# 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.

Effects of mammalian target of rapamycin complex (mTORC) 1 and mTORC2 inhibition on different immune cell types

| 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] |

iNOS, inducible nitric oxide synthase; MDSC, myeloid-derived suppressor cells; NKT, natural killer T cells; Th, T helper cell; Tmem, T memory cell;

# mTOR inhibitors in transplantation



**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 blockade97,98.

**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 TORKinbs 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 *et al.* (1999)[24] | Multi-centre, open-label (1 year) | 83 | Steroid + AZA + CsA or SRL | Similar graft survival, patient survival ar creatinine levels lower and pneumonia SRL group |
| Kahan *et al.* (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 stand CsA group; no difference in graft or pat haematologic and lipid abnormalities ir hypertension and NODAT in CsA group |
| Kreis *et al.* (2000)[106] | Multi-centre, open-label (1 year) | 78 | Steroid + MMF + CsA or SRL | Graft survival, patient survival and BPAI creatinine lower in SRL group |
| Rapamune US (2000)[105] | Multi-centre, double blind trial (1 year) | 719 | Steroid + CsA + AZA or SRL | Reduced occurrence and severity of BP 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 rejectio |
| Johnson *et al.* (2001)[302] | Open-label (1 year) | 525 | Steroid + CsA (maintenance or withdrawal at 3 months) + SRL | Improved renal function and lower bloo when CsA withdrawn; thrombocytoper hypokalaemia and abnormal LFTS in Cs group |
| Gonwa *et al.* (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 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<br>• 4 years: Non-significant increase in acute rejection rates with CsA withdrawal; higher incidence of adverse effects with t |
| Larson *et al.* (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<br>• 5 years: 133 | Polyclonal antilymphocyte antibodies + steroid + MMF + CsA or SRL | • 1 year: BPAR, graft sur different; SRL group h (bronchopneumonia, rates |

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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 *et al.* (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<br>• 8 years: 128 | MMF + CNI or MMF + SRL | • 2 years: similar renal function between groups<br>• 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<br>• Group 2: SRL + MMF<br>• Group 3: TAC + MMF<br>• 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 *et al.* (2012 (REF. 320), 2015 (REF. 321)) | Multi-centre, open-label (3 years) | • 1 year: 202<br>• 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<br>• 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 risk**118.

**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 γδ 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.

# mTOR in glomerular disease



**Exposure of healthy rats to an mTOR inhibitor (temsirolimus) increased early glomerular permeability, but reduced late permeability**158.

**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 administration**175.

**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 warranted**184

# 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.