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## Roles of mTOR complexes in the kidney: implications for renal disease and transplantation

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### Abstract

The mTOR pathway has a central role in the regulation of cell metabolism, growth and proliferation. Studies involving selective gene targeting of mTOR complexes (mTORC1 and mTORC2) in renal cell populations and/or pharmacologic mTOR inhibition have revealed important roles of mTOR in podocyte homeostasis and tubular transport. Important advances have also been made in understanding the role of mTOR in renal injury, polycystic kidney disease and glomerular diseases, including diabetic nephropathy. Novel insights into the roles of mTORC1 and mTORC2 in regulation of immune cell homeostasis and function are helping to improve understanding of the complex effects of mTOR targeting on immune responses, including those that impact both *de novo* renal disease and renal allograft outcomes. Extensive experience in clinical renal transplantation has resulted in successful conversion of patients from calcineurin inhibitors to mTOR inhibitors at various times post-transplantation, with excellent long-term graft function. Widespread use of this practice has, however, been limited owing to mTOR-inhibitor-related toxicities. Unique attributes of mTOR inhibitors include reduced rates of squamous cell carcinoma and cytomegalovirus infection compared to other regimens. As understanding of the mechanisms by which mTORC1 and mTORC2 drive the pathogenesis of renal disease progresses, clinical studies of mTOR pathway targeting will enable testing of evolving hypotheses.

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The authors declare no competing interests.

## Introduction

Since the discovery of rapamycin (also known as sirolimus more than 40 years ago,<sup>1</sup> advances in the understanding of its molecular mode of action as well as the functional biology of its primary target — mTOR — have permeated many areas of medicine, including cardiovascular disease, autoimmunity and cancer. mTOR is an evolutionarily-conserved serine-threonine kinase that regulates cell growth, proliferation and metabolism. Increasing evidence indicates that mTOR has an important role in the regulation of renal cell homeostasis and autophagy. Moreover, this kinase has been implicated in the development of glomerular disease, polycystic kidney disease (PKD), acute kidney injury (AKI) and kidney transplant rejection.

The development of rapamycin and its analogues (known as rapalogstemsirrolimus and everolimus, has expanded the pharmacological armamentarium for treatment of renal disease. Owing to its ability to potently inhibit T cell proliferation, rapamycin was initially developed as an immunosuppressive agent in kidney transplantation.<sup>2</sup> Rapalogs have now also been added to the immunosuppressive repertoire for glomerulonephritides (although not a therapeutic mainstay for these conditions) and renal cell carcinoma. In this Review, we discuss aspects of mTOR function and its inhibition in relation to renal physiology, kidney disease including malignancy, and the role of mTOR complexes and their inhibitors in renal transplantation.

## mTOR complexes

mTOR operates in at least two distinct, multi-protein complexes: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) (FIG. 1). Details of the structural biochemistry of mTOR and role in cellular signalling have been reviewed in detail elsewhere.<sup>3–5</sup> mTORC1 is often described as a ‘nutrient sensor’ as it can be activated by amino acids and inhibited by severe oxidative stress and energy depletion. The primary roles of mTOR are to facilitate cell growth and anabolism as well as to prevent autophagy. Although mTORC1 was localized initially to the cytoplasm, this complex has since been identified in association with endosomal compartments (outer mitochondrial membranes and nuclei<sup>6–8</sup>) and has been shown to have a role in stress granule formation.<sup>9</sup> These findings provide further evidence that mTOR is a ‘metabolic rheostat’ for eukaryotic cells.

Growth factors and cytokines can activate mTORC1 via upstream signalling through phosphoinositide 3-kinase (PI3K). Generation of phosphatidylinositol (3,4,5) triphosphate (PIP3) by PI3K activates 3-phosphoinositide-dependent protein kinase-1 (PDK1), which enhances Akt (also known as protein kinase B) activity by phosphorylating the activation loop at threonine 308. Interestingly, mTORC2 uniquely stabilizes Akt via phosphorylation of the turn motif at serine 450, and further stimulates Akt kinase activity by phosphorylating the hydrophobic motif at serine 473. The activity of tuberous sclerosis complex 1 (TSC1) and TSC2, the major upstream inhibitors of mTORC1, is dampened directly by Akt-dependent phosphorylation and provides a functional link between the PI3K–Akt signalling axis and mTOR. TSC1 stabilizes and TSC2 inhibits the activity of the guanosine triphosphate (GTP)-ase Rheb (RAS homolog enriched in brain), through its GTPase-

activating protein activity. Rheb enhances the catalytic activity of mTORC1 when they are in close proximity.

Tools to study mTORC2 are limited owing to a lack of mTORC2-specific inhibitors. Substrates of mTORC2 include Akt, protein kinase C $\alpha$  (PKC $\alpha$ ) and serum and glucocorticoid-induced kinase-1 (SGK-1). Furthermore, phosphorylation and cytoplasmic sequestration of the forkhead box proteins O1 (FOXO1) and FOXO3 by Akt has been shown to require mTORC2.<sup>10</sup> Consistent with these signalling cascades, mTORC2-orchestrated processes include cell survival, protein synthesis, re-organization of the actin cytoskeleton and sodium homeostasis. Although active mTORC2 physically associates with ribosomes in mammalian cells, the intricacies of this pathway have not been completely characterized.<sup>11</sup> Importantly, crosstalk between the mTORC1 and mTORC2 pathways<sup>12</sup> adds an extra layer of complexity in dissecting mTORC1 and mTORC2 biology. Thus, mTORC1 inhibits the PI3K–mTORC2 pathway through a negative feedback mechanism.

## mTOR inhibitors

Although rapamycin immediately inhibits mTORC1, its ability to destabilize and inhibit mTORC2 requires prolonged exposure and is more sensitive to fluctuations in concentrations of the immunophilin FK506 binding protein 12 (FKBP12), which binds rapamycin and mediates its interaction with mTOR.<sup>13</sup> The negligible effect of rapamycin on mTORC2 function has been disputed, however, with evidence that this agent might inhibit mTORC2 assembly and signalling.<sup>14</sup> The C-terminal part of Avo3, a subunit unique to TORC2 in yeast, is located close to the FKBP12/rapamycin-binding domain of mTORC2 and removal of Avo3 from TORC2 confers sensitivity to rapamycin.<sup>15</sup> Although less is known about how rapalogs affect mTORC1 and 2, inhibition of mTORC2 has been demonstrated in acute myeloid leukemia cells exposed to everolimus and temsirolimus.<sup>16</sup> Novel dual inhibitors of TORC1 and TORC2 (TORKinibs) that compete for the adenosine triphosphate (ATP)-binding site of mTOR were developed to limit activation of both mTOR complexes<sup>17</sup> and provide broader clinical efficacy than the currently available rapalogs. These molecules were first described to inhibit PI3K,<sup>17</sup> a critical component upstream of the Akt/mTOR pathway, and subsequently also found to inhibit mTOR, presumably owing to sequence homology. Initial TORKinibs (mTOR and PI3K dual inhibitors) failed to selectively inhibit either signalling moiety,<sup>18</sup> but newer molecular inhibitors (mTORC1 and mTORC2 dual inhibitors) exhibit greater potency for mTOR.<sup>19</sup> These agents are being investigated for the treatment of malignancy in phase I and II clinical trials<sup>20</sup> but have yet to be tested in clinical organ transplantation.

## mTOR in renal physiology

Given the ubiquitous expression of mTORC1 and mTORC2 in the kidney, elucidating the roles of mTOR in renal physiology is a formidable undertaking. Nonetheless, pharmacologic inhibition and targeted cell-specific genetic deletion have enabled analyses of mTOR function in the rodent and human kidney (FIG 2). Studies of patients with TSC, a syndrome with autosomal dominant inheritance characterized by upregulation of mTORC1, have also increased understanding of the consequences of enhanced mTORC1 activity on renal

physiology. Although much of the current understanding of mTOR function pertains to the podocyte or tubular epithelial cell, renal endothelial and immune cells are emerging as important players in the regulation of renal homeostasis and metabolism.

## Podocytes

Glomerular podocytes are highly-differentiated, voluminous cells with numerous foot processes that line the outer aspects of the glomerular basement membrane (GBM). The foot processes interdigitate with each other and form the slit diaphragm, an intercellular junction composed of several membrane proteins that regulate protein movement into the urinary space.<sup>21</sup> Although glomerular diseases can be caused by disturbances in various barrier components, podocyte loss and effacement is a critical event that, if not constrained, leads to proteinuria, renal fibrosis and end-stage renal disease (ESRD).

Much of the understanding of podocyte mTORC1 and mTORC2 function stems from genetic deletion studies. In mice, podocyte-specific embryonic knockout of mTORC1 (owing to deletion of the regulatory-associated protein of mTOR (Raptor) (Ed: Yes) resulted in early albuminuria, later development of glomerulosclerosis, weight loss and increased mortality.<sup>22</sup> Loss of podocyte mTORC1 in adult mice failed to induce similar deleterious effects. Surprisingly, heterozygous deletion of Raptor in podocytes expression and podocyte volume. Mice with podocyte-specific loss of mTORC2 (owing to deletion of rapamycin-insensitive companion of mTOR (Rictor) did not exhibit significant phenotypic differences compared to littermate controls, with the exception of transient albuminuria following protein overload. However, combined deletion of both mTOR complexes from podocytes precipitated an early (6 weeks of age) fulminant proteinuric phenotype, suggesting some degree of interaction between mTORC1 and mTORC2 in the regulation of podocyte development and homeostasis.

Excessive mTORC1 activity can also result in severe pathologic effects, including hallmarks of diabetic nephropathy. In a murine study, podocyte-specific deletion of *Tsc1* led to aberrant mTORC1 activation, enhanced pS6 expression, mesangial expansion, GBM thickening, podocyte loss, podocyte foot-process effacement and proteinuria (that was attenuated by rapamycin), as well as glomerular expression of transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1), type IV collagen and fibronectin.<sup>23</sup> The remaining podocytes of the transgenic mice had a fibroblastic phenotype with markers of endoplasmic reticulum stress. Immunofluorescence studies demonstrated altered distribution of podocyte nephrin and synaptopodin that was corrected by rapamycin administration.

## Tubular epithelial cells

Tubular epithelial cells (TECs) are bathed in urine via their apical membranes and have an important role in maintaining salt and water balance, typically via their basolateral surfaces. This role is accomplished largely via energy-dependent cellular transporters. Initial evidence that mTOR might regulate tubular homeostasis came from repeated observations of electrolyte disturbances in renal transplant recipients treated with rapamycin, although the underlying cause of these disturbances is still disputed. The renal-related adverse effects of rapamycin are typically hypokalaemia,<sup>24</sup> hypomagnesaemia and hypophosphataemia,<sup>25, 26</sup>

as well as decreased urinary calcium excretion.<sup>27</sup> Sirolimus decreases the expression of the apical Na-K-2Cl co-transporter (NKCC2) in the thick ascending limb (TAL), resulting in renal tubular wasting of sodium, magnesium and potassium.<sup>26</sup> The phosphaturic actions of rapamycin might be linked to the induction of klotho, Rictor and mTORC2, which has been shown *in vivo* and in an immortalized renal tubular cell line.<sup>28</sup> Post-transplant phosphaturia is aggravated by administration of sirolimus, although this effect is reportedly not the result of altered phosphate uptake via sodium–phosphate co-transporters in the brush border of the proximal tubule.<sup>29</sup> Nevertheless, in *Xenopus* oocytes expressing sodium–phosphate transporters, co-transfection with mTOR increased phosphate currents and this effect was abrogated by exposure to rapamycin.<sup>30</sup> The past few years have seen several reports linking mTORC1 to proximal tubular transport mechanisms. Work performed mainly in *Drosophila melanogaster* showed that the proton pump V-ATPase responsible for intracellular compartment acidification and apical amino acid uptake requires mTORC1. This interplay seems to be regulated through the multi-ligand-binding receptor megalin.<sup>31</sup> Indeed, in C57BL/6 mice, long-term rapamycin exposure reduced proximal tubular megalin expression and caused concurrent low molecular weight proteinuria, consistent with renal proximal TEC dysfunction.

Most recently, however, it has been demonstrated that mice deficient in proximal tubular mTORC1 present with a Fanconi Syndrome-like phenotype consisting of phosphaturia, glucosuria, low-molecular weight proteinuria, albuminuria and aminoaciduria.<sup>32</sup> Deep proteomic and phosphoproteomic analysis shows that mTORC1 deficiency affects the translation and phosphorylation of specific transport systems, its regulators as well as proteins involved in endocytosis in a kinase-dependent manner. Interestingly, and in contrast to the earlier study,<sup>31</sup> no alterations in the abundance of the two scavenger receptors cubilin and megalin could be detected.

In mice, specific deletion of Raptor (mTORC1) in distal TECs did not cause significant developmental defects, but these animals developed polyuria and hypercalciuria after weaning as a result of a defect in TAL countercurrent multiplication<sup>33</sup>. Consistent with these perturbations, transcriptional profiling identified reduced levels of NKCC2 in these mice. Generation of mTORC1/2-deficient animals resulted in aggravated pathology and increased mortality, suggesting that these complexes have complimentary roles.

Although the molecular pathways downstream of mTORC1 and mTORC2 that regulate ion channels in the TAL (such as NKCC2) remain to be determined, significant advances have occurred in the understanding of how mTOR regulates the aldosterone-sensitive epithelial sodium channel (ENaC), a protein that is most abundant in the distal tubule. Serum and glucocorticoid inducible kinase 1 (SGK1) acts as a central driver of this process by increasing cell surface expression of ENaC via inhibition of the E3 ubiquitin-protein ligase Nedd4–2<sup>34</sup> and mTORC2 has been found to activate SGK1 via phosphorylation of its hydrophobic motif.<sup>35</sup>

To investigate the physiological significance of the mTORC2–SGK1–ENaC axis *in vivo*, researchers compared the effect on electrolyte homeostasis of targeting mTORC1 (using rapamycin) with that of dual inhibition of mTORC1 and mTORC2 (using the TORKinibs

PP242 and AZD8055).<sup>36</sup> Interestingly, the TORKinibs uniquely induced polyuria and substantial natriuresis without kaliuresis (compared to rapamycin) in wild-type mice. This effect was lost, however, when PP242 and the ENaC blocker amiloride were administered together, suggesting that the natriuretic effects of TORKinibs occur upstream of the ENaC. Conflicting data came from a study that used a mouse genetic approach to examine the role of mTORC2 in the aldosterone-sensitive distal nephron.<sup>37</sup> In contrast to the findings of the subtractive pharmacological inhibitor experiments, mice with mTORC2 deficiency (as a result of Rictor deletion) developed life-threatening hyperkalaemia when challenged with a high K<sup>+</sup> diet or the K<sup>+</sup>-sparing diuretic triamterene. Patch-clamp recordings of cortical collecting ducts revealed an absence of barium-sensitive K<sup>+</sup> currents. Phosphorylation of the hydrophobic motif of SGK1 and PKC $\alpha$  was virtually absent and membrane expression of renal outer medullary K<sup>+</sup> channels greatly reduced in the Rictor-deficient animals. The findings of Grahammer et al<sup>37</sup> essentially show that mTORC2 drives kaliuresis and is not a prerequisite for Na reabsorption. The equation: effect with TORKinib minus effect with rapamycin= mTORC2-specific effect, is certainly too simplistic and in most instances is wrong and misleading. It rather seems that inhibiting both mTORC1 and mTORC2 vitally challenges any cell examined so far. To obtain true insight into mTORC2-specific effects, the only possibility is to use a genetic approach, at least until mTORC2-specific inhibitors are available. The findings underline the necessity to combine genetic deletion and pharmacological inhibition approaches for a full appraisal of the functional importance of mTOR complexes.

### Renal endothelial cells

Diseases such as thrombocytopaenic purpura (TTP), haemolytic uraemic syndrome (HUS), pre-eclampsia, anti-phospholipid antibody syndrome (APS) and antibody-mediated kidney allograft rejection are driven partially by endothelial cell activation and injury.

Conceptualizing mTOR as a driver of vascular endothelial proliferation and immune activation has developed over the past ten years. Activation of the mTORC pathway has been observed in the intra-renal vessels of patients with APS nephropathy.<sup>38</sup>

Immunofluorescent staining of endothelium from the renal vessels (but excluding glomerular endothelium of individuals with primary or secondary APS identified phosphorylation of S6 ribosomal protein (S6RP, indicating mTORC1 activation) and Akt (indicating mTORC2 activation),<sup>38</sup> often with colocalization. Incubation of human microvascular endothelial cells with antiphospholipid antibody led to upregulation of SR6P and Akt phosphorylation *in vitro*, a phenomenon that was prevented by treatment with the TORkinib PP242. Interestingly, patients who received sirolimus after transplantation showed reduced vascular proliferation and lesion recurrence on biopsy, compared to those receiving alternative immunosuppression. Use of mTOR inhibitors was also associated with a higher incidence of functioning allografts up to 12 years post-transplantation.

The development of transplant vasculopathy is induced by antibody binding to HLA class I molecules expressed by endothelial and smooth muscle cells, which promotes their proliferation. The effect of these antibodies on endothelial cell proliferation has been verified *in vitro* and is mediated by formation of mTORC1 and mTORC2.<sup>39</sup> Downregulation of endothelial mTOR using small interfering RNA (siRNA) against Rictor or Raptor blocked



HLA class I antibody-induced endothelial cell proliferation, whereas administration of rapamycin prevented HLA class I antibody-induced Akt phosphorylation. Additional studies have demonstrated TORC2-mediated extracellular kinase (ERK) phosphorylation induced by endothelial-cell-based MHC class I and integrin ligation,<sup>40</sup> which was inhibited by rapamycin.

Although the role of mTOR in endothelial cell proliferation might imply a benefit of mTOR inhibitors in kidney transplant recipients, these agents have also been implicated in the development of donor-specific antibodies (DSAs) that might precipitate antibody-mediated rejection (ABMR). Both sirolimus<sup>41</sup> and everolimus<sup>42</sup> are associated with the development of *de novo* DSAs and subsequent onset of AMR.<sup>42, 43</sup> Although endothelial mTOR pathway activation has been implicated in acute and chronic AMR in rodent and human studies,<sup>20, 39, 44, 45</sup> the therapeutic efficacy of mTOR inhibitors in these settings remains unclear. Potential explanations for these conflicting findings might include reduced potency of mTOR inhibitors compared to CNIs, for T cell subsets that regulate AMR and higher rates of intolerable side effects with mTOR inhibitors that may limit compliance and, as a consequence, increase allograft rejection.

### Regulation of autophagy

Autophagy is a catabolic process that degrades various substrates including misfolded proteins, damaged organelles and microorganisms. This material is sequestered in autophagosomes prior to breakdown in lysosomes. In most circumstances autophagy promotes cell survival and is important when a cell faces environmental stress and must use cellular components as an energy source.<sup>46</sup> By contrast, the mTOR pathway is most active in nutrient-replete environments. Thus, autophagy and mTOR can be envisaged as opposing molecular pathways that negatively regulate each another.

Importantly, mechanisms by which mTOR regulates autophagy have been described over the past ten years. Three groups have demonstrated that mTORC1 inhibits the UNC-51-like autophagy-activating kinase (ULK) complex by phosphorylating ULK1/2 and other components<sup>47–49</sup>. In mammalian cells phosphorylation of ULK1/2 at Ser758 by mTORC1 prevents ULK1/2 activation by the energy sensor AMP-activated protein kinase (AMPK). mTORC1 has also been found to regulate autophagy at the transcriptional level via phosphorylation and cytoplasmic sequestration of transcription factor EB (a regulator of lysosomal and autophagy genes) and by inhibition of PI3K catalytic subunit type 3 (also known as VPS34), which has an important role in autophagosome formation.<sup>46</sup> Whether mTORC2 regulates autophagy directly, independent of mTORC1, remains unclear.

Given the high metabolic demands and autophagic flux that exist in podocytes and TEC, the role of autophagy in renal health and disease is now under investigation.<sup>50</sup> In ageing mice, podocyte-specific deletion of the autophagy inducer, autophagy protein 5 (Atg5), led to the accumulation of oxidized protein, podocyte loss, proteinuria and glomerulosclerosis.<sup>51</sup> Similarly deletion of Atg5 from the renal epithelial cells (tubules and podocytes) of mice resulted in the development of a disease phenotype resembling human focal segmental glomerulosclerosis (FSGS) by the age of 2–4 months.<sup>52</sup> Thus, the concept of ‘autophagic failure’ has become an attractive hypothesis to explain podocytopathies in humans.

Renoprotective effects of autophagy in acute kidney injury (AKI) have also been characterized.<sup>53</sup> Nonetheless, the beneficial effects of enhanced autophagy induced by mTOR inhibitors are likely to be offset by detrimental effects of these agents on cell growth and survival.

## mTOR in renal ageing

Regulation of ageing by mTOR was first noted in studies of *S. cerevisiae*,<sup>54</sup> *C. elegans*<sup>55</sup> and *Drosophila melanogaster*.<sup>56</sup> A subsequent report showed that feeding aged mice with rapamycin extended their lifespan.<sup>57</sup> The role of mTOR in ageing has been reviewed previously.<sup>58</sup> Age-related alterations in the kidney include accumulation of lipofuscin, damaged mitochondria and protein aggregates, as well as loss of podocytes and autophagosome formation. Atg5, a component of the autophagic pathway regulated by mTOR, is critical for autophagosome creation, and its deletion in specific renal compartments (podocytes or proximal tubules) phenocopies findings seen in aged kidneys.<sup>51, 53</sup> Studies in ageing rodent kidneys have shown elevated mTOR expression in whole tissue and isolated primary mesangial cells,<sup>59</sup> possibly through regulation of cell cycling by p21 and/or the class III histone deacetylase sirtuin.<sup>60</sup> These features are ameliorated, at least *in vitro*, by treatment with rapamycin. Whether direct manipulation of renal mTOR expression and signal transduction ameliorates features of renal ageing remains to be shown.

## mTOR in immune cells

The role of mTOR in regulation of immune cell metabolism, function and reactivity<sup>3, 4, 61</sup> has implications for kidney transplant rejection, glomerulonephritides and their treatment.<sup>62, 63</sup> Both innate (dendritic cells [DCs] and macrophages) and adaptive immune cells (T and B lymphocytes) reside in the kidney and can promote acute and chronic renal disease,<sup>64–66</sup> but their dependence on mTOR activity remains unexplored. Our understanding of the role of mTOR in renal immunity must therefore be extrapolated largely from studies of lymphoid tissue. These observations (FIG. 3 and Table 1) provide insight into the potential of rapalogs, TORKinibs and other PI3K-mTOR pathway inhibitors in transplantation. In parallel, studies in experimental organ transplantation using rodent and non-human primate models, have increased understanding of the complex effects of rapamycin on allograft function.

## Dendritic cells

DCs are professional APCs that link the innate and adaptive immune responses by regulating T-cell responses to antigen. In the context of transplantation, they orchestrate both direct and indirect allorecognition. DC density in biopsy samples has been reported to correlate with T-cell proliferation and diminished graft survival in human kidney transplantation.<sup>67</sup> The understanding of the role of mTOR in shaping DC function stems largely from studies of the influence of rapamycin on these cells<sup>3, 61</sup>. Protracted mTORC1 inhibition by rapamycin inhibits maturation of DCs and their ability to promote effector T cell proliferation, while promoting regulatory T cell (Treg) enrichment.<sup>68, 69</sup> In a rodent heart transplant model, rapamycin-treated DCs promoted allograft survival following their systemic administration. Paradoxically however, exposure to rapamycin can also enhance the T-cell stimulatory



function of DCs with enhanced IL-12p40/p70 production following lipopolysaccharide stimulation.<sup>70</sup>

Evidence for an immunostimulatory effect of rapamycin on DC function also stems from studies of therapeutic vaccination. Pulsing DCs *in vitro* with a combination of rapamycin and Toll-like receptor (TLR) agonists enhanced antigen-specific CD8+ T-cell responses and anti-tumour immunity, as well as prolonged DC survival.<sup>71</sup> Whether these effects are mediated by mTORC1 or mTORC2 remains unclear, but both genetic deletion and RNA targeting of mTORC2 (Rictor) in DCs induces a hyperinflammatory phenotype<sup>72, 73</sup> and promotes alloreactive Th1 and Th17 responses *in vitro* and *in vivo*, suggesting that the effects may be mediated by mTORC2.<sup>74</sup>

## T cells

The transition from naive to effector T cells in response to antigen stimulation involves a metabolic switch from primarily oxidative phosphorylation to a more glycolytic pathway.<sup>75</sup> This switch enables activated T cells to consume large amounts of glucose and glutamine and undergo rapid proliferation and growth. By contrast, Treg cells, memory T cells and T follicular helper cells (Tfh; a T cell subset that stimulates germinal centre B cells) engage metabolism differently with a propensity to oxidize fatty acids and suppress mTOR through AMPK.

As understanding of T cell metabolism has progressed, distinct roles of mTORC1 and mTORC2 in T cell subset development have also emerged. In mice, both mTORC1 and mTORC2 promote thymic T cell development.<sup>76</sup> Administration of rapamycin promotes thymic atrophy, accelerates apoptosis of CD4+CD8+ (immature) T cells and spares CD4+CD25+ Treg cells.<sup>77</sup> The 'sparing' of Treg cells exposed to mTOR inhibitors has been observed *in vivo*, in mice and in the peripheral blood of kidney transplant recipients, and might be explained by the differing metabolism of these cells compared to effector T cells.<sup>78,79</sup> Paradoxically, specific deletion of mTORC1 (Raptor) in Treg cells results in loss of suppressive activity and fatal inflammation *in vivo*.<sup>80</sup> These results suggest that a basal level of mTOR activity, which might be rapamycin-resistant mTORC1 activity, is required by Treg cells to maintain their function. Furthermore, deletion of mTOR in T cells results in failure to generate Th1, Th2 or Th17 effector cells under skewing conditions, whereas Treg cell differentiation is preserved.<sup>81</sup> Skewing of Th1 and Th17 cells has been attributed to mTORC1, whereas Th2 cell differentiation is attributed to mTORC2.<sup>82</sup> The phenotype of mTORC2 deletion in T cells somewhat mirrors that in DCs and would be expected to promote rather than inhibit acute transplant rejection. Short hairpin RNA vectors targeted against mTORC1 (Raptor) and mTORC2 (Rictor) differentially altered the distribution of Th1 and Tfh cells; Raptor silencing augmented Tfh differentiation and reduced Th1 cell differentiation, whereas Rictor silencing minimally affected Tfh cells but promoted Th1 cell development.<sup>83</sup> These results are relevant to renal transplantation as they could account for the elevations in DSA levels seen in some kidney transplant recipients on rapamycin.

## B cells

Everolimus is a potent inhibitor of human B-cell activation and function (that is, T cell-dependent immunoglobulin production).<sup>84</sup> Marginal zone (MZ) B cells and humoral immune responses are also affected by mTOR manipulation. Thus, conditional deletion of TSC1 in murine B cells leads to loss of MZ B cells,<sup>85</sup> whereas conditional deletion of Rictor (mTORC2) impairs B cell survival and antibody responses.<sup>86</sup> On the other hand, two studies that used mTOR inhibitors to dissect the role of mTOR in antibody class switching have produced conflicting results. In mice rapamycin administration promoted cross-strain protection against lethal influenza infection by inhibiting B cell class switching,<sup>87</sup> whereas transient treatment with TORKinibs increased titres of class-switched high-affinity antibodies to a hapten-protein conjugate.<sup>88</sup> Given these results, coupled with clinical data on the relationship between mTOR inhibitors and DSA production, more basic and clinical studies are necessary to ascertain whether mTOR pathway manipulation protects or promotes ABMR. Data suggest that everolimus inhibits anti-HLA class I antibody-mediated endothelial cell signalling, migration and proliferation more potently than does sirolimus<sup>89</sup> pointing to a potential beneficial effect of everolimus in the prevention of chronic ABMR.

## NK cells and NKT cells

Studies of the role of mTOR in natural killer (NK) cells and NKT cells have extended our understanding of mTOR immunobiology. Given the important roles that these cells play in DSA formation and chronic ABMR, insights from these studies have direct implications for kidney transplant rejection.

mTOR is an essential component of the IL-15 signalling pathway in NK cells and drives both their development in the bone marrow and their activation in the periphery.<sup>90</sup> However, only minimal effects of mTOR inhibition on anti-HLA antibody-dependent NK cell activation have been reported.<sup>91</sup> On the other hand, exposure to either rapamycin or the dual mTORC1 and mTORC2 inhibitor PP242 inhibits inflammation-induced priming of induced (i) NK cells. Genetic deletion of either mTORC1 or mTORC2 in iNKT cells indicates that these complexes have important, non-overlapping roles. While mTORC2 is essential for transition from stage 1 to 2 development in the thymus, stage 3 thymic-resident iNKT cell subsets depend more on mTORC1 for survival.<sup>92</sup>

## 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.<sup>93, 94</sup> Importantly, administration of rapamycin late post-transplant halted the progression of allograft vasculopathy in rats and non-human primates (NHPs).<sup>95, 96</sup> 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 mixed chimerism when combined with costimulation blockade.<sup>97, 98</sup>

New generation mTOR inhibitors are of considerable potential interest in transplant models. TORKinibs were developed initially to overcome the limitations of rapamycin in clinical oncology. Thus “rapalog resistance”, seen in a variety of tumors treated with rapalogs, 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. The dual mTOR inhibitor INK128 has been shown to regulate NF- $\kappa$ B signalling and suppress inflammatory cytokines in a murine macrophage cell line (RAW 264.7 cells).<sup>99</sup> Moreover, the ATP-competitive mTOR inhibitor AZD8055 was shown to prevent acute rejection of mouse heart allografts and increase the numbers of graft-infiltrating Treg cells.<sup>100</sup> In a rat transplant model, modulation of both mTORC1 and mTORC2 eliminated chronic rejection when combined with subtherapeutic ciclosporin.<sup>101</sup> These data, coupled with the unacceptably high rates of late renal allograft failure secondary to chronic ABMR and vasculopathy, provide justification for further investigation of novel mTOR pathway inhibitors in transplantation.

### Clinical organ transplantation

Initial studies that combined sirolimus (versus placebo or titrated dosage<sup>102</sup>) with ciclosporin and prednisolone in renal transplantation demonstrated lower biopsy-proven acute rejection (BPAR) with sirolimus compared to ciclosporin and prednisone alone,<sup>103, 104</sup> even in the context of reduced ciclosporin dosage.<sup>104</sup> Sirolimus was also clearly superior to azathioprine<sup>105</sup> and has been investigated as a possible calcineurin-sparing agent.<sup>24, 106</sup> However, a subsequent study found a higher rate of BPAR in patients on prednisone and sirolimus who withdrew ciclosporin at 3 months compared to those who remained on this agent.<sup>107</sup> Furthermore, in the Elite-Symphony study, patients assigned to sirolimus, MMF and prednisone had higher BPAR rates (37.2%) than those treated with either low dose ciclosporin (24%) or tacrolimus (12.3%). Therefore, the use of Sirolimus as baseline immunosuppression in the absence of CNI has limitations, including higher rates of acute rejection.<sup>108,109</sup>, albeit offset by better GFR.<sup>107</sup>

The majority of studies and meta-analyses show that patient-based outcomes and the incidence of treatment withdrawal are worse following mTOR inhibition with sirolimus or everolimus compared to CNI-based regimens.<sup>110–112</sup> It is generally accepted that mTOR inhibitors provide inferior initial post-transplant immunosuppression compared to conventional CNI-based regimens. In addition, the propensity of mTOR inhibitors to impair wound healing, induce wound dehiscence and promote lymphocoele 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. The documented adverse effects associated with mTOR inhibitors include bone marrow suppression, hypertriglyceridaemia, peripheral oedema, gastrointestinal disturbance and stomatitis, new-onset diabetes after transplantation, proteinuria, gonadal toxicity, cough, dyspnoea and interstitial pneumonitis.<sup>113</sup> The ACE inhibitor ramipril has proven effective in reducing the incidence of proteinuria for up to 1

year after conversion to sirolimus in renal transplant recipients on maintenance immunosuppression.<sup>114</sup> However, the underlying pathobiology contributing to these adverse effects is incompletely understood. The development of aphthous ulcers might represent a breach of mucosal immunity and a lack of CD4+CD25+ Treg cells,<sup>115</sup> but this mechanism is counterintuitive as treatment with mTOR inhibitors tends to spare Treg from depletion in pre-clinical models and human kidney graft recipients.<sup>116</sup> Impaired wound healing through inhibition of cell proliferation and neoangiogenesis, or impaired glucose tolerance might be contributing factors. Similarly, interstitial pneumonitis might have an immunological aetiology, with increased CD4+ T cells found in bronchoalveolar lavage.<sup>117</sup>

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 CNIs 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 transplant have been conducted (Table 3).

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 endothelin-1 and oxidative stress, suggesting a protective effect on the arterial wall that might be translated into reduced cardiovascular risk.<sup>118</sup> On the other hand, among patients randomly assigned to either sirolimus or ciclosporin at 3-months post-transplantation, the incidence of subclinical inflammation in protocol biopsy samples 1 year post-transplantation was greater in the sirolimus group.<sup>119</sup> Tacrolimus is currently the most commonly used CNI in kidney transplantation. In a prospective randomized trial, kidney transplant recipients receiving rapid corticosteroid withdrawal, tacrolimus and mycophenolate mofetil (MMF) for 1 month were randomly assigned to either sirolimus plus MMF or tacrolimus plus MMF maintenance therapy.<sup>120</sup> Although graft survival at 2 years was similar between the groups, 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. Most studies comparing tacrolimus and sirolimus have involved *de novo* use of these agents rather than conversion.<sup>108, 109, 121</sup>

Other than their anti-neoplastic effects, a theoretical advantage of mTOR inhibitors in transplantation lies in their anti-viral properties. mTOR inhibitors stimulate CMV-specific Th1 cells and  $\gamma\delta$  T cells against CMV and translation of CMV viral proteins relies on host mTOR activity.<sup>122–124</sup> Furthermore, sirolimus inhibits and tacrolimus activates BK polyomavirus replication in renal TECs.<sup>125</sup> Despite these paradoxical anti-viral properties, exposure to rapamycin enhances the quality and quantity of pathogen-specific but not graft-reactive CD8+ T cells.<sup>126</sup> 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. Nonetheless, many comparative studies have demonstrated that early use of mTOR-inhibitor-based regimens can reduce the incidence of CMV infection.<sup>127, 128</sup> In a single centre 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 survival.<sup>129</sup> 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 beneficial effects on kidney function by enabling CNI sparing and their association with reduced rates of malignancies and non-melanoma skin cancer,<sup>130</sup> less viral infections and less weight gain<sup>131</sup> compared to CNI-based regimens.<sup>132</sup> Nonetheless, for the many renal transplant recipients with a documented history of sensitization, these agents remain inferior to CNIs. For these reasons, coupled with adverse effects and the availability of anti-viral agents, mTOR inhibitors are currently not used extensively post-transplantation. As malignancy and infection remain two of the most common causes of death among kidney transplant recipients, establishing novel and less toxic ways to manipulate the mTOR pathway is an important goal in transplantation.

**mTOR in AKI and ischaemic injury**—AKI is an important health problem that affects approximately one in five adults and one in three children who are hospitalized.<sup>133</sup> As well as increasing morbidity and mortality in the acute setting, AKI increases the long-term risk of developing CKD.<sup>134</sup> The underlying aetiologies that precipitate AKI are numerous and can be categorized into pre-renal, intrarenal and post-renal causes.<sup>135</sup> When adequately addressed, pre-renal and post-renal AKI resolve rapidly. Management of intrarenal AKI is more difficult; this disease is often the result of ischaemic or toxic injury to renal tubules, which typically requires days to weeks to resolve. A special situation arises during kidney transplantation in which donated organs inevitably undergo both cold and warm ischaemia. Indeed, the potential positive and negative sequelae of mTOR inhibitor therapy first became evident in renal allograft recipients.<sup>136–138</sup> The findings of delayed graft function (DGF) and cast nephropathy in humans by Smith *et al.* suggested that rapamycin therapy exerts increased toxicity on TEC and/or retards healing after ischaemic injury.<sup>136</sup> In all prospective randomized trials published to date, however, no association between rapamycin therapy and a reduction in GFR has been reported. To further elucidate the effects of mTOR inhibitors on TECs and vascular endothelial cells, as well as on local and systemic immune cells, rodent models of ischaemia–reperfusion (I/R) and various renal transplant models have been utilized.

Although several lines of evidence initially pointed to aggravation and delayed recovery from renal I/R injury with rapamycin,<sup>139, 140</sup> this finding was later challenged.<sup>141, 142</sup> The three most probable confounders contributing to these contradictory findings were likely the control group selected, the time of mTOR inhibitor administration and the timing of the analysis.<sup>141, 143</sup> Most studies that used a placebo group have reported adverse outcomes after administration of mTOR inhibitor.<sup>140, 144</sup> This delayed functional recovery could be reproduced in a mouse model by conditional deletion of mTORC1 in tubular cells before

inflicting I/R injury.<sup>33</sup> By contrast, studies that have compared mTORC1 inhibitors to calcineurin inhibitors (CNIs) have reported beneficial outcomes of mTOR inhibition that can be explained mainly as the result of avoidance of the deleterious effects of ciclosporin on the renal microcirculation.<sup>141, 145</sup> Moreover, a preconditioning-like effect of mTOR inhibition prior to transplantation has been observed in organs such as the heart.<sup>146</sup> This effect has also been reported in experimental renal transplantation; treatment of the donor with a single dose of rapamycin before nephrectomy improved allograft function.<sup>147</sup>

Another important confounder in studies of mTOR inhibitors in AKI is the timing of analysis after I/R. Studies that reported an adverse outcome of mTOR inhibition investigated early time-points (within several days of transplantation), whereas those that analysed much later time points (up to 16 weeks following the initial I/R) reported beneficial effects in normotensive rats and to a lesser degree in hypertensive animals.<sup>139, 145, 148, 149</sup>

Unfortunately, only a few studies have explored the mechanisms that underlie the observed effects. While reduced proliferation and increased apoptosis are suggested as the most likely underlying cause for the initial adverse effects in I/R<sup>33</sup> directly linked to the role of the mTOR kinase, secondary effects of mTOR inhibition that influence dependent pathways (such as autophagy) or effects on the anti-oxidative system may be responsible for late beneficial effects of mTOR inhibitors in I/R.<sup>150, 151</sup>

Few studies have investigated the role of mTOR in AKI unrelated to transplantation. Interestingly, AKI concurrent with the start of mTOR inhibitor therapy has been noted in patients with cancer, in whom it can present as an acute-tubular-necrosis-type injury.<sup>152</sup> By contrast, mTOR inhibition was found to be beneficial in a mouse model of AKI induced by HIV-associated nephropathy.<sup>153</sup> This effect was most likely mediated by downregulation of mTOR-induced p53 expression resulting in a reduction in oxidative cell injury. Hence in this model, reduced apoptosis provided a functional and histological benefit.

In summary, the risk of delayed graft function seems to be increased in patients who are treated with mTOR inhibitors directly after transplantation. As this risk might complicate the immediate post-transplant phase with regard to the need for additional dialysis and graft biopsy to exclude acute rejection in the context of delayed graft function, 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

The glomerulus regulates filtration by size and charge. Changes in glomerular permeability owing to hyperglycaemia,<sup>154</sup> induction of reactive oxygen species<sup>155</sup> or pharmacologic challenge<sup>156, 157</sup> underpin a number of disease processes. Exposure of healthy rats to mTOR inhibitor (temsirolimus) increased early glomerular permeability, but reduced late permeability.<sup>158</sup> In BALB/c mice without renal pathology, rapamycin induced a mild deterioration in renal function, increased albuminuria and podocyte foot-process width, and induced a short-term reduction in nephrin and podocin expression; these effects resolved



after 8 weeks of treatment.<sup>159</sup> Although previous studies had demonstrated no gross histologic changes in the renal glomerulus in response to mTOR inhibition,<sup>160, 161</sup> pathologic abnormalities were seen in the rodent renal tubular compartment<sup>162</sup> or vessels.<sup>163</sup>

Glomerulonephritides comprise a substantial proportion of human renal diseases and several rodent models have been developed to mimic these disorders. mTOR is increasingly recognized as having a fundamental role in the development of glomerular pathology, particularly in terms of dysregulation of rodent podocyte function.<sup>164</sup> Activation of mTORC1 in podocytes led to the development of glomerular crescents,<sup>165</sup> which was abolished following treatment with rapamycin. Podocyte homeostasis is disrupted by mTOR inhibitors that alter the cytoskeleton via RhoA signalling,<sup>166</sup> reduce vascular endothelial growth factor (VEGF)<sup>167</sup> synthesis and Akt phosphorylation,<sup>168</sup> downregulate the expression of nephrin,<sup>169</sup> podocin and synaptopodin<sup>168</sup> and affect cell motility. Additional studies have identified activation of Akt2 downstream of mTORC2 as critical for podocyte survival.<sup>170</sup>

### Diabetes mellitus

Diabetes mellitus is a leading cause of chronic kidney disease (CKD) in developed countries and the causative aetiology of ESRD in 30–40% of patients seeking renal replacement therapy.<sup>171</sup> Early renal changes in response to hyperglycaemia include mesangial and basement membrane expansion, glomerular hypertrophy and loss of capillary surface area.<sup>172</sup> Diabetes is now thought to represent a state of mTORC1 hyperactivation.<sup>173</sup> The molecular mechanism underlying compensatory hypertrophy is not well-characterized, but involves increased phosphorylation of 40S ribosomal protein (rpS6), downstream of S6 kinase 1 (S6K1).<sup>174</sup> This kinase in turn regulates protein synthesis and cellular growth downstream of mTORC1. The role of pS6 as a molecular signature of mTORC1 activation in podocytes has been confirmed in the glomeruli of patients<sup>22</sup> and mice<sup>23</sup> with diabetes. Moreover, knockout of S6K1 protected against the development of renal hypertrophy in diabetic mice.<sup>23</sup> Inhibition of mTOR signalling has also been shown to ameliorate some renal compensatory mechanisms following 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.<sup>175</sup> Similarly in diabetic rats, rapamycin ameliorated albuminuria (but had no effect on glomerular hypertrophy) and downregulated the expression of mTOR, pAkt, TGF- $\beta$ 1 and connective tissue growth factor<sup>176</sup>. These effects occurred without normalization of serum glucose and blood pressure levels and paralleled achievement of normoglycaemia<sup>177</sup>. High glucose levels have been shown to induce podocyte apoptosis, activate mTOR and promote NADPH oxidase (Nox)<sup>1/4</sup> expression; these effects are attenuated by rapamycin.<sup>178</sup>

### Systemic lupus erythematosus

The chronic autoimmune disease systemic lupus erythematosus (SLE) commonly affects the kidney through immune complex deposition, complement activation and infiltration of innate immune cells. MRL/lpr and female NZBW/F1 mice develop overt SLE and renal disease that can be mitigated by rapamycin treatment.<sup>179–183</sup> Activation of the PI3K/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 Ser 2448 pTOR is co-localized in podocytes and endothelial cells. Mice treated with rapamycin (either 1 week after development of severe proteinuria or prior to 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 pre-clinical lupus models.<sup>179</sup> Despite the anti-proliferative properties of rapamycin, few clinical studies have used this agent to treat lupus nephritis. A recent report of 7 patients suggests further investigation may be warranted.<sup>184</sup> 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 podocytes.<sup>186</sup> Endoplasmic reticulum stress triggers the unfolded protein response, which is preceded by activation of mTORC1 and dysregulated energy production<sup>187</sup>; both of these effects can be inhibited by everolimus administration.

In rodents with adriamycin nephropathy, rapamycin reduces proteinuria, preserves renal function and ameliorates glomerulosclerosis and tubular dilatation, with reductions in the intrarenal expression of C-C motif chemokine 5 (CCL5, also known as RANTES) and collagen.<sup>188</sup> These renoprotective and antiproteinuric effects can be recapitulated with everolimus, which also restores glomerular nephrin and podocin expression.<sup>189</sup>

Administration of everolimus slowed recovery from endothelial cell injury in a mouse model of thrombotic microangiopathy; this agent inhibited endothelial cell proliferation, but had no effect on endothelial cell apoptosis.<sup>190</sup> These data are in keeping with *in vitro* findings of dose-dependent inhibition of glomerular endothelial cell proliferation. Everolimus also inhibits the expression of VEGF and S6K in cultured podocytes and *in vivo*.

The rat chronic Thy1.1 nephritis model (generated by injection of antibodies against glomerular mesangial cells) mimics membranoproliferative glomerulonephritis.<sup>191</sup> In this model, administration of everolimus, particularly at low doses, prevented compensatory glomerular hypertrophy, reduced fibronectin and VEGF expression and reduced renal influx of monocytic lineage cells.<sup>192</sup> Rapamycin similarly abrogated a rise in blood pressure attributable to CKD in these rats, but had a detrimental effect at high doses in the acute injury phase<sup>193, 194</sup> owing to inhibition of rat endothelial cell proliferation and glomerular neoangiogenesis.<sup>100,101</sup> A deleterious effect of everolimus has also been reported in a rat remnant kidney model; this agent induced proteinuria, interstitial fibrosis and glomerulosclerosis.<sup>195</sup> In a rat model of established IgAN, low-dose rapamycin reduced IgA deposition, prevented progression of proteinuria and limited deterioration of renal function.<sup>196</sup> These effects correlated with cell cycle arrest, upregulation of cyclin-dependent kinase inhibitor 1B and presumably inhibition of mesangial cell proliferation.

In Heymann nephritis<sup>197</sup> — a rodent model of human membranous nephropathy — production of antibodies directed against the target antigens megalin and receptor-associated protein<sup>198</sup> leads to the development of glomerular deposits. Rapamycin mitigates proteinuria<sup>199</sup> and histologic lesions in this model,<sup>200</sup> including CD8+ T cell inflammation, with restoration of glomerular expression of podocin and nephrin<sup>200</sup> and a reduction in immunoglobulin deposits.<sup>199</sup>

A nephrotoxic serum nephritis model, induced by immunization with heterologous (sheep or rabbit) GBM proteins, is analogous to Goodpasture's disease in humans. In this model the development of glomerular crescents is preceded by mTORC1 activation.<sup>201</sup> Rapamycin administration concurrent with disease initiation decreased proteinuria, inflammatory infiltrate and type 1 T helper (Th1) cytokine production. By contrast, when commenced after disease induction,<sup>202,203</sup> rapamycin treatment led to increased albuminuria and accompanying macrophage and CD4+ T cell infiltration with increased Foxp3 transcripts suggesting accumulation of Treg cells, and glomerular pro-inflammatory cytokine production. These divergent, time-dependent effects are thought to be related to an initial, beneficial, down-regulation of T and B cell responses, followed by inhibition of podocyte VEGF-A production, reducing endothelial proliferation and thus the integrity of the glomerulus.

Few clinical studies have investigated the influence of mTOR modulation in glomerular disease (Table 1), possibly owing to concern about exacerbation of proteinuria. Moreover, low numbers of recruited patients limit the interpretation and generalizability of existing studies. Twenty-three patients with poor prognosis IgA nephropathy (>1g proteinuria per 24 h and glomerular filtration rate (GFR) 30–60 ml/min/1.73 m<sup>2</sup>) treated with angiotensin-converting-enzyme (ACE) inhibitor and statin were also assigned sirolimus or placebo for 12 months.<sup>204</sup> 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 FSGS;<sup>205</sup> the therapy induced complete remission in 19% of patients and partial remission in 38%, with maintenance of GFR and reduction in proteinuria. A phase II open-label trial in 6 patients with FSGS, however, reported a lack of responsiveness to sirolimus and an association of this therapy with nephrotoxicity.<sup>206</sup> In some kidney transplant recipients, high-dose sirolimus has been reported to induce *de novo* FSGS, characterized by decreased expression of synaptopodin and p57.<sup>207</sup> A case of successful treatment of minimal change nephropathy using combined tacrolimus and rapamycin has also been reported.<sup>208</sup>

## mTOR in polycystic kidney disease

Autosomal dominant polycystic kidney disease (ADPKD), the most common renal monogenetic disease, is caused by mutations in *PKD1* or *PKD2*.<sup>209</sup> Although this disease primarily affects the kidneys, the liver, cerebral blood vessels, gut, pancreas and cardiac valves can also be affected; PKD is thus a systemic disease.<sup>210</sup> Approximately half of affected patients require renal replacement therapy in their sixth or seventh decade.<sup>209</sup> In addition, several juvenile cystic kidney diseases caused by various gene defects commonly

lead to renal failure before adulthood.<sup>211</sup> Although key insights into disease mechanisms at a cellular level have been gained over the past two decades, treatment choices remain limited.<sup>212</sup>

mTOR has an important role during initiation and enlargement in PKD.<sup>213, 214</sup> Initial studies aimed to establish whether mTOR inhibitors could ameliorate PKD in rodent models.<sup>213, 214</sup> With very few exceptions (female Han:SPRD rats, PCK rats),<sup>215, 216</sup> these agents elicited a profound and long-lasting reduction in kidney size and an improvement in renal function. The gene product of *PKD1*, polycystin-1, interacts with TSC2, which forms a complex with the GTP-binding protein RHEB upstream of mTOR. When polycystin-1 was mutated this multiprotein complex led to aberrant activation of mTORC1 in tubular epithelial cells in polycystic mice and in patients with PKD.<sup>214</sup> Loss of *PKD1* is also associated with increased expression and activity of ERK, which phosphorylates TSC2 and thereby increases the stimulatory effect of the TSC2/RHEB complex on mTORC1.<sup>217</sup> This molecular mechanism brings together several known signalling and cellular perturbations related to *PKD1* insufficiency: increased proliferation, increased cell size and increased apoptosis<sup>217</sup>.

Aberrant signalling in the primary cilia is a hallmark and initiating feature of PKD. Loss of cilia inhibits the basal body compartment and reduces serine/threonine-protein kinase STK11–AMPK-mediated inhibition of mTORC1.<sup>218</sup> This reduction in inhibition in turn, leads to increased activation of mTORC1 and increased cell size. Reduced AMPK signalling, together with increased ERK signalling, have also been identified as the cause of the high anaerobic glycolytic activity observed in human *PKD1* knockout cells and kidneys of PKD1 knockout mice.<sup>219</sup> Administration of metformin, AICAR (an analogue of AMP) or 2-deoxyglucose reduced glycolytic activity and restored AMPK phosphorylation, thereby inhibiting mTORC1 and ameliorating cyst growth and functional decline in PKD.<sup>219, 220</sup>

Clinical trials of mTOR inhibitors in PKD were initiated based on the central pathomechanistic role of mTORC1 in this disease, encouraging preclinical results in rodent models, and anecdotal reports of shrinkage of enlarged polycystic kidneys in transplant recipients receiving mTOR inhibitors.<sup>214, 221</sup> The two largest clinical trials to date enrolled a total of >500 patients. The Swiss ADPKD study focused on patients with early stage ADPKD who had only slightly limited renal function and fairly small kidneys<sup>222</sup>, whereas the Everolimus in ADPKD Study enrolled patients with progressive disease, moderate to severe impairment of renal function and enlarged kidneys<sup>223</sup>. Both trials failed to demonstrate improvement in renal function with mTOR inhibition. Moreover, in the everolimus in ADPKD Study, an initial significant increase in GFR followed by a trend towards worse renal function was reported. In the Swiss ADPKD study, change in kidney size did not differ between mTOR inhibitor and placebo after 18 months; the everolimus in ADPKD Study demonstrated a reduction in kidney size with everolimus versus placebo after 6 months and a trend towards a reduction in kidney size at 12 months, which likely failed to reach significance owing to the drop-out rate. Several explanations as to why these bench-to-bedside approaches did not yield positive results have been suggested,<sup>224, 225</sup> these relate to the short follow-up period for a slowly-progressive disease and, in the Swiss ADPKD study, low blood trough levels of rapamycin that might have been insufficient to achieve inhibition

of mTORC1 within the cystic tissue.<sup>222, 226</sup> In the Everolimus in ADPKD trial, the heterogeneous patient population with advanced disease, frequent adverse effects (angioedema, aphthous stomatitis, proteinuria) and hence a high drop-out rate, might have obscured subgroups that could have benefitted from an mTOR-targeted approach.<sup>223</sup> A smaller Italian study (SIRENA) in 21 patients with ADPKD reported positive outcomes with sirolimus in terms of arresting kidney enlargement and reducing loss of renal parenchymal tissue.<sup>227</sup> Again possibly due to the short study period and small sample size, no improvement in renal function was detected. Another caveat was the high blood trough levels of rapamycin that would certainly cause substantial adverse events in the long-term.

Despite the rather disappointing clinical outcomes of the adequately powered studies published to date, several smaller trials entailing different treatment protocols and more specific patient cohorts have been initiated.<sup>228, 229</sup> Despite the compelling pre-clinical data, mTOR inhibitors cannot currently be advocated as a therapeutic option in ADPKD outside of clinical trials. Further experimental work is needed to define optimal treatment time-points and appropriate drug levels for mTOR inhibitors during the course of ADPKD. Organ-specific drug targeting approaches would likely enable a reduction in adverse events and could enhance the desired effects of mTOR inhibitors within polycystic kidneys. Whether combined mTORC1/2 inhibition could have a role in future trials remains to be determined.<sup>230</sup>

## mTOR in renal fibrosis and CKD

The role of mTOR in the induction and progression of pathology in fibrotic kidney disease is less well described than the role of mTOR in AKI. Concurrent findings indicate that the use of rapamycin following kidney injury, regardless of aetiology, reduces the subsequent development of interstitial fibrosis and/or the expression of fibrosis-related genes in IRI,<sup>231</sup> transplantation,<sup>232</sup> adriamycin nephropathy,<sup>233</sup> unilateral ureteral obstruction (UUO)<sup>234</sup> and glomerulopathy.<sup>235, 236</sup>

Treatment of normal rat kidney cells (NRK-49F cell line) with TGF- $\beta$ 1 activates Rictor and mTORC2 signalling in a time-dependent fashion, whereas knockdown of Rictor (using siRNA) inhibits the expression of fibronectin and smooth muscle actin<sup>237</sup>. In rats subjected to UUO (a standard model of renal fibrosis that causes phenotypic transition of renal tubular epithelial cells, endothelial cells and pericytes), Rictor was upregulated in comparison to the levels in sham-operated controls and co-localized with cells expressing smooth muscle actin, consistent with myofibroblast expression. Fibroblast-specific deletion of Rictor produced phenotypically and functionally normal kidneys that demonstrated lower collagen content and fibrosis, apoptosis and inflammatory cell infiltration following UUO<sup>237</sup>. Despite the utility of this animal model, one must consider that UUO results in acute fibrosis, whereas interstitial fibrosis, at least in humans, usually occurs as a chronic consequence of renal injury.<sup>238</sup>

Initial findings of mTOR activation in kidney fibrosis were limited to macrophages and myofibroblasts.<sup>239</sup> Administration of rapamycin mitigated the fibrotic and macrophage-based inflammation in response to UUO, and *in vitro* induction of fibroblast-mediated

fibrogenic activity in response to TGF- $\beta$ . Indeed, TGF- $\beta$  activates mTOR exclusively through the TSC2 pathway in fibroblasts<sup>240</sup> and upregulation of TGF- $\beta$ -induced Rheb/mTORC1 signalling in interstitial myofibroblasts was abolished by rapamycin.<sup>241</sup> Activation of mTOR also inhibited microRNA-21 suppression of mesangial cell protein synthesis and hypertrophy by TGF- $\beta$ .<sup>242</sup>

Fully-mismatched rodent models of kidney transplantation have also been used to evaluate the role of mTOR in chronic allograft nephropathy. As in other chronic kidney injury models, mTOR inhibition in these animals resulted in improvements in proteinuria and glomerulosclerosis,<sup>243, 244</sup> reduced inflammatory infiltrate<sup>245</sup> and decreased expression of fibrotic factors (TGF- $\beta$ , platelet-derived growth factor and CTGF).<sup>245, 246</sup> Multiple mechanisms that might underlie these beneficial effects of mTOR inhibitors have been reported. Several studies noted alterations in podocyte VEGF transcript and protein expression *in vivo* following treatment with mTOR inhibitors, and this finding has been replicated *in vitro*.<sup>247</sup> Treatment with sirolimus reduced VEGF-C/VEGFR-3 signalling (which is critical to lymphangiogenesis and lymphatic endothelial cells, and inhibited lymphangiogenesis might represent the mechanism of nephroprotection.<sup>248</sup> Plasmin activator inhibitor (PAI)-1 has been implicated in fibrosis and is thought to have a critical role in extracellular matrix deposition and matrix metalloproteinase activity.<sup>249</sup> In renal transplant recipients with chronic allograft nephropathy treated with rapamycin, glomerular and tubulointerstitial expression of plasmin activator inhibitor (PAI)-1 was reduced compared to those maintained on calcineurin inhibitors.<sup>250</sup>

The rodent remnant kidney model involves unilateral nephrectomy and partial infarction of the remaining kidney leading to CKD, which is characterized by a progressive rise in systolic blood pressure and serum creatinine levels. In this model, everolimus and sirolimus have both been reported to limit the progression of renal disease,<sup>251, 252</sup> with decreased proteinuria, fibrosis and TGF- $\beta$  expression, fibroblast activation<sup>252</sup> and VEGF signalling.<sup>251</sup> However, deleterious effects of everolimus (induction of proteinuria, interstitial fibrosis and glomerulosclerosis) have also been demonstrated in the same model.<sup>195</sup> - These divergent findings are puzzling and it is not clear whether the observed differences are simply related to the rodent genotype.

## mTOR in malignancies

### Renal angiomyolipomas

Angiomyolipomas (AMLs) resulting from clonal proliferation of epithelioid cells, are the most common renal lesions in patients with TSC. These lesions manifest as multiple and bilateral benign kidney tumours that can bleed spontaneously or cause life-threatening haemorrhage if >4 cm in diameter.<sup>253</sup> Less common renal manifestations of TSC include renal cysts, renal cell carcinomas, oncocytomas and FSGS. TSC is caused by mutations in either *TSC1* on chromosome 9 or *TSC2* on chromosome 16.<sup>254, 255</sup> Their gene products, hamartin and tuberlin respectively, dimerize and function as a GTPase-activating protein for Rheb, explaining the variety of benign tumors that form across multiple organs.



Given the genetic and molecular basis of TSC, use of mTOR inhibitors has been investigated as a systemic treatment.<sup>253, 256</sup> Early mTOR inhibition in patients with TSC might prevent development of TSC lesions and alter the natural history of the disease.<sup>257</sup> Multiple non-randomized studies have found that sirolimus reduces AML tumour volume.<sup>258, 259</sup> In one study, rapamycin treatment reduced mean tumour volume by 55% at 6 months and 66% at 1 year,<sup>260</sup> with no difference in mean tumour volume seen between years 1 and 2. Neoadjuvant use of mTOR inhibitors to facilitate nephron-sparing resection has also been reported.<sup>261</sup>

The largest study of mTOR inhibitors in TSC to date is the EXIST-II trial.<sup>262</sup> In this study, 118 patients were randomly assigned in 2:1 ratio to receive everolimus or placebo. Inclusion criteria included age >18 years, diagnosis of TSC (or sporadic lymphangioleiomyomatosis (LAM)) and at least one AML  $\geq$  3 cm in diameter. The rate of response (defined as a 50% reduction in total AML volume) was 42% for everolimus and 0% for placebo. As a result of these findings, everolimus was FDA-approved for treatment of adults with renal AMLs and TSC who do not require immediate surgery. Long-term everolimus treatment seems to be safe and effective in patients with TSC or sporadic LAM-associated renal AML-related bleeding.<sup>263</sup> Currently, mTOR inhibitors are recommended as first-line therapy for patients with AML volume >3 cm who are not candidates for immediate surgery.

### Renal cell carcinoma

Renal cell carcinomas (RCCs) originate in the renal cortex and constitute 80–85% of primary renal neoplasms. The role of mTOR inhibitors in the treatment of advanced RCC has been studied extensively,<sup>264</sup> although their use has been restricted largely to patients refractory to anti-VEGF therapy or with mutations in PI3K. In the INTORSECT trial, 500 patients refractory to the protein kinase inhibitor (PKI) sunitinib were randomly assigned to receive either the PKI sorafenib or temsirolimus.<sup>265</sup> Overall survival was shorter with temsirolimus. In the BEST trial, treatment-naïve patients were randomly assigned to various molecular therapies including combinations of mTOR and VEGF pathway inhibitors<sup>266</sup>. However, none of these combinations outperformed anti-VEGF (bevacizumab) monotherapy. In the RECORD-1 trial, everolimus therapy prolonged progression-free survival (PFS) in comparison to placebo in patients with metastatic RCC that had progressed on sunitinib, sorafenib, both therapies, or cytokines<sup>208</sup>. In the RECORD-4 trial everolimus was assessed in a second-line setting that confirmed the PFS benefit<sup>209</sup>.

Several TORKinibs have emerged as attractive alternatives to rapalogs in advanced RCC, owing to their dual inhibition of mTORC1 and mTORC2 and their favourable pharmacokinetic profile. Targeting of both complexes is considered theoretically advantageous, as this approach would prevent ‘rapalog resistance’ caused by activation of feedback loops that arise from mTORC1 inhibition alone and produce greater cytotoxicity against malignant cells.<sup>267</sup> The novel TORKinib AZD2014 inhibited RCC cell survival and growth and enhanced autophagy *in vitro*, and inhibited RCC cell growth in a xenograft model.<sup>268</sup> However, a randomized Phase II study of AZD2014 versus everolimus in anti-VEGF-refractory metastatic RCC, showed inferior PFS and overall survival with the TORKinib despite favourable toxicity and pharmacokinetic profiles.<sup>269</sup> At present, a role for

these novel agents in advanced anti-VEGF-refractory RCC remains uncertain. With the advent of molecular profiling of tumours, targeted mTOR inhibition might, however, still prove beneficial in subgroups of patients with mTOR-driven neoplasms.

### Post-transplant malignancy

In the setting of post-transplant malignancy mTOR inhibitors have theoretical advantages over other established immunosuppressive agents owing to their simultaneous stimulatory effects on certain immune cells and growth suppressive effects on malignant cells. In various animal models, sirolimus suppresses the growth and proliferation of tumours.<sup>270</sup> As a nutrient sensor and regulator of cell growth, tumours rely on the mTOR pathway to acquire and catabolize nutrients. Indeed, many tumours are characterized by upregulated mTOR signalling as a result of mutations in tumour suppressors (*PTEN*, *TSC1/2*) or oncogenes (*PI3K*, *S6K*, *eIF4E*).<sup>271</sup> Hence, most tumours are susceptible to mTOR inhibitors to varying degrees. The ability of mTOR inhibitors to directly inhibit the replication of viruses such as cytomegalovirus (CMV) and human herpesvirus 8 is another potential advantage of these agents.<sup>272, 273</sup>

Despite the potential advantages of mTOR inhibitors and some evidence of anti-cancer effects in transplant recipients,<sup>274, 275</sup> including an anti-tumour effect of sirolimus early after transplantation in subgroups of liver recipients with hepatocellular carcinoma,<sup>276</sup> these agents have not replaced CNIs in the clinic. Reasons for this failure include a plethora of adverse effects that limit tolerability and a lack of randomized control trials showing a clear benefit of conversion to mTOR inhibitors on hard clinical outcomes, such as *de novo* malignancy or cancer-related death. Nonetheless, conversion to mTOR inhibitors seems to be a reasonable choice for patients with either prior non-melanoma skin cancer<sup>277</sup> or newly-diagnosed Kaposi's sarcoma.<sup>278</sup> Several randomized controlled trials have demonstrated lower recurrence rates of squamous cell carcinoma (SCC) in patients with skin tumours converted to sirolimus than in those remaining on CNIs.<sup>277, 279</sup> In one study, conversion of kidney transplant recipients with at least one cutaneous SCC to sirolimus reduced the incidence of new SCC at 2 years (22% in the sirolimus group versus 39% in the CNI group)<sup>280</sup>. In all studies, however, discontinuation of sirolimus because of adverse events was common (20–50%). Given these high discontinuation rates, balancing the optimal therapeutic dose to prevent graft rejection whilst avoiding dose-related toxicities remains a challenge.

In kidney transplant recipients with Kaposi's sarcoma, conversion from ciclosporin to sirolimus was associated with complete regression of lesions by 3 months.<sup>274</sup> Patients with Kaposi's sarcoma show markedly increased basal P70 (S6K) activation and depressed Akt phosphorylation in peripheral blood mononuclear cells, and long-term treatment with rapamycin is associated with marked inhibition of basal and stimulated phosphorylation of both Akt and P70 (S6K) in parallel with regression of the skin neoplasm.<sup>281</sup>

Two meta-analyses have examined the efficacy of mTOR inhibitors for prevention of *de novo* malignancy in kidney transplant recipients. In the first study, Knoll et al showed a decreased incidence of cancer with sirolimus therapy (40% reduction in risk of malignancy and 56% reduction in risk of non-melanoma skin cancer)<sup>130</sup>. However, excess mortality

largely owing to cardiovascular disease and infection was also seen in the sirolimus group. By contrast, the second analysis (of cancer registry data) showed no benefit of sirolimus on cancer incidence, although this finding was attributed largely to an unexplained significantly increased incidence of prostate cancer in patients who received this therapy.<sup>282</sup> These findings, particularly the excess mortality in the first study, have dampened enthusiasm for mTOR inhibitors.

Thus, although mTOR inhibitors continue to show promise for the treatment of post-kidney-transplant malignancies, their use for malignancies other than Kaposi's sarcoma and non-melanoma skin cancer remains controversial and understudied in well-powered randomized controlled trials. Furthermore, no role is apparent for the *de novo* use of these agents in the prevention of cancer.

## Conclusions and future perspectives

Although considerable insights have been gained, much remains to be uncovered regarding the roles of mTOR in renal physiology and disease. Moreover, the potential of mTOR inhibitors to alleviate kidney pathology and improve outcomes in kidney transplantation needs more extensive assessment, both in the laboratory and in the clinic. The advent of mTOR complex gene targeting in specific renal and immune cell populations and the availability of new generation pharmacologic mTOR inhibitors is enhancing our understanding of the diverse roles of mTORC1 and mTORC2 in regulation of kidney function, pathology and the autoimmune and alloimmune responses that impact on renal disease, patient care and transplant survival. Areas particularly worthy of investigation (Box 1) and future directions (Box 2) include the roles of mTOR in control of tissue ageing, growth of certain cancers and resistance to specific viral infections, as well as the optimal ways to use mTOR antagonists in conjunction with other therapeutic agents in the clinic.

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## References

1. Vezina C, Kudelski A & Sehgal SN Rapamycin (AY-22,989), a new antifungal antibiotic. I. Taxonomy of the producing streptomycete and isolation of the active principle. *J Antibiot (Tokyo)* 28, 721–6 (1975). [PubMed: 1102508]
2. Calne RY et al. Rapamycin for immunosuppression in organ allografting. *Lancet* 2, 227 (1989).
3. Thomson AW, Turnquist HR & Raimondi G Immunoregulatory functions of mTOR inhibition. *Nat Rev Immunol* 9, 324–37 (2009). [PubMed: 19390566]
4. Powell JD, Pollizzi KN, Heikamp EB & Horton MR Regulation of immune responses by mTOR. *Annu Rev Immunol* 30, 39–68 (2012). [PubMed: 22136167]

5. Shimobayashi M & Hall MN Making new contacts: the mTOR network in metabolism and signalling crosstalk. *Nat Rev Mol Cell Biol* 15, 155–62 (2014). [PubMed: 24556838]
6. Flinn RJ, Yan Y, Goswami S, Parker PJ & Backer JM The late endosome is essential for mTORC1 signaling. *Mol Biol Cell* 21, 833–41 (2010). [PubMed: 20053679]
7. Kim SG et al. Metabolic stress controls mTORC1 lysosomal localization and dimerization by regulating the TTT-RUVBL1/2 complex. *Mol Cell* 49, 172–85 (2013). [PubMed: 23142078]
8. Yadav RB et al. mTOR direct interactions with Rheb-GTPase and raptor: sub-cellular localization using fluorescence lifetime imaging. *BMC Cell Biol* 14, 3 (2013). [PubMed: 23311891]
9. Fournier MJ et al. Inactivation of the mTORC1-eukaryotic translation initiation factor 4E pathway alters stress granule formation. *Mol Cell Biol* 33, 2285–301 (2013). [PubMed: 23547259]
10. Guertin DA et al. Ablation in mice of the mTORC components raptor, rictor, or mLST8 reveals that mTORC2 is required for signaling to Akt-FOXO and PKC $\alpha$ , but not S6K1. *Dev Cell* 11, 859–71 (2006). [PubMed: 17141160]
11. Zinzalla V, Stracka D, Oppliger W & Hall MN Activation of mTORC2 by association with the ribosome. *Cell* 144, 757–68 (2011). [PubMed: 21376236]
12. Xie J & Proud CG Crosstalk between mTOR complexes. *Nat Cell Biol* 15, 1263–5 (2013). [PubMed: 24189516]
13. Schreiber KH et al. Rapamycin-mediated mTORC2 inhibition is determined by the relative expression of FK506-binding proteins. *Aging Cell* 14, 265–73 (2015). [PubMed: 25652038]
14. Sarbassov DD et al. Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. *Mol Cell* 22, 159–68 (2006). [PubMed: 16603397]
15. Gaubitz C et al. Molecular basis of the rapamycin insensitivity of target of rapamycin complex 2. *Mol Cell* 58, 977–88 (2015). [PubMed: 26028537]
16. Zeng Z et al. Rapamycin derivatives reduce mTORC2 signaling and inhibit AKT activation in AML. *Blood* 109, 3509–12 (2007). [PubMed: 17179228]
17. Feldman ME et al. Active-site inhibitors of mTOR target rapamycin-resistant outputs of mTORC1 and mTORC2. *PLoS Biol* 7, e38 (2009). [PubMed: 19209957]
18. Brunn GJ et al. Direct inhibition of the signaling functions of the mammalian target of rapamycin by the phosphoinositide 3-kinase inhibitors, wortmannin and LY294002. *EMBO J* 15, 5256–67 (1996). [PubMed: 8895571]
19. Yu K et al. Biochemical, cellular, and in vivo activity of novel ATP-competitive and selective inhibitors of the mammalian target of rapamycin. *Cancer Res* 69, 6232–40 (2009). [PubMed: 19584280]
20. Bendell JC et al. A phase I dose-escalation study to assess safety, tolerability, pharmacokinetics, and preliminary efficacy of the dual mTORC1/mTORC2 kinase inhibitor CC-223 in patients with advanced solid tumors or multiple myeloma. *Cancer* 121, 3481–90 (2015). [PubMed: 26177599]
21. Pavenstadt H, Kriz W & Kretzler M Cell biology of the glomerular podocyte. *Physiol Rev* 83, 253–307 (2003). [PubMed: 12506131]
22. Godel M et al. Role of mTOR in podocyte function and diabetic nephropathy in humans and mice. *J Clin Invest* 121, 2197–209 (2011). [PubMed: 21606591]
23. Inoki K et al. mTORC1 activation in podocytes is a critical step in the development of diabetic nephropathy in mice. *J Clin Invest* 121, 2181–96 (2011). [PubMed: 21606597]
24. Groth CG et al. Sirolimus (rapamycin)-based therapy in human renal transplantation: similar efficacy and different toxicity compared with cyclosporine. Sirolimus European Renal Transplant Study Group. *Transplantation* 67, 1036–42 (1999). [PubMed: 10221490]
25. Schwarz C, Bohmig GA, Steininger R, Mayer G & Oberbauer R Impaired phosphate handling of renal allografts is aggravated under rapamycin-based immunosuppression. *Nephrol Dial Transplant* 16, 378–82 (2001). [PubMed: 11158416]
26. da Silva CA et al. Rosiglitazone prevents sirolimus-induced hypomagnesemia, hypokalemia, and downregulation of NKCC2 protein expression. *Am J Physiol Renal Physiol* 297, F916–22 (2009). [PubMed: 19656910]
27. Wu MS, Hung CC & Chang CT Renal calcium handling after rapamycin conversion in chronic allograft dysfunction. *Transpl Int* 19, 140–5 (2006). [PubMed: 16441363]

28. Tataranni T et al. Rapamycin-induced hypophosphatemia and insulin resistance are associated with mTORC2 activation and Klotho expression. *Am J Transplant* 11, 1656–64 (2011). [PubMed: 21672148]
29. Haller M et al. Sirolimus induced phosphaturia is not caused by inhibition of renal apical sodium phosphate cotransporters. *PLoS One* 7, e39229 (2012). [PubMed: 22859939]
30. Kempe DS et al. Rapamycin-induced phosphaturia. *Nephrol Dial Transplant* 25, 2938–44 (2010). [PubMed: 20368307]
31. Gleixner EM et al. V-ATPase/mTOR signaling regulates megalin-mediated apical endocytosis. *Cell Rep* 8, 10–9 (2014). [PubMed: 24953654]
32. Grahammer F et al. mTOR Regulates Endocytosis and Nutrient Transport in Proximal Tubular Cells. *J Am Soc Nephrol* In press (2016).
33. Grahammer F et al. mTORC1 maintains renal tubular homeostasis and is essential in response to ischemic stress. *Proc Natl Acad Sci U S A* 111, E2817–26 (2014). [PubMed: 24958889]
34. Lang F & Pearce D Regulation of the epithelial Na<sup>+</sup> channel by the mTORC2/SGK1 pathway. *Nephrol Dial Transplant* (2015).
35. Lu M et al. mTOR complex-2 activates ENaC by phosphorylating SGK1. *J Am Soc Nephrol* 21, 811–8 (2010). [PubMed: 20338997]
36. Gleason CE et al. mTORC2 regulates renal tubule sodium uptake by promoting ENaC activity. *J Clin Invest* 125, 117–28 (2015). [PubMed: 25415435]
37. Grahammer F et al. mTORC2 critically regulates renal potassium handling. *J Clin Invest* 126, 1773–82 (2016). [PubMed: 27043284]
38. Canaud G et al. Inhibition of the mTORC pathway in the antiphospholipid syndrome. *N Engl J Med* 371, 303–12 (2014). [PubMed: 25054716]
39. Jindra PT, Jin YP, Rozengurt E & Reed EF HLA class I antibody-mediated endothelial cell proliferation via the mTOR pathway. *J Immunol* 180, 2357–66 (2008). [PubMed: 18250445]
40. Jindra PT, Jin YP, Jacamo R, Rozengurt E & Reed EF MHC class I and integrin ligation induce ERK activation via an mTORC2-dependent pathway. *Biochem Biophys Res Commun* 369, 781–7 (2008). [PubMed: 18312854]
41. de Sandes-Freitas TV et al. Subclinical lesions and donor-specific antibodies in kidney transplant recipients receiving tacrolimus-based immunosuppressive regimen followed by early conversion to sirolimus. *Transplantation* 99, 2372–81 (2015). [PubMed: 25929604]
42. Liefeldt L et al. Donor-specific HLA antibodies in a cohort comparing everolimus with cyclosporine after kidney transplantation. *Am J Transplant* 12, 1192–8 (2012). [PubMed: 22300538]
43. Croze LE et al. Conversion to mammalian target of rapamycin inhibitors increases risk of de novo donor-specific antibodies. *Transpl Int* 27, 775–83 (2014). [PubMed: 24684741]
44. Lepin EJ et al. Phosphorylated S6 ribosomal protein: a novel biomarker of antibody-mediated rejection in heart allografts. *Am J Transplant* 6, 1560–71 (2006). [PubMed: 16827856]
45. Jindra PT et al. Anti-MHC class I antibody activation of proliferation and survival signaling in murine cardiac allografts. *J Immunol* 180, 2214–24 (2008). [PubMed: 18250428]
46. Kim YC & Guan KL mTOR: a pharmacologic target for autophagy regulation. *J Clin Invest* 125, 25–32 (2015). [PubMed: 25654547]
47. Ganley IG et al. ULK1.ATG13.FIP200 complex mediates mTOR signaling and is essential for autophagy. *J Biol Chem* 284, 12297–305 (2009). [PubMed: 19258318]
48. Hosokawa N et al. Nutrient-dependent mTORC1 association with the ULK1-Atg13-FIP200 complex required for autophagy. *Mol Biol Cell* 20, 1981–91 (2009). [PubMed: 19211835]
49. Jung CH et al. ULK-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery. *Mol Biol Cell* 20, 1992–2003 (2009). [PubMed: 19225151]
50. Fougeray S & Pallet N Mechanisms and biological functions of autophagy in diseased and ageing kidneys. *Nat Rev Nephrol* 11, 34–45 (2015). [PubMed: 25385287]
51. Hartleben B et al. Autophagy influences glomerular disease susceptibility and maintains podocyte homeostasis in aging mice. *J Clin Invest* 120, 1084–96 (2010). [PubMed: 20200449]

52. Kawakami T et al. Deficient autophagy results in mitochondrial dysfunction and FSGS. *J Am Soc Nephrol* 26, 1040–52 (2015). [PubMed: 25406339]
53. Kimura T et al. Autophagy protects the proximal tubule from degeneration and acute ischemic injury. *J Am Soc Nephrol* 22, 902–13 (2011). [PubMed: 21493778]
54. Fabrizio P, Pozza F, Pletcher SD, Gendron CM & Longo VD Regulation of longevity and stress resistance by Sch9 in yeast. *Science* 292, 288–90 (2001). [PubMed: 11292860]
55. Vellai T et al. Genetics: influence of TOR kinase on lifespan in *C. elegans*. *Nature* 426, 620 (2003).
56. Kapahi P et al. Regulation of lifespan in *Drosophila* by modulation of genes in the TOR signaling pathway. *Curr Biol* 14, 885–90 (2004). [PubMed: 15186745]
57. Harrison DE et al. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. *Nature* 460, 392–5 (2009). [PubMed: 19587680]
58. Johnson SC, Rabinovitch PS & Kaeberlein M mTOR is a key modulator of ageing and age-related disease. *Nature* 493, 338–45 (2013). [PubMed: 23325216]
59. Zhuo L et al. Expression and mechanism of mammalian target of rapamycin in age-related renal cell senescence and organ aging. *Mech Ageing Dev* 130, 700–8 (2009). [PubMed: 19698731]
60. Zhang S et al. SIRT1 is required for the effects of rapamycin on high glucose-inducing mesangial cells senescence. *Mech Ageing Dev* 133, 387–400 (2012). [PubMed: 22561310]
61. Weichhart T, Hengstschlager M & Linke M Regulation of innate immune cell function by mTOR. *Nat Rev Immunol* 15, 599–614 (2015). [PubMed: 26403194]
62. Fantus D & Thomson AW Evolving perspectives of mTOR complexes in immunity and transplantation. *Am J Transplant* 15, 891–902 (2015). [PubMed: 25737114]
63. Lo YC, Lee CF & Powell JD Insight into the role of mTOR and metabolism in T cells reveals new potential approaches to preventing graft rejection. *Curr Opin Organ Transplant* 19, 363–71 (2014). [PubMed: 24991977]
64. Turner JE, Paust HJ, Steinmetz OM & Panzer U The Th17 immune response in renal inflammation. *Kidney Int* 77, 1070–5 (2010). [PubMed: 20375986]
65. Rogers NM, Ferenbach DA, Isenberg JS, Thomson AW & Hughes J Dendritic cells and macrophages in the kidney: a spectrum of good and evil. *Nat Rev Nephrol* 10, 625–43 (2014). [PubMed: 25266210]
66. Park CO & Kupper TS The emerging role of resident memory T cells in protective immunity and inflammatory disease. *Nat Med* 21, 688–97 (2015). [PubMed: 26121195]
67. Batal I et al. Dendritic cells in kidney transplant biopsy samples are associated with T cell infiltration and poor allograft survival. *J Am Soc Nephrol* 26, 3102–13 (2015). [PubMed: 25855773]
68. Turnquist HR et al. Rapamycin-conditioned dendritic cells are poor stimulators of allogeneic CD4+ T cells, but enrich for antigen-specific Foxp3+ T regulatory cells and promote organ transplant tolerance. *J Immunol* 178, 7018–31 (2007). [PubMed: 17513751]
69. Hackstein H et al. Rapamycin inhibits IL-4--induced dendritic cell maturation in vitro and dendritic cell mobilization and function in vivo. *Blood* 101, 4457–63 (2003). [PubMed: 12531798]
70. Turnquist HR et al. mTOR and GSK-3 shape the CD4+ T-cell stimulatory and differentiation capacity of myeloid DCs after exposure to LPS. *Blood* 115, 4758–69 (2010). [PubMed: 20335217]
71. Amiel E et al. Inhibition of mechanistic target of rapamycin promotes dendritic cell activation and enhances therapeutic autologous vaccination in mice. *J Immunol* 189, 2151–8 (2012). [PubMed: 22826320]
72. Brown J, Wang H, Suttles J, Graves DT & Martin M Mammalian target of rapamycin complex 2 (mTORC2) negatively regulates Toll-like receptor 4-mediated inflammatory response via FoxO1. *J Biol Chem* 286, 44295–305 (2011). [PubMed: 22045807]
73. Raich-Regue D et al. mTORC2 deficiency in myeloid dendritic cells enhances their allogeneic Th1 and Th17 stimulatory ability after TLR4 ligation in vitro and in vivo. *J Immunol* 194, 4767–76 (2015). [PubMed: 25840913]
74. Raich-Regue D et al. Intratumoral delivery of mTORC2-deficient dendritic cells inhibits B16 melanoma growth by promoting CD8C effector T cell responses. *Oncoimmunology* (In Press).



75. Chi H Regulation and function of mTOR signalling in T cell fate decisions. *Nat Rev Immunol* 12, 325–38 (2012). [PubMed: 22517423]
76. Lee K et al. Vital roles of mTOR complex 2 in Notch-driven thymocyte differentiation and leukemia. *J Exp Med* 209, 713–28 (2012). [PubMed: 22473959]
77. Tian L, Lu L, Yuan Z, Lamb JR & Tam PK Acceleration of apoptosis in CD4+CD8+ thymocytes by rapamycin accompanied by increased CD4+CD25+ T cells in the periphery. *Transplantation* 77, 183–9 (2004). [PubMed: 14742978]
78. Haxhinasto S, Mathis D & Benoist C The AKT-mTOR axis regulates de novo differentiation of CD4+Foxp3+ cells. *J Exp Med* 205, 565–74 (2008). [PubMed: 18283119]
79. Battaglia M, Stabilini A & Roncarolo MG Rapamycin selectively expands CD4+CD25+FoxP3+ regulatory T cells. *Blood* 105, 4743–8 (2005). [PubMed: 15746082]
80. Zeng H et al. mTORC1 couples immune signals and metabolic programming to establish T(reg)-cell function. *Nature* 499, 485–90 (2013). [PubMed: 23812589]
81. Delgoffe GM et al. The mTOR kinase differentially regulates effector and regulatory T cell lineage commitment. *Immunity* 30, 832–44 (2009). [PubMed: 19538929]
82. Delgoffe GM et al. The kinase mTOR regulates the differentiation of helper T cells through the selective activation of signaling by mTORC1 and mTORC2. *Nat Immunol* 12, 295–303 (2011). [PubMed: 21358638]
83. Ray JP et al. The Interleukin-2-mTORc1 Kinase Axis Defines the Signaling, Differentiation, and Metabolism of T Helper 1 and Follicular B Helper T Cells. *Immunity* 43, 690–702 (2015). [PubMed: 26410627]
84. Matz M et al. Effects of sotrastaurin, mycophenolic acid and everolimus on human B-lymphocyte function and activation. *Transpl Int* 25, 1106–16 (2012). [PubMed: 22816666]
85. Benhamron S & Tirosh B Direct activation of mTOR in B lymphocytes confers impairment in B-cell maturation and loss of marginal zone B cells. *Eur J Immunol* 41, 2390–6 (2011). [PubMed: 21674478]
86. Lee K et al. Requirement for Rictor in homeostasis and function of mature B lymphoid cells. *Blood* 122, 2369–79 (2013). [PubMed: 23958952]
87. Keating R et al. The kinase mTOR modulates the antibody response to provide cross-protective immunity to lethal infection with influenza virus. *Nat Immunol* 14, 1266–76 (2013). [PubMed: 24141387]
88. Limon JJ et al. mTOR kinase inhibitors promote antibody class switching via mTORC2 inhibition. *Proc Natl Acad Sci U S A* 111, E5076–85 (2014). [PubMed: 25385646]
89. Jin YP, Valenzuela NM, Ziegler ME, Rozengurt E & Reed EF Everolimus inhibits anti-HLA I antibody-mediated endothelial cell signaling, migration and proliferation more potently than sirolimus. *Am J Transplant* 14, 806–19 (2014). [PubMed: 24580843]
90. Marcais A et al. The metabolic checkpoint kinase mTOR is essential for IL-15 signaling during the development and activation of NK cells. *Nat Immunol* 15, 749–57 (2014). [PubMed: 24973821]
91. Shin BH et al. Regulation of anti-HLA antibody-dependent natural killer cell activation by immunosuppressive agents. *Transplantation* 97, 294–300 (2014). [PubMed: 24342979]
92. Prevot N et al. Mammalian target of rapamycin complex 2 regulates invariant NKT cell development and function independent of promyelocytic leukemia zinc-finger. *J Immunol* 194, 223–30 (2015). [PubMed: 25404366]
93. Ochiai T et al. Effects of rapamycin in experimental organ allografting. *Transplantation* 56, 15–9 (1993). [PubMed: 8333037]
94. Stepkowski SM, Chen H, Daloze P & Kahan BD Rapamycin, a potent immunosuppressive drug for vascularized heart, kidney, and small bowel transplantation in the rat. *Transplantation* 51, 22–6 (1991). [PubMed: 1987691]
95. Poston RS et al. Rapamycin reverses chronic graft vascular disease in a novel cardiac allograft model. *Circulation* 100, 67–74 (1999). [PubMed: 10393683]
96. Ikonen TS et al. Sirolimus (rapamycin) halts and reverses progression of allograft vascular disease in non-human primates. *Transplantation* 70, 969–75 (2000). [PubMed: 11014651]

97. Nakamura T, Nakao T, Yoshimura N & Ashihara E Rapamycin prolongs cardiac allograft survival in a mouse model by inducing myeloid-derived suppressor cells. *Am J Transplant* 15, 2364–77 (2015). [PubMed: 25943210]
98. Page A et al. CD40 blockade combines with CTLA4Ig and sirolimus to produce mixed chimerism in an MHC-defined rhesus macaque transplant model. *Am J Transplant* 12, 115–25 (2012). [PubMed: 21929643]
99. Pan H et al. The second-generation mTOR kinase inhibitor INK128 exhibits anti-inflammatory activity in lipopolysaccharide-activated RAW 264.7 cells. *Inflammation* 37, 756–65 (2014). [PubMed: 24385238]
100. Rosborough BR et al. Adenosine triphosphate-competitive mTOR inhibitors: a new class of immunosuppressive agents that inhibit allograft rejection. *Am J Transplant* 14, 2173–80 (2014). [PubMed: 25307040]
101. Zhang L et al. Abrogation of chronic rejection in rat model system involves modulation of the mTORC1 and mTORC2 pathways. *Transplantation* 96, 782–90 (2013). [PubMed: 23985719]
102. Murgia MG, Jordan S & Kahan BD The side effect profile of sirolimus: a phase I study in quiescent cyclosporine-prednisone-treated renal transplant patients. *Kidney Int* 49, 209–16 (1996). [PubMed: 8770969]
103. MacDonald AS A worldwide, phase III, randomized, controlled, safety and efficacy study of a sirolimus/cyclosporine regimen for prevention of acute rejection in recipients of primary mismatched renal allografts. *Transplantation* 71, 271–80 (2001). [PubMed: 11213073]
104. Kahan BD, Julian BA, Pescovitz MD, Vanrenterghem Y & Neylan J Sirolimus reduces the incidence of acute rejection episodes despite lower cyclosporine doses in caucasian recipients of mismatched primary renal allografts: a phase II trial. Rapamune Study Group. *Transplantation* 68, 1526–32 (1999). [PubMed: 10589950]
105. Kahan BD Efficacy of sirolimus compared with azathioprine for reduction of acute renal allograft rejection: a randomised multicentre study. The Rapamune US Study Group. *Lancet* 356, 194–202 (2000). [PubMed: 10963197]
106. Kreis H et al. Sirolimus in association with mycophenolate mofetil induction for the prevention of acute graft rejection in renal allograft recipients. *Transplantation* 69, 1252–60 (2000). [PubMed: 10798738]
107. Kreis H et al. Long-term benefits with sirolimus-based therapy after early cyclosporine withdrawal. *J Am Soc Nephrol* 15, 809–17 (2004). [PubMed: 14978184]
108. Ekberg H et al. Reduced exposure to calcineurin inhibitors in renal transplantation. *N Engl J Med* 357, 2562–75 (2007). [PubMed: 18094377]
109. Flechner SM et al. The ORION study: comparison of two sirolimus-based regimens versus tacrolimus and mycophenolate mofetil in renal allograft recipients. *Am J Transplant* 11, 1633–44 (2011). [PubMed: 21668635]
110. Webster AC, Lee VW, Chapman JR & Craig JC Target of rapamycin inhibitors (TOR-I; sirolimus and everolimus) for primary immunosuppression in kidney transplant recipients. *Cochrane Database Syst Rev*, CD004290 (2006). [PubMed: 16625599]
111. Lim WH et al. A systematic review of conversion from calcineurin inhibitor to mammalian target of rapamycin inhibitors for maintenance immunosuppression in kidney transplant recipients. *Am J Transplant* 14, 2106–19 (2014). [PubMed: 25088685]
112. Su L et al. Everolimus-based calcineurin-inhibitor sparing regimens for kidney transplant recipients: a systematic review and meta-analysis. *Int Urol Nephrol* 46, 2035–44 (2014). [PubMed: 25027805]
113. Murakami N, Riella LV & Funakoshi T Risk of metabolic complications in kidney transplantation after conversion to mTOR inhibitor: a systematic review and meta-analysis. *Am J Transplant* 14, 2317–27 (2014). [PubMed: 25146383]
114. Mandelbrot DA et al. Effect of Ramipril on Urinary Protein Excretion in Maintenance Renal Transplant Patients Converted to Sirolimus. *Am J Transplant* 15, 3174–84 (2015). [PubMed: 26176342]
115. Lewkowicz N et al. Dysfunction of CD4+CD25<sup>high</sup> T regulatory cells in patients with recurrent aphthous stomatitis. *J Oral Pathol Med* 37, 454–61 (2008). [PubMed: 18318707]

116. Sabbatini M et al. Oscillatory mTOR inhibition and Treg increase in kidney transplantation. *Clin Exp Immunol* 182, 230–40 (2015). [PubMed: 26077103]
117. Morelon E et al. Characteristics of sirolimus-associated interstitial pneumonitis in renal transplant patients. *Transplantation* 72, 787–90 (2001). [PubMed: 11571438]
118. Joannides R et al. Immunosuppressant regimen based on sirolimus decreases aortic stiffness in renal transplant recipients in comparison to cyclosporine. *Am J Transplant* 11, 2414–22 (2011). [PubMed: 21929645]
119. Thierry A et al. Long-term impact of subclinical inflammation diagnosed by protocol biopsy one year after renal transplantation. *Am J Transplant* 11, 2153–61 (2011). [PubMed: 21883902]
120. Heilman RL et al. Results of a prospective randomized trial of sirolimus conversion in kidney transplant recipients on early corticosteroid withdrawal. *Transplantation* 92, 767–73 (2011). [PubMed: 21775930]
121. Glotz D et al. Thymoglobulin induction and sirolimus versus tacrolimus in kidney transplant recipients receiving mycophenolate mofetil and steroids. *Transplantation* 89, 1511–7 (2010). [PubMed: 20386144]
122. Li H & Pauza CD Rapamycin increases the yield and effector function of human gammadelta T cells stimulated in vitro. *Cancer Immunol Immunother* 60, 361–70 (2011). [PubMed: 21107834]
123. Clippinger AJ, Maguire TG & Alwine JC The changing role of mTOR kinase in the maintenance of protein synthesis during human cytomegalovirus infection. *J Virol* 85, 3930–9 (2011). [PubMed: 21307192]
124. Araki K et al. mTOR regulates memory CD8 T-cell differentiation. *Nature* 460, 108–12 (2009). [PubMed: 19543266]
125. Hirsch HH, Yakhontova K, Lu M & Manzetti J BK polyomavirus replication in renal tubular epithelial cells is inhibited by sirolimus, but activated by tacrolimus through a pathway involving FKBP-12. *Am J Transplant* (2015).
126. Ferrer IR et al. Cutting edge: Rapamycin augments pathogen-specific but not graft-reactive CD8+ T cell responses. *J Immunol* 185, 2004–8 (2010). [PubMed: 20631309]
127. Nashan B et al. Review of cytomegalovirus infection findings with mammalian target of rapamycin inhibitor-based immunosuppressive therapy in de novo renal transplant recipients. *Transplantation* 93, 1075–85 (2012). [PubMed: 22683823]
128. Tedesco-Silva H et al. Reduced incidence of cytomegalovirus infection in kidney transplant recipients receiving everolimus and reduced tacrolimus doses. *Am J Transplant* 15, 2655–64 (2015). [PubMed: 25988935]
129. Polanco N et al. Everolimus-based immunosuppression therapy for BK virus nephropathy. *Transplant Proc* 47, 57–61 (2015). [PubMed: 25645770]
130. Knoll GA et al. Effect of sirolimus on malignancy and survival after kidney transplantation: systematic review and meta-analysis of individual patient data. *BMJ* 349, g6679 (2014). [PubMed: 25422259]
131. Diekmann F et al. Treatment with sirolimus is associated with less weight gain after kidney transplantation. *Transplantation* 96, 480–6 (2013). [PubMed: 23912169]
132. Halleck F et al. An evaluation of sirolimus in renal transplantation. *Expert Opin Drug Metab Toxicol* 8, 1337–56 (2012). [PubMed: 22928953]
133. Susantitaphong P et al. World incidence of AKI: a meta-analysis. *Clin J Am Soc Nephrol* 8, 1482–93 (2013). [PubMed: 23744003]
134. Rimes-Stigare C et al. Evolution of chronic renal impairment and long-term mortality after de novo acute kidney injury in the critically ill; a Swedish multi-centre cohort study. *Crit Care* 19, 221 (2015). [PubMed: 25944032]
135. Himmelfarb J & Ikizler TA Acute kidney injury: changing lexicography, definitions, and epidemiology. *Kidney Int* 71, 971–6 (2007). [PubMed: 17396117]
136. Smith KD et al. Delayed graft function and cast nephropathy associated with tacrolimus plus rapamycin use. *J Am Soc Nephrol* 14, 1037–45 (2003). [PubMed: 12660339]
137. McTaggart RA et al. Sirolimus prolongs recovery from delayed graft function after cadaveric renal transplantation. *Am J Transplant* 3, 416–23 (2003). [PubMed: 12694063]

138. Loverre A et al. Ischemia-reperfusion induces glomerular and tubular activation of proinflammatory and antiapoptotic pathways: differential modulation by rapamycin. *J Am Soc Nephrol* 15, 2675–86 (2004). [PubMed: 15466272]
139. Goncalves GM et al. The role of immunosuppressive drugs in aggravating renal ischemia and reperfusion injury. *Transplant Proc* 39, 417–20 (2007). [PubMed: 17362745]
140. Fuller TF et al. Sirolimus delays recovery of rat kidney transplants after ischemia-reperfusion injury. *Transplantation* 76, 1594–9 (2003). [PubMed: 14702530]
141. Inman SR et al. Rapamycin preserves renal function compared with cyclosporine A after ischemia/reperfusion injury. *Urology* 62, 750–4 (2003). [PubMed: 14550466]
142. Cicora F et al. Sirolimus in kidney transplant donors and clinical and histologic improvement in recipients: rat model. *Transplant Proc* 42, 365–70 (2010). [PubMed: 20172351]
143. Lieberthal W et al. Rapamycin impairs recovery from acute renal failure: role of cell-cycle arrest and apoptosis of tubular cells. *Am J Physiol Renal Physiol* 281, F693–706 (2001). [PubMed: 11553517]
144. Lui SL et al. Effect of rapamycin on renal ischemia-reperfusion injury in mice. *Transpl Int* 19, 834–9 (2006). [PubMed: 16961776]
145. Yang B et al. Inflammation and caspase activation in long-term renal ischemia/reperfusion injury and immunosuppression in rats. *Kidney Int* 68, 2050–67 (2005). [PubMed: 16221205]
146. Khan S et al. Rapamycin confers preconditioning-like protection against ischemia-reperfusion injury in isolated mouse heart and cardiomyocytes. *J Mol Cell Cardiol* 41, 256–64 (2006). [PubMed: 16769083]
147. Cicora F et al. Preconditioning donor with a combination of tacrolimus and rapamycin to decrease ischaemia-reperfusion injury in a rat syngenic kidney transplantation model. *Clin Exp Immunol* 167, 169–77 (2012). [PubMed: 22132896]
148. Viklicky O et al. Effect of sirolimus on renal ischaemia/reperfusion injury in normotensive and hypertensive rats. *Transpl Int* 17, 432–41 (2004). [PubMed: 15338121]
149. Goncalves GM et al. The role of heme oxygenase 1 in rapamycin-induced renal dysfunction after ischemia and reperfusion injury. *Kidney Int* 70, 1742–9 (2006). [PubMed: 17003813]
150. Nakagawa S, Nishihara K, Inui K & Masuda S Involvement of autophagy in the pharmacological effects of the mTOR inhibitor everolimus in acute kidney injury. *Eur J Pharmacol* 696, 143–54 (2012). [PubMed: 23022334]
151. Kezic A, Thaiss F, Becker JU, Tsui TY & Bajcetic M Effects of everolimus on oxidative stress in kidney model of ischemia/reperfusion injury. *Am J Nephrol* 37, 291–301 (2013). [PubMed: 23548777]
152. Izzedine H et al. Acute tubular necrosis associated with mTOR inhibitor therapy: a real entity biopsy-proven. *Ann Oncol* 24, 2421–5 (2013). [PubMed: 23798615]
153. Rai P et al. mTOR plays a critical role in p53-induced oxidative kidney cell injury in HIVAN. *Am J Physiol Renal Physiol* 305, F343–54 (2013). [PubMed: 23678040]
154. Axelsson J, Rippe A & Rippe B Acute hyperglycemia induces rapid, reversible increases in glomerular permeability in nondiabetic rats. *Am J Physiol Renal Physiol* 298, F1306–12 (2010). [PubMed: 20237233]
155. Kuwabara A, Satoh M, Tomita N, Sasaki T & Kashihara N Deterioration of glomerular endothelial surface layer induced by oxidative stress is implicated in altered permeability of macromolecules in Zucker fatty rats. *Diabetologia* 53, 2056–65 (2010). [PubMed: 20526760]
156. McCarthy ET et al. TNF-alpha increases albumin permeability of isolated rat glomeruli through the generation of superoxide. *J Am Soc Nephrol* 9, 433–8 (1998). [PubMed: 9513905]
157. Axelsson J, Rippe A, Oberg CM & Rippe B Rapid, dynamic changes in glomerular permeability to macromolecules during systemic angiotensin II (ANG II) infusion in rats. *Am J Physiol Renal Physiol* 303, F790–9 (2012). [PubMed: 22718887]
158. Axelsson J, Rippe A & Rippe B mTOR inhibition with temsirolimus causes acute increases in glomerular permeability, but inhibits the dynamic permeability actions of puromycin aminonucleoside. *Am J Physiol Renal Physiol* 308, F1056–64 (2015). [PubMed: 25740597]
159. Stylianou K et al. Rapamycin induced ultrastructural and molecular alterations in glomerular podocytes in healthy mice. *Nephrol Dial Transplant* 27, 3141–8 (2012). [PubMed: 22290989]

160. DiJoseph JF, Sharma RN & Chang JY The effect of rapamycin on kidney function in the Sprague-Dawley rat. *Transplantation* 53, 507–13 (1992). [PubMed: 1549839]
161. Di Joseph JF & Sehgal SN Functional and histopathologic effects of rapamycin on mouse kidney. *Immunopharmacol Immunotoxicol* 15, 45–56 (1993). [PubMed: 8450180]
162. Andoh TF, Burdmann EA, Fransechini N, Houghton DC & Bennett WM Comparison of acute rapamycin nephrotoxicity with cyclosporine and FK506. *Kidney Int* 50, 1110–7 (1996). [PubMed: 8887267]
163. DiJoseph JF, Mihatsch MJ & Sehgal SN Renal effects of rapamycin in the spontaneously hypertensive rat. *Transpl Int* 7, 83–8 (1994). [PubMed: 8179808]
164. Grahmmer F, Wanner N & Huber TB mTOR controls kidney epithelia in health and disease. *Nephrol Dial Transplant* 29 Suppl 1, i9–i18 (2014). [PubMed: 24493874]
165. Mao J et al. Mammalian target of rapamycin complex 1 activation in podocytes promotes cellular crescent formation. *Am J Physiol Renal Physiol* 307, F1023–32 (2014). [PubMed: 24990893]
166. Jeruschke S et al. Protective effects of the mTOR inhibitor everolimus on cytoskeletal injury in human podocytes are mediated by RhoA signaling. *PLoS One* 8, e55980 (2013). [PubMed: 23418489]
167. Letavernier E et al. Sirolimus interacts with pathways essential for podocyte integrity. *Nephrol Dial Transplant* 24, 630–8 (2009). [PubMed: 18927120]
168. Muller-Krebs S et al. Cellular effects of everolimus and sirolimus on podocytes. *PLoS One* 8, e80340 (2013). [PubMed: 24260371]
169. Vollenbroeker B et al. mTOR regulates expression of slit diaphragm proteins and cytoskeleton structure in podocytes. *Am J Physiol Renal Physiol* 296, F418–26 (2009). [PubMed: 19019920]
170. Canaud G et al. AKT2 is essential to maintain podocyte viability and function during chronic kidney disease. *Nat Med* 19, 1288–96 (2013). [PubMed: 24056770]
171. Narres M et al. The incidence of end-stage renal disease in the diabetic (compared to the non-diabetic) population: A systematic review. *PLoS One* 11, e0147329 (2016). [PubMed: 26812415]
172. Ibrahim HN & Hostetter TH Diabetic nephropathy. *J Am Soc Nephrol* 8, 487–93 (1997). [PubMed: 9071718]
173. Mori H et al. The mTOR pathway is highly activated in diabetic nephropathy and rapamycin has a strong therapeutic potential. *Biochem Biophys Res Commun* 384, 471–5 (2009). [PubMed: 19422788]
174. Chen JK, Chen J, Thomas G, Kozma SC & Harris RC S6 kinase 1 knockout inhibits uninephrectomy- or diabetes-induced renal hypertrophy. *Am J Physiol Renal Physiol* 297, F585–93 (2009). [PubMed: 19474189]
175. Sakaguchi M et al. Inhibition of mTOR signaling with rapamycin attenuates renal hypertrophy in the early diabetic mice. *Biochem Biophys Res Commun* 340, 296–301 (2006). [PubMed: 16364254]
176. Yang Y et al. Rapamycin prevents early steps of the development of diabetic nephropathy in rats. *Am J Nephrol* 27, 495–502 (2007). [PubMed: 17671379]
177. Lloberas N et al. Mammalian target of rapamycin pathway blockade slows progression of diabetic kidney disease in rats. *J Am Soc Nephrol* 17, 1395–404 (2006). [PubMed: 16597691]
178. Eid AA et al. Mammalian target of rapamycin regulates Nox4-mediated podocyte depletion in diabetic renal injury. *Diabetes* 62, 2935–47 (2013). [PubMed: 23557706]
179. Stylianou K et al. The PI3K/Akt/mTOR pathway is activated in murine lupus nephritis and downregulated by rapamycin. *Nephrol Dial Transplant* 26, 498–508 (2011). [PubMed: 20709738]
180. Lui SL et al. Rapamycin attenuates the severity of established nephritis in lupus-prone NZB/W F1 mice. *Nephrol Dial Transplant* 23, 2768–76 (2008). [PubMed: 18445640]
181. Lui SL et al. Rapamycin prevents the development of nephritis in lupus-prone NZB/W F1 mice. *Lupus* 17, 305–13 (2008). [PubMed: 18413412]
182. Ramos-Barron A et al. Prevention of murine lupus disease in (NZBxNZW)F1 mice by sirolimus treatment. *Lupus* 16, 775–81 (2007). [PubMed: 17895299]



183. Warner LM, Adams LM & Sehgal SN Rapamycin prolongs survival and arrests pathophysiologic changes in murine systemic lupus erythematosus. *Arthritis Rheum* 37, 289–97 (1994). [PubMed: 8129783]
184. Yap DY, Ma MK, Tang CS & Chan TM Proliferation signal inhibitors in the treatment of lupus nephritis: preliminary experience. *Nephrology (Carlton)* 17, 676–80 (2012). [PubMed: 22817609]
185. Fernandez D, Bonilla E, Mirza N, Niland B & Perl A Rapamycin reduces disease activity and normalizes T cell activation-induced calcium fluxing in patients with systemic lupus erythematosus. *Arthritis Rheum* 54, 2983–8 (2006). [PubMed: 16947529]
186. Greka A & Mundel P Cell biology and pathology of podocytes. *Annu Rev Physiol* 74, 299–323 (2012). [PubMed: 22054238]
187. Ito N et al. mTORC1 activation triggers the unfolded protein response in podocytes and leads to nephrotic syndrome. *Lab Invest* 91, 1584–95 (2011). [PubMed: 21876538]
188. Lui SL et al. Rapamycin attenuates the severity of murine adriamycin nephropathy. *Am J Nephrol* 29, 342–52 (2009). [PubMed: 18948688]
189. Ramadan R et al. Early treatment with everolimus exerts nephroprotective effect in rats with adriamycin-induced nephrotic syndrome. *Nephrol Dial Transplant* 27, 2231–41 (2012). [PubMed: 22036940]
190. Keller K et al. Everolimus inhibits glomerular endothelial cell proliferation and VEGF, but not long-term recovery in experimental thrombotic microangiopathy. *Nephrol Dial Transplant* 21, 2724–35 (2006). [PubMed: 16861242]
191. Bagchus WM, Hoedemaeker PJ, Rozing J & Bakker WW Glomerulonephritis induced by monoclonal anti-Thy 1.1 antibodies. A sequential histological and ultrastructural study in the rat. *Lab Invest* 55, 680–7 (1986). [PubMed: 3537521]
192. Wittmann S et al. The mTOR inhibitor everolimus attenuates the time course of chronic anti-Thy1 nephritis in the rat. *Nephron Exp Nephrol* 108, e45–56 (2008). [PubMed: 18270495]
193. Daniel C, Ziswiler R, Frey B, Pfister M & Marti HP Proinflammatory effects in experimental mesangial proliferative glomerulonephritis of the immunosuppressive agent SDZ RAD, a rapamycin derivative. *Exp Nephrol* 8, 52–62 (2000). [PubMed: 10644883]
194. Daniel C et al. Mechanisms of everolimus-induced glomerulosclerosis after glomerular injury in the rat. *Am J Transplant* 5, 2849–61 (2005). [PubMed: 16302997]
195. Vogelbacher R, Wittmann S, Braun A, Daniel C & Hugo C The mTOR inhibitor everolimus induces proteinuria and renal deterioration in the remnant kidney model in the rat. *Transplantation* 84, 1492–9 (2007). [PubMed: 18091526]
196. Tian J, Wang Y, Liu X, Zhou X & Li R Rapamycin ameliorates IgA nephropathy via cell cycle-dependent mechanisms. *Exp Biol Med* (Maywood) 240, 936–45 (2015). [PubMed: 25349217]
197. Heymann W, Hackel DB, Harwood S, Wilson SG & Hunter JL Production of nephrotic syndrome in rats by Freund's adjuvants and rat kidney suspensions. *Proc Soc Exp Biol Med* 100, 660–4 (1959). [PubMed: 13645677]
198. Farquhar MG, Saito A, Kerjaschki D & Orlando RA The Heymann nephritis antigenic complex: megalin (gp330) and RAP. *J Am Soc Nephrol* 6, 35–47 (1995). [PubMed: 7579068]
199. Naumovic R et al. Effects of rapamycin on active Heymann nephritis. *Am J Nephrol* 27, 379–89 (2007). [PubMed: 17570905]
200. Stratakis S et al. Rapamycin ameliorates proteinuria and restores nephrin and podocin expression in experimental membranous nephropathy. *Clin Dev Immunol* 2013, 941893. [PubMed: 24069045]
201. Kurayama R et al. Role of amino acid transporter LAT2 in the activation of mTORC1 pathway and the pathogenesis of crescentic glomerulonephritis. *Lab Invest* 91, 992–1006 (2011). [PubMed: 21403644]
202. Hochegger K et al. Differential effects of rapamycin in anti-GBM glomerulonephritis. *J Am Soc Nephrol* 19, 1520–9 (2008). [PubMed: 18480312]
203. Kirsch AH et al. The mTOR-inhibitor rapamycin mediates proteinuria in nephrotoxic serum nephritis by activating the innate immune response. *Am J Physiol Renal Physiol* 303, F569–75 (2012). [PubMed: 22696604]



204. Cruzado JM et al. Low-dose sirolimus combined with angiotensin-converting enzyme inhibitor and statin stabilizes renal function and reduces glomerular proliferation in poor prognosis IgA nephropathy. *Nephrol Dial Transplant* 26, 3596–602 (2011). [PubMed: 21393611]
205. Tumlin JA et al. A prospective, open-label trial of sirolimus in the treatment of focal segmental glomerulosclerosis. *Clin J Am Soc Nephrol* 1, 109–16 (2006). [PubMed: 17699197]
206. Cho ME, Hurley JK & Kopp JB Sirolimus therapy of focal segmental glomerulosclerosis is associated with nephrotoxicity. *Am J Kidney Dis* 49, 310–7 (2007). [PubMed: 17261434]
207. Letavernier E et al. High sirolimus levels may induce focal segmental glomerulosclerosis de novo. *Clin J Am Soc Nephrol* 2, 326–33 (2007). [PubMed: 17699432]
208. Patel P, Pal S, Ashley C, Sweny P & Burns A Combination therapy with sirolimus (rapamycin) and tacrolimus (FK-506) in treatment of refractory minimal change nephropathy, a clinical case report. *Nephrol Dial Transplant* 20, 985–7 (2005). [PubMed: 15741203]
209. Ong AC, Devuyst O, Knebelmann B, Walz G & Diseases E.-E.W.G.f.I.K. Autosomal dominant polycystic kidney disease: the changing face of clinical management. *Lancet* 385, 1993–2002 (2015). [PubMed: 26090645]
210. Grantham JJ Clinical practice. Autosomal dominant polycystic kidney disease. *N Engl J Med* 359, 1477–85 (2008). [PubMed: 18832246]
211. Hildebrandt F, Benzing T & Katsanis N Ciliopathies. *N Engl J Med* 364, 1533–43 (2011). [PubMed: 21506742]
212. Torres VE et al. Tolvaptan in patients with autosomal dominant polycystic kidney disease. *N Engl J Med* 367, 2407–18 (2012). [PubMed: 23121377]
213. Tao Y, Kim J, Schrier RW & Edelstein CL Rapamycin markedly slows disease progression in a rat model of polycystic kidney disease. *J Am Soc Nephrol* 16, 46–51 (2005). [PubMed: 15563559]
214. Shillingford JM et al. The mTOR pathway is regulated by polycystin-1, and its inhibition reverses renal cystogenesis in polycystic kidney disease. *Proc Natl Acad Sci U S A* 103, 5466–71 (2006). [PubMed: 16567633]
215. Renken C, Fischer DC, Kundt G, Gretz N & Haffner D Inhibition of mTOR with sirolimus does not attenuate progression of liver and kidney disease in PCK rats. *Nephrol Dial Transplant* 26, 92–100 (2011). [PubMed: 20615907]
216. Belibi F, Ravichandran K, Zafar I, He Z & Edelstein CL mTORC1/2 and rapamycin in female Han:SPRD rats with polycystic kidney disease. *Am J Physiol Renal Physiol* 300, F236–44 (2011). [PubMed: 20943770]
217. Distefano G et al. Polycystin-1 regulates extracellular signal-regulated kinase-dependent phosphorylation of tuberin to control cell size through mTOR and its downstream effectors S6K and 4EBP1. *Mol Cell Biol* 29, 2359–71 (2009). [PubMed: 19255143]
218. Boehlke C et al. Primary cilia regulate mTORC1 activity and cell size through Lkb1. *Nat Cell Biol* 12, 1115–22 (2010). [PubMed: 20972424]
219. Boletta A Emerging evidence of a link between the polycystins and the mTOR pathways. *Pathogenetics* 2, 6 (2009). [PubMed: 19863783]
220. Takiar V et al. Activating AMP-activated protein kinase (AMPK) slows renal cystogenesis. *Proc Natl Acad Sci U S A* 108, 2462–7 (2011). [PubMed: 21262823]
221. Huber TB, Walz G & Kuehn EW mTOR and rapamycin in the kidney: signaling and therapeutic implications beyond immunosuppression. *Kidney Int* 79, 502–11 (2011). [PubMed: 21085109]
222. Serra AL et al. Sirolimus and kidney growth in autosomal dominant polycystic kidney disease. *N Engl J Med* 363, 820–9 (2010). [PubMed: 20581391]
223. Walz G et al. Everolimus in patients with autosomal dominant polycystic kidney disease. *N Engl J Med* 363, 830–40 (2010). [PubMed: 20581392]
224. Grantham JJ, Bennett WM & Perrone RD mTOR inhibitors and autosomal dominant polycystic kidney disease. *N Engl J Med* 364, 286–7; author reply 287–9 (2011). [PubMed: 21247328]
225. Levey AS & Stevens LA mTOR inhibitors and autosomal dominant polycystic kidney disease. *N Engl J Med* 364, 287; author reply 287–9 (2011).

226. Novalic Z et al. Dose-dependent effects of sirolimus on mTOR signaling and polycystic kidney disease. *J Am Soc Nephrol* 23, 842–53 (2012). [PubMed: 22343118]
227. Perico N et al. Sirolimus therapy to halt the progression of ADPKD. *J Am Soc Nephrol* 21, 1031–40 (2010). [PubMed: 20466742]
228. Riegersperger M, Herkner H & Sunder-Plassmann G Pulsed oral sirolimus in advanced autosomal-dominant polycystic kidney disease (Vienna RAP Study): study protocol for a randomized controlled trial. *Trials* 16, 182 (2015). [PubMed: 25899445]
229. Braun WE, Schold JD, Stephany BR, Spirko RA & Herts BR Low-dose rapamycin (sirolimus) effects in autosomal dominant polycystic kidney disease: an open-label randomized controlled pilot study. *Clin J Am Soc Nephrol* 9, 881–8 (2014). [PubMed: 24721888]
230. Ravichandran K et al. An mTOR anti-sense oligonucleotide decreases polycystic kidney disease in mice with a targeted mutation in *Pkd2*. *Hum Mol Genet* 23, 4919–31 (2014). [PubMed: 24847003]
231. Jain S, Bicknell GR, Whiting PH & Nicholson ML Rapamycin reduces expression of fibrosis-associated genes in an experimental model of renal ischaemia reperfusion injury. *Transplant Proc* 33, 556–8 (2001). [PubMed: 11266957]
232. Jolicoeur EM et al. Combination therapy of mycophenolate mofetil and rapamycin in prevention of chronic renal allograft rejection in the rat. *Transplantation* 75, 54–9 (2003). [PubMed: 12544871]
233. Rangan GK & Coombes JD Renoprotective effects of sirolimus in non-immune initiated focal segmental glomerulosclerosis. *Nephrol Dial Transplant* 22, 2175–82 (2007). [PubMed: 17550925]
234. Wu MJ et al. Rapamycin attenuates unilateral ureteral obstruction-induced renal fibrosis. *Kidney Int* 69, 2029–36 (2006). [PubMed: 16732193]
235. Bonegio RG et al. Rapamycin ameliorates proteinuria-associated tubulointerstitial inflammation and fibrosis in experimental membranous nephropathy. *J Am Soc Nephrol* 16, 2063–72 (2005). [PubMed: 15917339]
236. Kramer S et al. Low-dose mTOR inhibition by rapamycin attenuates progression in anti-thy1-induced chronic glomerulosclerosis of the rat. *Am J Physiol Renal Physiol* 294, F440–9 (2008). [PubMed: 18094032]
237. Li J et al. Rictor/mTORC2 signaling mediates TGFβ1-induced fibroblast activation and kidney fibrosis. *Kidney Int* 88, 515–27 (2015). [PubMed: 25970154]
238. Grahmmer F Halting renal fibrosis: an unexpected role for mTORC2 signaling. *Kidney Int* 88, 437–9 (2015). [PubMed: 26323069]
239. Chen G et al. Rapamycin ameliorates kidney fibrosis by inhibiting the activation of mTOR signaling in interstitial macrophages and myofibroblasts. *PLoS One* 7, e33626 (2012). [PubMed: 22470459]
240. Rahimi RA et al. Distinct roles for mammalian target of rapamycin complexes in the fibroblast response to transforming growth factor-beta. *Cancer Res* 69, 84–93 (2009). [PubMed: 19117990]
241. Jiang L et al. Rheb/mTORC1 signaling promotes kidney fibroblast activation and fibrosis. *J Am Soc Nephrol* 24, 1114–26 (2013). [PubMed: 23661807]
242. Dey N, Ghosh-Choudhury N, Kasinath BS & Choudhury GG TGFβ-stimulated microRNA-21 utilizes PTEN to orchestrate AKT/mTORC1 signaling for mesangial cell hypertrophy and matrix expansion. *PLoS One* 7, e42316 (2012). [PubMed: 22879939]
243. Koch M, Mengel M, Poehnert D & Nashan B Effects of everolimus on cellular and humoral immune processes leading to chronic allograft nephropathy in a rat model with sensitized recipients. *Transplantation* 83, 498–505 (2007). [PubMed: 17318083]
244. Viklicky O et al. SDZ-RAD prevents manifestation of chronic rejection in rat renal allografts. *Transplantation* 69, 497–502 (2000). [PubMed: 10708101]
245. Lutz J, Zou H, Liu S, Antus B & Heemann U Apoptosis and treatment of chronic allograft nephropathy with everolimus. *Transplantation* 76, 508–15 (2003). [PubMed: 12923436]
246. Luo L, Sun Z & Luo G Rapamycin is less fibrogenic than Cyclosporin A as demonstrated in a rat model of chronic allograft nephropathy. *J Surg Res* 179, e255–63 (2013). [PubMed: 22480837]

247. Liu Q et al. Characterization of Torin2, an ATP-competitive inhibitor of mTOR, ATM, and ATR. *Cancer Res* 73, 2574–86 (2013). [PubMed: 23436801]
248. Palin NK, Savikko J & Koskinen PK Sirolimus inhibits lymphangiogenesis in rat renal allografts, a novel mechanism to prevent chronic kidney allograft injury. *Transpl Int* 26, 195–205 (2013). [PubMed: 23167600]
249. Ghosh AK & Vaughan DE PAI-1 in tissue fibrosis. *J Cell Physiol* 227, 493–507 (2012). [PubMed: 21465481]
250. Pontrelli P et al. Rapamycin inhibits PAI-1 expression and reduces interstitial fibrosis and glomerulosclerosis in chronic allograft nephropathy. *Transplantation* 85, 125–34 (2008). [PubMed: 18192922]
251. Diekmann F et al. Mammalian target of rapamycin inhibition halts the progression of proteinuria in a rat model of reduced renal mass. *J Am Soc Nephrol* 18, 2653–60 (2007). [PubMed: 17804674]
252. Kurdian M et al. Delayed mTOR inhibition with low dose of everolimus reduces TGFbeta expression, attenuates proteinuria and renal damage in the renal mass reduction model. *PLoS One* 7, e32516 (2012). [PubMed: 22427849]
253. Budde K & Gaedeke J Tuberous sclerosis complex-associated angiomyolipomas: focus on mTOR inhibition. *Am J Kidney Dis* 59, 276–83 (2012). [PubMed: 22130643]
254. Hallett L, Foster T, Liu Z, Blieden M & Valentim J Burden of disease and unmet needs in tuberous sclerosis complex with neurological manifestations: systematic review. *Curr Med Res Opin* 27, 1571–83 (2011). [PubMed: 21692602]
255. Osborne JP, Fryer A & Webb D Epidemiology of tuberous sclerosis. *Ann N Y Acad Sci* 615, 125–7 (1991). [PubMed: 2039137]
256. Curatolo P et al. The role of mTOR inhibitors in the treatment of patients with tuberous sclerosis complex: Evidence-based and expert opinions. *Drugs* 76, 551–65 (2016). [PubMed: 26927950]
257. Kotulska K, Borkowska J & Jozwiak S Possible prevention of tuberous sclerosis complex lesions. *Pediatrics* 132, e239–42 (2013). [PubMed: 23733802]
258. Bissler JJ et al. Sirolimus for angiomyolipoma in tuberous sclerosis complex or lymphangioleiomyomatosis. *N Engl J Med* 358, 140–51 (2008). [PubMed: 18184959]
259. Dabora SL et al. Multicenter phase 2 trial of sirolimus for tuberous sclerosis: kidney angiomyolipomas and other tumors regress and VEGF-D levels decrease. *PLoS One* 6, e23379 (2011). [PubMed: 21915260]
260. Cabrera-Lopez C et al. Assessing the effectiveness of rapamycin on angiomyolipoma in tuberous sclerosis: a two years trial. *Orphanet J Rare Dis* 7, 87 (2012). [PubMed: 23140536]
261. Staehler M et al. Nephron-sparing resection of angiomyolipoma after sirolimus pretreatment in patients with tuberous sclerosis. *Int Urol Nephrol* 44, 1657–61 (2012). [PubMed: 23054313]
262. Bissler JJ et al. Everolimus for angiomyolipoma associated with tuberous sclerosis complex or sporadic lymphangioleiomyomatosis (EXIST-2): a multicentre, randomised, double-blind, placebo-controlled trial. *Lancet* 381, 817–24 (2013). [PubMed: 23312829]
263. Bissler JJ et al. Everolimus for renal angiomyolipoma in patients with tuberous sclerosis complex or sporadic lymphangioleiomyomatosis: extension of a randomized controlled trial. *Nephrol Dial Transplant* 31, 111–9 (2016). [PubMed: 26156073]
264. Pal SK & Quinn DI Differentiating mTOR inhibitors in renal cell carcinoma. *Cancer Treat Rev* 39, 709–19 (2013). [PubMed: 23433636]
265. Hutson TE et al. Randomized phase III trial of temsirolimus versus sorafenib as second-line therapy after sunitinib in patients with metastatic renal cell carcinoma. *J Clin Oncol* 32, 760–7 (2014). [PubMed: 24297950]
266. Flaherty KT et al. BEST: A Randomized Phase II study of vascular endothelial growth factor, RAF kinase, and mammalian target of rapamycin combination targeted therapy with bevacizumab, sorafenib, and temsirolimus in advanced renal cell carcinoma—a trial of the ECOG-ACRIN Cancer Research Group (E2804). *J Clin Oncol* 33, 2384–91 (2015). [PubMed: 26077237]

267. Wander SA, Hennessy BT & Slingerland JM Next-generation mTOR inhibitors in clinical oncology: how pathway complexity informs therapeutic strategy. *J Clin Invest* 121, 1231–41 (2011). [PubMed: 21490404]
268. Zheng B et al. Pre-clinical evaluation of AZD-2014, a novel mTORC1/2 dual inhibitor, against renal cell carcinoma. *Cancer Lett* 357, 468–75 (2015). [PubMed: 25444920]
269. Powles T et al. A randomised Phase 2 Study of AZD2014 versus everolimus in patients with VEGF-refractory metastatic clear cell renal cancer. *Eur Urol* (2015).
270. Guba M et al. Rapamycin inhibits primary and metastatic tumor growth by antiangiogenesis: involvement of vascular endothelial growth factor. *Nat Med* 8, 128–35 (2002). [PubMed: 11821896]
271. Ghosh AP et al. Point mutations of the mTOR-RHEB pathway in renal cell carcinoma. *Oncotarget* 6, 17895–910 (2015). [PubMed: 26255626]
272. Nichols LA, Adang LA & Kedes DH Rapamycin blocks production of KSHV/HHV8: insights into the anti-tumor activity of an immunosuppressant drug. *PLoS One* 6, e14535 (2011). [PubMed: 21264294]
273. Roy S & Arav-Boger R New cell-signaling pathways for controlling cytomegalovirus replication. *Am J Transplant* 14, 1249–58 (2014). [PubMed: 24839861]
274. Stallone G et al. Sirolimus for Kaposi's sarcoma in renal-transplant recipients. *N Engl J Med* 352, 1317–23 (2005). [PubMed: 15800227]
275. Geissler EK Post-transplantation malignancies: here today, gone tomorrow? *Nat Rev Clin Oncol* 12, 705–17 (2015). [PubMed: 26483298]
276. Geissler EK et al. Sirolimus Use in Liver Transplant Recipients With Hepatocellular Carcinoma: A Randomized, Multicenter, Open-Label Phase 3 Trial. *Transplantation* 100, 116–25 (2016). [PubMed: 26555945]
277. Halleck F & Budde K Transplantation: Sirolimus for secondary SCC prevention in renal transplantation. *Nat Rev Nephrol* 8, 687–9 (2012). [PubMed: 23026948]
278. Hosseini-Moghaddam SM, Soleimanirahbar A, Mazzulli T, Rotstein C & Husain S Post renal transplantation Kaposi's sarcoma: a review of its epidemiology, pathogenesis, diagnosis, clinical aspects, and therapy. *Transpl Infect Dis* 14, 338–45 (2012). [PubMed: 22316356]
279. Campbell SB, Walker R, Tai SS, Jiang Q & Russ GR Randomized controlled trial of sirolimus for renal transplant recipients at high risk for nonmelanoma skin cancer. *Am J Transplant* 12, 1146–56 (2012). [PubMed: 22420843]
280. Euvrard S et al. Sirolimus and secondary skin-cancer prevention in kidney transplantation. *N Engl J Med* 367, 329–39 (2012). [PubMed: 22830463]
281. Di Paolo S, Teutonico A, Ranieri E, Gesualdo L & Schena PF Monitoring antitumor efficacy of rapamycin in Kaposi sarcoma. *Am J Kidney Dis* 49, 462–70 (2007). [PubMed: 17336708]
282. Yanik EL et al. Sirolimus use and cancer incidence among US kidney transplant recipients. *Am J Transplant* 15, 129–36 (2015). [PubMed: 25522018]
283. Reitamo S et al. Efficacy of sirolimus (rapamycin) administered concomitantly with a subtherapeutic dose of cyclosporin in the treatment of severe psoriasis: a randomized controlled trial. *Br J Dermatol* 145, 438–45 (2001). [PubMed: 11531834]
284. Senior PA, Paty BW, Cockfield SM, Ryan EA & Shapiro AM Proteinuria developing after clinical islet transplantation resolves with sirolimus withdrawal and increased tacrolimus dosing. *Am J Transplant* 5, 2318–23 (2005). [PubMed: 16095517]
285. Constantinescu AR, Liang M & Laskow DA Sirolimus lowers myeloperoxidase and p-ANCA titers in a pediatric patient before kidney transplantation. *Am J Kidney Dis* 40, 407–10 (2002). [PubMed: 12148116]
286. Koenig CL, Hernandez-Rodriguez J, Molloy ES, Clark TM & Hoffman GS Limited utility of rapamycin in severe, refractory Wegener's granulomatosis. *J Rheumatol* 36, 116–9 (2009). [PubMed: 19012360]
287. Fervenza FC et al. Acute rapamycin nephrotoxicity in native kidneys of patients with chronic glomerulopathies. *Nephrol Dial Transplant* 19, 1288–92 (2004). [PubMed: 15102967]
288. Kim HJ & Edelstein CL Mammalian target of rapamycin inhibition in polycystic kidney disease: From bench to bedside. *Kidney Res Clin Pract* 31, 132–8 (2012). [PubMed: 26894018]

289. Lieberthal W, Fuhro R, Andry C, Patel V & Levine JS Rapamycin delays but does not prevent recovery from acute renal failure: role of acquired tubular resistance. *Transplantation* 82, 17–22 (2006). [PubMed: 16861936]
290. Lee L et al. Efficacy of a rapamycin analog (CCI-779) and IFN-gamma in tuberous sclerosis mouse models. *Genes Chromosomes Cancer* 42, 213–27 (2005). [PubMed: 15578690]
291. Zhang H et al. A comparison of Ku0063794, a dual mTORC1 and mTORC2 inhibitor, and temsirolimus in preclinical renal cell carcinoma models. *PLoS One* 8, e54918 (2013). [PubMed: 23349989]
292. Chiarini F, Evangelisti C, McCubrey JA & Martelli AM Current treatment strategies for inhibiting mTOR in cancer. *Trends Pharmacol Sci* 36, 124–35 (2015). [PubMed: 25497227]
293. Hudes G et al. Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. *N Engl J Med* 356, 2271–81 (2007). [PubMed: 17538086]
294. Motzer RJ et al. Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomised, placebo-controlled phase III trial. *Lancet* 372, 449–56 (2008). [PubMed: 18653228]
295. Mita MM et al. Phase I trial of the novel mammalian target of rapamycin inhibitor deforolimus (AP23573; MK-8669) administered intravenously daily for 5 days every 2 weeks to patients with advanced malignancies. *J Clin Oncol* 26, 361–7 (2008). [PubMed: 18202410]
296. Atkins MB et al. Randomized phase II study of multiple dose levels of CCI-779, a novel mammalian target of rapamycin kinase inhibitor, in patients with advanced refractory renal cell carcinoma. *J Clin Oncol* 22, 909–18 (2004). [PubMed: 14990647]
297. Raich-Regue D, Rosborough BR, Turnquist HR & Thomson AW Myeloid dendritic cell-specific mTORC2 deficiency enhances alloreactive Th1 and Th17 cell responses and skin graft rejection. *Am J Transplant* 14, 18 (Abstract #584) (2014).
298. Weichhart T et al. The TSC-mTOR signaling pathway regulates the innate inflammatory response. *Immunity* 29, 565–77 (2008). [PubMed: 18848473]
299. Haidinger M et al. A versatile role of mammalian target of rapamycin in human dendritic cell function and differentiation. *J Immunol* 185, 3919–31 (2010). [PubMed: 20805416]
300. Cao W et al. Toll-like receptor-mediated induction of type I interferon in plasmacytoid dendritic cells requires the rapamycin-sensitive PI(3)K-mTOR-p70S6K pathway. *Nat Immunol* 9, 1157–64 (2008). [PubMed: 18758466]
301. Boor PP, Metselaar HJ, Mancham S, van der Laan LJ & Kwekkeboom J Rapamycin has suppressive and stimulatory effects on human plasmacytoid dendritic cell functions. *Clin Exp Immunol* 174, 389–401 (2013). [PubMed: 23968562]
302. Lee K et al. Mammalian target of rapamycin protein complex 2 regulates differentiation of Th1 and Th2 cell subsets via distinct signaling pathways. *Immunity* 32, 743–53 (2010). [PubMed: 20620941]
303. Berezhnoy A, Castro I, Levay A, Malek TR & Gilboa E Aptamer-targeted inhibition of mTOR in T cells enhances antitumor immunity. *J Clin Invest* 124, 188–97 (2014). [PubMed: 24292708]
304. Liacini A, Seamone ME, Muruve DA & Tibbles LA Anti-BK virus mechanisms of sirolimus and leflunomide alone and in combination: toward a new therapy for BK virus infection. *Transplantation* 90, 1450–7 (2010). [PubMed: 21079551]
305. McInturff AM et al. Mammalian target of rapamycin regulates neutrophil extracellular trap formation via induction of hypoxia-inducible factor 1 alpha. *Blood* 120, 3118–25 (2012). [PubMed: 22919032]
306. Vitiello D, Neagoe PE, Sirois MG & White M Effect of everolimus on the immunomodulation of the human neutrophil inflammatory response and activation. *Cell Mol Immunol* 12, 40–52 (2015). [PubMed: 24882386]
307. He Y et al. Mammalian target of rapamycin and Rictor control neutrophil chemotaxis by regulating Rac/Cdc42 activity and the actin cytoskeleton. *Mol Biol Cell* 24, 3369–80 (2013). [PubMed: 24006489]
308. Liu L, Gritz D & Parent CA PKCbetaII acts downstream of chemoattractant receptors and mTORC2 to regulate cAMP production and myosin II activity in neutrophils. *Mol Biol Cell* 25, 1446–57 (2014). [PubMed: 24600048]



309. Zhang L et al. Mammalian target of rapamycin complex 1 orchestrates invariant NKT cell differentiation and effector function. *J Immunol* 193, 1759–65 (2014). [PubMed: 25015820]
310. Wei J, Yang K & Chi H Cutting edge: Discrete functions of mTOR signaling in invariant NKT cell development and NKT17 fate decision. *J Immunol* 193, 4297–301 (2014). [PubMed: 25261481]
311. Zhang S et al. Constitutive reductions in mTOR alter cell size, immune cell development, and antibody production. *Blood* 117, 1228–38 (2011). [PubMed: 21079150]
312. Zhang Y et al. Rictor is required for early B cell development in bone marrow. *PLoS One* 9, e103970 (2014). [PubMed: 25084011]
313. Lazorchak AS et al. Sin1-mTORC2 suppresses rag and il7r gene expression through Akt2 in B cells. *Mol Cell* 39, 433–43 (2010). [PubMed: 20705244]
314. Wang C et al. Rapamycin-treated human endothelial cells preferentially activate allogeneic regulatory T cells. *J Clin Invest* 123, 1677–93 (2013). [PubMed: 23478407]
315. Wang C et al. Rapamycin antagonizes TNF induction of VCAM-1 on endothelial cells by inhibiting mTORC2. *J Exp Med* 211, 395–404 (2014). [PubMed: 24516119]
316. Johnson RW et al. Sirolimus allows early cyclosporine withdrawal in renal transplantation resulting in improved renal function and lower blood pressure. *Transplantation* 72, 777–86 (2001). [PubMed: 11571437]
317. Gonwa TA, Hricik DE, Brinker K, Grinyo JM & Schena FP Improved renal function in sirolimus-treated renal transplant patients after early cyclosporine elimination. *Transplantation* 74, 1560–7 (2002). [PubMed: 12490789]
318. Oberbauer R et al. Long-term improvement in renal function with sirolimus after early cyclosporine withdrawal in renal transplant recipients: 2-year results of the Rapamune Maintenance Regimen Study. *Transplantation* 76, 364–70 (2003). [PubMed: 12883194]
319. Oberbauer R et al. Early cyclosporine withdrawal from a sirolimus-based regimen results in better renal allograft survival and renal function at 48 months after transplantation. *Transpl Int* 18, 22–8 (2005). [PubMed: 15612979]
320. Larson TS et al. Complete avoidance of calcineurin inhibitors in renal transplantation: a randomized trial comparing sirolimus and tacrolimus. *Am J Transplant* 6, 514–22 (2006). [PubMed: 16468960]
321. Buchler M et al. Sirolimus versus cyclosporine in kidney recipients receiving thymoglobulin, mycophenolate mofetil and a 6-month course of steroids. *Am J Transplant* 7, 2522–31 (2007). [PubMed: 17868057]
322. Lebranchu Y et al. Five-year results of a randomized trial comparing de novo sirolimus and cyclosporine in renal transplantation: the SPIESSER study. *Am J Transplant* 12, 1801–10 (2012). [PubMed: 22486815]
323. Gatault P et al. Eight-year results of the Spiesser study, a randomized trial comparing de novo sirolimus and cyclosporine in renal transplantation. *Transpl Int* 29, 41–50 (2016). [PubMed: 26285161]
324. Ekberg H et al. Calcineurin inhibitor minimization in the Symphony study: observational results 3 years after transplantation. *Am J Transplant* 9, 1876–85 (2009). [PubMed: 19563339]
325. Lebranchu Y et al. Efficacy on renal function of early conversion from cyclosporine to sirolimus 3 months after renal transplantation: concept study. *Am J Transplant* 9, 1115–23 (2009). [PubMed: 19422337]
326. Lebranchu Y et al. Efficacy and safety of early cyclosporine conversion to sirolimus with continued MMF-four-year results of the Postconcept study. *Am J Transplant* 11, 1665–75 (2011). [PubMed: 21797975]
327. Guba M et al. Renal function, efficacy, and safety of sirolimus and mycophenolate mofetil after short-term calcineurin inhibitor-based quadruple therapy in de novo renal transplant patients: one-year analysis of a randomized multicenter trial. *Transplantation* 90, 175–83 (2010). [PubMed: 20463641]
328. Guba M et al. Early conversion to a sirolimus-based, calcineurin-inhibitor-free immunosuppression in the SMART trial: observational results at 24 and 36 months after transplantation. *Transpl Int* 25, 416–23 (2012). [PubMed: 22320241]



329. Budde K et al. Everolimus-based, calcineurin-inhibitor-free regimen in recipients of de-novo kidney transplants: an open-label, randomised, controlled trial. *Lancet* 377, 837–47 (2011). [PubMed: 21334736]
330. Budde K et al. Five-year outcomes in kidney transplant patients converted from cyclosporine to everolimus: the randomized ZEUS study. *Am J Transplant* 15, 119–28 (2015). [PubMed: 25521535]
331. Holdaas H et al. Conversion of long-term kidney transplant recipients from calcineurin inhibitor therapy to everolimus: a randomized, multicenter, 24-month study. *Transplantation* 92, 410–8 (2011). [PubMed: 21697773]
332. Weir MR et al. Mycophenolate mofetil-based immunosuppression with sirolimus in renal transplantation: a randomized, controlled Spare-the-Nephron trial. *Kidney Int* 79, 897–907 (2011). [PubMed: 21191361]
333. Weir MR et al. Long-term follow-up of kidney transplant recipients in the spare-the-nephron-trial. *Transplantation* In press (2016).
334. Mjornstedt L et al. Improved renal function after early conversion from a calcineurin inhibitor to everolimus: a randomized trial in kidney transplantation. *Am J Transplant* 12, 2744–53 (2012). [PubMed: 22812414]
335. Mjornstedt L et al. Renal function three years after early conversion from a calcineurin inhibitor to everolimus: results from a randomized trial in kidney transplantation. *Transpl Int* 28, 42–51 (2015). [PubMed: 25176389]
336. Budde K et al. Renal, efficacy and safety outcomes following late conversion of kidney transplant patients from calcineurin inhibitor therapy to everolimus: the randomized APOLLO study. *Clin Nephrol* 83, 11–21 (2015).

**Key points**

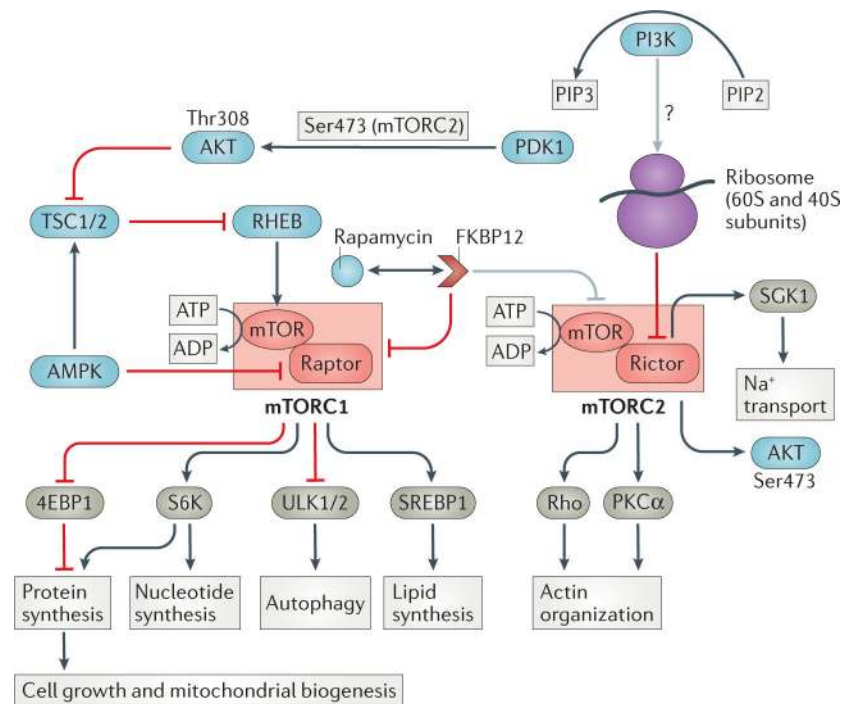
- The mTOR pathway has a central role in regulation of cell metabolism, growth and proliferation
- Pharmacological inhibition of mTOR and selective gene targeting of mTORC1 or mTORC2 in podocytes and tubular epithelial cells has begun to elucidate their role in renal cell homeostasis, including autophagy
- mTOR is increasingly recognized as having a fundamental role in the development of glomerular disease and in acute kidney injury; its role in fibrotic kidney disease is less certain
- Mechanistic insights into the roles of mTOR complexes in regulating immune cell function are helping to improve understanding of the effects of mTOR inhibitors in renal disease and transplant rejection
- New generation dual mTORC1 and mTORC2 inhibitors offer potential for treatment of renal cell carcinoma
- mTOR inhibitors are associated with reduced rates of skin cancer and cytomegalovirus infection in renal transplant recipients

**Box 1 |****Gaps in knowledge regarding mTOR**

- A complete interactome of mTORC1 and mTORC2 within the different cells of the kidney is lacking and hence physiological function of these two complexes is only partially known
- Several findings regarding mTOR inhibition e.g. promotion of immune regulation and tolerance are well-documented in pre-clinical models of transplantation, yet proof in patients is lacking
- Influence of mTOR inhibitors on immune cells, for example B cells and Breg cells
- The underlying mechanisms by which mTOR inhibition promotes donor-specific antibody generation in transplant recipients need further exploration.
- Prospective CRTs are lacking to substantiate the benefits of using mTOR inhibitors to avoid or reduce certain cancers and viral infections in transplant patients.
- While there is evidence in invertebrate and rodent models that mTOR inhibition can prolong life by ameliorating ageing-associated cellular decline, it is unclear whether this also applies in humans

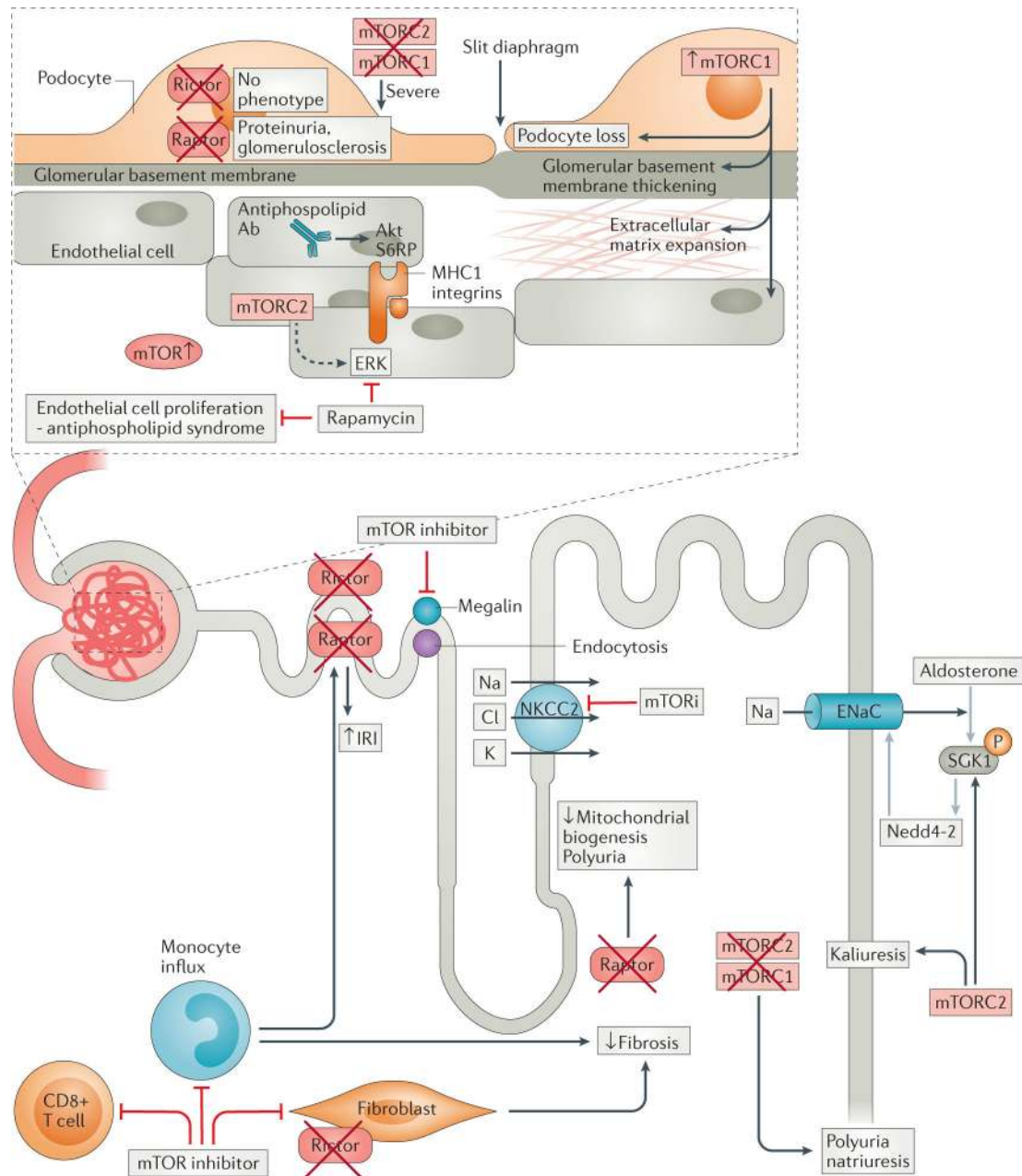
**Box 2 |****Future directions**

- From a pathophysiological perspective it is paramount to improve understanding of how mTORC1 and mTORC2 drive pathogenesis of renal disease
- Clinical trials in transplantation should aim at delineating the optimal drug combinations accompanying mTOR inhibition on both a temporal and intensity basis
- Future drug development should not only aim to test the potential superiority of TORKinibs over mTOR inhibitors, but should also identify downstream mediators of mTORC1 and mTORC2 action which might improve the side effect profile by specifically targeting pathological activation loops



**Figure 1. mTOR complex biology.**

RAPA-sensitive mTOR complex 1 (mTORC1) is composed of mTOR in association with regulatory associated protein of mTOR (RAPTOR) as well as other proteins not shown here (mammalian lethal with Sec13 protein 8, proline-rich substrate of Akt of 40 kD and DEP domain-containing mTOR-interacting protein). mTORC1 is regulated by environmental cues (nutrients, growth factors and energy) to drive cell growth and metabolism. Many signalling pathways converge on the tumour suppressors tuberous sclerosis complex 1 (TSC1) and TSC2, a GTPase activating protein and major negative regulator of RHEB (Ras homologue enriched in brain), that directly stimulates mTORC1. The two main downstream targets of mTORC1 are p70 ribosomal S6 kinase (S6K) and 4E-binding protein 1 (4EBP1); their phosphorylation by mTORC1 drives ribosome synthesis, cap-dependent translation and cell growth. The transcription factor sterol regulatory element binding protein 1 (SREBP1) is also activated by mTORC1 and regulates lipid synthesis. Rapamycin-insensitive mTOR-containing complex 2 (mTORC2) lacks rAPTOR but has rapamycin-insensitive companion of mTOR (RICTOR) as an essential component. Known substrates of mTORC2 include AKT and the serum and glucocorticoid-induced kinase-1 (SGK1). PDK1 enhances Akt activity by phosphorylating the activation loop at threonine 308. mTORC2 uniquely stabilizes Akt via phosphorylation of the turn motif at serine 450 (not shown), and further stimulates Akt kinase activity by phosphorylating the hydrophobic motif at serine 473. mTORC2 controls fundamental cellular processes including metabolism, differentiation, cell cycle arrest and DNA repair. Ribosomes have been found to physically associate with mTORC2. Rapamycin and rapalogs form complexes with FKBP12 and acutely inhibit mTORC1 assembly, whereas inhibition of mTORC2 assembly requires chronic exposure and is inconsistent across cell types.



**Figure 2. Roles of mTOR complexes in the kidney as revealed using pharmacological inhibition or cell-specific genetic deletion of mTOR1 or mTOR2.**

mTOR complexes are expressed throughout the nephron and regulate homeostasis of all resident parenchymal and non-parenchymal cells. Disruption of mTOR signalling results in various pathologies that are dependent on cellular location and whether TORC1 or TORC2 is targeted: proximal TEC downregulate megalin resulting in proteinuria, while distal TEC exposure leads to polyuria and natriuresis, and inflammatory cell infiltrate and fibroblastic responses are reduced following IRI. Within the glomerulus, podocyte function is disrupted leading to frank proteinuria and glomerulosclerosis, while endothelial cell proliferation is limited. Abbreviations: ERK, extracellular signal-regulated kinase; NKCC, Na-K-Cl co-transporter; MR, mineralocorticoid receptor; SGK1, serum and glucocorticoid inducible



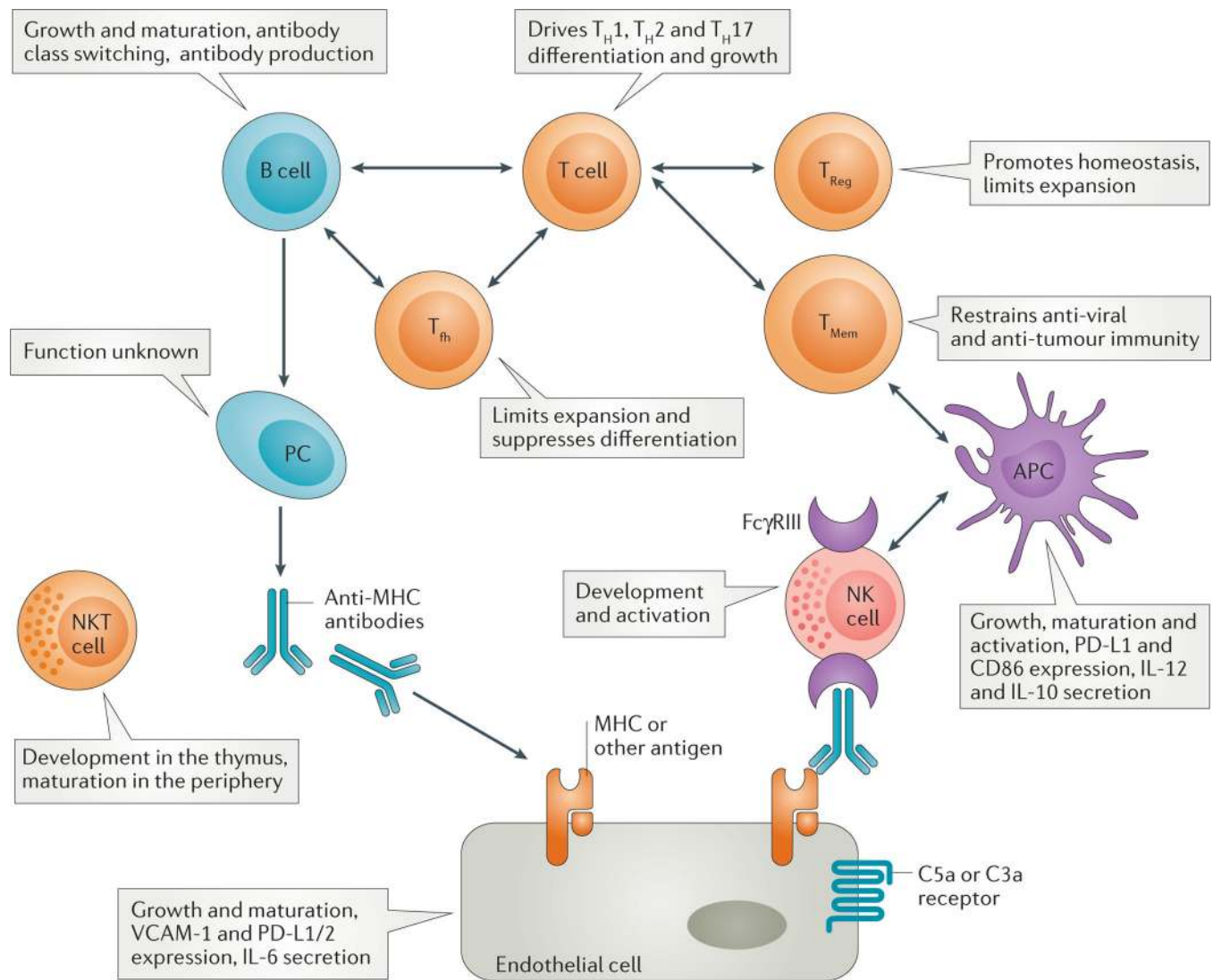
kinase 1; ENaC, Epithelial sodium channel; IRI, ischemia-reperfusion injury; mTORi, mTOR inhibition.

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**Figure 3. Roles of mTOR in shaping immune and vascular endothelial cell homeostasis.** mTOR shapes antigen-presenting cell (APC), natural killer (NK) cell, T cell, B cell and endothelial cell homeostasis, growth and function. Depicted is the role of mTOR in cells known to be key mediators in the pathogenesis of cell- and antibody-mediated rejection. While our knowledge of mTOR function in T cells and DC is extensive, less is known about how mTOR modulates B cells, T<sub>fh</sub> and NK cells. As a result of the pivotal role of mTOR in metabolic programming of all immune cells, global inhibition of this pathway with pharmacologic inhibitors will modulate the immune response in a manner that remains incompletely understood. Given the importance of complement in AMR, C3a and C5a receptor engagement on endothelial cells may affect their proliferation via mTOR-dependent events. Abbreviations: PC=plasma cell; APC encompasses dendritic cells and macrophages; PD-L1, programmed death ligand-1; VCAM, vascular cell adhesion molecule.

**Table 1:**

Roles of mTOR complexes in immune cell populations and their putative roles in clinical organ transplantation

Immune cell	Role of mTOR	Putative Role of mTOR in Clinical transplantation
cDC	Promotes growth, proliferation, maturation, alters pro/anti-inflammatory cytokine secretion, <sup>68, 70</sup> suppresses inflammation (mTORC2) <sup>72, 297</sup> and restrains T cell responses in human myeloid DC (mTORC1) <sup>298, 299</sup>	May either promote (eg rejection: ACR) or restrain (eg pneumonitis) immune hyper-responsiveness
pDC	Promotes activation (TLR9), type-I IFN production and restrains (TLR7) Tmem and Treg proliferation <sup>300, 301</sup>	May regulate function including interaction with Treg
Effector T cells	Drives Th1 (mTORC1), Th2 (mTORC2+/-mTORC1), and Th17 (mTORC1) cell differentiation and growth <sup>81, 82, 302</sup>	Promotes expansion with role in rejection (ACR, AMR, CAMR)
TFH	Restrains TFH differentiation (mTORC1) <sup>83</sup>	May prevent alloantibody formation and AMR
CD8 memory T cells	Restrains anti-viral and anti-tumor immunity <sup>124, 126, 303</sup>	May promote viral infections, including CMV, human herpesvirus 8 and BK virus <sup>127, 304</sup>
Treg	Promotes homeostasis and function (mTORC1) <sup>80</sup> , limits expansion (mTORC1+/-mTORC2) <sup>79</sup>	May restrict role in promotion of tolerance
Neutrophils	Formation of neutrophil extracellular traps (NETs), <sup>305</sup> promotes endothelial cell and extracellular matrix adhesion <sup>306</sup> (mTORC1), promotes chemotaxis (mTORC2) <sup>307, 308</sup>	May promote role in rejection (ACR, AMR, CAMR)
NK cells	Promotes development in the BM, activation in the periphery and IL-15-induced function <sup>90</sup>	May promote role in rejection (ACR, AMR, CAMR)
NKT Cells	Promotes thymic and peripheral iNKT development (mTORC1) <sup>309</sup> , promotes thymic and peripheral iNKT development and fate of NKT17 cells (mTORC2) <sup>310</sup>	May promote role in rejection (ACR, AMR, CAMR) and restrict role in tolerance
B cells	Promotes growth, proliferation, maturation, antibody class switching and production (mTORC1 +mTORC2) <sup>86, 87, 311, 312</sup> but decreases IL-7 receptor and RAG recombinase gene expression (mTORC2) <sup>313</sup>	May promote role in rejection (ACR, AMR, CAMR)
Breg	Unknown	Unknown
Plasma Cells	Unknown	Alloantibody generation in AMR/CAMR
Endothelial Cells	Promotes growth, proliferation, maturation, alters pro/anti-inflammatory cytokine secretion, negatively regulates Treg expansion and increases VCAM-1 expression (mTORC2) <sup>314, 315</sup>	May promote role in rejection (ACR, AMR, CAMR)

Abbreviations: ACR: acute cell-mediated rejection; AMR: antibody-mediated rejection; BM: bone marrow; Breg: regulatory B cell; CAMR: chronic antibody-mediated rejection; cDC: conventional dendritic cell; CMV: cytomegalovirus; EC: endothelial cell; IFN: interferon; iNKT: Invariant Natural Killer T cell; NK: Natural Killer; NKT: Natural Killer T cell; pDC: plasmacytoid DC; RAG: Recombination-activating gene; RAPA: Rapamycin; TFH: Follicular Helper CD4 T cells; Tmem: memory T cell; TLR: Toll-like receptor; Treg: regulatory T cell; VCAM-1: vascular cell adhesion molecule-1

**Table 2:**

## Clinical trials of mTORi in renal transplantation

Study	Type and duration of follow-up	n	Treatment groups	Outcomes
Groth et al (1999) <sup>24</sup>	Multi-centre, open-label (1 year)	83	Steroid + AZA + CsA or SRL	Graft survival, patient survival and BPAR similar; serum creatinine lower in SRL group; higher pneumonia rates in SRL group
Kahan et al (1999) <sup>104</sup>	Phase II trial (1 year)	149	Steroid + CsA + placebo or low- or high-dose SRL, steroid + low-dose CsA + low- or high-dose SRL	Addition of SRL reduced BPAR in standard dose CsA group; no difference in graft or patient survival; haematologic and lipid abnormalities in SRL group, HT and NODAT in CsA group
Kreis et al (2000) <sup>106</sup>	Multi-centre, open-label (1 year)	78	Steroid + MMF + CsA or SRL	Graft survival, patient survival and BPAR similar; serum creatinine lower in SRL group
Rapamune US (2000) <sup>105</sup>	(1 year)	719	Steroid + CsA + AZA or SRL	Reduced occurrence and severity of BPAR in SRL group at 6 months
Rapamune Global (2001) <sup>103</sup>	Phase III (1 year)	576	Steroid + CsA + placebo, low- or high-dose SRL	Addition of SRL reduced acute rejection rates
Johnson et al (2001) <sup>316</sup>	Open-label (1 year)	525	Steroid + CsA + SRL, 3 month withdrawal CsA or CsA maintenance	Improved renal function and lower BP when CsA withdrawn; thrombocytopenia, hypokalemia and abnormal LFTS in SRL group
Gonwa et al (2001) <sup>317</sup>	Phase II, open-label (1 year)	246	CsA + SRL or reduced-dose CsA (taper at 2 months)+ SRL	Renal function better in CsA elimination arm; graft and patient survival similar, BPAR similar
Rapamune Maintenance Study (2003 <sup>318</sup> , 2005 <sup>319</sup> )	Phase III (4 years)	525	Steroid + CsA + SRL, random assignment to CsA withdrawal at 3 months	2 years: CsA withdrawal 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 triple therapy
Larson et al (2006) <sup>320</sup>	(1 year)	165	Steroid + MMF + TAC or Steroid + MMF + SRL	Similar acute rejection, graft survival and renal function
SPIESSER Study (2007 <sup>321</sup> , 2012 <sup>322</sup> , 2016 <sup>323</sup> )	(8 years)	1 year: 145 5 years: 133 8 years: 119	Polyclonal antilymphocyte antibodies + steroid + MMF + CsA or SRL	1 year: BPAR, graft survival and patient survival not different; SRL group had higher adverse events (bronchopneumonia, proteinuria) and discontinuation rates 5 years: eGFR higher in SRL group; graft and patient survival no different, adverse effects more common in SRL group 8 years: No difference in graft survival, eGFR greater in SRL group, no detrimental impact in patients in whom SRL was withdrawn. No difference in malignancy
Symphony (2007 <sup>108</sup> , 2009 <sup>324</sup> )	(3 years)	1 year: 1,645	Steroid + CsA + MMF or daclizumab + MMF + low-dose CsA/low-dose	1 year: GFR and allograft survival highest and BPAR lowest in low-dose TAC; adverse

Study	Type and duration of follow-up	n	Treatment groups	Outcomes
		3 years: 958	TAC or low-dose SRL	effects most common in low-dose SRL 3 years: highest GFR and graft survival in MMF+TAC group
CONCEPT Study (2009 <sup>325</sup> , 2011 <sup>326</sup> )	(4 years)	1 year: 192 (237 enrolled) 4 years: 162	Steroid+MMF + CsA, ± converted to SRL at 3 months	1 year: patient and graft survival similar, GFR better in SRL group, ACR rates not significantly higher in SRL group, more adverse events in SRL group 4 years: mean benefits in renal function maintained
Glottz et al (2010) <sup>121</sup>	1 year	141	Steroid + MMF + SRL or TAC	No difference in GFR or patient survival, graft loss, withdrawal and adverse events higher in SRL group
SMART trial (2010 <sup>327</sup> , 2012 <sup>328</sup> )	3 years	1 year: 141 2 & 3 years: 132	ATG induction, steroids + MMF + CsA, conversion to SRL at 10–24 days	1 year: GFR better in SRL group, BPAR, patient and graft survival not different, lower incidence CMV infection, more adverse events in SRL group 2 & 3 years: SRL conversion associated with sustained improvement of renal function; discontinuation of SRL due to adverse events was common
ZEUS Study (2011 <sup>329</sup> , 2015 <sup>330</sup> )	5 years	1 year: 503 enrolled, 5 year follow-up: 245	Basiliximab induction, steroids + MMF + CsA, at 4–5 months conversion to EVL or stay on CsA	1 year: GFR better in EVL group, higher BPAR, lipidaemia and proteinuria, lower haemoglobin and greater adverse events 5 years: EVL group: better GFR Higher BPAR (grade I), did not affect long-term graft function. No difference in graft loss, mortality, adverse events and neoplasm
ASCERTAIN study (2011) <sup>331</sup>	Multi-centre, open-label, 2 years	n=394	Randomization at >6 months to EVL with CNI elimination, EVL with CNI minimization or CNI unchanged	Conversion to EVL with CNI elimination or minimization had no renal benefit; more frequent adverse events and discontinuation
Heilman et al (2011) <sup>120</sup>	(2 years)	122	MMF+TAC or MMF+SRL	63% withdrawal from SRL group
STN Study (2011 <sup>332</sup> , 2016 <sup>333</sup> )	(8 years)	2 years: 229 8 years: 128	MMF+CNI 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) <sup>109</sup>	Phase IV trial (2 years)	443	(1) SRL+TAC, TAC eliminated week 13; (2) SRL+MMF; (3) TAC+MMF All patients also received steroids and daclizumab	Group 2 had high BPAR (>30%), SRL assoc with hyperlipidemia and delayed wound healing, SRL assoc w greater proteinuria and discontinuation, TAC assoc with NODAT, SRL not associated with improved outcomes
Mjörnstedt et al (2012 <sup>334</sup> , 2015) <sup>335</sup>	(3 years)	1 year: 202 3 years: 182	Steroid+MMF + CsA, at 6 weeks convert to EVL or stay on CsA	1 year: GFR better 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

Study	Type and duration of follow-up	<i>n</i>	Treatment groups	Outcomes
APOLLO Study (2015) <sup>336</sup>	(1 year)	93	Remain on CsA or convert to EVL	Premature termination due to slow recruitment. Higher rate of discontinuation with EVL

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



**Table 3:**

Summary of the effects of mTOR inhibitors in renal diseases

Setting	Effect of mTOR inhibitor	
	Pre-clinical models	Clinical studies
Healthy kidney	No histologic abnormalities <sup>160</sup> Deterioration in GFR in spontaneously hypertensive rat <sup>163</sup>	No effect on renal function (serum creatinine levels) after 8 weeks of treatment <sup>283</sup>
Diabetes mellitus	Attenuates renal hypertrophy mitigates albuminuria <sup>175–177</sup>	No direct studies; use of sirolimus post-islet transplant associated with proteinuria <sup>284</sup>
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 <sup>184</sup>
Adriamycin nephropathy	Preservation of renal function, amelioration of glomerulosclerosis and tubular dilatation <sup>180, 189</sup>	No human disease equivalent
Anti-GBM disease, Goodpasture's disease and crescentic GN	Concurrent with disease induction: improved proteinuria and renal histology After disease induction: worsening proteinuria and inflammatory infiltrates <sup>201</sup>	Case report of SRL reducing ANCA titre <sup>285</sup> , another case report suggesting limited utility owing to adverse events <sup>286</sup>
Thrombotic microangiopathy	Impaired recovery <sup>190</sup>	No human studies; SRL 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 <sup>192</sup>	6 out of 11 patients with chronic glomerulonephritis and pre-existing proteinuria were treated with rapamycin developed acute renal failure <sup>287</sup>
Chronic kidney disease	Induces proteinuria, interstitial fibrosis and glomerulosclerosis in a rat remnant kidney model <sup>251</sup>	- No formal human studies
Membranous nephropathy	Mitigated proteinuria, and reduced immunoglobulin deposits in rats with Heymann nephritis <sup>199</sup>	No formal human studies
IgA nephropathy	Protected kidney function, reduced IgA deposition and prevented proteinuria increase <sup>196</sup>	Improved GFR, decreased endocapillary proliferation <sup>204</sup>
FSGS	No studies	Evidence of complete and partial remission <sup>205</sup> , cases of nephrotoxicity reported <sup>287</sup>
Minimal change nephropathy	No studies	Complete remission when combined with tacrolimus <sup>208</sup>
Polycystic kidney disease	Decreased kidney enlargement and cyst volume; improved renal function <sup>213</sup>	Unimpressive results, high adverse effect profile <sup>288</sup>
Acute kidney injury	Delayed recovery <sup>289</sup>	Delayed recovery <sup>136, 137</sup>
Angiomyolipoma	Decreased tumour burden, cyst size and increased survival in a mouse model of TSC <sup>290</sup>	Long-term treatment effective in reducing tumour volume <sup>256, 263</sup> Neoadjuvant use of sirolimus facilitates nephron-sparing resection <sup>261</sup>
Renal cell carcinoma	Temsirolimus and the TORKinib Ku0063794 both inhibit tumor growth in a xenograft model of RCC <sup>291</sup>	Several inhibitors tested without great success in advanced disease <sup>292</sup> including temsirolimus, <sup>293</sup> everolimus, <sup>294</sup> deforolimus <sup>295</sup> and CCI-779 <sup>296</sup>

Abbreviations: ANCA, anti-neutrophil cytoplasmic antibody; FSGS, focal segmental glomerulosclerosis; GBM, glomerular basement membrane; GN, glomerulonephritis; NA, not applicable; TSC, tuberous sclerosis complex.