

mTOR inhibitors in kidney transplantation

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Rapamycin was isolated in 1975 as an antibiotic product of the actinomycete *Streptomyces hygroscopicus*, obtained from a soil probe collected on Easter Island (Rapa Nui), and was **investigated initially for its antifungal properties** [1].

Since the first description of its immunosuppressive activity in 1977 [2], much has been learned about the complex mechanisms of action of this macrolide and its site of action, **the mammalian target of rapamycin (mTOR)** [3].

mTOR is an evolutionarily conserved intracellular serine–threonine kinase that plays a central role in the regulation of cell growth, metabolism and proliferation

The catalytic activity of mTOR occurs via at least two distinct complexes mTOR complex (mTORC) 1 and mTORC2.

Compared with mTORC1, little is known about the function of mTORC2. To exert its function, rapamycin forms a complex with the intracellular immunophilin FK506 binding protein 1 A 12 kDa (FKBP12)

This complex inhibits the kinase activity of mTOR by directly blocking substrate recruitment and restricting active site access .

While rapamycin and its analogues, or ‘rapalogs’, almost completely inhibit mTORC1, mTORC2 is affected only after long exposure [10].

Specific deletion of genes encoding mTORC1 or mTORC2, and the use of new-generation dual mTOR kinase inhibitors, known as ‘TORKinibs, have opened up new possibilities to investigate the discrete functions of each mTOR subunit in immune cells, with implications for their roles in transplantation.

Cell type	mTORC 1 inhibition [References]	mTORC 2 inhibition [References]
Dendritic cells (DCs)		
- Conventional DCs	Suppresses maturation, antigen uptake and micropinocytosis, and induces apoptosis [24–26]; paradoxical augmentation of proinflammatory cytokine production [124]	Augments ability to polarize Th1 and Th17; mTORC2 restrains proinflammatory function of activated DCs [33]
- Plasmacytoid DCs	Inhibits activation, modifies cytokine production, enhances Tmem and Treg proliferation [38]	Unknown
T cells	Controls Th1 and Th 17 differentiation [38]	Controls Th2 differentiation [125]
- Effector T cells		
- CD8⁺ memory cells	Augments CD8 ⁺ Tmem responses in infection [126]	Regulates development of CD8 ⁺ cells, altering the quantity and quality of receptors important for cell differentiation [45]
- Tregs	Promotes Treg expansion, differentiation and function [50, 78]	Maintains Treg cell stability and coordinates Treg-mediated control of effector responses [127]
NKT cells	Decreases terminal differentiation, reduces peripheral invariant NKT cells, impairs cytokine production [54]	Reduces NKT-17 cell differentiation, reduces thymic and peripheral NKT cells [55]
B cells	Reduces marginal zone formation, decreases antibody (Ab) class switching, alters Ab repertoire [128]	Affects development, survival and function of mature B lineage cells, impairs Ab production [58]
MDSCs	Induces T cell suppression by MDSCs, higher expression of iNOS, upregulation of Tregs [42]	Unknown
Endothelial cells	Lessens proliferation and cytokine secretion by allogeneic CD4 ⁺ , upregulates Tregs and reduces infiltration of allogeneic effector T cells into the arterial intima [62]	Antagonizes TNF induction of VCAM-1 [63]

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Experimental organ transplantation:

The majority of studies investigating mTOR in experimental organ transplantation have involved administration of rapamycin, either alone or in combination with other agents.

Initially, rapamycin monotherapy was shown to prolong organ allograft survival in rodents^{93,94}.

Importantly, administration of rapamycin late post-transplantation halted the progression of allograft vasculopathy in rats and non-human primates^{95,96}. **Attributes of rapamycin** reported in murine and NHP transplant models include an ability to **induce myeloid-derived suppressor cells, preserve Treg half-life** and phenotype post-infusion, and **produce mixed chimerism** when combined with co-stimulation blockade^{97,98}.

New generation mTOR inhibitors are of considerable interest in transplant models. TORKinibs were developed initially to overcome the limitations of rapamycin in clinical oncology. Thus 'rapalogue resistance', seen in a variety of tumours treated with rapalogues, is largely attributed to incomplete inhibition of mTORC1 and compensatory activation of mTORC2. Despite rapid incorporation of these drugs into early phase clinical trials in oncology, **little is known about their efficacy in transplantation.** **Theoretical advantages of TORKinibs over rapamycin in this setting include greater inhibition of mTORC2 in Tfh cells, B cells and endothelial cells, all of which orchestrate both DSA formation and chronic allograft vasculopathy.**

Clinical organ transplantation:

Initial studies that combined sirolimus (versus placebo or titrated dosage¹⁰²) with ciclosporin and prednisolone in renal transplantation demonstrated lower biopsy proven acute rejection (BPAR) even in the context of reduced ciclosporin dosage¹⁰⁴.

Sirolimus was also clearly superior to azathioprine¹⁰⁵ and has been investigated as a possible

calcineurin-sparing agent. A subsequent study, however, found a higher rate of BPAR in patients on prednisone and sirolimus who **withdrew ciclosporin at 3 months posttransplantation** compared to those who remained on this agent. Furthermore, in the Elite-Symphony study, patients assigned to sirolimus, mycophenolate mofetil and prednisone had higher rates of BPAR (37.2%) than those treated with either low dose ciclosporin (24%) or tacrolimus (12.3%). These findings demonstrate that use of sirolimus as baseline immunosuppression in the absence of a calcineurin inhibitor (CNI) has limitations, including higher rates of acute rejection, albeit offset by better glomerular filtration rate (GFR).

Study	Type (follow-up)	n	Treatment groups	Outcomes
Groth <i>et al.</i> (1999) ²⁴	Multi-centre, open-label (1 year)	83	Steroid + AZA + CsA or SRL	Similar graft survival, patient survival and creatinine levels lower and pneumonia and BPAR; serum SRL group rates higher in
Kahan <i>et al.</i> (1999) ¹⁰⁴	Phase II trial (1 year)	149	Steroid + CsA (normal or reduced dose) + placebo or SRL (low or high dose)	Addition of SRL reduced BPAR in standard CsA group; no difference in graft or patient survival; haematologic and lipid abnormalities in CsA group; hypertension and NODAT in CsA group; SRL group,
Kreis <i>et al.</i> (2000) ¹⁰⁶	Multi-centre, open-label (1 year)	78	Steroid + MMF + CsA or SRL	Graft survival, patient survival and BPAR; creatinine lower in SRL group; similar; serum
Rapamune US (2000) ¹⁰⁵	Multi-centre, double blind trial (1 year)	719	Steroid + CsA + AZA or SRL	Reduced occurrence and severity of BPAR in SRL group at 6 months
Rapamune Global (2001) ¹⁰³	Phase III (1 year)	576	Steroid + CsA + placebo or SRL (low or high dose)	Addition of SRL reduced acute rejection rates
Johnson <i>et al.</i> (2001) ³⁰²	Open-label (1 year)	525	Steroid + CsA (maintenance or withdrawal at 3 months) + SRL	Improved renal function and lower blood pressure when CsA withdrawn; thrombocytopenia, hypokalaemia and abnormal LFTS in CsA withdrawal group
Gonwa <i>et al.</i> (2001) ³⁰³	Phase II, open-label (1 year)	246	CsA + SRL or reduced-dose CsA (taper at 2 months) + SRL	Renal function better in CsA elimination group; BPAR, graft and patient survival similar
Rapamune Maintenance Study (2003 (REF. 304), 2005 (REF. 305))	Phase III (4 years)	525	Steroid + CsA (maintenance or withdrawal at 3 months) + SRL	• 2 years: CsA withdrawal group showed improved renal function and blood pressure, no change in graft loss or late acute rejection rates • 4 years: Non-significant increase in acute rejection rates with CsA withdrawal; higher incidence of adverse effects with treatment
Larson <i>et al.</i> (2006) ³⁰⁶	Phase II (1 year)	165	Steroid + MMF + TAC or SRL	Similar acute rejection,
SPIESSER Study (2007 (REF. 307), 2012 (REF. 308), 2016 (REF. 309))	Phase III (8 years)	• 1 year: 145 • 5 years: 133	Polyclonal antilymphocyte antibodies + steroid + MMF + CsA or SRL	• 1 year: BPAR, graft survival different; SRL group had lower rates (bronchopneumonia, rates

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What I thought of after the steam declaration announcement

Table 2 (cont.) | Clinical trials of mTOR inhibitors in renal transplantation

Study	Type (follow-up)	n	Treatment groups	Outcomes
ASCERTAIN study (2011) ³¹⁷	Multi-centre, open-label (2 years)	394	Randomization at >6 months to EVL with CNI maintenance, minimization or elimination	Conversion to EVL with CNI elimination or minimization had no renal benefit; more frequent adverse events and discontinuation
Heilman <i>et al.</i> (2011) ¹²⁰	Phase III (2 years)	122	MMF + TAC or SRL	63% withdrawal from SRL group
STN Study (2011 (REF. 318), 2016 (REF. 319))	Phase III (8 years)	• 2 years: 229 • 8 years: 128	MMF + CNI or MMF + SRL	• 2 years: similar renal function between groups • 8 years: improved long-term renal function with SRL + MMF compared to CNI + MMF
Orion (2011) ¹⁰⁹	Phase IV trial (2 years)	443	• Group 1: SRL + TAC with elimination of TAC at week 13 • Group 2: SRL + MMF • Group 3: TAC + MMF • All patients received steroids and daclizumab	Group 2 had high BPAR (>30%), SRL associated with hyperlipidaemia, delayed wound healing, greater proteinuria and discontinuation; TAC associated with NODAT; SRL not associated with improved outcomes
Mjörnstedt <i>et al.</i> (2012 (REF. 320), 2015 (REF. 321))	Multi-centre, open-label (3 years)	• 1 year: 202 • 3 years: 182	Steroid + MMF + CsA with conversion to EVL or maintenance of CsA at 6 weeks	• 1 year: higher GFR in EVL group, but higher incidence of BPAR and adverse events leading to discontinuation • 3 years: EVL associated with significant benefit in renal function but drug discontinuation more common
APOLLO Study (2015) ³²²	Multi-centre, open label (1 year)	93	Remain on CsA or convert to EVL	Premature termination due to slow recruitment; higher rate of discontinuation with EVL

ACR, acute cellular rejection; ATG, anti-thymocyte globulin; AZA, azathioprine; BPAR, biopsy proven acute rejection; CMV, cytomegalovirus; CNI, calcineurin inhibitor; CsA, ciclosporin; GFR, glomerular filtration rate; LFTS, liver function tests; MMF, mycophenolate mofetil; NODAT, new onset diabetes after transplantation; EVL, Everolimus; SRL, Sirolimus; TAC, Tacrolimus

mTOR inhibitors are generally accepted to provide inferior initial posttransplantation immunosuppression than conventional CNI-based regimens.

In addition, the propensity of mTOR inhibitors to impair wound healing, induce wound dehiscence and promote lymphocele development further precludes their use as a primary immunosuppressant.

mTOR inhibitors might be best utilized for conversion therapy to avoid CNI toxicity, or to provide flexibility for patients who develop CNI-related complications

Since the FDA approval of rapamycin (sirolimus) for use in clinical kidney transplantation in 1999, an extensive literature has emerged on its effects on graft survival, mortality and other important clinical outcomes, such as malignancy, cardiovascular disease and infection. The majority of studies of mTOR inhibitors involve conversion from CNI either early (2–6 months) or late (>6 months) post-transplantation. Few studies on the de novo use of mTOR inhibitors and mTOR inhibitor monotherapy post kidney transplantation have been conducted

In the majority of the conversion trials published to date, mTOR inhibition has been compared with ciclosporin.

In one study of renal transplant recipients, a ciclosporin-free regimen based on sirolimus reduced aortic stiffness, plasma endothelin1 and oxidative stress, suggesting a protective effect on the arterial wall that might be translated into reduced cardiovascular risk¹¹⁸.

On the other hand, among patients randomly assigned to either sirolimus or ciclosporin at 3months post-transplantation, the incidence of subclinical inflammation in protocol biopsy samples 1 year post-transplantation was greater in the sirolimus group¹¹

In a prospective randomized trial, kidney transplant recipients receiving rapid corticosteroid withdrawal, tacrolimus and mycophenolate mofetil (MMF) for 1 month were randomly assigned to receive either sirolimus plus MMF or tacrolimus plus MMF maintenance therapy, withdrawal from the study owing to rejection or adverse effects was a major problem in the sirolimus group (63% versus 18% in the tacrolimus group). These data indicate that in the absence of steroids, sirolimus and tacrolimus are not interchangeable.

Other than their anti-neoplastic effects, a theoretical advantage of mTOR inhibitors in transplantation lies in their anti-viral properties. mTOR inhibitors stimulate cytomegalovirus (CMV)-specific TH1 cells and $\gamma\delta$ T cells,
Furthermore, sirolimus inhibits and tacrolimus activates BK polyomavirus replication in renal TECs.

As BK and CMV infections cause considerable excess morbidity and mortality, the immunomodulatory effects of these mTOR inhibitors provide a rationale for conversion from CNIs post-transplantation.

Unfortunately, many studies have not been adequately powered to compare the influence of different immunosuppressive regimens on the incidence of either CMV or BK virus infection

Many comparative studies have demonstrated that early use of mTOR inhibitor-based regimens can reduce the incidence of CMV infection.

In a single center case series that included 15 patients with BK nephropathy, suspension of mycophenolate and conversion from tacrolimus to everolimus immunotherapy was associated with decreased viraemia and increased graft survival¹²⁹.

Although a role for mTOR inhibitors in biopsy-proven BK nephropathy is plausible, prospective large randomized studies are needed to adequately address this question

Overall, mTOR inhibitors have some attractive characteristics, particularly their

1-beneficial effects on kidney function by enabling CNI sparing

2-reduced rates of malignancies and non-melanoma skin cancer¹³⁰,

3-fewer viral infections and 4- reduced weight gain compared to CNI-based regimens¹³².

Nonetheless, for the many renal transplant recipients with a documented history of sensitization, these agents remain inferior to CNIs.

mTOR in AKI and ischemic injury

A special situation arises during kidney transplantation in which donated organs inevitably undergo both cold and warm ischaemia.

In summary, **the risk of delayed graft function seems to be increased in patients who are treated with mTOR inhibitors directly after transplantation.**

, early treatment with mTOR inhibitors should be avoided.

Most injury mechanisms seem to be related to the anti-proliferative and pro-apoptotic effects of mTOR inhibitors on the renal epithelium, whereas specific effects on local and systemic immune cells that might affect the development of AKI have not been thoroughly explored.

Exposure of healthy rats to an mTOR inhibitor (temsirolimus) increased early glomerular permeability, but reduced late permeability¹⁵⁸.

In mice without renal pathology, rapamycin induced a mild deterioration in renal function, increased albuminuria and podocyte foot-process width, and short-term reduction in nephrin and podocin expression; these effects resolved after 8 weeks of treatment. Although previous studies had demonstrated no gross histologic changes in the renal glomerulus in response to mTOR inhibition, pathologic abnormalities were seen in the rodent renal tubular compartment and in vessels.

mTOR is increasingly recognized as having a fundamental role in the development of glomerular pathology.

Activation of mTORC1 in podocytes led to the development of glomerular crescents, which was abolished following treatment with rapamycin.

Diabetes mellitus

Diabetes is now thought to represent a state of mTORC1 hyperactivation¹⁷³.

Inhibition of mTOR signalling has also been shown to ameliorate some renal compensatory mechanisms following the induction of diabetes.

In mice with streptozotocin-induced diabetes, renal hypertrophy was accompanied by upregulation of S6K1 kinase expression that could be attenuated by daily rapamycin administration¹⁷⁵.

Similarly in diabetic rats, **rapamycin ameliorated albuminuria** (but had no effect on glomerular hypertrophy) and **downregulated the expression of mTOR**,. These effects occurred without normalization of serum glucose and blood pressure levels and paralleled achievement of normoglycaemia.

Systemic lupus erythematosus

female NZBW/F1 mice develop overt SLE and renal disease that can be mitigated by rapamycin treatment. Activation of the P13K–Akt–mTOR pathway has been demonstrated in the affected glomeruli of NZBW/F1 mice, with upregulation of phosphorylated Akt (at residues 308 and 473) and concurrent downregulation of podocin and nephrin.

In the glomerulus, expression of mTOR and of phosphorylated mTOR (at Ser2448) is colocalized in podocytes and endothelial cells. **Mice treated with rapamycin (either 1 week after development of severe proteinuria or before SLE development) showed preservation of remaining renal mass and function, reduced levels of anti-double-stranded-DNA antibodies, mitigated pathognomonic histological lesions and maintenance of podocin and nephrin expression compared with untreated controls.**

Treatment with rapamycin or other mTOR inhibitors also suppressed interstitial inflammatory infiltrate (T cells, B cells and macrophages) in preclinical lupus models¹⁷⁹.

Despite the anti-proliferative properties of rapamycin, few clinical studies have used this agent to treat lupus nephritis^{184,185}.

Preliminary data showing improvements in serology, renal function and proteinuria with sirolimus in seven patients with this disease suggest that further investigation may be warranted¹⁸⁴

General glomerulopathies

mTOR inhibitors have been investigated only sparingly in animal models of other glomerular diseases (TABLE 3). Moreover few studies have explored the mechanisms involving mTOR and glomerular pathophysiology, as well as beneficial effects related to mTOR inhibitors. Proteinuria is a key clinical feature of glomerular disease and urinary protein loss is regulated predominantly by podocytes¹⁸⁶. Endoplasmic reticulum stress triggers the unfolded protein response, which is preceded by activation of mTORC1 and dysregulated energy production¹⁸⁷; both of these effects can be inhibited by everolimus

In rodents with **adriamycin nephropathy**, rapamycin reduces proteinuria, preserves renal function and ameliorates glomerulosclerosis and tubular dilatation, with reductions in the intrarenal expression of CC motif chemokine 5 (CCL5, also known as RANTES) and collagen¹⁸⁸. These renoprotective and antiproteinuric effects can be recapitulated with everolimus, which also restores glomerular nephrin and podocin expression¹⁸⁹.

in the rat remnant kidney model; this agent induced proteinuria, interstitial fibrosis and glomerulosclerosis¹⁹⁵. **In a rat model of established IgA nephropathy, low-dose rapamycin reduced IgA deposition, prevented progression of proteinuria and limited deterioration of renal function**¹⁹⁶. These effects correlated with cell cycle arrest, upregulation of cyclin-dependent kinase inhibitor 1B and presumably inhibition of mesangial cell proliferation. In Heymann nephritis¹⁹⁷ — a rodent model of human membranous nephropathy — production of antibodies directed against the target antigens megalin and receptor associated protein¹⁹⁸ leads to the development of glomerular deposits. Rapamycin mitigates proteinuria¹⁹⁹ and histologic lesions in this model²⁰⁰, including CD8⁺-T cell inflammation, with restoration of glomerular expression of podocin and nephrin²⁰⁰ and a reduction in immunoglobulin deposits¹⁹⁹

Few clinical studies have investigated the influence of mTOR modulation in glomerular disease (TABLE 3), possibly owing to concern about exacerbation of proteinuria. Moreover, low numbers of recruited patients limit the interpretation and generalizability of existing studies. **In 23 patients with poor-prognosis IgA nephropathy (>1g proteinuria per 24 h and GFR 30–60 ml/min/1.73 m²) treated with ACE inhibitor and a statin, who were also assigned sirolimus or placebo for 12 months²⁰⁴, those treated with sirolimus showed improvements in GFR and decreased endocapillary proliferation compared with the placebo group.** Proteinuria, glomerulosclerosis and interstitial fibrosis were unchanged, regardless of treatment

The efficacy of sirolimus has also been evaluated in 21 patients with FSGS²⁰⁵; the therapy induced complete remission in 19% of patients and partial remission in 38%, with maintenance of GFR and a reduction in proteinuria. A phase II open-label trial in six patients with FSGS, however, reported a lack of responsiveness to sirolimus and an association of this therapy with nephrotoxicity²⁰⁶. In some kidney transplant recipients, high-dose sirolimus has been reported to induce de novo FSGS, characterized by decreased expression of synaptopodin and p57 (REF. 207). **A case of successful treatment of minimal change nephropathy using combined tacrolimus and rapamycin has also been reported**²⁰⁸.

Table 3 | The effects of mTOR inhibitors in renal diseases

Setting	Effect of mTOR inhibitor	
	Pre-clinical models	Clinical studies
Healthy kidney	No histologic abnormalities ¹⁶⁰ ; deterioration in GFR in spontaneously hypertensive rat ¹⁶³	No effect on renal function (serum creatinine levels) after 8 weeks of treatment ³²³
Diabetes mellitus	Attenuates renal hypertrophy, mitigates albuminuria ^{175–177}	No direct studies; use of sirolimus post-islet transplantation associated with proteinuria ³²⁴
Systemic lupus erythematosus	Preservation of renal mass and renal function, improved glomerular histological findings, decreased anti-double stranded DNA antibodies	One human study, improvements in renal function and proteinuria in 3 of 5 patients ¹⁸⁴
Adriamycin nephropathy	Preservation of renal function, amelioration of glomerulosclerosis and tubular dilatation ^{180,189}	No human disease equivalent
Anti-GBM disease, Goodpasture disease and crescentic GN	Concurrent with disease induction: improved proteinuria and renal histology; after disease induction: worsening proteinuria and inflammatory infiltrates ²⁰¹	Case report of sirolimus reducing ANCA titre ³²⁵ , another case report suggesting limited utility owing to adverse events ³²⁶
Thrombotic microangiopathy	Impaired recovery ¹⁹⁰	No human studies; sirolimus has been associated with TMA in renal allografts
Chronic glomerulonephritis	In Thy 1.1 nephritis, low dose prevents compensatory glomerular hypertrophy, renal inflammatory cell infiltration ¹⁹²	6 out of 11 patients with chronic glomerulonephritis and pre-existing proteinuria who were treated with rapamycin developed acute renal failure ³²⁷
Chronic kidney disease	Induces proteinuria, interstitial fibrosis and glomerulosclerosis in a rat remnant kidney model ²⁵¹	No formal human studies

Membranous nephropathy	Mitigated proteinuria, and reduced immunoglobulin deposits in rats with Heymann nephritis ¹⁹⁹	No formal human studies
IgA nephropathy	Protected kidney function, reduced IgA deposition and prevented proteinuria increase ¹⁹⁶	Improved GFR, decreased endocapillary proliferation ²⁰⁴
Focal segmental glomerulosclerosis	No studies	Evidence of complete and partial remission ²⁰⁵ , cases of nephrotoxicity reported ³²⁷
Minimal change nephropathy	No studies	Complete remission when combined with tacrolimus ²⁰⁸
Polycystic kidney disease	Decreased kidney enlargement and cyst volume; improved renal function ²¹³	Unimpressive results, high adverse effect profile ³²⁸
Acute kidney injury	Delayed recovery ³²⁹	Delayed recovery ^{136,137}
Angiomyolipoma	Decreased tumour burden, cyst size and increased survival in a mouse model of TSC ³³⁰	Long-term treatment effective in reducing tumour volume ^{256,263} ; neoadjuvant use of sirolimus facilitates nephron-sparing resection ²⁶¹
Renal cell carcinoma	Temsirolimus and the TORKinib Ku0063794 inhibit tumour growth in a xenograft model of renal cell carcinoma ³³¹	Several inhibitors tested without great success in advanced disease ³³² including temsirolimus ³³³ , everolimus ³³⁴ , deforolimus ³³⁵ and CCI-779 (REF. 336)

ANCA, anti-neutrophil cytoplasmic antibody; GBM, glomerular basement membrane; GFR, glomerular filtration rate; GN, glomerulonephritis; TORKinib, novel dual inhibitor of TORC1 and TORC2; TSC, tuberous sclerosis complex.