporin and corticosteroids alone.[35] Full exposure of ciclosporin was achieved based on trough blood concentrations. These promising results prompted a randomised, controlled, multicentre phase II trial in which sirolimus was used in combination with either full-dose or reduced-dose ciclosporin (based on lower target trough concentrations) in recipients of deceased donor renal allografts.[36] In non-African American patients, the incidence of rejection was 8.5% in the first 6 months post-transplant in the reduced-dose ciclosporin arm, a result that was not statistically different from that achieved in the full-dose ciclosporin arm.[36] On the other hand, the rejection rate was substantially higher (39%) in African Americans treated with reduced-dose ciclosporin.[36]

Subsequently, two large phase III randomised, double-blind, prospective trials compared the efficacy and safety of two sirolimus dosages (2 or 5 mg/day) versus either azathioprine (US study)[24] or placebo (global study)[37] in patients also treated with ciclosporin and corticosteroids. Results of these trials, together enrolling more than 1300 patients, are summarised in table I. In the US study, the cumulative incidence of biopsy-proven acute rejection post-transplant was significantly reduced in the sirolimus arms (2mg 16.9%; 5mg 12.0%; azathioprine 29.8%).[24] The incidence of acute rejection was reduced in a similar fashion in the global study (2mg 24.7%; 5mg 19.2%; placebo 41.5%).[37] In each study, use of sirolimus also was associated with lower rates of corticosteroid-resistant acute rejection, lower histological grades of rejection and delay

Table I. Results of phase III trials comparing sirolimus with either azathioprine or placebo in kidney transplant patients also receiving ciclosporin and prednisone

Treatment	Graft survival (%)	Patient survival (%)	Incidence of acute rejection (%)	p-Value
US study: 12-month follow-up data[24]				
Sirolimus 2mg	94	97	16.9	0.002[a]
Sirolimus 5mg	93	96	12	<0.001[a]
Azathioprine	94	97	29.8	
Global study: 6-month follow-up data[37]				
Sirolimus 2mg	93	98	24.7	0.003[b]
Sirolimus 5mg	93	96	19.2	<0.001[b]
Placebo	88	95	41.5	

a Versus azathioprine.

b Versus placebo.

in onset to the time of first rejection episode. In the African American subgroup of the US study, a statistically significant benefit of sirolimus on cumulative incidence of biopsy-proven rejection was demonstrated only in patients receiving the 5mg dose. Overall, 12-month patient and graft survival rates were equal to the control arms of each trial (table I).

The immunosuppressive potency of the sirolimus plus ciclosporin combination was further demonstrated in a single-centre analysis of 137 high-risk African American kidney transplant recipients.[38] When compared with an historical control group of African Americans treated with ciclosporin and prednisone alone, those treated with sirolimus, ciclosporin and prednisone exhibited a significantly lower cumulative incidence of acute rejection (43.3% vs 19.2%), and better graft survival (85.6% vs 97.9%) 2 years post-transplant. Graft survival in the sirolimus-treated group of African Americans was comparable to that in a concurrent cohort of 120 Caucasian patients treated with the same regimen.

Consistent with observations from preclinical and phase I/II trials, hyperlipidaemia and thrombocytopenia (sections 4.1 and 4.2) were among the most common adverse effects associated with sirolimus therapy in the phase III trials.[24,37] Somewhat unexpected was the observation that serum creatinine levels at 12 months were significantly higher than those in control patients in each study (figure 3). Although sirolimus dosage in these trials was not concentration-controlled, *post hoc* analyses studying the relationship between drug concentrations and outcomes indicated pharmacodynamic synergy between ciclosporin and sirolimus,[13] suggesting that the immunosuppressive potency of the combination may be counterbalanced by enhanced ciclosporin toxicities (section 4.1). This consistent observation has motivated the use of sirolimus with lower dosages of ciclosporin designed to reduce exposure, and preliminary experiences suggest that such an approach is associated with better long-term renal function.[39,40]

3.1.2 Use with Tacrolimus

Early experiences with the combination of sirolimus and tacrolimus in kidney,[41-44] liver,[41] pancreas[41] and islet cell[45] transplantation generally reported low rates of acute rejection in small uncontrolled studies. In most of these early studies, blood tacrolimus concentrations were intentionally lowered based on earlier experiences with the sirolimus plus ciclosporin combination.

A small prospective, randomised, multicentre European study shed additional light on the potency of the tacrolimus plus sirolimus combination in preventing acute rejection.[46] Primary kidney transplant recipients were randomised to receive corticosteroids and either tacrolimus alone (n = 28) or corticosteroids, tacrolimus and one of three daily

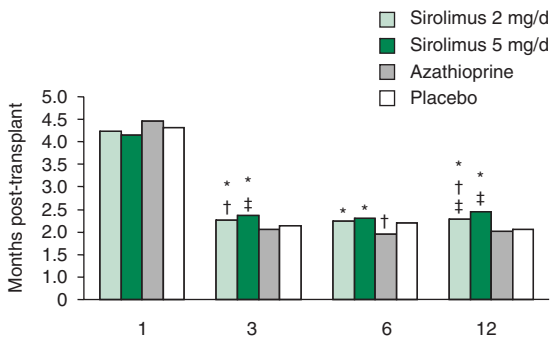

Fig. 3. Renal function over time estimated by serum creatinine concentration in patients receiving fixed-dose sirolimus in combination with ciclosporin and corticosteroids. Pooled results from two phase II trials (data derived from Kahan[24] and MacDonald[37]). * $p < 0.001$ vs azathioprine; † $p < 0.001$ vs sirolimus 5 mg/day; ‡ $p < 0.001$ vs placebo.

doses of sirolimus: 0.5mg (n = 25), 1mg (n = 25) or 2mg (n = 25). Exposure to tacrolimus was adjusted to trough blood concentrations that were identical in each group. The 3-month incidence of biopsy-proven acute rejection was significantly higher in the control group than in the sirolimus groups (28.6%, 8%, 8% and 3.8%, respectively; p = 0.014).

Two randomised studies subsequently have compared the efficacy and safety of the combination of tacrolimus and sirolimus with that of the combination of tacrolimus and mycophenolate mofetil (MMF) in kidney transplant recipients. In a multicentre study, living and deceased donor kidney transplant recipients were randomised to receive maintenance therapy with corticosteroids and either tacrolimus and sirolimus (n = 185) or tacrolimus and MMF (n = 176). After 6 months of follow-up,[47] there were no differences in the incidence of acute rejection (13.0% vs 11.4%) nor in overall patient or graft survival. However, renal function estimated by serum creatinine levels was significantly worse in the sirolimus arm. Analysis after 12 months of follow-up[48] again showed higher serum creatinine levels in the patients receiving tacrolimus and sirolimus (1.5 vs 1.3 mg/dL; p = 0.03). Although overall patient and graft survival were not different, 1-year graft survival was significantly better among patients in the MMF arm when the analysis was limited to patients without delayed graft function (99% vs 93%; p = 0.01). Patients receiving sirolimus had a significantly higher incidence of study discontinuation (26.5% vs 14.8%). Similar results have been reported from a single-centre experience in which patients were randomised to receive maintenance therapy with corticosteroids and either tacrolimus plus sirolimus, tacrolimus plus MMF, or ciclosporin plus sirolimus.[49] After 6 months of follow-up, there were no differences in the incidence of acute rejection. However, there were more protocol discontinuations and trends towards decreasing creatinine clearances in the sirolimus arms of the study. Importantly, and in contrast to most of the earlier uncontrolled experiences with the tacrolimus plus sirolimus combination, no effort was made to lower target blood concentrations for tacrolimus in the sirolimus arms of these latter randomised studies.

Collectively, these experiences suggest that the combination of tacrolimus and sirolimus yields short-term clinical outcomes comparable to those achieved with tacrolimus and MMF, albeit with a higher incidence of adverse effects, including nephrotoxicity that is reminiscent of earlier experiences with ciclosporin and sirolimus. It appears that long-term outcomes are affected in a similar fashion. A recent analysis of the Scientific Renal Transplant Registry examined the outcomes of 44 915 patients who received kidney transplants between 2000 and 2004 and showed that the 3-year graft survival rate in patients receiving an initial discharge regimen of tacrolimus and sirolimus was inferior to the rates in patients receiving either ciclosporin plus sirolimus or ciclosporin plus MMF, despite relatively low rates of acute rejection.[50] The best long-term graft survival rate, after adjustment for a number of factors known to affect long-term graft survival, was achieved in patients initially treated with tacrolimus and MMF. By 24 months post-transplant, more than 55% of patients initially treated with sirolimus-based immunosuppression had been converted to a different regimen.[50]

3.1.3 Calcineurin Inhibitor-Sparing Regimens

Conversion from Calcineurin Inhibitors to Sirolimus

Several small studies have reported the outcomes of kidney transplant recipients converted from a CNI to sirolimus, most often because of chronic graft dysfunction and a desire to eliminate the nephrotoxic effects of the CNIs. Dominguez et al.[51] reported the results of conversion to sirolimus to facilitate marked reduction in dose or elimination of a CNI in 20 patients, 15 of whom had either acute or chronic CNI nephrotoxicity. Twelve patients with chronic nephrotoxicity exhibited a significant decrease in serum creatinine levels after 7–24 months of follow-up. However, during the conversion, overlapping doses of sirolimus and a CNI was accompanied by either pneumonitis or bronchiolitis in seven patients. Wyzgal et al.[52] converted 13 kidney transplant recipients with biopsy-proven CNI toxicity to sirolimus. After 6 months, serum creatinine levels

decreased. However, one patient experienced an acute rejection episode after the conversion and two patients developed pneumonia. Interestingly, an increase in urinary protein excretion was noted despite improvement in renal function (section 4.3.3). Diekmann et al.[53] converted 20 patients from a CNI to sirolimus because of suspected CNI nephrotoxicity. After 1 year, renal function improved in 55% of patients. However, in the remaining 45% of patients, serum creatinine levels actually rose from 3.2 to 4.4 mg/dL ($p < 0.01$) and there was an associated increase in proteinuria. The authors suggested that patients with heavy proteinuria or baseline serum creatinine levels exceeding 3.0 mg/dL may not benefit from conversion. Egidi et al.[54] reported the outcomes of CNI to sirolimus conversion in kidney, kidney-pancreas and liver transplant recipients. The indications for conversion included CNI nephrotoxicity (n = 20), haemolytic uraemic syndrome (n = 12), chronic allograft nephropathy (n = 13) and glucose intolerance (n = 19). A unique feature of their conversion protocol was the abrupt discontinuation of the CNI with sirolimus initially administered as an 8–12mg loading dose 24–72 hours later. To avoid under-immunosuppression, pancreas transplant recipients were treated with the IL-2 receptor antibody daclizumab at the time of the conversion. Serum creatinine fell from 2.9 to 2.2 mg/dL ($p = 0.01$) in the patients with renal indications for conversion and glucose intolerance resolved in 11 of the 19 patients who were converted because of hyperglycaemia.

The CONVERT trial is an ongoing study representing the largest experience with CNI to sirolimus conversion to date. In this study, kidney transplant recipients were randomised to maintain treatment with a CNI (n = 275) or to convert to sirolimus (n = 555) within 16 weeks of a baseline transplant biopsy, usually performed because of chronic graft dysfunction. Study participants were initially stratified according to baseline glomerular filtration rate (GFR) [estimated by the Nankivell formula] of 20–40 mL/min or >40 mL/min. The 12-month data from this 4-year study have been presented preliminarily.[55] Enrolment of patients with baseline GFRs between 20 and 40 mL/min was halted because of higher rates of safety events including acute rejection and graft loss. In this subset of 90 patients, there was no significant difference in GFR in patients randomised to CNI continuation compared with those converted to sirolimus over time. In patients with baseline GFR >40 mL/min, a statistically significant improvement in GFR was detectable within 12 months of conversion to sirolimus compared with that of patients maintained on a CNI. In this larger group of patients, the mean change from baseline GFR was +1.4 mL/min for the sirolimus group and −1.3 mL/min for the CNI group at 12 months ($p = 0.003$). Interestingly, patients achieving the greatest improvement in GFR over time were those with the lowest rates of urine protein excretion at baseline. If confirmed with longer follow-up, results of this trial will suggest that conversion from CNIs to sirolimus for the sake of improving long-term renal function yields optimal results when performed when renal function is still relatively well preserved.

Calcineurin Inhibitor Withdrawal

Several randomised, prospective trials have examined the outcomes of stable kidney transplant patients initially maintained on corticosteroids, sirolimus and a CNI, and randomised either to continue CNI therapy or to discontinue either ciclosporin[56-60] or tacrolimus[61] approximately 2–3 months after transplantation. In the largest of these trials, Johnson et al.[56] enrolled 525 patients who were treated initially with sirolimus, ciclosporin and corticosteroids. At approximately 3 months post-transplant, 430 of these patients were deemed eligible for randomisation to CNI continuation or gradual ciclosporin elimination. After 12 months of follow-up, calculated mean GFR was 63 mL/min in the ciclosporin withdrawal group and 57 mL/min in the ciclosporin continuation group ($p < 0.001$) among patients who remained on their randomly assigned therapy. Both systolic and diastolic blood pressures were significantly better in the group withdrawn from ciclosporin. After 12 months of follow-up, the cumulative incidence of acute rejection following randomisation was higher in the group of patients withdrawn from ciclosporin (9.8% vs 4.2%; p =

0.035). However, the difference in cumulative rejection rates was no longer statistically significant after 3 years of follow-up.[62]

In a similar but smaller trial, Gonwa et al.[57] randomised 197 patients initially treated with corticosteroids, sirolimus and ciclosporin to either ciclosporin withdrawal (n = 100) or continuation (n = 97) 2 months after transplantation. In this study, patients with severe delayed graft function were excluded from randomisation, which occurred shortly after transplantation. Patient survival, graft survival and the incidence of acute rejection did not differ between the two groups at 12 months, but patients withdrawn from ciclosporin exhibited better renal function based on calculated GFRs.

These two large randomised trials recently were combined with four smaller randomised trials[58-61] as part of a meta-analysis[63] that examined the outcomes of CNI withdrawal in 1047 patients initially treated with sirolimus and ciclosporin. Overall, CNI withdrawal was associated with a 6% incremental risk of acute rejection (p = 0.002). Graft loss and death occurred at rates similar to those in the control groups who were maintained on a CNI. CNI withdrawal was associated with higher creatinine clearances and lower blood pressures at 1 year.

Calcineurin Inhibitor Avoidance

Two phase II studies performed in multiple European centres compared sirolimus to ciclosporin in kidney transplant recipients who also received corticosteroids and either azathioprine or MMF.[64,65] At 12 months, patient and graft survival rates were similar. In addition, there were no short-term differences in the incidence of acute rejection (41% sirolimus vs 38% ciclosporin with azathioprine;[64] 27.5% sirolimus vs 18.4% ciclosporin with MMF[65]). The pooled 2-year data from these two studies showed that calculated GFR was significantly higher among sirolimus- than ciclosporin-treated patients (69.3 vs 56.8 mL/min at 2 years; p = 0.004).[66]

Flechner et al.[67] also compared the use of corticosteroids, sirolimus and MMF to corticosteroids, ciclosporin and MMF in primary kidney transplant recipients from a single centre. In contrast to the earlier study by Kreis et al.,[65] all patients received induction antibody therapy with basiliximab. After a mean follow-up at 18 months, the cumulative incidence of acute rejection was low and not significantly different between groups (6.4% in the sirolimus arm and 16.6% in the ciclosporin arm). At both 6 and 12 months, the sirolimus-treated patients demonstrated significantly better renal function. In a 2-year follow-up, the difference in renal function persisted.[68] Protocol biopsies were performed at 2 years in 87% of the original patient cohort. Using Banff criteria for histological changes compatible with chronic allograft nephropathy, scores of zero were obtained in 66.6% of the sirolimus-treated patients versus 20.8% of the ciclosporin-treated patients (p = 0.013).[68]

Lo et al.[69] reviewed their sequential experience with sirolimus-based immunosuppression for cadaveric kidney transplant recipients treated with a CNI minimisation (n = 41) protocol and a later CNI avoidance (n = 29) protocol. Notably, 71% of patients were African American and 47% exhibited delayed graft function. In this relatively high-risk population, the cumulative incidence of acute rejection at 1 year was 10% in the CNI minimisation arm and 7% in the CNI-free arm. At 1 year, mean creatinine clearance was higher in the CNI-free group (72 vs 51 mL/min; p < 0.01).

3.1.4 Corticosteroid-Sparing Regimens

To date, experience with corticosteroid-free, sirolimus-based immunosuppression for kidney transplant recipients has been limited to uncontrolled trials. Included among these are several experimental efforts to use T cell-depleting antibodies to facilitate minimisation of corticosteroids and other immunosuppressants with sirolimus-based maintenance therapy.[70-72] An open-label observational study of 156 kidney transplant recipients treated with sirolimus and ciclosporin examined the outcomes of corticosteroid withdrawal between 1 week and >2 years following transplantation.[73] With a mean follow-up of >3 years, over 75% of patients remained off corticosteroids, and corticosteroids were renewed because of acute rejection in only 6.4% of patients.

Woodle et al.[74] reported the results of a prospective, uncontrolled study of 77 low-risk kidney transplant recipients treated with an anti-IL-2 receptor antibody for induction therapy, tacrolimus, sirolimus and early withdrawal of corticosteroids 4 days post-transplant. African Americans and highly sensitised patients were excluded from the study. At 1 year, patient and graft survival were 100%. The incidence of biopsy-proven acute rejection was 13%.

Two uncontrolled experiences have been reported with sirolimus-based corticosteroid-free protocols limited to high-risk African American patients. Hricik et al.[75] reported the outcomes of 30 African American kidney transplant recipients withdrawn from prednisone 3 months post-transplant and maintained on tacrolimus and sirolimus. Notably, induction antibody therapy was generally not administered. After 14 months of follow-up, the cumulative incidence of acute rejection was 13% and more than 80% of patients remained off corticosteroid therapy. However, long-term follow-up has suggested a significant deterioration of renal function, even among patients who remain off corticosteroids.[76] Kumar et al.[77] reported the outcomes of early corticosteroid withdrawal in 103 African American kidney transplant recipients, 35 of whom received sirolimus with either tacrolimus or ciclosporin. All patients received induction antibody therapy with basiliximab. Outcomes were compared with those of 103 concurrent non-African Americans treated with similar immunosuppression regimens. Surveillance biopsies were performed at 1, 6 and 12 months. The incidence of subclinical acute rejection detected on 1-month surveillance biopsies was significantly higher in the African American group (23% vs 11%; $p = 0.04$). Interestingly, although corticosteroids were used as initial treatment for all clinical and subclinical rejection episodes, all patients were withdrawn from corticosteroid therapy after treatment for rejection so that 100% of the patients in each group were corticosteroid-free after 1 year. Patient and graft survival and the incidence of biopsy-proven chronic allograft nephropathy at 1 year were equivalent in the two groups.

3.2 Extrarenal Organ Transplantation and Paediatric Kidney Transplantation

3.2.1 Pancreas Transplantation

Sirolimus has been used with success both *de novo* and as rescue therapy for pancreas transplant recipients.[78,79] Vincenti and Stock[78] described a regimen of induction therapy with rabbit antithymocyte globulin followed by maintenance treatment with sirolimus, MMF and low-dose tacrolimus in a corticosteroid-sparing regimen for kidney-pancreas recipients and reported rejection rates of <10% for each organ. Rogers et al.[79] described successful conversion from tacrolimus plus MMF to tacrolimus plus sirolimus in kidney-pancreas recipients, with a low incidence of rejection despite a reduction in tacrolimus concentrations. In pancreatic islet cell transplantation, sirolimus has been used with tacrolimus as part of the original Edmonton protocol in a corticosteroid-free regimen along with induction therapy with daclizumab.[45] With this protocol, reported rates of insulin independence at 1–2 years are as high as 80%.[80]

3.2.2 Liver Transplantation

Enthusiasm for the use of sirolimus in *de novo* therapy for liver transplantation was tempered by early reports of wound complications, infections and hepatic arterial thrombosis, leading to early termination of a multicentre trial and a 'black box' labelling by the FDA.[81] Despite this experience, there is growing interest in the use of sirolimus for liver transplant recipients, particularly as a strategy to avoid or eliminate the nephrotoxic effects of CNIs. Smaller studies of *de novo* therapy reported excellent patient and graft survival early post-transplant when used with lower-dose CNIs.[81,82] There are many reports of successful conversion from CNI-based therapy to sirolimus with low rates of rejection after conversion.[83,84] Improvement in renal function was demonstrated in both early and late converters, with greatest improvement in the first 30 days after conversion. Anaemia was a common problem after conversion.[85]

Sirolimus may have potential benefit in liver transplant recipients with hepatocellular carcinoma

(HCC) as it exhibits antiproliferative effects against HCC *in vitro*.[86] Kneteman et al.[87] reported on 40 patients with HCC who underwent liver transplant with a sirolimus-based regimen. Although there was no control group, the rate of tumour recurrence appeared low (5/40, 13%), and four cases of recurrence were in patients with more extensive malignant disease at baseline. Some have advocated use of *de novo* sirolimus therapy in all liver transplant recipients with HCC, although larger studies are needed to determine if the use of sirolimus conveys a survival advantage in these patients.

3.2.3 Heart Transplantation

There are also reports of successful use of sirolimus in *de novo* treatment of heart transplant recipients. Everolimus, a chemical analogue of sirolimus,[88] has been studied extensively in combination with ciclosporin and results have been promising. In addition to low rates of acute rejection, the use of everolimus has been associated with relatively low rates of cardiac transplant vasculopathy as measured by intravascular ultrasound.[89] Sirolimus has been used less commonly, but reports of *de novo* use with reduced-dose CNI therapy have shown excellent efficacy and safety in phase II trials.[90] In cardiac recipients with chronic renal failure, conversion to sirolimus plus MMF was successful in a cohort of 31 patients. Serum creatinine levels decreased significantly after conversion and there were no episodes of acute rejection.[91] More recently, Meiser et al.[92] reported on a pilot study of eight heart transplant recipients given sirolimus/MMF with prednisone *de novo* after antithymocyte globulin induction. Acute rejection occurred in 2/8 (25%). However, complications occurred in the majority of patients, and included pleural and pericardial fluid collections and delayed wound healing.

One potential benefit of sirolimus (and everolimus) therapy in heart transplantation is the prevention of allograft vasculopathy. Sirolimus inhibits vascular smooth muscle proliferation.[93] Studies in animal models have demonstrated an exposure-dependent association between sirolimus treatment and regression of cardiac allograft vasculopathy, and these effects have been shown to correlate with sirolimus concentrations.[94] Thus, mTOR inhibiting agents may have long-term protective effects in cardiac allografts.[95]

3.2.4 Lung Transplantation

Enthusiasm for sirolimus as primary immunosuppression in lung transplant has been diminished by reports of postoperative airway dehiscence. In two recent case series, airway dehiscence occurred in 6 of 19 patients (32%) on sirolimus therapy, with four deaths postoperatively.[96,97] Better outcomes have been seen with conversion to sirolimus later post-transplant. Minimisation of CNI in conjunction with sirolimus was shown to improve renal function in one small series,[98] while the addition of sirolimus to tacrolimus led to improvement in bronchiolitis obliterans in 8 of 23 patients in another series.[99] Two small series also report on complete CNI elimination in selected patients with improvement in renal function and no increase in pulmonary complications.[100,101]

3.2.5 Paediatric Transplantation

Finally, there have been reports of success with sirolimus in a paediatric population. Hymes and Warshaw[102] used sirolimus in conjunction with CNI and corticosteroid therapy in 66 paediatric renal transplant recipients after IL-2 antibody induction therapy. Patient and graft survival were excellent, with a low 11% rate of rejection at 6 months. However, viral infection with Epstein-Barr or cytomegalovirus occurred in a total of 12 patients, and post-transplant lymphoproliferative disease (PTLD) occurred in three. Thirteen patients (20%) discontinued sirolimus because of significant adverse effects including wound dehiscence and pneumonitis. Fewer adverse effects have been described with conversion to sirolimus late post-transplant in paediatric lung and heart transplant recipients.[103,104]

4. Tolerability

While sirolimus has been shown to be a potent and highly effective immunosuppressive drug in solid organ transplant recipients, it does have multiple adverse effects. This is due primarily to the ubiquitous nature of the mTOR-p70^{S6} kinase path-

way which is common to multiple cell lines. Most adverse effects are related to drug exposure,[105] leading to the need for careful drug monitoring for dose adjustments. Akin to other immunosuppressive agents, sirolimus increases the risk of infectious complications, and this risk is dependent on dose, concomitant immunosuppression and patient factors. Other adverse effects are described in this section.

4.1 Hyperlipidaemia

Experience from early trials indicated that use of sirolimus was associated with a dose-dependent increase in serum triglyceride levels and in the levels of total, low-density lipoprotein (LDL)- and high-density lipoprotein (HDL)-cholesterol.[24,37] In these trials, all lipid levels tended to decrease with time after transplantation, probably coincident with gradual reduction in the doses of corticosteroids and ciclosporin and, more importantly, related to the frequent use of lipid-lowering therapy. Metabolic balance studies have shown that the defect in lipid metabolism is characterised by a dose-dependent decrease in the catabolism of apoB100-containing lipoproteins.[106] The defect is reversible with discontinuation of the drug.

A pilot study of six patients converted from ciclosporin-based therapy to sirolimus noted consistent, reversible and dose-dependent increases in total cholesterol, LDL cholesterol, triglycerides and apolipoproteins, with little effect on HDL cholesterol.[107] The same study found an increase in free fatty acids in circulation, leading to increased hepatic synthesis of triglycerides. This appeared to counter the effects of insulin and the authors theorised that sirolimus may alter insulin-signalling pathways leading to increased fatty acid pooling in circulation. Supporting this notion was a recent report describing a worsening of insulin resistance in patients converted to sirolimus from CNI.[108] Insulin resistance correlated with a rise in triglycerides after conversion to sirolimus therapy.

Sirolimus-induced hyperlipidaemia responds to conventional treatment with HMG-CoA reductase inhibitors (statins) and fibric acid derivatives (fibrates).[109] However, there may be significant interactions with sirolimus and these drugs as a result of competitive metabolism via CYP3A.[110] Therefore, heightened awareness is necessary for potential hepatic and muscular toxicity with statins or fibrates in conjunction with sirolimus.

The tendency of sirolimus to promote hyperlipidaemia must be balanced against the putative antiatherogenic effects of the mTOR inhibitors. By inhibiting vascular smooth muscle cell proliferation these agents may block the fundamental pathophysiology responsible for a number of arterial injuries. This forms the basis for sirolimus-coated stents, now shown to be effective in preventing restenosis after stenting of coronary arteries.[111,112] In apoE-deficient mice, normally prone to premature death from severe atherosclerosis when fed a high-fat diet, treatment with sirolimus during the neonatal period prevents the development of atherosclerotic lesions despite worsening hyperlipidaemia.[113] Because smooth muscle cell proliferation also plays a role in the pathophysiology of the vascular lesions that represent the hallmarks of 'chronic rejection' in organs such as the heart and kidney, the promise of sirolimus is that the drug may mollify the lesion underlying many cases of chronic renal allograft nephropathy and transplant vasculopathy. In a study by Ikonen et al.,[114] sirolimus was shown to inhibit the progression of graft vascular disease in cynomolgus monkey recipients of aortic allografts. In contrast, monkeys treated with ciclosporin or placebo showed progressive arterial intimal thickening. All of these observations suggest that sirolimus-induced hyperlipidaemia may pose less of a threat of cardiovascular disease than comparable degrees of hyperlipidaemia in the general population or in transplant recipients treated with other immunosuppressants. Indeed, in a single-centre analysis comparing 280 patients receiving sirolimus, ciclosporin and prednisone with 118 patients receiving ciclosporin and prednisone, there was no increase in the 4-year risk of cardiovascular events among the sirolimus-treated patients for any level of hyperlipidaemia.[115]

4.2 Myelosuppression

Thrombocytopenia and leukopenia are also common adverse effects of sirolimus.[116] The occurrence of each often correlates with sirolimus trough concentrations. The majority of patients in one experience had normalisation of blood counts either spontaneously or with dose reduction.[117] In a multicentre trial of ciclosporin withdrawal from patients previously receiving ciclosporin plus sirolimus and corticosteroids, the incidence of thrombocytopenia was 4.7% in the 215 patients who remained on combination therapy versus 12.1% ($p = 0.008$) in patients who converted to sirolimus alone with corticosteroids. The mean sirolimus trough concentration was 11.2 ng/mL in the ciclosporin plus sirolimus group versus 22.5 ng/mL in the ciclosporin withdrawal group, suggesting a dose-related effect.[62]

Anaemia has been recognised recently as a common problem after organ transplantation.[118] Because $p70^{S6}$ kinase is a downstream effector that modifies cellular translation in response to various growth factors including erythropoietin, thrombopoietin and IL-3,[119,120] it is logical that sirolimus and other mTOR inhibitors may have a negative effect on erythropoiesis. We recently reviewed risk factors for anaemia in renal transplant recipients receiving either tacrolimus plus sirolimus or tacrolimus plus MMF.[121] At 12 months post-transplant, the prevalence of anaemia was 57% in the sirolimus group ($n = 87$) versus 31% in the MMF group ($n = 127$) [$p < 0.001$]. This difference remained significant in a multivariate model controlling for factors known to influence anaemia including creatinine clearance, recipient age, donor age, infection and sex.

4.3 Nephrotoxicity

4.3.1 Enhancement of Calcineurin Inhibitor Nephrotoxicity

As noted previously, sirolimus appears to increase the risk of nephrotoxicity when used in combination with CNIs. The drug potentiates ciclosporin nephrotoxicity in rodent models[122,123] and appears to exert similar effects in humans.[24,37,124,125] One strategy to overcome nephrotoxicity with ciclosporin plus sirolimus is to minimise ciclosporin exposure. This has been reported with success in clinical trials.[39] In addition, multicentre trials of ciclosporin elimination with sirolimus therapy have demonstrated significant and lasting improvements in renal allograft function and histology[57,59] (see in section 3.1.3 Calcineurin Inhibitor-Sparing Regimens).

While sirolimus has traditionally been considered a non-nephrotoxic drug by itself, some reports have challenged this contention. Supratherapeutic dosages of sirolimus caused tubular toxicity in a rat model.[126] A more recent animal study demonstrated increased transforming growth factor (TGF)-β messenger RNA (mRNA) and protein in the kidney with therapeutic dosages of sirolimus.[127] This latter study also demonstrated increased biglycan and type I collagen in renal tissue, although immediate renal function and histology were not affected. Because TGFβ has been demonstrated to cause interstitial fibrosis and tubular apoptosis in the kidney, it may be a causative factor in the development of chronic allograft nephropathy.[127]

We have recently described a cohort of kidney transplant recipients with impaired renal function while treated with tacrolimus plus sirolimus therapy, despite relatively low target trough concentrations for tacrolimus (5–8 ng/mL).[128] We identified a subgroup of patients who converted from sirolimus to MMF for nonrenal complications. By 18 months there was a significant improvement in renal function in converters, despite higher tacrolimus concentrations. Conversion to MMF remained an independent correlate of superior renal function at 18 months in a multivariable analysis. These clinical observations suggest that sirolimus may exert nephrotoxic effects independent of the CNIs.

4.3.2 Delayed Graft Function

Sirolimus also has been shown to inhibit growth factor-induced proliferation of cultured proximal tubular cells, leading to apoptosis that results in prolonged recovery from acute tubular necrosis in a rodent model of acute renal failure.[129] Two studies

suggest that this phenomenon might be relevant in human kidney transplantation. McTaggart et al.[130] retrospectively reviewed factors influencing the duration of delayed graft function in 132 kidney transplant recipients, including 55 patients treated initially with sirolimus. Patients receiving sirolimus were twice as likely to remain on dialysis and the authors concluded that the drug prolongs recovery from delayed graft function. Smith et al.[131] observed a higher incidence of delayed graft function in patients receiving sirolimus and also suggested a relationship between the dose of the drug and the development of delayed graft function. However, in a multivariate analysis, sirolimus was not associated with prolonged recovery from delayed graft function.[131] These observations suggest that sirolimus may impair renal recovery after ischaemic or toxic injury in transplant recipients. However, it is important to note that the incidence of delayed graft function has not been higher than that observed in control populations in recent randomised trials.[47,49]

4.3.3 Proteinuria

There have been several recent reports of proteinuria related to sirolimus use in transplant recipients. Most studies show an increase in urinary protein in subgroups of patients converted from CNI-based therapy to sirolimus therapy.[132,133] Some have theorised that the aggravation of proteinuria is linked not to sirolimus but to the haemodynamic renal effects resulting from CNI withdrawal.[134] However, proteinuria recently was reported with *de novo* use of sirolimus and tacrolimus in islet cell transplant recipients.[135] Proteinuria resolved in these three patients after discontinuation of sirolimus and simultaneous increase in tacrolimus dosage, suggesting that sirolimus might exert an independent effect on glomerular permeability. Future studies are needed to clarify the cause-effect relationship of sirolimus and proteinuria, and to explore possible pathophysiological mechanisms. Interestingly, and as noted previously (see in section 3.1.3 Conversion from Calcineurin Inhibitors to Sirolimus), pre-existing proteinuria in renal transplant recipients has been identified as an independent predictor of worse renal outcomes after conversion from CNI-based to sirolimus-based therapy.[136]

4.4 Impaired Wound Healing

Most, but not all, studies indicate that sirolimus impairs wound healing and is associated with postoperative fluid collections and anastomotic complications. One analysis did not find sirolimus to be an independent risk factor for wound complications when comparing a sirolimus plus MMF plus corticosteroid regimen with two other historical groups given ciclosporin-based therapy.[137] The authors defined wound complications as any wound opened or draining at 3 weeks post-transplant. The incidence was 19.7% in the sirolimus group, not significantly different from the ciclosporin groups. Alternatively, Valente et al.[138] retrospectively studied 158 patients receiving either MMF or sirolimus in conjunction with tacrolimus and prednisone and examined the association of a number of risk factors for impaired wound healing. Only use of sirolimus and hypoalbuminaemia independently correlated with wound complications. In this study the difference in the rate of superficial or deep wound breakdown was highly significant at 20.1% in sirolimus patients versus 0% in MMF patients.

Dean et al.[139] studied the incidence of wound complications in a prospective, randomised trial comparing sirolimus plus MMF plus prednisone with tacrolimus plus MMF plus prednisone. Initially, patients were randomised regardless of body mass index (BMI), with targeted sirolimus trough concentrations of 15–20 ng/mL. Because of the frequency of wound complications in the early phase of the trial, the protocol was modified to exclude patients with BMI >32 kg/m^2 from the sirolimus arm and sirolimus target concentrations were reduced to 10–15 ng/mL. Overall, wound complications including fluid collections, were 47% in the sirolimus group versus 8% in the tacrolimus group ($p < 0.001$). Superficial wound infections and perigraft fluid collections were significantly more common in the sirolimus group.

One study of early corticosteroid withdrawal in patients receiving sirolimus-based therapy found no

increase in wound complications or fluid collections compared with an historical control,[140] an observation suggesting that sirolimus and corticosteroids may have at least additive effects on impaired wound healing. However, a recent randomised trial using early corticosteroid withdrawal at 5 days compared a ciclosporin plus MMF regimen against two tacrolimus plus sirolimus regimens.[141] Wound complications, including dehiscences and infections, occurred in 25% in the higher-dose sirolimus group, 18% in the lower-dose sirolimus group and 8% in the ciclosporin group ($p = 0.02$), despite successful early elimination of corticosteroids.

Abdominal lymphoceles have been found with greater frequency with sirolimus therapy. Goel et al.[142] noted a 23% incidence in lymphoceles requiring drainage in 152 patients receiving sirolimus versus approximately a 12% incidence in 361 patients receiving ciclosporin-based therapy ($p = 0.003$). Similarly, a retrospective study found a 16% incidence of lymphoceles requiring drainage in 354 patients treated with a ciclosporin plus sirolimus combination versus a 4% incidence in 136 patients treated with ciclosporin ± azathioprine ($p < 0.001$).[143]

The aetiology of fluid collections and poor wound healing is probably a result of sirolimus-induced cell-cycle arrest which inhibits proliferation of fibroblasts and other cells during wound healing. Sirolimus inhibits mouse fibroblast entry into S phase of the cell cycle.[144] The drug also reduces collagen type I mRNA levels by inhibiting mTOR in human dermal fibroblasts.[145] Finally, the negative effects of mTOR inhibition on angiogenesis probably play a contributory role.[146]

4.5 Miscellaneous Adverse Effects

Liver functional abnormalities can occur with sirolimus therapy, although elevations of liver enzymes are typically mild and respond to dose reduction or discontinuation of the drug. In the previously described randomised trial of ciclosporin elimination from ciclosporin plus sirolimus-based therapy, the 24-month incidence of elevated liver function tests at 2 years post-transplant was 3.7% in a ciclosporin plus sirolimus group versus 9.8% in patients converted off ciclosporin with higher targeted sirolimus concentrations ($p = 0.02$).[63] In liver transplant recipients, sirolimus-associated hepatotoxicity may create clinical uncertainty and mandate additional liver biopsies.[147] Mild increases in liver function tests may not warrant discontinuation of sirolimus, but may make instituting other potentially hepatotoxic drugs such as statins difficult in cases of sirolimus-induced hyperlipidaemia.

Oedema is another adverse effect increasingly recognised during treatment with sirolimus. In our renal transplant population, severe oedema has led to conversion from sirolimus to other agents in 5 of 127 patients (4%). Leg oedema was noted in 57% of 175 liver transplant patients from a single centre, with generalised, facial and upper extremity oedema occurring in 6%, 2% and 1%, respectively.[148] Others have reported diffuse angioedema.[149,150] Isolated eyelid oedema also has been reported, with resolution after discontinuation of the drug.[151] We have seen occasional cases of marked lymphoedema with sirolimus therapy, often involving multiple limbs. Vascular obstruction is typically absent in such cases,[150,151] but evidence of lymphatic obstruction can sometimes be identified by lymphoscintigraphy. The mechanism of lymphatic obstruction is unclear but lymphoedema can be reversible with early discontinuation of sirolimus.

Many oral and dermatological adverse effects have been reported with sirolimus. These include aphthous ulcerations, lip fissures, acne, dermatitis, onychopathy and folliculitis.[152,153] A 60% incidence of oral ulcers was reported in a cohort of patients converted to sirolimus plus MMF in the absence of corticosteroids,[154] and this combination may particularly predispose to this painful complication. Skin and oral lesions developed in the majority of renal transplant patients in a recent randomised study of sirolimus conversion, although most lesions resolved with reduction in dose and blood concentrations.[155]

Decreased testosterone levels in male recipients of heart and kidney transplants have been reported, with increased luteinising hormone and follicle-

stimulating hormone (FSH) levels relative to patients receiving other immunosuppressive regimens.[156,157] Sertoli cells in the testes are dependent on FSH stimulation for germ cell survival and function in adults.[158] FSH binds to a transmembrane receptor with a $p70^{S6}$ kinase downstream from this receptor which is partially mTOR dependent.[159] Sirolimus has been shown to inhibit spermatogenesis *in vitro* via blockade of the same pathway in response to mitogenic factors.[160]

Sirolimus was originally touted as a safe replacement for CNIs in patients with post-transplant haemolytic uraemic syndrome, traditionally considered to be a complication of treatment with ciclosporin or tacrolimus. However, several recent reports suggest that sirolimus may either potentiate the effects of the CNIs[161] or lead independently to thrombotic microangiopathy.[162,163] One recent study suggested that the effect may be mediated by sirolimus-mediated downregulation of vascular endothelial growth factor (VEGF).[164]

Finally, one idiosyncratic reaction associated with sirolimus therapy is interstitial pneumonitis, characterised by diffuse pulmonary infiltrates in the absence of infection. A recent review noted over 50 cases in the literature in renal transplant recipients, along with a number of cases in lung, liver, heart, heart-lung and islet cell recipients.[165] This review also noted three deaths reported from this complication in heart transplant recipients. Characteristics of sirolimus-associated pneumonitis include fever, dyspnoea, fatigue, non-productive cough and occasional haemoptysis. Diffuse interstitial and ground-glass parenchymal opacities are typically evident by chest radiograph and computed tomography. Bronchiolar lavage and biopsy reveal a lymphocytic alveolitis, with a pathological picture often consistent with bronchiolitis obliterans organising pneumonia (BOOP).[166] Cases have been reported most commonly within 6 months of initiating treatment with sirolimus, but a significant number have also occurred more than 1 year after initiation of therapy or after late conversion to sirolimus. A dose-related effect has been suggested, with some patients improving after dose reduction alone. However, cases have been reported even with moderate, therapeutic sirolimus concentrations. Sirolimus-associated pulmonary toxicity remains a diagnosis of exclusion. When suspected, discontinuation of the drug is recommended, as most cases report improvement within 3 weeks of discontinuing the drug.[167]

5. Antineoplastic and Antiviral Effects

One striking finding in long-term studies of patients receiving sirolimus-based immunosuppression is a relatively low incidence of malignancy compared with the high rates of neoplasia associated with other immunosuppressants. In animal models, both ciclosporin and tacrolimus have been associated with cancer progression and significant increases in pulmonary metastases, perhaps related to enhanced expression of TGFβ, which is known to enhance the metastatic growth of certain cancer cells.[168,169] In contrast, the mTOR inhibitors have a negative effect on tumour cells in multiple animal models. Sirolimus alone, or in combination with ciclosporin, prevents metastatic tumour progression and prolongs the survival of mice inoculated with renal cancer cells, while ciclosporin alone increases the number of metastases.[170,171] The effects of sirolimus on the metastatic potential of renal cancer cells has been attributed to some combination of direct antiproliferative effects and reduced expression of both TGFβ and VEGF,[172] which may influence angiogenesis within tumours. In addition, preliminary studies suggest that sirolimus may inhibit the proliferation of Epstein-Barr virus-infected cell lines from patients with PTLD.[173]

Low rates of malignancy have now been reported in both single-centre and multicentre experiences with kidney transplant patients receiving sirolimus-based therapy. In 1008 kidney transplant recipients treated with sirolimus plus ciclosporin at a single centre and followed for a mean 60.3 months (range 1–10 years), only 30 cases of malignancy were observed in 29 patients,[37] substantially lower than rates in historical controls. A recent report of pooled data from five multicentre trials indicated that sirolimus-based immunosuppression was associated with a reduced 2-year incidence of malignancy.[174]

Preliminary data from a large randomised trial of CNI-to-sirolimus-based therapy for patients with chronic renal allograft dysfunction suggests lower rates of both skin and non-skin malignancies in the cohort converted to sirolimus after 18 months of follow-up.[55] A recent analysis of 33 249 deceased donor primary kidney transplant recipients reported to the Organ Procurement and Transplantation/United Network for Organ Sharing (OPTN/UNOS) convincingly demonstrated a reduced risk for both skin and non-skin malignancies in patients receiving mTOR inhibitor-based therapy[175] (table II).

Additional evidence supporting the ability of mTOR inhibitors to suppress cancer progression in humans comes from reports suggesting that conversion to sirolimus-based therapy may have beneficial effects in patients with existing post-transplant malignancies. One report indicated complete remission of Kaposi's sarcoma in two patients converted from CNI-based to sirolimus-based immunosuppression.[176] Another report indicated that 12 of 13 patients with PTLD were free of cancer for a mean follow-up of 2.3 years after conversion to sirolimus-based therapy.[177] Taken together, the bulk of evidence suggests that sirolimus may display beneficial antineoplastic effects in kidney transplant recipients. Given the activity of this agent in animal models of renal cell carcinoma, particular consideration should be given to the use of this agent in kidney transplant patients with a history of renal cell carcinoma. Further studies are needed to verify the benefit of converting transplant patients with other malignancies to sirolimus-based therapy.

Table II. Incidence of all *de novo* malignancies and *de novo* non-skin malignancies within 963 days of transplantation by drug regimen (data derived from Campistol et al.[176])

Regimen (no. pts)	All *de novo* malignancies		Non-skin solid malignancies[a]	
	no.	%	no.	%
SRL or EVE alone[b] (504)	3	0.60	0	0
SRL or EVE + CsA or TAC[c] (2321)	14	0.60	11	0.47
CsA or TAC alone (30 424)	552	1.81	304	1.00

a Non-skin malignancies excluded squamous and basal cell cancers but included melanomas and Kaposi's sarcoma.

b $p = 0.041$ for *de novo* malignancies and $p = 0.011$ for non-skin malignancies vs CsA or TAC alone.

c $p < 0.0001$ for *de novo* malignancies and $p = 0.0125$ for non-skin malignancies vs CsA or TAC alone.

CsA = ciclosporin; **EVE** = everolimus; **SRL** = sirolimus; **TAC** = tacrolimus.

Indirect evidence from clinical studies suggests that sirolimus may exhibit additional antiviral properties. Relatively low rates of cytomegalovirus infection have been reported in both kidney[178,179] and liver transplant recipients[180] receiving sirolimus-based immunosuppression. In one of the larger experiences with kidney transplant recipients, Gruber et al.[179] reported cytomegalovirus infection in 6 of 37 patients treated with non-sirolimus therapy versus 0 of 43 patients treated with sirolimus ($p = 0.006$).

6. Conclusions

Sirolimus represents an important addition to the armamentarium of immunosuppressive drugs available for the prevention of allograft rejection in solid organ transplant recipients. Originally designed to be an adjunct to treatment with CNIs, the drug is now considered to be the cornerstone of many CNI-free regimens. However, further studies with long-term follow-up are needed to verify the efficacy and safety of sirolimus-based, CNI-free regimens. Encouraging data suggest that sirolimus may have significant advantages over existing immunosuppressive agents including promotion of tolerance, reduction in the incidence of chronic allograft vasculopathy and avoidance of nephrotoxicity. These potential benefits must be weighed against a number of important adverse effects, including some that are putative (e.g. proteinuria, oedema, thrombotic microangiopathy, pneumonitis) and require further study. The antiatherogenic, antiviral and antineoplastic effects of sirolimus are novel and provide the potential for several niches for use of this drug.

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Correspondence: Dr *Donald E. Hricik*, University Hospitals of Cleveland, 11100 Euclid Avenue, Room 8124 Lakeside Building, Cleveland, OH 44106, USA.
E-mail: dhricik@aol.com