

# Sirolimus: A Current Perspective

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Sirolimus, a macrocyclic lactone that displays a novel mechanism of immunosuppressive action, is a critical-dose drug requiring therapeutic drug monitoring for optimal outcomes. The compound was documented in two multicenter, blinded clinical trials to reduce the incidence of acute rejection episodes when used in combination with cyclosporine and steroids vs. azathioprine or placebo comparators. Furthermore, studies utilizing cyclosporine withdrawal documented a long-term benefit on renal function of chronic sirolimus therapy, albeit with a modestly enhanced incidence of acute rejection episodes. Although this application may be useful in selected cases, we believe that minimal initial cyclosporine exposures *de novo* mitigate the need for eventual withdrawal for chronic nephropathy, while preserving the immunosuppressive synergy during the maintenance phase.

Recipients treated *de novo* with a sirolimus-cyclosporine combination tolerate steroid withdrawal

at 1 month after living-donor or at 3 to 6 months after cadaveric kidney transplantation with only a 5% risk of acute rejection episodes and 6% incidence of chronic reactions within 3 years. However, sirolimus exacerbates the hypertriglyceridemic and hypercholesterolemic proclivities of transplant recipients, as well as exerts myelosuppressive effects, which are augmented by concomitant therapy with azathioprine or, particularly, with mycophenolate mofetil. Due to its apparent lack of nephrotoxicity, sirolimus has been employed for induction therapy in a calcineurin antagonist-free regimen in combination with either basiliximab or rabbit antilymphocyte sera for weak or strong immune responders, respectively, followed by introduction of a calcineurin antagonist upon resolution of the ischemia-reperfusion injury. Therefore, sirolimus proffers a potent and unique platform for new immunosuppressive strategies in organ transplantation.

**Keywords:** *Sirolimus; cyclosporine; immunosuppression; renal transplantation*

Sirolimus (rapamycin; Rapamune; Wyeth Research, Philadelphia, PA), a macrocyclic lactone produced by the *Streptomyces hygroscopicus*, was isolated from the soil of the Vai Atari region of Rapa Nui (Easter Island) [1,2]. By blocking signals 2 and 3 in the evolution of the immune response [3], the drug displays a novel mechanism of immunosuppressive action. In the past decade, sirolimus has

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undergone clinical trials that progressed from Phase I safety, tolerability, and pharmacokinetic investigations to Phase II dose-finding and combination drug studies to large, blinded, randomized, Phase III international trials that demonstrated a reduced occurrence of acute rejection episodes leading to approval of the drug in combination with cyclosporine and steroids by The U. S. Food and Drug Administration in September 1999. In November 2000, the drug was registered by the European Agency as an alternative to calcineurin antagonists for maintenance therapy. Effective treatment of transplant patients with sirolimus requires knowledge of its immunosuppressive mechanisms of action, exposure targets, as well as toxicities and drug-drug interactions.

#### **Mechanism of Immunosuppressive Action**

Sirolimus binds to the cytosolic immunophilin FKBP12 forming an immunosuppressive complex [4,5] that inhibits the multifunctional serine-threonine kinase, mammalian target of rapamycin [6], thereby blocking lymphocyte responses to costimulatory signal 2 of the  $G_0$  to  $G_1$  transition and to cytokine signal 3 triggering  $G_1$  progression. During signal 2, sirolimus prevents activation of the inhibitory factor kappa kinase necessary for generation of the c-Rel transcription factors of the NF- $\kappa$ B complex and possibly also modulates protein kinase C activity [7,8]. During the  $G_1$  build-up, four cytokine-driven signaling pathways are blockaded: (1) p27<sup>kip1</sup> degradation [9,10], which is required for cyclin and kinase activation necessary for entry into the S phase [11,12]; (2) p70<sup>S6</sup> kinase phosphorylation, necessary for the synthesis of endoplasmic reticulum structural proteins [5,13]; (3) release of elongation factor (eIF) 4A from its association with PHAS-I, facilitating protein

synthesis by ribosomes [14-16]; (4) transcriptional upregulation of the anti-apoptotic proteins bcl and p21Ras (Figure 1) [17-19]. These effects inhibit both  $Ca^{2+}$ -dependent and  $Ca^{2+}$ -independent activation pathways that mediate transduction of proliferative and differentiation signals delivered by cytokines, including the lymphokines that act on T and B cells: interleukins (IL)-2, -3, -5, -6, and -15, as well as the hematologic and vascular families of humoral growth factors. In addition, sirolimus interrupts B cell responses, reducing immunoglobulin production both *in vitro* and *in vivo* [20-22].

#### **Pharmacokinetics and Therapeutic**

##### **Drug Monitoring**

Sirolimus, like the calcineurin antagonists cyclosporine and tacrolimus, is a critical-dose drug [23]. Although uniform doses were administered during the randomized, placebo-controlled pivotal trials to maintain blinding, post-approval use has been based on the application of therapeutic drug monitoring. The active moiety—parent compound sirolimus—may be detected in whole blood samples using high-performance liquid chromatography methods coupled to ultraviolet detection [24,25] or to mass spectroscopy [26]. A microparticle enzyme assay (IMx<sup>®</sup>, Abbott Laboratories, N. Chicago, IL) tested in the pivotal trials showed less sensitivity and specificity due to a 25% to 42.5% cross-reactivity with metabolites [27].

The well-described pharmacokinetic properties of sirolimus cannot be predicted based on an individual patient's demographic characteristics [28-30]. The oral bioavailability of sirolimus is approximately 14%, with a time to peak concentration at about 1 hr after dosing [31]. Sirolimus is

widely distributed in tissues (19 L/kg), partitioning more extensively into blood cells compared to plasma [32]. Because sirolimus is metabolized by the cytochrome P450 3A4 (CYP3A4), its potential for drug-drug interactions is similar to that of cyclosporine and tacrolimus, both of which serve as substrates for the same system [33].

Interestingly, sirolimus pharmacokinetics displayed by African-American patients are similar to those of other ethnic groups [34]. Because concurrent administration of sirolimus with high doses of the microemulsion, but not the oil-based formulation of cyclosporine, results in a variable (up to 80%) increase in sirolimus area under the concentration-time curve [35], the blinded pivotal trials employed separation of the administration of the two drugs by 4 hr [36]. However, present strategies utilizing low doses of a calcineurin antagonist are benefited by the augmented concentration (and reduced cost) achieved by simultaneous drug delivery.

## Clinical Efficacy

### Phase I and Phase II Studies

The Phase I study showed neither nephrotoxic nor hypertensive effects among 40 stable renal transplant recipients who received a 2-week course of twice-daily doses of sirolimus compared with placebo added to a regimen of cyclosporine and steroids [37]. The dose-dependent collateral toxicities included thrombocytopenia, granulocytopenia, and hypercholesterolemia. The initial Phase I/II open-label, single-center, ascending-dose trial of the sirolimus-cyclosporine-prednisone combination in living-related renal transplants documented a reduced incidence of acute rejection episodes from 32% to 7.5% when compared with an historical cyclosporine-prednisone group. Steroids were withdrawn from the regimens of most patients as early as one week post-transplant [38].

As a test of the concept of synergy, a multicenter Phase IIB study revealed similar rates of acute rejection episodes (12%) among non-African

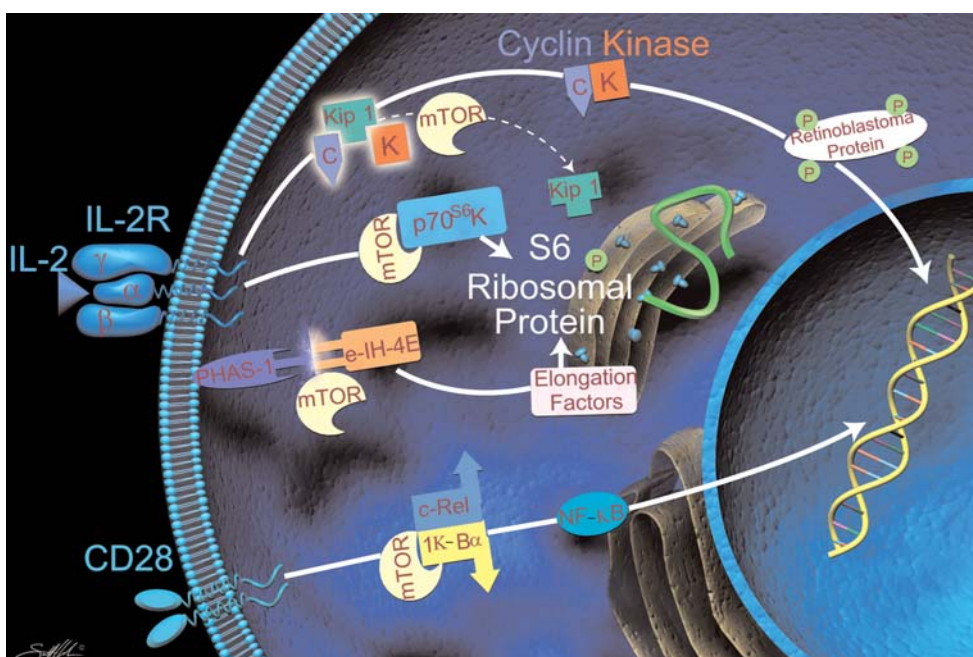


Figure 1. Sirolimus: Mechanism of immunosuppressive action

American recipients treated with sirolimus combined with reduced compared to full doses of the oil-based cyclosporine formulation [34].

In an open-labeled, multicenter European trial, first cadaveric renal allograft recipients were randomized to receive concentration-controlled immunosuppressive regimens based upon cyclosporine vs. sirolimus in addition to corticosteroids and azathioprine. The graft survival rates of 98% vs. 90%, patient survivals of 100% vs. 98%, and biopsy-confirmed acute rejection rates of 41% vs. 38%, respectively, were similar between the groups. On the one hand, the mean serum creatinine was significantly lower among the sirolimus-treated arm ( $P \leq 0.05$ ) at 3 and 4 months. On the other hand, laboratory abnormalities were reported significantly more often with sirolimus, including hypertriglyceridemia (51% vs. 12%), hypercholesterolemia (44% vs. 14%), thrombocytopenia (37% vs. 0%), and leukopenia (39% vs. 14%) [39].

In another open-labeled European trial at 14 centers, first cadaveric renal allograft recipients were randomized to receive concentration-controlled exposure to sirolimus vs. cyclosporine in addition to corticosteroids and mycophenolate mofetil (2 g/day). At 12 months, the endpoints of graft survival, patient survival, and the incidence of biopsy-proven acute rejection episodes were not statistically different between the 2 groups. However, the safety profiles of the 2 regimens were different: after 2 months, the calculated glomerular filtration rate was consistently higher among the sirolimus-treated patients. Adverse events reported more frequently with sirolimus included thrombocytopenia and diarrhea. The cyclosporine-only group showed significantly more frequent occurrences of increased creatinine, hyperuricemia,

cytomegalovirus infection, and tremor [40].

#### *Phase III Studies*

Two pivotal Phase III, multicenter, randomized, double-blind trials in patients receiving cyclosporine and sirolimus evaluated the efficacy of sirolimus for prophylaxis of acute allograft rejection episodes in mismatched living or cadaveric donor renal transplants: the Rapamune U.S. Multicenter Study [41,42] compared sirolimus to azathioprine, and the Rapamune Global Study Group compared sirolimus to placebo [43] (Table 1). The results of the 80 centers, which enrolled 1295 patients, showed a significant reduction in the composite endpoint, namely, the incidence of a biopsy-proven acute rejection episodes, graft loss, patient death within the first 6 months after transplantation, or loss to follow-up compared to either azathioprine or placebo treatment. While both studies showed the efficacy of the higher sirolimus exposure, the Global study showed a benefit of both the sirolimus 2 and 5 mg doses at two years, the end of the study; graft and patient survivals were similar in all treatment groups [41].

#### **Phase IV Explorations of Therapeutic**

##### **Indications**

##### *Cyclosporine Elimination*

Two open-labeled trials were conducted to exploit the immunosuppressive efficacy of a cyclosporine-sirolimus combination during the initial postoperative period with withdrawal of cyclosporine after 3 months to minimize the risk of long-term nephrotoxic complications. The renal function of patients withdrawn from cyclosporine was superior to that of subjects continuing on the drug. Although the incidence of acute rejection episodes was higher among patients from whom cyclosporine was withdrawn, all episodes

responded to augmented steroid treatment [44,45]. While a withdrawal strategy may be useful for patients with calcineurin antagonist toxicity, we believe that the use of minimal cyclosporine exposures *de novo* reduces the need for eventual withdrawal and preserves the immunosuppressive synergy obtained with the combination.

#### *Steroid Withdrawal*

Steroids were successfully withdrawn from a cyclosporine-sirolimus-prednisone regimen in 18 of 20 (90%) living-donor recipients of mismatched renal transplants within 1 month and in all 40 patients in the Phase I/II trial after 5 months [38]. Among 124 renal transplant recipients withdrawn from steroids at various times, 77% remained steroid-free at a mean follow-up of 25 months [46]. Histopathological evidence suggesting chronic rejection to mandate reinstatement of steroids was only observed in 6 patients. During a 1 to 6 year follow-up, five grafts were lost: one patient died, three patients were noncompliant, and one trans-

plant failed because of intractable gastrointestinal bleeding. Recipients treated *de novo* with a sirolimus-cyclosporine combination can tolerate steroid withdrawal at 1 month after living-donor or 3 to 6 months after cadaveric kidney transplants with only a modest risk of acute rejection episodes or chronic nephropathy.

#### *Induction Therapy for Delayed Graft Function*

The use of marginal donor kidneys has increased the occurrence of delayed graft function, which is associated with acute rejection episodes and decreased long-term graft survival [47]. Calcineurin antagonists may exacerbate this condition due to their intrinsic nephrotoxicity, which rarely produces irreversible damage to the kidney. Because antilymphocyte preparations only provide a two-week window for recovery of function, sirolimus seems to be a promising alternative to provide calcineurin antagonist-free immunosuppression. In contrast, a daclizumab-mycophenolate mofetil-prednisone regimen administered to

**Table 1.** Etiology of graft loss and death at 24 months: U.S. and global trials composite data

	Number (%) at 24 Months <sup>a</sup>			
	Azathioprine/ placebo (n=291)	Sirolimus 2 mg/day (n=511)	Sirolimus 5 mg/day (n=493)	Total (n=1295)
<b>A. Graft Loss</b>				
Death with function	14 (4.8)	30 (5.9)	22 (4.5)	66 (5.1)
Acute rejection	9 (3.1)	11 (2.2)	10 (2.0)	30 (2.3)
Acute tubular necrosis	7 (2.4)	3 (0.6)	11 (2.2)	21 (1.6)
Vein/artery thrombosis	3 (1.0)	5 (0.98)	3 (0.6)	11 (0.8)
Other	4 (1.4)	9 (1.8)	13 (2.6)	26 (2.0)
<b>Total</b>	<b>37 (12.7)</b>	<b>58 (11.4)</b>	<b>59 (12.0)</b>	<b>154 (11.9)</b>
<b>B. Death</b>				
Infection	2 (0.7)	11 (2.2)	9 (1.8)	22 (1.7)
Malignancy	3 (1.0)	7 (1.4)	2 (0.4)	12 (0.9)
Cardiovascular events	8 (2.7)	11 (2.2)	10 (2.0)	29 (2.2)
Other	3 (1.0)	6 (1.2)	7 (1.4)	16 (1.2)
<b>Total</b>	<b>16 (5.5)</b>	<b>35 (6.8)</b>	<b>28 (5.7)</b>	<b>79 (6.1)</b>

<sup>a</sup> None of the differences were significant.  
Data derived from [42].

low-risk patients [48] was associated with a 48% rate of biopsy-proven acute rejection episodes within 6 months.

A basiliximab-sirolimus-prednisone combination with delayed introduction of cyclosporine when serum creatinine levels reached  $<2.5$  mg/dL [49] engendered only a 4% incidence of acute rejection episodes among low-risk recipients. However, high-risk African-American or retransplant patients displayed a 30% incidence. A recent strategy substituting rabbit antilymphocyte antibody preparations for basiliximab has resulted in a rejection rate of  $<10\%$  [49]. Although McTaggart et al. [50] believe that sirolimus use may prolong recovery from delayed graft function, this evolution is infrequent in our experience and readily reversible by a bridge of 14 days of muromonab antibody therapy while withholding sirolimus.

#### *Refractory Renal Allograft Rejection*

Acute rejection episodes refractory to antilymphocyte antibodies represent serious complications of renal transplantation likely to lead to graft loss. To minimize graft loss, sirolimus was administered at a dose of  $7$  mg/m<sup>2</sup> for five days and then at  $5$  mg/m<sup>2</sup> in combination with moderately reduced doses of cyclosporine and withdrawn or tapered doses of baseline steroids. This regimen yielded a significantly greater reversal rate of refractory rejection episodes compared with patients treated with mycophenolate mofetil and continued steroids (96% vs. 67%;  $P=0.03$ ) [51].

#### *Combinations with Tacrolimus*

Although initial *in vitro* studies suggested a pharmacodynamic antagonism between sirolimus and tacrolimus, possibly due to their competition for FKBP12 in the cytoplasm, this drug combination has been subjected to preclinical studies [52-54]

and to early clinical use. Khanna et al. [55] showed that addition of sirolimus to the blood of stable renal transplant recipients on a tacrolimus regimen decreased lymphocyte proliferation as well as mRNA expression of the pro-inflammatory cytokines—tumor necrosis factor- $\alpha$ , and interferon- $\gamma$  and of cyclins—G<sub>1</sub> and E—with increased expression of transforming growth factor- $\beta$  and p21 [55].

In a limited number of liver recipients, Peltekian et al. demonstrated that sirolimus therapy targeted to  $C_0=7$  ng/mL allows administration of low exposures to tacrolimus ( $C_0=5$  ng/mL), yielding a 9.5% rate of acute rejection episodes [56]. Also, a preliminary experience with pediatric liver recipients revealed that sirolimus may facilitate early elimination of steroids and late discontinuation of tacrolimus in a significant number of children after transplantation [57]. However, because of an apparently increased incidence of hepatic artery thrombosis with combined sirolimus-calcineurin antagonist therapy, the major application of the mTOR inhibitor has been after one month in patients with renal dysfunction.

### **Safety and Toxicity**

#### *Hyperlipidemia*

Hyperlipidemia, a serious adverse effect of sirolimus treatment, occurs beginning in the second or third month after transplantation among more than 40% of renal transplant recipients. Sirolimus exacerbates the tendency of calcineurin antagonists to induce hypercholesterolemia and steroids to induce hypertriglyceridemia, as well as the dyslipidemias associated with chronic renal disease. Sirolimus apparently delays the clearance of circulating low-, intermediate-, and very-low-

density lipoproteins [58]. When the prescription of a low-fat diet and exercise fails to ameliorate hyperlipidemia, namely, triglyceride or cholesterol values exceeding 300 or 200 mg/dL, respectively, countermeasure therapy is necessary [58,59]. In some studies, hyperlipidemia has been implicated in an increased incidence of chronic rejection [60,61]. In a multivariate analysis, Ponticelli et al. [62] demonstrated that elevated low-density lipoproteins at one year represented a major risk factor for late graft failure. The U.S. Phase III sirolimus trial failed to show a significant difference between the sirolimus and azathioprine arms in the incidence of conditions attributed to elevated blood lipids; namely, pancreatitis, myocardial infarction, or stroke at either one or two years [41]. However, sirolimus patients more frequently required lipid countermeasure therapy.

#### *Bone Marrow Suppression*

Many immunosuppressive drugs produce myelosuppression. Although myelosuppression is common among patients treated with nucleoside synthesis inhibitors or polyclonal antilymphocyte antibodies, approximately 60% of sirolimus treated patients display this complication, particularly when the trough value is  $\geq 16$  ng/mL during the first 4 weeks of therapy [63]. One hypothesis suggests that sirolimus blockades critical cytokine signals promoting maturation and/or proliferation of bone marrow elements, including IL-11 on platelets, granulocyte colony stimulating factor on leukocytes, and erythropoietin on red blood cell precursors. Concomitant therapy with azathioprine or particularly mycophenolate mofetil may augment the myelosuppression, especially in the presence of postoperative infections, antiviral chemoprophylaxis, and/or alloimmune reactions

to the graft [63,64].

#### *Infections and Malignancies*

Although initial studies suggested that herpes simplex virus, cytomegalovirus, and Epstein-Barr virus infections, as well as *Pneumocystis carinii* pneumonia occurred at a greater frequency among sirolimus-treated renal transplant recipients, the multicenter Phase III trials only revealed aphthous mucosal ulcers to occur more frequently, particularly among patients receiving sirolimus 5 mg/day than those receiving 2 mg/day or placebo in the Global ( $P=0.002$ ) but not the U.S. trial. The incidences of bacterial, viral, or fungal infections were similar among all groups at 24 months [42].

Sirolimus treatment was not associated with an increased risk of malignancy within 24 months (Table 2). Among 34 patients in the U.S. trial who experienced histologically confirmed malignancies, 25/558 (4%) had been randomly assigned to the sirolimus treatment groups. In the Global trial, among 44 patients with biopsy-proven malignancy, 33/446 (7.4%) had been assigned to sirolimus treatment groups. The distribution of malignancies and the incidence of posttransplant lymphoproliferative disorder across treatment groups were similar at 24 months [42].

We examined neoplasms among 1008 renal transplant recipients treated with sirolimus-cyclosporine-prednisone at our center for up to 10 years. The overall incidence of malignancy throughout follow-up was 30/1008 (3%) with presentation at  $32.4 \pm 31.6$  months (range: 1-135). The incidences of various malignancies common among transplant patients were: posttransplant lymphoproliferative disorder 0.4%, renal cell carcinoma 0.2%, and skin tumors 1.9%, the last group

**Table 2.** Malignancies diagnosed within 24 months: U.S. and Global trials by treatment arm

Type	Azathioprine (n=161)	U.S. Trial		Placebo (n=130)	Global Trial	
		Sirolimus 2 mg/day (n=284)	Sirolimus 5 mg/day (n=274)		Sirolimus 2 mg/day (n=227)	Sirolimus 5 mg/day (n=219)
Lymphoma	1	1	3	0	3	3
PTLD	0	1	0	1	1	4
Other	0	0	0	0	0	0
Basal cell	4	2	4	6	2	3
Melanoma	0	1	0	0	1	2
Prostate	0	1	0	0	0	0
Lung	0	1	0	0	1	1
Squamous cell	3	1	4	3	5	2
Testicular	0	0	1	0	0	0
Adenocarcinoma	0	0	0	0	2	0
Other skin Carcinoma	0	0	1	0	1	0
Breast	0	0	0	0	1	0
Renal	0	0	0	0	0	1
Pancreas	0	0	0	1	0	0
Cervical	0	0	1	0	0	0
Transitional	0	0	2	0	0	0
Small cell	0	0	1	0	0	0
Adeno-esophageal	1	0	0	0	0	0
<b>Total (%)</b>	<b>9 (5.6)</b>	<b>8 (2.8)</b>	<b>17 (6.2)</b>	<b>11 (8.5)</b>	<b>17 (7.5)</b>	<b>16 (7.3)</b>

including squamous cell (0.9%), basal cell (0.5%), melanoma (0.2%), Merkel cell (0.2%), and basosquamous cell (0.1%). The other malignancies were single cases of breast, bladder, endometrial, lung, and brain neoplasms. Furthermore, when our data were compared to the Surveillance, Epidemiology, and End Results database over a 5-year period, the sirolimus-cyclosporine cohort showed a similar incidence of skin tumors (1.9% vs. 1.5%), which is significantly less than the 7% reported among other renal transplant patient cohorts. Our patients showed a 4-fold increase in posttransplant lymphoproliferative disorder (0.4% vs. 0.1%) and renal cell carcinoma (0.2% vs. 0.05%) compared to the general U.S. population, figures that were far less than the previously reported 27.2- and 8-fold increases, respectively, using tacrolimus plus mycophenolate mofetil [65].

### Conclusion:

Both *in vitro* and *in vivo* evidence supports the use

of sirolimus in renal transplant recipients, since it reduces the incidence of acute rejection episodes in *de novo* transplants and appears to be more effective when given in combination with calcineurin antagonist therapy than alone. Regimens utilizing a strategy of cyclosporine elimination are under investigation to reduce nephrotoxicity. Maximum unanticipated benefits uncovered during Phase IV trials have emerged from use in combination with an antibody induction agent, such as basiliximab or thymoglobulin, for kidneys displaying delayed graft function, as well as the substitution of sirolimus for cyclosporine in patients with histopathologic evidence of nephrotoxicity. The former regimen achieves a low (6.4%) rate of acute rejection episodes and allows maximal recovery of renal function: mean calculated creatinine clearances increased to over 80 mL/min during the first year [66]. In order to determine the efficacy of sirolimus-based maintenance therapy, longer follow-up with protocol biopsies is essen-

tial. Sirolimus thus proffers a potent and unique platform for new immunosuppressive strategies in organ transplantation.

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