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Benefit-Risk Assessment of Sirolimus in Renal Transplantation

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Abstract

Sirolimus (rapamycin) is a macrocyclic lactone isolated from a strain of *Streptomyces hygroscopicus* that inhibits the mammalian target of rapamycin (mTOR)-mediated signal-transduction pathways, resulting in the arrest of cell cycle of various cell types, including T- and B-lymphocytes. Sirolimus has been demonstrated to prolong graft survival in various animal models of transplantation, ranging from rodents to primates for both heterotopic, as well as orthotopic organ grafting, bone marrow transplantation and islet cell grafting.

In human clinical renal transplantation, sirolimus in combination with ciclosporin (cyclosporine) efficiently reduces the incidence of acute allograft rejection. Because of the synergistic effect of sirolimus on ciclosporin-induced nephrotoxicity, a prolonged combination of the two drugs inevitably leads to progressive irreversible renal allograft damage. Early elimination of calcineurin inhibitor therapy or complete avoidance of the latter by using sirolimus therapy is the optimal strategy for this drug. Prospective randomised phase II and III clinical studies have confirmed this approach, at least for recipients with a low to moderate immunological risk. For patients with a high immunological risk or

recipients exposed to delayed graft function, sirolimus might not constitute the best therapeutic choice – despite its ability to enable calcineurin inhibitor sparing in the latter situation – because of its anti-proliferative effects on recovering renal tubular cells. Whether lower doses of sirolimus or a combination with a reduced dose of tacrolimus would be advantageous in these high risk situations remains to be determined.

Clinically relevant adverse effects of sirolimus that require a specific therapeutic response or can potentially influence short- and long-term patient morbidity and mortality as well as graft survival include hypercholesterolaemia, hypertriglyceridaemia, infectious and non-infectious pneumonia, anaemia, lymphocele formation and impaired wound healing. These drug-related adverse effects are important determinants in the choice of a tailor-made immunosuppressive drug regimen that complies with the individual patient risk profile. Equally important in the latter decision is the lack of severe intrinsic nephrotoxicity associated with sirolimus and its advantageous effects on arterial hypertension, post-transplantation diabetes mellitus and esthetic changes induced by calcineurin inhibitors. Mild and transient thrombocytopenia, leukopenia, gastrointestinal adverse effects and mucosal ulcerations are all minor complications of sirolimus therapy that have less impact on the decision for choosing this drug as the basis for tailor-made immunosuppressive therapy.

It is clear that sirolimus has gained a proper place in the present-day immunosuppressive armament used in renal transplantation and will contribute to the development of a tailor-made immunosuppressive therapy aimed at fulfilling the requirements outlined by the individual patient profile.

Sirolimus (rapamycin) is a macrocyclic lactone fermentation product isolated from a strain of *Streptomyces hygroscopicus* in soil samples collected from the Vai Atare region of Rapa Nui. Sirolimus complexes with a family of intracellular immunophilins known as the FK-binding proteins (FKBPs). The sirolimus/FKBP-12 complex binds directly to mTOR (mammalian target of rapamycin) and blocks its function. mTOR is a 289kD protein that has a C-terminal 600-amino-acid domain with homology to several protease inhibitor (PI) kinases, including mammalian PI 3 kinase, and activates S6K1 (p70 ribosomal S6 kinase). By interfering with the function of mTOR, sirolimus inhibits the mTOR-mediated signal transduction pathways, resulting in the arrest of cell cycle in the G1-S phase in various cell types. The increase in protein synthesis by growth factor-mediated activation of mTOR is blocked by sirolimus.[1]

The immunosuppressive effects of sirolimus result from its inhibition of T and B cell activity. Sirolimus inhibits T-lymphocyte proliferation induced by mitogens that induce either calcium-dependent or -independent signal transduction pathways. Sirolimus blocks interleukin (IL)-2-induced proliferation of T cells, but it does not affect the signal that results in the activation-induced apoptosis.[2] The effects of sirolimus are limited not only to IL-2- or IL-4-mediated proliferation of T cells, but also include inhibition of IL-12, IL-7 and IL-15 driven proliferation of activated T cells.[3,4] Sirolimus has been shown to inhibit growth factor-mediated proliferation of non-immune cells. It blocks basic fibroblast growth factor (bFGF)-induced proliferation of aortic and umbilical vein endothelial cells. Sirolimus also inhibits bFGF and platelet-derived growth factor-stimulated proliferation of smooth muscle cells.[3,4]

Sirolimus has been demonstrated to prolong graft survival in many animal models of transplantation, ranging from rodents to primates for both heterotopic, as well as orthotopic organ grafting, bone marrow transplantation and islet cell grafting.[4]

In human clinical transplantation sirolimus has proven to be a powerful immunosuppressive compound that is capable of preventing acute graft rejec-

Table I. Pharmacokinetic characteristics of sirolimus

Pharmacokinetic parameter	Value
Apparent oral bioavailability: F (%)	± 15
T_{max} (h)	1–2
Blood : plasma partitioning coefficient	35 : 1
Plasma binding (%)	92 (lipoproteins: 60, lipoprotein-free plasma: 40)
Apparent volume of distribution: V_d/F (L/kg)	5.6–16.7
Metabolism	Hepatic and intestinal (CYP3A4/P-glycoprotein)
Metabolites	>16 metabolites (demethylation, hydroxylation): <10% pharmacological activity
Apparent oral clearance: CL/F (L/h/kg):	0.042–0.416
Terminal half-life: (h)	62 ± 16
Elimination	
biliary (faeces) [%]	91
urinary (%)	2.2
Quantification	HPLC-MSMS, HPLC-UV, microparticle enzyme immunoassay
Drug interactions	CYP3A4, P-glycoprotein mediated

CYP = cytochrome P450; **HPLC-MSMS =** high performance liquid chromatography-mass spectrometry; **HPLC-UV** = high performance liquid chromatography-ultraviolet detection; $\mathbf{T}_{max}$ = time to reach maximum concentration following drug administration.

tion in different combinations with other immunosuppressive drugs. Its anti-proliferative action on human vascular smooth muscle cells[5,6] and the ability to reduce intimal thickening in models of vascular injury[7] have led to its development as an anti-restenotic agent in sirolimus-eluting coronary stents.[8,9] Further unraveling of the exact molecular mechanism of action of sirolimus has opened a new, interesting field of research that focuses on the anti-tumour effects of this agent.[2,10,11]

Similar to calcineurin inhibitors, mTOR inhibitors are characterised by a narrow therapeutic window, highly variable absorption and large intra- and interindividual variability in pharmacokinetics (see table I).[12,13] Therapeutic drug monitoring is therefore mandatory for the clinical application of sirolimus in solid organ transplantation.[14,15] In addition, the significant pharmacokinetic interaction between sirolimus and ciclosporin,[16] mycophenolate mofetil[17] and possibly tacrolimus,[18] necessitates concentration monitoring each time dose adjustments are performed. Defining a clinically useful therapeutic window for sirolimus has proven to be a rather complex and ongoing task (table II). A minimal obtained blood sirolimus trough concentration of 5 ng/mL signifies a clinical threshold differentiating for acute rejection, at least in combination with ciclosporin.[19,20] The upper limit of sirolimus exposure, discriminating the onset of adverse events, was determined to be around 15 ng/mL in several studies that combined sirolimus with ciclosporin.[19,20] From this, especially for thrombocytopenia and hypertriglyceridaemia, an exposure-response relationship could be defined. Using receiver operating characteristic curves, an inflection point of 14 ng/mL for thrombocytopenia, 11 ng/mL for hypertriglyceridaemia and 13 ng/mL for hypercholesterolaemia was determined.[20] Multiple clinical studies have been performed examining the role of sirolimus in calcineurin inhibitor free protocols.[17,21,22] The target concentration ranges for sirolimus in these trials have not yet been completely validated but seem to concur with those established in the elimination trials[23] (see section 1.2). Compared with calcineurin inhibitors, a better correlation seems to exist between pre-dose trough blood sirolimus concentration and total dose-interval steady-state measurement of the area under the concentration-time curve $(AUC)_{0\text{-}24}$,[18,20,24] which makes trough concentration monitoring more feasible in practice.

This review assesses the evidence-based clinical therapeutic benefits of sirolimus in the prevention of renal allograft rejection and in optimising graft function. Moreover, a review is conducted of the relevant adverse effects and safety profile of the drug that can have bearings on its clinical application. Ideally, this will enable clinicians to construct a

Table II. Suggested target therapeutic trough blood concentrations for different immunosuppressive drug combinations based on randomised controlled trials

Immunosuppressive drug combination	Target therapeutic trough blood concentrations (ng/mL)
Sirolimus + sCic + corticosteroids	8–10
Sirolimus + rCic + corticosteroids	10–12
Sirolimus after early Cic elimination	12–20
Sirolimus + sTac + corticosteroids	5–10
Sirolimus + rTac + corticosteroids	8–12
Sirolimus + MMF + IL-2 receptor blockade	10–12

IL-2 = interleukin-2; **MMF** = mycophenolate mofetil; **rCic** = reduced dose ciclosporin (cyclosporine); **rTac** = reduced dose tacrolimus; **sCic** = standard dose ciclosporin; **sTac** = standard dose tacrolimus.

balanced perspective view of the drug and its use in clinical transplantation medicine that is characterised by an increasing recognition of the need for tailor-made immunosuppressive therapy.

A search was conducted in Medline (1966–June 2004) using the keywords 'sirolimus (SRL)', 'rapamycin(e)', 'rapamune', '(m)TOR inhibitor(s)', 'transplantation', 'renal' and 'kidney'.

1. Efficacy

1.1 Randomised Comparative Trials

It was first shown in a large randomized, phase III, double-blind, multi-centre study in *de novo* renal allograft recipients, that adding a fixed daily dosage of sirolimus 2mg or 5mg to ciclosporin and corticosteroids resulted in a significant reduction of biopsy-confirmed acute rejection rates at 1 year after transplantation compared with a standard regimen of azathioprine, ciclosporin and corticosteroids (see table III); at least in non-Black renal recipients.[25] In addition, the histological severity of acute rejection episodes, evaluated according to the Banff 1993 criteria,[26] was significantly less in sirolimus-treated recipients, resulting in lower requirements for T cell antibody therapy. Surprisingly, renal allograft function at 1 year was significantly lower in sirolimus-treated patients, irrespective of ciclosporin blood concentrations.

In another phase III study of similar design, 5mg of sirolimus together with a standard dose of ciclosporin (target trough blood concentrations between 150–250 ng/mL from 3 months onwards) produced an identical significant reduction in glomerular filtration rate 6 months post-transplantation despite the fact that the acute rejection rate was substantially lower (19.2% vs 41.5%; $p < 0.001$) compared with control patients treated with ciclosporin and corticosteroids.[27] These unexpected findings were the first indication that the combination of sirolimus with a standard dose of ciclosporin had deleterious effects on allograft function (see section 2.1). Short-term patient and graft survival in both studies were not affected by adding sirolimus to a ciclosporin-based regimen. Assigned treatment was not discontinued more frequently in the sirolimus study arms; in fact, in the former of the two trials, fewer patients who received sirolimus 2mg (32.4%) were prematurely withdrawn from the study compared with control patients who received azathioprine (44.7%; $p = 0.011$), mainly because of unsatisfactory response and adverse events.[25,27]

In an earlier smaller phase II study, the association of sirolimus 1, 3 or 5 mg/m^2/day with a reduced dose of ciclosporin (initial target trough blood concentration between 100–175 ng/mL) produced significantly lower acute rejection rates at 1 year; again only in Caucasian recipients but this time without negative effects on allograft function.[28] More patients discontinued sirolimus therapy because of adverse events (25% vs 4% in the control group). Two important issues surfaced in these early clinical trials: the standard dose of sirolimus seemed inadequate for prevention of acute rejection for Black recipients and a standard dose of ciclosporin in combination with sirolimus was detrimental for allograft function. Preliminary pharmacokinetic studies had already shown that the total body clearance of sirolimus was 20% and 44% higher in *de novo* and stable chronic Black transplant patients, respectively, compared with non-Black patients.[30,31] However, in the settings of a prospective clinical

trial these differences in drug clearance were not confirmed; the addition of sirolimus to a ciclosporin-based regimen significantly reduced acute rejection rates from 43.3% to 19.2% and improved 2-year graft survival in African-American recipients.[29]

1.2 Elimination Studies

The pharmacokinetic interaction between sirolimus and ciclosporin, whereby both drugs increase the other's blood concentration,[32] led to studies demonstrating augmented calcineurin inhibitor-induced nephrotoxicity in animal models.[16,33,34] The impaired allograft function in patients receiving sirolimus in combination with a standard dose of ciclosporin triggered new attempts to safely reduce or minimise the dose of calcineurin inhibitor[35,36] or to delay its introduction in recipients at risk for nephrotoxicity.[37,38] However, the results of these non-randomised studies were unequivocal and a novel approach to optimise the use of sirolimus was construed by eliminating calcineurin inhibitor therapy early after grafting, preferably around 3 months when the acute immunological damage to the graft had subdued.[39] This latter method proved very successful, both in terms of efficacy (prevention of acute rejection) and preservation of allograft function (table IV).

In the largest of these elimination studies, 525 *de novo* patients were initially treated with a fixed daily dosage of sirolimus 2mg in combination with ciclosporin and corticosteroids.[23,43] After 3 months, 430 eligible recipients were randomly (1:1) assigned to remain on triple therapy (with ciclosporin target trough concentration of 75–200 ng/mL) or to have ciclosporin withdrawn and continue on bi-therapy with higher target sirolimus trough blood concentrations (20–30 ng/mL in the first year; 15–25 ng/mL thereafter). The incidence of primary biopsy-confirmed acute graft rejection was 13.1% in the pre-randomisation period (first 3 months of the trial). Acute rejection rate after randomisation was 4.7% higher with ciclosporin withdrawal (9.8% vs 5.1%; $p = 0.09$). Graft function became significantly better after elimination of ciclosporin, with a persistently higher calculated glomerular filtration rate at 2 years of 58.3 ± 1.6 mL/min versus 48.4 ± 1.6 mL/min for patients who continued on triple therapy ($p < 0.001$). Patient and graft survival were excellent in both study arms (patient survival 94% vs 95.3% and graft survival 91.2% vs 93.5% for patients receiving triple- and bi-therapy, respectively). Ninety-five patients (18%) in this study were not randomised at 3 months because of severe acute graft rejection in the 4 weeks preceding random assignment, dialysis dependency, a serum creatinine level >400 μmol/L, or inadequate renal function to support ciclosporin elimination. The group of patients that were not randomised did not differ from the randomised patients in terms of age, ethnicity, gender, primary

Table III. Prospective controlled studies of sirolimus in combination with standard (sCic) or reduced (rCic) dose ciclosporin (cyclosporine)

Study	No. of patients	Follow-up (months)	Study design	Biopsy-confirmed AR incidence (%)		Graft function GFR (mL/min)	
				sirolimus	Cic	sirolimus	Cic
Kahan[25]a	719	12	2 and 5mg sirolimus + sCic vs sCic + Aza	21.8 (2mg); 14.6 (5mg)b	31.1	61.9 (2mg); 55.5 (5mg)b	67.5
MacDonald et al.[27]	576	6	2 and 5mg sirolimus + sCic vs sCic	24.7 (2mg); 19.2 (5mg)b	41.5	59.6 (2mg); 56.4 (5mg)b	62.6
Kahan et al.[28]	149	6	Placebo, 1 and 3 mg/m^2 sirolimus + sCic vs 1, 3 and 5 mg/m^2 sirolimus + rCic	8.5 (sCic); 19.5 (rCic)bc	32	60.7 (sCic); 62.5 (rCic)c	65.4
Podder et al.[29]d	137	24	Sirolimus + sCic vs sCic	19.2b	43.3	NAe	NAe

a Acute rejection rates not significantly different in subgroup of Black recipients.

b $p < 0.05$ between sirolimus and Cic.

c 1 and 3mg sirolimus (+ sCic) groups and 1, 3, 5mg sirolimus (+rCic) groups combined.

d Exclusively African-American recipients.

e Serum creatinine levels not different between groups.

AR = acute rejection; **Aza** = azathioprine; **GFR** = glomerular filtration rate; **NA** = not applicable.

Table IV. Ciclosporin (cyclosporine) elimination studies

Study	No. of patients	Follow-up (months)	Study design	Biopsy-confirmed AR incidence (%)		Graft function GFR (mL/min)	
				sirolimus	Cic	sirolimus	Cic
Kreis et al.[40]	525	36	Sirolimus + sCic (sCic stop at month 3) vs sirolimus + sCic	20.5	14.9	59.4[a]	47.3
Gonwa et al.[41]	197	12	Sirolimus + rCic (rCic stop at month 2) vs 2mg sirolimus + sCic	22	18.6	63.2[a]	49.1
Baboolal[42]	133	6	Sirolimus + sCic (sCic stop at month 3) vs sirolimus + sCic (rCic start at month 3)	19[b]	6.7[b]	65[a]	57

a $p < 0.05$ between sirolimus and Cic.

b Rejection rates for randomised recipients only; 15/22 acute rejections in the first 3 months occurred in non-randomised patients (46/133).

AR = acute rejection; **GFR** = glomerular filtration rate; **rCic** = reduced dose ciclosporin; **sCic** = standard dose ciclosporin.

versus secondary graft, cadaveric donor use, donor age, cold ischaemia time and human leukocyte antigen mismatches. However, the incidence of delayed graft function (DGF) was significantly higher in the non-randomised patients (48.4% vs 21.9% vs 19.1%; $p < 0.001$) as was the pre-randomisation rate of acute rejections (28.4% vs 9.3% vs 10.2%; $p < 0.001$), indicating that non-randomised patients were characterised by a higher immunological risk.

In this large study it was clearly demonstrated that for recipients with a low-to-moderate immunological risk profile, early calcineurin inhibitor withdrawal was safe and translated into a persistently better long-term allograft function. However, the selection bias represented by the non-randomised patient group warrants against extrapolating these conclusions to high-risk transplant populations. The 3-year follow-up data of this study continue to show a clear benefit of calcineurin inhibitor elimination in terms of graft function (59.4 ± 1.8 mL/min vs 47.3 ± 1.8 mL/min; $p < 0.001$) despite further reductions of ciclosporin target trough concentrations (50–150 ng/mL) in the control arm from 2 years onwards.[40] Protocol biopsies performed at baseline, 12 months and 3 years confirm that early ciclosporin withdrawal leads to significantly less chronic allograft damage assessed at 3 years post-transplantation in concordance with the clinical course.[44] Moreover, the fact that the histological chronic allograft damage index (CADI) used in this trial is a surrogate end point for long-term graft survival,[45] strengthens the strategic approach of eliminating ciclosporin therapy early after grafting and maintaining these patients with sirolimus treatment. Three years after grafting, discontinuations from the assigned therapy were significantly more frequent in the ciclosporin continuation group (47.9% vs 37.7%; $p = 0.041$), predominantly because of adverse events. Ciclosporin toxicity, nervous system disorders and abnormal kidney function were the most frequent adverse events associated with discontinuation in the ciclosporin/sirolimus group while abnormal liver function tests and hypertriglyceridaemia caused patients to discontinue sirolimus therapy.[40]

In the second ciclosporin elimination trial, 197 *de novo* recipients were randomised to either triple therapy with full-dose ciclosporin plus a daily low and fixed dosage of sirolimus (2 mg/day; $n = 97$) or reduced dose ciclosporin plus concentration-controlled sirolimus (target trough blood concentrations of 10–20 ng/mL; $n = 100$).[41] In the second part of the trial, patients with stable graft function who had received a reduced-dose ciclosporin and achieved target trough sirolimus concentrations were eligible for calcineurin inhibitor elimination at the end of 2 months post-transplantation if they remained free of acute rejection in the preceding 3 weeks. Again, ciclosporin elimination did not result in an excess of biopsy-confirmed acute rejections at 12 months (22% vs 18.6%; $p = 0.59$) but led to a significant improvement of 1-year graft function (63.2 ± 3.4 mL/min vs 49.1 ± 3.3 mL/min; $p < 0.001$) for both Black and non-Black recipients.[41] Patient and graft survival were high in both study arms (patient survival 96.9% vs 96% and graft survival 92.8% vs 95%). In analogy with the previous elimination trial, patients with DGF extending beyond day 7 postop-

erative and patients experiencing acute rejection in the 3 weeks prior to ciclosporin withdrawal (n = 49) were excluded from randomisation. Consequently, the same restrictions applied when the results were interpreted because of this important selection bias.

Whether ciclosporin elimination at 3 months post-transplantation was advantageous in terms of allograft function compared with dose minimisation (target trough concentrations of 50–100 ng/mL) was examined in a third larger trial involving 133 recipients.[42] Pre-randomisation target trough ciclosporin concentrations of 200–400 ng/mL in the first month and 125–250 ng/mL up until 3 months, in combination with sirolimus trough concentrations of 4–12 ng/mL, were associated with a 16.5% incidence of biopsy-proven acute graft rejection at 3 months. After randomisation at month 3, three more acute rejections occurred in the ciclosporin elimination group versus one episode in the minimisation group. Ciclosporin withdrawal was associated with better graft function at 6 months (glomerular filtration rate 65 ± 14 mL/min vs 57 ± 13 mL/min; p = 0.027) compared with dose reduction of the calcineurin inhibitor. Important in this trial was the fact that 46 (34.6%) patients who were not randomised because of severe (vascular) rejection or a serum creatinine level >400 μmol/L, accounted for two-thirds (68.2%) of all pre-randomisation rejections; again making the randomised and analysed groups highly selected.

The general conclusion that can be drawn from these randomised controlled elimination studies is clear: early ciclosporin elimination results in improved allograft function compared with continued full- or reduced-dose ciclosporin. This beneficial effect could be established without the sacrifice of an unacceptable high rate of extra acute rejection episodes, but only for patients with a low-to-moderate immunological risk. Based on these studies, recipients that experienced severe (vascular) repetitive acute rejection episodes, DGF or had persistent suboptimal graft function, cannot be considered as candidates for early calcineurin inhibitor elimination.

1.3 Comparative Trials on Calcineurin Inhibitor Avoidance

The improvement of long-term allograft function after calcineurin inhibitor elimination opened the way to new trials testing the feasibility of transplanting without calcineurin inhibitors from the start. Groth et al.[21] compared sirolimus with ciclosporin therapy, administered in combination with azathioprine and corticosteroids in a randomised trial. Although 1-year patient and graft survival were excellent in this trial, the incidence of acute rejection was high in both groups (41% vs 38%, see table V) and this was despite the high initial target trough concentrations of sirolimus (30 ng/mL until 2 months and 15 ng/mL thereafter) that led to numerous sirolimus-related adverse effects (see section 2). Twenty-four (58.2%) patients in the sirolimus arm and 19 (45.2%) patients in the control arm prematurely discontinued the assigned treatment. Probably because of the high rate of acute rejections leading to permanent graft damage,[46] no difference in graft function was detected 1 year post-transplantation, although glomerular filtration rate was numerically

Table V. Prospective controlled studies on calcineurin inhibitor avoidance

Study	No. of patients	Follow-up (months)	Study design	Biopsy-confirmed AR incidence (%)		Graft function GFR (mL/min)	
				sirolimus	Cic/tacrolimus	sirolimus	Cic/tacrolimus
Groth et al.[21]	83	12	Sirolimus + Aza vs Cic + Aza	41	38	69.5	58.7
Kreis et al.[17]	78	12	Sirolimus + MMF vs Cic + MMF	27.4	18.4	67.8	60.5
Flechner et al.[22]	61	12	Sirolimus + MMF + basiliximab vs Cic + MMF + basiliximab	6.4	16.6	81.1[a]	61.1
Stegall et al.[48]	85	4	Sirolimus + MMF + thymoglob vs tacrolimus + MMF + thymoglob	6.7	7.5	±60[b]	±53[b]

a p < 0.05 between sirolimus and Cic group.

b Glomerular filtration rate determined by iothalamate clearance at 1 month post-transplantation.

AR = acute rejection; **Aza** = azathioprine; **Cic** = ciclosporin (cyclosporine); **GFR** = glomerular filtration rate; **MMF** = mycophenolate mofetil; **thymoglob** = thymoglobulin induction therapy.

higher when calcineurin inhibitor therapy could be avoided (see table V). In a trial of similar design using identical target trough blood concentrations for sirolimus but with azathioprine substituted by the more potent mycophenolate mofetil, the incidence of biopsy-proven acute rejection became lower in both groups (27.5% sirolimus vs 18.4% ciclosporin; not significant [ns]).[17] Again, excellent patient and graft survival was obtained but without a significantly better graft function 1 year post-transplantation. Seventeen (43%) patients in the sirolimus/mycophenolate mofetil arm and 10 (26%) patients in the control arm had stopped therapy by 12 months. Analysis of the pooled 2-year follow-up data from these two comparative calcineurin inhibitor-free trials did show that graft function was significantly better after 2 years in patients not receiving ciclosporin therapy (69.3 ± mL/min vs 56.8 mL/min; p = 0.004) and irrespective of prior acute rejection episodes.[47] The 2-year pooled data analysis comprised only patients remaining on their randomly assigned initial therapy, which meant that a substantial number of patients (>50%) had dropped out of the study.

In order to reduce early acute rejection rates and their undeniable negative impact on long-term graft function,[39,46] a more powerful initial immunosuppressive drug regimen was necessary, at least when complete calcineurin inhibitor avoidance was desired. Patients who theoretically would benefit the most from calcineurin inhibitor-free therapy or delayed introduction of the drug, were recipients receiving grafts from marginal donors or experiencing delayed or impaired graft function. The combination of sirolimus with mycophenolate mofetil, corticosteroids and induction therapy with monoclonal antibodies against the IL-2 receptor (basiliximab) was associated with low acute rejection rates, both in patients with DGF (16%)[49] and recipients of marginal donor kidneys (14%),[50] allowing for late introduction of ciclosporin in the latter group. This calcineurin inhibitor-free immunosuppressive regimen was subsequently compared with ciclosporin-based therapy in a prospective, randomised trial[22] (see table V). Thirty-one primary kidney graft recipients were treated with sirolimus aimed at target trough concentrations of 10–12 ng/mL for the first 6 months and 5–10 ng/mL thereafter. Ciclosporin was administered to keep blood trough concentrations of 200 and 250 ng/mL in combination with 2g mycophenolate mofetil, corticosteroids and induction treatment with basiliximab (30 patients). 25.8% and 23.3% respectively, of recipients in this trial were African-American. One-year patient survival and graft function were excellent in both study groups while the incidence of biopsy-confirmed acute rejection was 6.4% in sirolimus-treated recipients versus 16.6% in patients receiving ciclosporin (ns). More importantly, from 3 months post-transplantation onwards, patients not receiving calcineurin inhibitor therapy had significantly better graft function measured as serum creatinine level and glomerular filtration rate and they continued to do so with prolonged follow-up.[22] Routine histological examination after 2 years confirmed that calcineurin inhibitor avoidance not only caused better graft function but was also associated with significantly less signs of chronic allograft nephropathy and diminished intra-graft expression of genes involved in tissue injury, remodeling and inflammation.[51]

In an analogue trial, sirolimus-based therapy (target trough concentrations 12–18 ng/mL) compared with tacrolimus was associated with even lower acute rejection rates (at 4 months: sirolimus 7.5% vs tacrolimus 6.7%; ns) but without differences in early (1 month) graft function.[48] A multitude of uncontrolled, usually single-centre experiences have been communicated recently, confirming the principle that calcineurin inhibitor avoidance can be achieved by using sirolimus in various combinations with other non-nephrotoxic drugs.[52-54] This goal has been attempted many times in the past by employing different immunosuppressive strategies other than sirolimus and with similar variable success.[55,56] Whether the combination of sirolimus with mycophenolate mofetil specifically is beneficial for long-term graft survival is still unclear. The finding that mycophenolate mofetil can inhibit sirolimus-induced pro-fibrotic effects in renal grafts, such as the upregulation of transforming growth factor (TGF)-β-1, plasminogen activator inhibitor-1 and extracellular matrix proteins, could constitute a potentially protective mechanism against the development of chronic allograft nephropathy while at the

Table VI. Prospective controlled studies of sirolimus in combination with standard (sTac) or reduced (rTac) dose tacrolimus

Study	No. of patients	Follow-up (months)	Study design		Biopsy-confirmed AR incidence (%)		Graft function GFR (mL/min)	
			study arm	comparator	study arm	comparator	study arm	comparator
van Hooff et al.[59]	104	6	0.5, 1, 2mg sirolimus + sTac	sTac	8 (0.5mg); 8 (1mg); 3.8 (2mg)[a]	28.6	52.7 (0.5mg); 51.4 (1mg); 49.0 (2mg)	54.1
Paczek et al.[63]	128	6	Sirolimus + rTac	Sirolimus + sTac	17.5	7.7	63.8[a]	52.7
Russ et al.[64]	64	6	Sirolimus + rTac	Sirolimus + sTac	21	19	68	62
Ciancio et al.[66,67]	150[b]	12	Sirolimus + rTac	rTac + MMF Sirolimus + sCic	4[a]	4 (rTac)[a] 14 (sCic)	73	84 (rTac) 71 (sCic)
Grinyo et al.[68]	87	12	Sirolimus + rTac (rTac stop at month 3)	Sirolimus + sTac	11.1[c]	10.3	72.9[acd]	58.4

a $p < 0.05$ between sirolimus and CsA/tacrolimus group.

b All patients received induction with basiliximab.

c Post-amendment.

d Patients on protocol therapy only.

AR = acute rejection; **GFR** = glomerular filtration rate; **MMF** = mycophenolate mofetil; **sCic** = standard dose ciclosporin (cyclosporine).

same time avoiding chronic calcineurin inhibitor-induced nephrotoxicity.[57]

1.4 Randomised Comparative Trials of Sirolimus and Tacrolimus

Attention has focused on tacrolimus as an alternative calcineurin inhibitor in combination with sirolimus, mainly because of an apparent lack of pharmacokinetic interaction between the two drugs[24] and hence the lower risk for nephrotoxicity. Whether long-term association of sirolimus and tacrolimus does indeed not cause alterations of both drugs' blood concentration remains unclear. One report that showed an effect of standard dose tacrolimus on maintenance low dose sirolimus implied an interaction.[18] Alternatively, significant decreases in tacrolimus exposure were observed early after transplantation in patients receiving low doses of sirolimus.[58]

Nevertheless, early findings of randomised clinical trials using this combination are still sparse. In a 6-month prospective study, 104 *de novo* recipients were randomised into groups with three different fixed dosages of sirolimus (0.5, 1 and 2 mg/day) in combination with standard-dose tacrolimus (target trough concentrations 10–20 ng/mL in the first 2 weeks, 10–15 ng/mL until week 6 and 5–15 ng/mL thereafter) and compared with a control group receiving standard-dose tacrolimus and corticosteroids.[59] The incidence of early (month 3) biopsy-confirmed acute rejection was low in all treatment arms (8.0%, 8.0% and 3.8%, respectively) compared with controls (28.6%; $p = 0.014$); taking into account that the control arm was receiving an immunosuppressive regimen without mycophenolate mofetil (see table VI). Sirolimus trough blood concentrations ranged between 1 and 3 ng/mL (high performance liquid chromatography-mass spectrometry [HPLC-MS/MS]) while blood tacrolimus concentrations were on target during the study. Surprisingly, early graft loss in the treatment arms was quite high (graft survival 84%, 88%, 84.6%, respectively, and 96.4% in the control group) and was attributed to the use of 15 kidney grafts coming from non-heart beating donors. One quarter of these kidneys (26.7%) were lost compared with eight (8.9%) grafts from heart-beating donors. At 6 months, no significant difference in graft function was noted across the groups and this was not completely unexpected. The short follow-up time, the fact that acute rejection rates were unacceptably high in the control group according to current standards where tacrolimus is combined with mycophenolate mofetil[60,61] and the bias introduced by using grafts from non-heart beating donors – known to be a risk for early transplant failure and sub-optimal function[62] – clearly make interpretation of the results difficult. In

another trial, 128 *de novo* patients were randomised to receive either standard-dose sirolimus (target trough concentrations 8–15 ng/mL) with reduced-dose tacrolimus (3–7 ng/mL) [n = 63] or reduced-dose sirolimus (5–10 ng/mL) in combination with standard-dose tacrolimus (8–12 ng/mL) [n = 65].[63] The initial sub-optimal doses in the reduced-dose tacrolimus study arm led to five early acute rejection episodes and, subsequently, the study protocol was amended to ensure sufficient early drug exposure. Post-amendment, at 6 months, the incidence of biopsy-confirmed acute rejection was not significantly different between the groups (13.6% vs 10.4%). Including the pre-amendment rejection episodes did not alter these findings (17.5% vs 7.7%; ns). Patient and graft survival was similar in both study arms (95.2% vs 92.1% and 96.9% vs 95.4%, respectively). Interestingly, despite the numerically higher number of rejection episodes in the group of recipients receiving a reduced-dose of tacrolimus and a standard-dose of sirolimus, graft function at 6 months was significantly better compared with patients treated with a full-dose of tacrolimus (63.8 ± 17.3 mL/min vs 52.7± 18.9 mL/min; p = 0.005). In a smaller Australian study of identical design and involving 64 patients, similar efficacy results were obtained but graft function was not found to be significantly better in the reduced-dose tacrolimus group (68 vs 62 mL/min; p = 0.23).[64] Finally, African-American recipients treated with reduced-dose tacrolimus and standard-dose sirolimus did benefit from calcineurin inhibitor minimisation in terms of graft function and without excess rejections.[65]

From the preliminary results of these comparative studies it seems that combining sirolimus with standard-dose tacrolimus is highly efficient, while reducing the dose of calcineurin inhibitor in combination with sirolimus leads to a small increment in acute rejection episodes but has an advantageous effect on short-term allograft function. However, whether standard-dose tacrolimus in combination with sirolimus is deleterious for short-term graft function and survival cannot be concluded from these early trials.

In a very recent large trial, reduced-dose tacrolimus (target trough concentrations 10, 8 and 6 ng/mL at month 1, 6 and 12, respectively) in combination with sirolimus (maintenance trough concentration 8 ng/mL) was compared with reduced-dose tacrolimus plus mycophenolate mofetil and with standard-dose ciclosporin (target trough concentrations 225, 175 ng/mL at month 1 and 12) and sirolimus.[66,67] All patients (n = 150) received induction therapy with basiliximab. The biopsy-confirmed acute rejection rate was identical in both tacrolimus-treated patient groups (4%) and significantly lower than in ciclosporin-treated patients (p = 0.03). At 12 months no difference in allograft function could be detected between the three study groups (creatinine clearance: sirolimus + reduced dose tacrolimus 73 ± 25 mL/min vs reduced dose tacrolimus + mycophenolate mofetil 84 ± 39 mL/min vs sirolimus + standard dose ciclosporin 71 ± 28 mL/min; p = 0.11), while patient and graft survival were comparable. Interestingly, sirolimus bioavailability was higher in this trial when coadministered with ciclosporin than with tacrolimus, explaining the lower dose requirements for sirolimus in ciclosporin-treated recipients. A true comparison in this study of calcineurin inhibitor effect on graft function was hampered because of the standard, rather than reduced-dose, ciclosporin employed and the higher incidence of acute rejection in the latter group. The combination of ciclosporin and sirolimus was associated with a significantly higher discontinuation and conversion rate (total of 52%) compared with the other study regimens (tacrolimus with sirolimus 38%, tacrolimus with mycophenolate mofetil 8%; p = 0.00001 across groups).

The first randomised tacrolimus elimination study (see table VI), designed in analogy with the ciclosporin elimination trials, confirmed that a reduced dose tacrolimus (target trough concentrations 3–8 ng/mL) could be withdrawn from a sirolimus combination therapy (target trough concentrations 8–16 ng/mL pre-withdrawal and 12–20 ng/mL post-elimination) at 3 months post-transplantation without excessive biopsy-proven acute rejection episodes, compared with recipients continuing on standard dose tacrolimus (target trough concentrations 8–12 ng/mL for 3 months and 5–10 ng/mL thereafter) in combination with sirolimus aimed at trough concentrations of 4–8 ng/mL (post-amendment biopsy-confirmed acute rejection rate 10.3% vs 11.1%; ns).[68] Because of initial difficulties in

achieving target trough concentrations, the study protocol was amended to increase the loading doses of both sirolimus and tacrolimus. Tacrolimus elimination resulted in better graft function at 1 year (only for patients receiving therapy and post-amendment: calculated glomerular filtration rate 72.9 mL/min vs 58.4 mL/min; p = 0.03). Patient and graft survival were excellent in both study groups (95.4% vs 95.3%; 90.9% vs 93%, respectively). More patients who were maintained on combination therapy discontinued from the trial (32% vs 22%) but this difference was not significant. Similar to the ciclosporin elimination trials, a selection bias was present in this tacrolimus elimination study because only recipients with a stable graft function who were free from acute rejection and obtained a sirolimus trough concentration of 12–20 ng/mL in the 3 weeks prior to withdrawal were eligible for elimination. Only 33 recipients randomised for elimination fulfilled these criteria, thereby excluding 25% (11/44) of high-risk patients from the analysis.

Several uncontrolled trials have recently focused on combining sirolimus and tacrolimus[69] in high-risk recipients such as African-Americans[70-72] or in order to facilitate early (3 months) withdrawal of corticosteroids.[71] The low rates of acute rejection achieved in these exploratory trials form an encouraging stimulus for studying these applications in controlled settings.

1.5 Sirolimus for Ongoing Acute Rejection ('Rescue'), Sirolimus Conversion Studies and Minimalisation Trials

Experimental animal data demonstrating the capability of sirolimus to reverse ongoing acute allograft rejection[73] preceded similar studies in humans. In one such study, 36 patients experiencing severe acute vascular rejection (grade IIB and III according to the Banff 1993 criteria) refractory to corticosteroid therapy and anti-lymphocyte treatment received either sirolimus (n = 24) or mycophenolate mofetil (n = 12) as rescue added to a baseline ciclosporin regimen.[74] In 96% of patients sirolimus rescue reversed allograft dysfunction compared with 67% in mycophenolate mofetil-treated recipients (p = 0.03) and this was despite more recurrent rejection episodes in the former group. Serum creatinine levels at 12 months was comparable between groups (sirolimus 2.8 mg/dL vs mycophenolate mofetil 3.2 mg/dL) as was graft survival (sirolimus 83% vs mycophenolate mofetil 67%). The results of this uncontrolled study confirm earlier anecdotal reports on the efficacy of sirolimus as rescue therapy for treatment-resistant acute kidney and cardiac graft rejection.[75-77] However, a definite indication for employing sirolimus in this situation cannot be concluded without evidence from controlled studies.

The beneficial effects of avoiding calcineurin inhibitor therapy on renal (allograft) function has triggered many clinical initiatives to evaluate sirolimus in conversion studies in stable recipients with allograft dysfunction because of calcineurin inhibitor nephrotoxicity or chronic allograft nephropathy (CAN). Experimental data show that sirolimus not only inhibits proliferation of smooth muscle, fibroblast and endothelial cells but also reduces intimal hyperplasia and reverses allograft vascular disease in animal models.[7,78,79] Because these chronic vascular changes play an important role in the development of CAN and chronic vasculopathy in other solid organ transplantation, sirolimus might be a key player in the prevention of the latter. Several uncontrolled studies in liver, heart, lung and kidney transplantation have been reported.[80-83] Conversion trials in kidney transplant recipients are relatively small and comprise heterogeneous patient populations with relatively short follow-up post-conversion of approximately 6 months.[84-88] Success, measured as short-term improvement in allograft function, is highly variable in these reports and seems to be the result of calcineurin inhibitor withdrawal in cases of biopsy-proven nephrotoxicity rather than an effect of sirolimus *per se*. True CAN is less responsive to sirolimus conversion or calcineurin inhibitor dose-reduction as was demonstrated in a small randomised study.[89] In this trial, patients with biopsy-confirmed CAN were treated with a 40% ciclosporin dose reduction and either the addition of sirolimus 2mg (n = 16) or not (n = 15). The addition of sirolimus 2mg had little functional, histological or molecular benefit, measured as intra-graft profibrotic gene expression, for recipients with established CAN. Reasons for this poor effect of sirolimus on CAN could have been the administration of too low an anti-proliferative dose of sirolimus, the higher toxic tissue concentrations of

ciclosporin caused by the addition of sirolimus,[90] or the severity of established chronic allograft damage that might have progressed beyond a point where any meaningful intervention was achievable. In favour of the latter hypothesis is the finding that early and complete ciclosporin withdrawal with sirolimus maintenance can prevent chronic allograft damage in 1-year protocol biopsies compared with recipients who continue on the combination of sirolimus and ciclosporin.[91] Similarly, in a cohort of 59 recipients converted from ciclosporin to sirolimus because of chronic allograft dysfunction, Diekmann et al.[92] tried to identify predictors of successful response, i.e. improvement of graft function 12 months after conversion. Pre-existing proteinuria of <800 mg/day at the time of conversion was the only independent predictor of successful outcome amongst other known risk factors, such as recipient age, prior acute rejection, grade of chronic allograft nephropathy and vasculopathy and serum creatinine levels. In order to assess the potential benefits of sirolimus conversion therapy on chronic allograft dysfunction in a prospective and controlled way, a large international multi-centre study of more than 800 patients has just been completed that compared the long-term effects of sirolimus conversion with calcineurin inhibitor dose reduction in recipients with biopsy-confirmed chronic allograft dysfunction. Preliminary results of this trial are expected in early 2005.

There is experimental evidence that sirolimus is advantageous for obtaining donor-specific immunological hyporesponsiveness.[93,94] Recently, this has led to several small uncontrolled clinical trials aimed at the minimisation of maintenance immunosuppression by using strong induction agents such as alemtuzumab (Campath-1H)[95,96] and antithymocyte globulin together with sirolimus.[97] Although none of these studies have yet succeeded in demonstrating graft tolerance or 'probe tolerance' ('near-tolerance'), sirolimus appears to be an agent that could potentially maintain recipients free of clinical rejection in monotherapy. Since long-term data from these trials are currently lacking, no projections can be made on long-term graft function and survival.

2. Safety Profile

2.1 Renal Effects

It is justified, on the basis of current evidence, to classify sirolimus as a mild nephrotoxic compound rather than a non-nephrotoxic agent. Early experimental, mostly animal, data already pointed towards there being undesired renal effects with sirolimus, albeit significantly less than with calcineurin inhibitors.[98-104] Sirolimus does not affect glomerular function[98,100,103] like calcineurin inhibitors but induces histological changes and tubular toxicity.[99,102,104] Tubular atrophy and proximal tubular intra-cytoplasmatic vacuolisations were a common finding in animals and to some extent the vasculopathy present in spontaneously hypertensive rats was accelerated by sirolimus.[99,102,104] More importantly, sirolimus impaired recovery from ischaemia/reperfusion injury in rat models by promoting apoptosis of the proximal tubular cells and reducing the proliferative repair response triggered by renal growth factors through inhibition of protein kinase $p70^{S6k}$.[105,106] When tested together, a synergistic effect of sirolimus on ciclosporin nephrotoxicity could be discerned in rats.[34] As to the cause of this nephrotoxic synergism, several hypothesis were postulated. Coadministration of sirolimus not only augments tissue concentrations of ciclosporin through pharmacokinetic interaction,[16,90] it also precipitates hyperglycaemia, at least in rats, which is a known promoter of fibrosis.[34] On a subcellular basis, the dose-dependent reduction (uncoupling) of oxidative phosphorylation caused by long-term ciclosporin treatment could render renal cells more dependent on anaerobic glycolysis for their energy supply, which in turn is suppressed by the coadministration of sirolimus through the inhibition of mTOR.[107-109]

Irrespective of the exact underlying mechanism, clinical phase II and III studies have confirmed the deleterious effects of sirolimus and ciclosporin on renal allograft function,[25,27-29] especially when standard doses of ciclosporin are administered.[25,27] Equally, the elimination studies have also made it clear that early withdrawal of ciclosporin from this combination results in long-term improvement of graft function in low risk recipients[40-42] and has

favourable effects on graft histology.[44,91] Finally, calcineurin inhibitor-free transplantation with sirolimus seems to be beneficial in terms of graft function, at least in one controlled trial.[22] Whether the combination of sirolimus with tacrolimus is equally nephrotoxic as with ciclosporin cannot be concluded from the current short-term studies.[59,63,64,66,67] Interestingly, reduction of tacrolimus dose does not appear to ameliorate graft function,[64,66,67] except in one report[63] and this raises questions as to the underlying mechanism responsible for the nephrotoxic effect of this combination. Experimental animal data suggest that the combination of sirolimus and tacrolimus produces less reduction in glomerular filtration rate and no significant increase in semi-quantitative kidney fibrosis scores compared with sirolimus in combination with ciclosporin.[110] Reports of severe acute renal graft failure after exposure to sirolimus and tacrolimus[111] put these animal data in perspective and warrant persistent vigilance for the nephrotoxic effects of this combination.

In a recent large study,[112] the incidence of DGF was significantly higher in patients treated with sirolimus than without and positively correlated with the initial sirolimus dose. More so, renal biopsies from patients with DGF receiving sirolimus together with tacrolimus showed extensive intratubular eosinophilic casts composed of degenerating renal tubular epithelial cells.[112] Biopsies performed early in the first 2 weeks post-transplantation revealed tubular injury consistent with acute tubular necrosis, and only in later biopsies did prominent features of intra-tubular cast formation develop. Interestingly, withdrawal of sirolimus and tacrolimus was followed by histological resolution of the cast nephropathy and coincided with clinical recuperation. This chain of events concurs with previous animal data[105,106] indicating sirolimus-enhanced tubular cell death, reduced epithelial regeneration and possibly altered clearance of apoptotic cells in renal grafts that sustain ischaemia-reperfusion injury. This finding has important implications as sirolimus has long been considered to be the agent of choice in recipients with DGF, allowing late introduction of calcineurin inhibitors while keeping acute allograft rejection under control.[49,50,74] In a retrospective multivariate analysis of clinical risk factors for prolonged DGF in 132 renal recipients, sirolimus was identified as being independently and negatively associated with time to graft function.[113] In the latter study calcineurin inhibitors were withheld until resolution of DGF and could not have played a role in prolonging DGF. In the first calcineurin inhibitor-free comparative studies[17,21,22] no higher incidence of DGF was observed, probably because of the selection of low risk recipients. Indeed, recipients of suboptimal kidney grafts receiving sirolimus therapy and a low dose ciclosporin had a similar incidence of DGF as patients treated with ciclosporin and mycophenolate mofetil (52.4% vs 58.3%) but the recovery from DGF was significantly prolonged by the use of sirolimus (19 ± 6 vs 10.3 ± 3.2 days; $p = 0.001$).[114] Interestingly, graft survival and function at 1 year post-transplantation was not affected by this initial delayed recovery from DGF.[114] African-American and non-African-American recipients with high immunological risk and treated with sirolimus in combination with a low-dose of tacrolimus, experienced significantly more DGF than patients on a sirolimus-based calcineurin inhibitor-free regimen (63% vs 34%; $p = 0.04$) and displayed a significantly worse 1-year graft function (52.9 ± 22.8 mL/min vs 72.4 mL/min ± 20 mL/min; $p = 0.03$).[72] Therefore, the temporal association of severe ischaemic allograft injury with high doses of a powerful anti-proliferative drug might not be ideal. Whether the use of a lower (and thus less anti-proliferative) dose of sirolimus without loading could ameliorate these effects remains to be determined.

Sirolimus causes tubular dysfunction resulting in early hypokalaemia[23,40,47] and hypophosphataemia.[47] The incidence of hypokalaemia varies from 8% to 27% in sirolimus-treated patients[23,40,47,115,116] and is characterised by an increased urinary potassium excretion in the presence of hypokalaemia with an elevated transtubular potassium gradient.[115] The exact tubular (secretory) mechanism by which renal potassium wasting takes place has not yet been elucidated, but other signs of tubular dysfunction are usually absent. Only two patients with sirolimus-induced hypokalaemia and simultaneous aminoaciduria were reported.[115] Sirolimus-induced hypokalaemia occurs more frequently during the initial 3 months post-transplantation

and is easily corrected by potassium supplementation. From 2 to 3 years post-transplantation, 10% to 15% of recipients receiving sirolimus remain hypokalaemic.[40,47] More importantly, clinical adverse effects that have arisen from sirolimus-associated hypokalaemia have not yet been described. Post-transplant hypophosphataemia is a relatively common problem of multi-factorial origin that tends to improve spontaneously with time, provided that adequate renal allograft function is attained.[117] Patients receiving sirolimus therapy are characterised by a decreased maximum tubular capacity for phosphate per unit volume of glomerular filtrate (TmP/GFR), resulting in a prolonged renal phosphate leak and consequently low serum phosphate concentrations, extending beyond 3 months post-transplantation.[47] No clinical consequences of prolonged sirolimus-induced renal phosphate leak have been reported to date. Contrary to sirolimus-induced hypokalaemia, oral phosphate supplementation should be avoided because of the risk of parathyroid stimulation and $1,25(OH)_2D$ suppression.[118] In contrast to calcineurin inhibitors, sirolimus does not cause hyperuricaemia or hypomagnesaemia as was repeatedly shown in phase III comparative clinical trials.[17,21,23]

In 50 recipients with chronic allograft nephropathy that were taken off calcineurin inhibitors and switched to sirolimus therapy, 64% developed marked proteinuria with nephrotic syndrome occurring in more than half of these patients.[87] Smaller anecdotal reports described similar findings.[85,119] Calcineurin inhibitor-induced vasoconstriction and reduced renal blood flow may of course obscure proteinuria in those patients with pre-existing chronic allograft nephropathy.[120] Renal biopsies performed after switch because of the development of proteinuria, revealed histological changes characteristic of focal segmental glomerular sclerosis in 5 of 15 biopsy cases.[87] Recurrence of the original renal disease as an explanation for this finding was ruled out in the five patients. Whether these focal segmental glomerular sclerosis lesions are part of the natural history of chronic allograft nephropathy, as was previously shown,[121] or are sirolimus induced, remains to be determined. The fact that in none of the randomised phase II and III trials excess *de novo* proteinuria was reported in sirolimus-treated recipients, makes the latter unlikely. However, although recent reports revealing a proinflammatory effect of sirolimus in experimental proliferative glomerulonephritis[122,123] contradict earlier findings of inhibition of mesangial cell proliferation by sirolimus, these clinical observations do not rule out sirolimus as the cause of primary glomerular changes and disease in transplanted kidneys, as well as in native kidneys treated with sirolimus for chronic glomerulopathies.[124] In fact, four cases of sirolimus-associated *de novo* and recurrent primary glomerulonephritis in transplanted kidneys have been documented recently by Dittrich et al.[125] In this study, report biopsies performed before and after the introduction of sirolimus revealed new onset histological glomerular lesions, thereby confirming a causal relationship and subsequent amelioration of proteinuria after reconversion back to calcineurin inhibitor treatment.

In conclusion, it is correct to describe sirolimus as an immunosuppressive compound with mild-to-moderate nephrotoxic properties compared with calcineurin inhibitors. The direct effects of sirolimus on the transplanted kidney justify a certain degree of caution when using this drug as induction therapy for recipients at risk for DGF or when applying sirolimus as switch therapy for patients with chronic allograft nephropathy. In combination with ciclosporin or tacrolimus, even in reduced dose, nephrotoxicity is to be expected, mainly as a result of the concomitant calcineurin inhibitor.

2.2 Lipids, Metabolic and Endocrine Effects

2.2.1 Hypercholesterolaemia and Hypertriglyceridaemia

Sirolimus induces dose-dependent reversible hyperlipidaemia necessitating the use of concomitant lipid-lowering medication,[17,21,25,27,43] especially in recipients with pre-existing hyperlipidaemia.[126] Sirolimus-associated hypertriglyceridaemia develops particularly in patients with pre-existing hyperbetalipoproteinaemia through expansion of the very low-density lipoprotein-apolipoprotein B100 (VLDL-apoB100) pool, not by increased synthesis but rather by inhibition of the fractional catabolic rate for VLDL-apoB100.[127] Interestingly, in recipients that remain normo-

triglyceridaemic during sirolimus treatment, both the synthesis rate and the catabolic rate for VLDL-apoB100 and intermediate density lipoprotein (IDL)-apoB100 are increased.[127] Hypercholesterolaemia in sirolimus-treated patients is mainly caused by reduced catabolism of low-density lipoprotein (LDL)-apoB100 resulting in elevated LDL-apoB100 concentrations.[127] However, direct conversion of VLDL-apoB100 to LDL-apoB100 is also augmented by sirolimus.[127] Transplant recipients with hyperlipidaemia are characterised by a strongly reduced baseline lipoprotein lipase activity. Sirolimus treatment has a variable effect on this reduced lipoprotein lipase activity[127] and one of the determinants of the latter is the level of *apo CIII* gene expression, a known endogenous inhibitor of lipoprotein lipase.[128] An attractive hypothesis to explain the effect of sirolimus on lipoprotein lipase activity involves the peroxisome proliferator activated receptor (PPAR)-α/9-*cis*-retinoid acid receptor (RXR) heterodimer that is able to downregulate *apo CIII* gene transcription. Ultimately, sirolimus could determine *apo CIII* gene transcription by blocking heterodimer formation trough inhibition of cytochrome P450 (CYP) 3A-mediated conversion of *trans*-retinoic acid into 9-*cis*-retinoic acid.[128] Blood concentrations of apo CII, an activator of lipoprotein lipase, stay unchanged during sirolimus treatment.[129] Compared with calcineurin inhibitors, the highest apo CIII and triglyceride concentrations were observed in sirolimus-treated recipients.[128] The free fatty acid pool is expanded by 42% with sirolimus, leading to an increased hepatic synthesis of triglycerides through the hormone sensitive lipase.[129] Whether this sirolimus-induced effect occurs via inhibition of the insulin-dependent pathway and release of free fatty acids into the circulation or as the result of decreased oxidation of free fatty acids is not yet determined.[129] Based on these findings, the use of fibrates that enhance triglyceride-rich lipoprotein clearance may therefore be the preferred therapy for patients with hypertriglyceridaemia receiving sirolimus treatment. On the contrary, patients with hypercholesterolaemia might benefit more from treatment with HMG CoA-reductase inhibitors because of an increased catabolism of LDL-apoB100 obtained by these agents.

Dyslipidaemia associated with sirolimus is a serious clinical problem that was initially underestimated in renal recipients.[130] When a cholesterol value of >240 mg/dL is used as a cut-off point for defining hypercholesterolaemia, the incidence increases from 11% pre-transplantation to 60–80% in the first 2 years in recipients treated with sirolimus in combination with ciclosporin and decreases to around 50% at 4 years.[126] Similarly, the incidence of hypertriglyceridaemia defined as an absolute value of >200 mg/dL increases from 32% pre-transplantation to 78% between 6 and 12 months and remains at 70% thereafter.[126] Expectedly, the use of statins and fibrates or fish oil to lower serum cholesterol or triglycerides is the highest in the first year post-transplantation (49% and 60%, respectively) and decreases slightly thereafter (40% and 50%, respectively).[126] The clinical evolution, whereby dyslipidaemia is worst during the first postoperative year in sirolimus-treated recipients while ameliorating thereafter but remaining clearly elevated compared with pre-transplant conditions, is determined by multiple factors such as progressive dose lowering of sirolimus, concomitant calcineurin inhibitors and corticosteroids, dietary modifications, exercise and the use of lipid-lowering drugs. Blum[131] estimated the cardiovascular risk associated with sirolimus therapy, analysing data from 1295 pooled patients extracted from the combined phase III randomised trials and applying the Framingham risk model. The use of sirolimus 2 and 5 mg/day was associated with a significantly higher cholesterol (17 and 30 mg/dL, respectively) and triglyceride (59 and 103 mg/dL, respectively) level compared with placebo or azathioprine.[131] The hypercholesterolaemia associated with sirolimus 2 and 5 mg/day would result, according to the model, in 0.7 and 1.2 additional cardiovascular deaths per 1000 patients per year, respectively.[131] However, this rather favourable risk assessment has to be interpreted cautiously since the applied Framingham model does not take into account additional cardiovascular risk factors typically related to transplantation.[132] Moreover, although relatively short-term follow-up data do not reveal an excess cardiovascular morbidity and mortality in sirolimus-treated hyperlipidaemic patients,[17,21,25,27,40,43] it is not yet possible to determine the ultimate clinical balance of

sirolimus-associated dyslipidaemia on the one hand and the postulated beneficial effects on vascular smooth muscle cell proliferation, chronic rejection, arterial blood pressure and apparent lack of increased thrombogenicity – at least in renal transplants[133] – on the other hand. Another important factor of which the impact has yet to be evaluated is the obvious learning curve for the dose adjustment of the drug in different immunosuppressive combinations, leading to better characterisation of the optimal target blood concentrations and guaranteeing adequate efficacy while avoiding over-exposure and adverse effects. Initial trials examining calcineurin inhibitor-free use of sirolimus demonstrated a significantly higher incidence of dyslipidaemia or use of lipid lowering medication, reflecting the high target trough concentrations.[17,21] Subsequent studies, aimed at lower sirolimus trough concentrations, still showed significant increases in cholesterol and triglyceride levels postgrafting but could not demonstrate any differences in the lipid profiles or use of lipid lowering medication between sirolimus- and ciclosporin-treated patients.[22,40]

Despite initial reports in renal and liver transplantation, suggesting that the combination of sirolimus and tacrolimus causes less hyperlipidaemia[134,135] compared with the combination with ciclosporin, not all short-term results of randomised trials seem to support this hypothesis.[59,63] Cianco et al.[66,67] not only showed that triglyceride and cholesterol levels were significantly lower during the first 6 months in patients treated with sirolimus in combination with tacrolimus but, more importantly, that lipid lowering therapy was required significantly less than for patients receiving sirolimus and ciclosporin (at 1 year 54% vs 80%; $p < 0.00001$).

Dyslipidaemia associated with sirolimus is a dose-dependent phenomena that clearly evolves as time elapses post-transplantation and tends to improve, partially because of dose reductions and the increased use of lipid lowering drugs. Clinical vigilance is mandatory as to whether the supposed beneficial vascular effects of sirolimus are strong enough to outweigh the negative cardiovascular risk associated with hyperlipidaemia.

2.2.2 Diabetes Mellitus

Despite contradicting data from animal and *in vitro* experiments showing a potentially diabetogenic effect of sirolimus[136] and the calcineurin inhibitor-like effects of this agent on the adenosine triphosphate (ATP)-dependent potassium channel-mediated insulin release from pancreatic β-cells,[137] it is clear from the phase II and III trials, the ciclosporin elimination studies and the calcineurin inhibitor-free trials that sirolimus has no negative influence on the clinical occurrence of post-transplantation diabetes mellitus (PTDM).[17,21,22,25,27,28,40-42] This lack of diabetogenic effect is an advantage in terms of diabetes-related cardiovascular risks and constitutes a potential strategy for attempting to reduce the incidence of this serious complication by allowing dose reductions of the concomitant calcineurin inhibitors.

Interestingly, Ciancio et al.[66,67] demonstrated a substantially higher incidence of PTDM in sirolimus and ciclosporin-treated recipients compared with patients receiving the sirolimus and tacrolimus combination (33% vs 17%; $p = 0.06$). Despite confounding factors such as ethnicity, corticosteroid dose, cross-over and discontinuation rate potentially influencing this discrepant finding, the most likely explanation lies in the initially lower tacrolimus trough concentrations (<10 ng/mL) and higher (standard) ciclosporin concentrations (225–175 ng/mL). Whether in humans a low dose sirolimus and tacrolimus could potentially prevent β-cell insulitis and subsequent diabetes through alteration of immunoregulatory cytokines, as was demonstrated in non-obese diabetic mice,[138] is an intriguing question that deserves further investigation. Although controlled trials are presently lacking, sirolimus could be considered as an alternative immunosuppressive therapy in renal transplant candidates at risk for developing PTDM. Pre-transplantation identification of risk factors such as age, obesity, impaired glucose tolerance, ethnicity, acute rejection probability and hepatitis C status could be used as clinical guidance for directing induction therapy.[139]

2.2.3 Fertility and Pregnancy

Sirolimus-induced oligospermia and infertility occurred in a young male who had successfully produced offspring prior to the initiation of sirolimus therapy.[140] Sperm analysis revealed dramatic

diminution of sperm count and motility. Replacement of sirolimus by tacrolimus was followed by the normalisation of sperm quality 6 months later. That sirolimus can affect testicular function, as previous reports of testicular atrophy in rats suggest,[141] was further demonstrated in a follow-up study of 28 male recipients.[142] Testosterone levels were significantly lower in sirolimus patients and more recipients had abnormally low hormone levels. Gonadotropin levels (follicle stimulating hormone and luteinising hormone) were significantly elevated, as expected, while prolactine levels remained normal. Similar hormonal alterations were reported in a pair-matched analysis of 66 cardiac patients receiving sirolimus therapy and gonadotropin levels correlated with sirolimus trough concentrations.[143] From its mechanism of action, it is conceivable that sirolimus blocks the early stages of spermatogenesis in the seminiferous tubules by interrupting the crucial stem cell factor/c-Kit system through disruption of PI 3 kinase-binding to cKit.[144] Whether these hormonal changes obligatory lead to male infertility or accelerated osteoporosis cannot be concluded, but attention has to be paid to sexual dysfunction, infertility and unexpected bone loss occurring in male patients receiving sirolimus therapy. Female diabetic recipients of allogeneic islets who were treated with sirolimus and tacrolimus developed more frequent menstrual cycle alterations and benign ovarian cysts after transplantation, possibly as a result of sirolimus-mediated inhibition of ovarian progesterone receptors. However, if similar changes occur in female renal recipients on sirolimus monotherapy is currently not known.[145]

With regard to pregnancy, consensus guidelines judge sirolimus as contraindicated in pregnancy.[146] Only one successful pregnancy after exposure to sirolimus at conception has been reported to the National Transplantation Pregnancy Registry (NTPR).[147] A healthy infant with no structural malformations was delivered after 36 weeks gestation. A formal contraindication for pregnancy and breast feeding is maintained for all sirolimus-treated recipients until more information becomes available.

2.2.4 Lymphoceles and Wound Healing Problems

The anti-proliferative properties of sirolimus are associated with adverse effects in transplant recipients of which wound healing problems and lymphoceles are the most common. Indeed, compared with ciclosporin and tacrolimus, the incidence of postoperative lymphoceles is significantly increased in sirolimus-treated patients.[25,66,67] A retrospective analysis clearly showed that perinephric fluid collections occurred more frequently in the group of recipients treated with sirolimus compared with the ciclosporin group (38.1% vs 17.6%; $p < 0.001$).[148] Moreover, sirolimus-treated patients required more therapeutic interventions for their lymphocele and more invasive surgical procedures compared with patients receiving ciclosporin.[148,149] Interestingly, especially in ciclosporin treated subjects, more acute rejection episodes preceded the development of lymphoceles, which confirmed earlier reports on the association between these two events.[150] In contrast to previous studies,[133] lymphoceles were not found to be associated with a higher incidence of thromboembolic events.[148]

Recent experimental[151] and clinical[152] reports describing lethal airway anastomotic dehiscence immediately after lung transplantation in recipients treated with sirolimus (together with HMG-CoA reductase inhibitors[153]), have directed attention back to this relatively infrequent but serious complication. The capability of sirolimus to inhibit growth factor-driven proliferation of various cell types involved in wound repair[154,155] is responsible for clinical wound problems, especially when additional risk factors for impaired wound healing such as obesity, diabetes, infection, rejection and older age, are simultaneously present.[156] Wound healing problems after renal transplantation are more frequently reported with mycophenolate mofetil[156] and sirolimus[157,158] next to other known risk factors such as re-operation, increasing recipient age and obesity.[156] Nevertheless, the overall incidence of fascial dehiscence or wound hernia remains relatively low (3.6% without sirolimus[156] and 5.6% with sirolimus[40]). A recent retrospective direct comparison between mycophenolate mofetil and sirolimus clearly pointed out that the use of sirolimus was associated with a higher incidence of lymphoceles, non-lymphocele perinephric fluid collections and poor wound healing.[158] At the same time, a retrospective study of 513 renal recipients did not show an increased rate of wound healing problems when

sirolimus in combination with mycophenolate mofetil was compared with ciclosporin-based regimens containing either mycophenolate mofetil or azathioprine.[159] Consistently, body weight (body mass index [BMI] >30) and DGF were the most important determinants of impaired wound healing. Finally, a prospective comparison between sirolimus with mycophenolate mofetil versus tacrolimus with mycophenolate mofetil demonstrated significantly more wound healing problems in sirolimus-treated recipients (47% vs 8%; $p < 0.0001$) and these differences remained significant even after correction for BMI and sirolimus blood concentrations.[160]

2.3 Infections and Pulmonary Complications

Sirolimus treatment, like any immunosuppressive drug, carries a risk for various types of infectious complications.[161] In some instances, sirolimus solicits a specific susceptibility for particular infectious agents in recipients. For example, initial animal experiments have shown a provocative effect of sirolimus on *Pneumocystis carinii* pneumonitis (PCP), in contrast to the protective effects of mycophenolate mofetil.[162] This experimental effect was later confirmed in human clinical trials, both in *de novo* transplant recipients and chronic patients who switched to sirolimus.[28,84] The higher incidence of PCP in sirolimus-treated recipients has led to the generally accepted recommendations for PCP-prophylaxis with co-trimoxazole (trimethoprim/sulfamethoxazole) during the first post-transplant year.[163] Other *ex vivo* experiments demonstrated a strong inhibition of IL-10 gene transcription in human peripheral mononuclear cells pre-treated with sirolimus in response to various bacterial products.[164] Also, neutrophil chemotaxis and chemokinesis induced by granulocyte-macrophage colony-stimulating factor (GM-CSF) was inhibited by sirolimus through blocking phosphorylation of ribosomal $p70^{S6K}$,[165] while neutrophil oxidative bursts were reduced,[166] indicating a potential increased risk for bacterial sepsis. Indeed, in both calcineurin inhibitor-free and combination protocols using sirolimus, a higher incidence of sepsis and bacterial infections was reported compared with ciclosporin; however, these differences did not reach statistical significance.[21,25] In primary cardiac transplant recipients a significantly higher rate of bacterial infections was noted.[167]

On the other hand, the documented anti-infectious properties of sirolimus have not yet resulted in clear advantages in clinical practice. Sirolimus bound to FKBP-12 is capable of inhibiting the Target of rapamycin 1 (TOR1) enzyme in yeast, explaining the anti-fungal properties of the drug against *Candida albicans*, *Cryptococcus neoformans* and other pathogenic fungi.[168] However, no reduced incidence of fungal infections with sirolimus has been reported in controlled trials to date. Oral *Herpes simplex* virus (HSV) infections are more frequently reported in sirolimus-treated patients, but no reason for this increased susceptibility has been found.[21,25,27] It is possible that these oral mucosal ulcers are in fact not HSV infections but are a direct adverse effect of sirolimus because in >80% of presumed HSV infections in these studies, the diagnosis was not confirmed by viral culture or biopsy.[27] Human herpesvirus-6 (HHV-6) seroconversion was also significantly more frequent in patients receiving sirolimus therapy[169] while cytomegalovirus or fungal infections, which are often facilitated through immunomodulation by HHV-6 seroconversion, do not occur more frequently in patients treated with sirolimus.[17,169,170]

Pneumonia constitutes a more serious and potentially fatal infectious complication of sirolimus and various aetiological infectious agents have been identified.[21,23,28,84,87] Since the worldwide acceptance of antimicrobial prophylaxis it appears that PCP is no longer a major cause of lung infections with sirolimus therapy,[17,21-23] even in patients at risk.[171] Morelon and the US FDA[172,173] reported the occurrence of a severe type of pneumonitis in patients treated with sirolimus without any evidence of an infectious cause. More recently, other anecdotal reports have described similar cases of sirolimus-associated pneumonitis in other types of solid organ transplantation including renal, liver, heart, lung and islets grafts.[174-179] Sirolimus-associated pneumonitis is characterised by bilateral alveolo-interstitial lung infiltrates accompanied by clinical symptoms of dyspnea on exertion, dry cough, fatigue, fever and signs of lymphocytic alveolitis (mainly CD4 type cells) or intra-alveolar haemorrhage in the bronchoalveolar fluid, without evidence of infec-

tion.[180,181] Histological features encountered in affected patients include bronchiolitis obliterans with organising pneumonia, lymphocytic interstitial pneumonitis, non-necrotising macrophagic granuloma and intra-alveolar haemorrhage.[172,173,180] Withdrawal of sirolimus or dose reduction leads to rapid clinical and radiological improvement and complete resolution within 3 months, suggesting dose dependency.[172,173,175,180] Some degree of skepticism is permitted as to the validity of the initial diagnostic criteria put forward by Morelon et al.[180] for defining sirolimus-associated pneumonia. It remains difficult to assess whether patients whose treatment for an infectious pneumonia fails also have underlying sirolimus-associated pneumonitis that can aggravate their pulmonary defense. Therefore, until more is known about the exact pathophysiology of this drug-induced pneumonitis, caution is advised, especially when a transplant patient is not sufficiently recovering despite adequate anti-infectious therapy. Prompt interruption of sirolimus treatment is warranted together with rapid invasive diagnostic re-evaluation. Lastly, other immunosuppressive drugs such as mycophenolate mofetil have to be considered as a possible cause of non-infectious pneumonitis[182,183] if discontinuation of sirolimus does not result in improvement of respiratory signs.

Epistaxis of unknown origin has been reported in several comparative trials involving 6–9% of *de novo* recipients[25,27] as well as in sirolimus conversion studies.[85] Whether these nose bleeds in sirolimus-treated patients are because of underlying infection of the nasal mucosa by viruses or opportunistic microbial agents is not known.

2.4 Bone Marrow

One of the most frequently reported adverse effects of sirolimus is probably thrombocytopenia, and it is by far the most benign of all drug-related adverse events. Thrombocytopenia (platelet count <150 x 10^3 cells/mm) occurs in 37–45% of sirolimus[17,21] and 9–23% of sirolimus/ciclosporin-treated recipients.[25,27] Usually within the first 4 weeks of treatment, the platelet count reaches its lowest point and resolves spontaneously in the majority of cases. Eleven percent of recipients require dose reduction or temporary suspension of sirolimus treatment because of persistent thrombocytopenia followed by a resolution of toxicity within 1–57 days.[184] The incidence, but not the severity, of thrombocytopenia correlates with sirolimus trough blood concentrations[19,20,184] and this self-limiting adverse effect disappears over time. Thrombocytopenia is often accompanied by a similarly benign degree of leukopenia (white blood cell count <5000 cells/mm) that also tends to resolve spontaneously as time elapses. One study[184] found that 63% of patients with thrombocytopenia developed leukopenia. It is important to note that permanent withdrawal of sirolimus therapy because of thrombocytopenia or leukopenia and clinical events occurring as a result of these adverse effects are extremely rare. Two hypotheses have been formulated regarding the mechanism by which sirolimus reduces platelet and white blood cell counts.[185,186] *In vitro* experiments have demonstrated an enhanced, dose-dependent, agonist-induced (adenosine diphosphate) platelet aggregation and granule secretion following sirolimus exposure,[185] while others have shown that sirolimus inhibits signal transduction via the gp130β chain shared by cytokine receptors for IL-11, granulocyte colony stimulating factor and erythropoetin, which are necessary for the production of platelets, leukocytes and erythrocytes.[186] Blocking the proliferative response of bone marrow cell lines and colony-forming cells to a variety of haematopoietic growth factors is probably also the pathway by which sirolimus induces anaemia. Although the incidence of anaemia at 12 months was numerically higher in studies comparing sirolimus plus azathioprine[21] (37% vs 24%) or sirolimus plus mycophenolate mofetil[17] (43% vs 29%) with ciclosporin-based regimens comprising either of both anti-metabolites, the differences were not significant. In trials in which sirolimus was associated with ciclosporin and compared with ciclosporin alone or in combination with azathioprine, anaemia occurred significantly more frequently in sirolimus-treated recipients, particularly in the 5mg dose groups, and not only immediately after transplantation but also at 1 year.[25,27,187] Direct comparison of sirolimus with mycophenolate mofetil, both in combination with calcineurin inhibitors, revealed that sirolimus was clearly associated with a higher prevalence of anaemia (57% vs 31%, $p < 0.001$) at 12 months post-transplanta-

tion.[188] None of the randomised controlled trials provided information about the incidence of anaemia stratified according to allograft function, nor about the use of erythropoietin. Since anaemia appears to be more frequent and severe in chronic transplant patients who switch to sirolimus therapy because of allograft dysfunction,[85,87,89] more attention should be paid to this high-risk group. In one of the latter switch trials, 17 of 22 patients were started on erythropoietin because of severe anaemia occurring after conversion to sirolimus-based therapy.[85] Associating sirolimus with other myelosuppressive immunosuppressive drugs might, therefore, not be the optimal choice for recipients with moderate or severe allograft dysfunction.

Replacing calcineurin inhibitor therapy with sirolimus for renal recipients with post-transplantation thrombotic microangiopathy (TMA) often results in complete remission of haemolytic signs and the recovery of graft function.[189-192] However, the development of TMA in patients receiving a combination of sirolimus and ciclosporin is well documented from the phase III trials[193] and case reports[194,195] and TMA was recently also associated with the coadministration of sirolimus and tacrolimus.[196] One possible explanation for this phenomenon is that calcineurin inhibitor-induced endothelial damage[197] worsens with higher intra-renal ciclosporin concentrations caused by the addition of sirolimus.[16] On the other hand, platelet aggregation is activated by sirolimus itself,[185] which can maintain pre-existing TMA. Sirolimus alone does not promote necrosis of endothelial cells *in vitro*, but when added to ciclosporin the pro-necrotic effects of the latter are further enhanced, while the anti-angiogenic and anti-proliferative effects of sirolimus can impair subsequent recovery from TMA-induced vascular endothelial injury.[198] It is, therefore, advisable to use sirolimus in case of TMA as an alternative for calcineurin inhibitors rather than adding it to a reduced dose of the latter. A retrospective analysis of a large renal transplant patient database, the United States Renal Data System (USRDS), identified amongst other risk factors, the immediate postoperative use of sirolimus as an independent risk factor for the development of *de novo* TMA.[199] Because TMA is usually the result of several coinciding risk factors, these retrospective data have to be interpreted cautiously as other evident risk factors for TMA were not included in this multivariate analysis. Nevertheless, a recent case report describing TMA occurring in a recipient on sirolimus monotherapy illustrates that alertness for this serious complication is mandatory.[200]

2.5 Gastrointestinal Effects

Sirolimus causes diarrhoea in 16–38% of recipients, particularly early after transplantation when trough blood concentrations are high[17,25,27,41] and early after conversion.[88] As time elapses, non-febrile diarrhoea becomes less of a clinical problem with sirolimus therapy. The real long-term incidence of diarrhoea associated with sirolimus is difficult to assess since treatment-emergent diarrhoea is often reported in controlled trials as adverse events without employing pre-defined diagnostic criteria. A more persistent gastrointestinal adverse effect of sirolimus is liver function disturbances, ranging between 7% and 16.3%, in situations where sirolimus is used alone or in combination with calcineurin inhibitors.[21,40,41,43] Data from animal experiments suggest that tacrolimus is probably the preferred calcineurin inhibitor to combine with sirolimus in cholestatic patients as the latter combination only marginally reduces bile flow and does not change biliary bile salt and cholesterol excretion, in contrast to sirolimus and ciclosporin.[201] Cholestasis secondary to sirolimus is accompanied by reduced expression of canalicular transport proteins (Mrp2) and hepatic CYP while sinusoidal transport proteins are upregulated (Oatp2), possibly leading to an accumulation of toxic drug metabolites in the hepatocytes.[202] Whether the dose-dependent anti-proliferative effects of sirolimus on hepatocytes[203] contributes to the drugs hepatotoxicity remains unknown. In this context a remarkable but yet unexplained finding in sirolimus-treated recipients is elevated lactate dehydrogenase (LDH) blood levels of unspecified origin.[17,204,205] Whether these LDH enzymes are derived from renal, bone marrow or hepatic tissue is not clear, although the latter seems less likely because in the majority of cases the isolated increase in LDH level is not accompanied by other liver function test disturbances.

Ileus and internal haemorrhoids have also been reported more frequently in sirolimus-treated

patients but without any lasting deleterious effects.[23,40]

Finally an interesting observation is the occurrence of aphthous mouth ulcers in 10–19% of patients receiving high-dose sirolimus (5mg) therapy as observed in the phase III controlled trials.[25,27] Especially following replacement of chronic calcineurin inhibitor therapy by sirolimus, these lesions are observed more frequently (up to 47%) in the early weeks after switch,[84,85,87,206] similar to non-renal transplant[207] and non-transplant patients.[208] It usually involves superficial ulcerations of the gingival and buccal mucosa and tongue without any evidence of viral infection. The true incidence of this adverse effect might actually be underestimated as these mucosal lesions can easily be mistaken for HSV infections,[27] especially when no viral culture or biopsy is performed (see section 2.3). In addition, other immunosuppressive drugs might also provoke mucosal ulcerations.[209] As to the cause of these lesions, many hypotheses have been forwarded without clear evidence. The strong anti-proliferative properties of sirolimus might function as the primary trigger for the development of these ulcers or hamper their secondary healing.[206] Toxicity caused by specific compounds in the oral emulsion formulation in contrast to tablets have also been suggested as a possible cause.[210]

2.6 Malignancies

Malignancies are the third leading cause of death in kidney transplant recipients with a functioning graft[211] and therefore of major concern when testing new drug combinations. Increasingly, evidence points towards important anti-tumour effects of sirolimus.[2,10,11] In a complex transplant tumour murine model of allogeneic cardiac transplantation and simultaneous inoculation of adenocarcinoma or melanoma, sirolimus was capable of effectively preventing graft rejection and at the same time inhibiting tumour growth.[212] Conversely, in the clinical phase II trials, no excess incidence of malignancies could be discerned in sirolimus-treated recipients.[25,27] Furthermore, the analysis of the 3 year data of the largest of the elimination trials revealed that the total incidence of malignancies was lower in patients who eliminated ciclosporin and were maintained on sirolimus therapy (5.6% vs 11.2%; $p = 0.054$), mainly because of a lower incidence of skin cancer.[40] Analysis of the combined 2-year results of 5 phase II/III multi-centre studies, comprising a total of 1295 randomised patients, indicated that the overall incidence of malignancy in patients receiving combined sirolimus/ciclosporin therapy was similar to standard therapy (ciclosporin with or without azathioprine or mycophenolate mofetil) while early elimination of ciclosporin tended to reduce the risk of malignancies.[213]

A first clinical case report describing successful treatment of post-transplantation Kaposi's sarcoma by converting two renal recipients from ciclosporin to sirolimus maintenance therapy,[214] indirectly illustrates that clinicians are already exploring the anti-tumour effects of sirolimus in uncontrolled trials.

2.7 Musculoskeletal, Skin and Connective Tissues

Despite the short-term beneficial effects of sirolimus on trabecular bone resorption in animals,[215] prolonged sirolimus administration leads to increased osteoclastic activity[216] and reduced serum $1,25(OH)_2D$[215] concentrations, that may adversely affect bone mineral metabolism in the long term. Among the risk factors for early loss of bone mineral density after transplantation are glucocorticosteroid treatment, alcohol consumption[217] and calcineurin inhibitor use, which is responsible for high-turnover osteoporosis.[218] Whether the combination of a low-dose ciclosporin with sirolimus is bone sparing in humans, as was shown in animals,[219] remains to be determined. Pain in weight-bearing bones and joints, suggestive of algodystrophy, were repeatedly observed with sirolimus, predominantly in switch studies.[27,87-89] The exact cause of this pain is not clear and its occurrence has also been reported with calcineurin inhibitors.[220,221] Avascular necrosis in two patients treated with sirolimus in combination with glucocorticosteroids and ciclosporin was recently described.[222]

A first case of sirolimus myopathy was reported in a patient who was switched from ciclosporin and shortly afterwards developed muscle pain and weakness, severe cramps and elevation of creatine kinase and LDH activity.[223] The myopathy resolved rapidly and completely after the withdrawal of sirolimus,

thereby suggesting a causal relationship. Other drugs (e.g. statins) potentially causing or aggravating myopathy were ruled out.

Sirolimus-associated oedema of the lower and upper limbs is not rare[87-89,224] and is possibly caused by some type of capillary leak as previously observed in psoriatic patients and lung transplant recipients treated with sirolimus.[225,226] This drug-induced oedema is sometimes painful and inflammatory[87] and often resistant to diuretic therapy. Sirolimus promotes prostacyclin release from endothelial cells and the resulting vasodilatation can facilitate the formation of oedema.[227] Similarly, uni- or bilateral eyelid oedema has been observed, both in *de novo* renal transplant recipients and chronic patients converted to sirolimus.[86,228] Angioedema related to sirolimus was recently reported in three African-American recipients.[229] These patients developed not only facial oedema after initial (n = 1) and re-exposure (n = 2) to sirolimus but had diffuse swelling of the mouth, tongue and epiglottis, necessitating prompt discontinuation of the drug and administration of high-dose corticosteroids and histamine receptor blockers.[229] Whether the angioedema is caused by complement- or bradykinin-driven processes or is antibody mediated, is not known.

Finally, cutaneous lesions caused by sirolimus are not infrequent; pustular eruptions on the scalp, face and trunk have been described,[87] as well as nonspecific rash[27] and acne.[87] Usually, male recipients are affected and lesions disappear spontaneously within 4–6 weeks without the need for specific therapeutic interventions.

Two cases of cutaneous leukocytoclastic vasculitis have been reported: one in a lung recipient and one in a renal transplant patient.[230,231] In both cases, the discontinuation of sirolimus was accompanied by complete resolution of skin lesions and in the former patient, re-challenge with the drug confirmed a causal relationship.

3. Conclusions

Sirolimus is an immunosuppressive drug capable of preventing acute renal allograft rejection, both alone and in combination with calcineurin inhibitors. Permanent association of sirolimus with a standard or, to a lesser extent, reduced dose of ciclosporin leads to progressive cumulative renal allograft damage, mainly as a result of augmented ciclosporin-related nephrotoxicity. Allowing early elimination of ciclosporin by employing sirolimus is the most effective strategy to exploit the beneficial effects of sirolimus on long-term graft function and histology; at least for recipients with a low to moderate immunological risk. Sirolimus-based immunosuppression accompanied by induction treatment enables complete avoidance of calcineurin inhibitor therapy with adequate control of rejection and better graft function. Whether use of sirolimus with a reduced dose of tacrolimus could accomplish similar long-term results remains to be determined. High immunological-risk patients and recipients prone to develop DGF cannot be considered for the approaches outlined, based on the current evidence. Sirolimus delays the recovery of ischaemia-reperfusion injury and is, therefore, not the ideal candidate drug to employ in this situation. Whether lower doses of sirolimus will provide adequate immunosuppression and facilitate the delayed introduction of nephrotoxic calcineurin inhibitors, without hampering the reparative proliferation of renal tubular cells, has not been determined. Finally, whether conversion to sirolimus maintenance treatment can be considered as an optimal choice in case of chronic calcineurin inhibitor nephrotoxicity and, to a lesser extent, chronic allograft nephropathy, will depend on the results of recently completed randomised controlled trials.

Clinical application of sirolimus in renal recipients is inevitably associated with treatment-emergent adverse effects, of which some are clinically relevant and require adequate therapeutic response (see table VII). Moderate to severe hyperlipidaemia is probably the most consistent complication of sirolimus therapy necessitating the chronic use of lipid lowering medication. The balance between the anti-proliferative and beneficial cardiovascular effects of the drug and the dyslipidaemia will determine the ultimate cardiovascular risk associated with prolonged use of sirolimus. Furthermore, this risk assessment has to be interpreted against the background of alternative immunosuppressive compounds and their proper profile in terms of nephrotoxicity, arterial hypertension, diabetes, hyperuri-

Table VII. Semi-quantitive comparison of safety profiles of current primary immunosuppressive compounds

	Sirolimus	Ciclosporin (cyclosporine)	Tacrolimus	Mycophenolate mofetil
Nephrotoxicity[a]	+	+++	++(+)	-
Hyperlipidaemia	+++	++	+(+)	-
Arterial hypertension	-	+++	++	-
Neurotoxicity	-	+++	+++	-
Post-transplant diabetes mellitus	-	++	+++	-
Bone marrow suppression	++	-	-	+++
Gastrointestinal adverse effects[b]	+	+	+	+++
Hepatotoxicity	+	+	+	-
Esthetical changes	-	++	+	-
Wound healing problems[c]	++	-	-	+
Pulmonary toxicity	+	-	-	-
Fetal toxicity	NA	+	+	?
Osteoporosis	?	+	+	-

a Sirolimus without calcineurin inhibitor.

b Gastrointestinal disorders: diarrhoea, abdominal pain, nausea and vomiting, ileus, rectal disorders, mucosal ulcerations.

c Wound healing problems including lymphocele formation.

- indicates the drug has no effect on this adverse effect; **+** indicates mild; **++** indicates moderate; **+++** indicates severe; **?** indicates clinical data available but insufficient to provide conclusions (see sections 2.2.3 and 2.7 for details); **NA** = no information available.

caemia, hyperlipidaemia and other cardiovascular risk factors.

Infectious complications predominantly relate to the lower respiratory tract with bacterial pneumonia remaining clinically the most debilitating and dangerous infection, while PCP no longer poses a clinical risk provided adequate antibacterial prophylaxis is adhered to. Sirolimus-induced pneumonitis of non-infectious origin is a rare but serious complication that requires prompt cessation of the drug and aggressive invasive diagnostic work-up. Although thrombocytopenia and, to a lesser extent, leukopenia are often observed in sirolimus-treated patients, these conditions rarely require intervention. Anaemia is a more clinically relevant adverse effect of sirolimus therapy, especially in situations of impaired graft function or concomitant use of other bone marrow suppressive drugs. The gastrointestinal adverse effects of sirolimus are mainly characterised by liver function disturbances – rarely demanding therapeutic interventions – and the development of superficial mucosal mouth ulcers of unknown origin, especially after conversion to long-term sirolimus maintenance therapy. Finally, the development of lymphoceles and wound healing problems often present a cumbersome surgical dilemma, especially in obese recipients, requiring prolonged hospitalisation and operative interventions.

It is clear that sirolimus has gained a proper place in the present-day immunosuppressive armament used in renal transplantation and will contribute to the development of a tailor-made immunosuppressive therapy aimed at fulfilling the requirements outlined by the individual patient profile.

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