Rapamycin: Something Old, Something New, Sometimes Borrowed and Now Renewed

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The molecular target of rapamycin (mTOR) is central to a complex intracellular signaling pathway and is involved in diverse processes including cell growth and proliferation, angiogenesis, autophagy, and metabolism. Although sirolimus (rapamycin), the oldest inhibitor of mTOR, was discovered more than 30 years ago, renewed interest in this pathway is evident by the numerous rapalogs recently developed. These newer agents borrow from the structure of sirolimus and, although there are some pharmacokinetic differences, they appear to differ little in terms of pharmacodynamic effects and overall tolerability. Given the multitude of potential applications for this class of agents and the decrease in cost that can be expected upon the expiration of sirolimus patents, renewed focus on this agent is warranted.

Rapamycin (or sirolimus, the official generic name) is the prototypical inhibitor of the molecular target of rapamycin (mTOR). It was discovered more than 30 years ago as an antifungal agent and was approved in 1999 as an immunosuppressant for the prevention of renal allograft rejection. Over the years, with the elucidation of the complex intracellular signaling network in which mTOR participates and the realization of the multitude of potential therapeutic applications of interfering with this network, interest in agents that can inhibit mTOR has increased. There are currently three mTOR inhibitors in clinical trials, all either prodrugs or analogs of sirolimus, including temsirolimus, which was recently approved by the Food and Drug Administration (FDA) for the treatment of renal cancer. Although these newer agents (rapalogs) exhibit slightly different pharmacokinetic properties, they appear to differ little pharmacodynamically from sirolimus. This review will provide an overview of the mTOR pathway, discuss the pharmacokinetics and tolerability of the different mTOR inhibitors, and highlight the many potential clinical applications of these agents.

THE mTOR PATHWAY

The mTOR protein is a serine-threonine kinase that is central to a complex intracellular signaling pathway (**Figure 1**) and is involved in a number of important processes such as cell growth and proliferation, cellular metabolism, autophagy, and angiogenesis. It responds to signals from the extracellular environment such as nutrient and growth factor supply, energy, and stress. Signaling through the pathway is promoted when there is an abundance of nutrients or energy and is downregulated in states of depletion and stress.[1]

mTOR exists within the cell complexed to the proteins GβL and either raptor or rictor.[2] The mTOR/rictor protein complex is not responsive to inhibition by rapalogs and will not be discussed in detail except to mention its interaction with mTOR/raptor. The mTOR/GβL/raptor complex can be activated by various stimuli through different upstream molecules. Insulin (via the insulin receptor substrate-1) or other growth factors affect mTOR via the phosphoinositide-3 kinase (PI3K)/Akt pathway[1] (**Figure 1**). After stimulation, PI3K initiates a cascade that ultimately results in the phosphorylation and activation of Akt. Akt then acts via the tuberous sclerosis complex (TSC), consisting of the proteins TSC1 and TSC2, to exert its effect on mTOR. Phosphorylation of TSC2 by Akt inhibits TSC2, thereby releasing the inhibition that TSC2 otherwise exerts on mTOR via inhibition of Rheb, an activator of mTOR.[1]

The energy status of the cell also affects mTOR signaling and acts via serine/threonine protein kinase 1 (LKB1) and AMP-activated kinase (AMPK). In states of energy depletion (increased AMP relative to ATP), LKB1 activates AMPK, which then activates TSC2, resulting in inhibition of the pathway.[1] Stress signals, for example, DNA damage and

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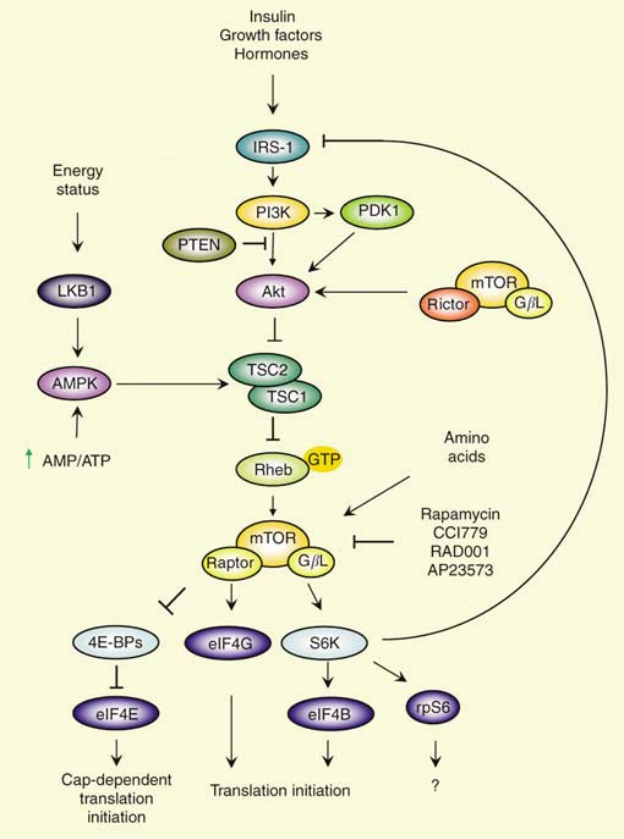

Figure 1 Schematic of the mTOR pathway. mTOR receives input from a number of upstream pathways, including PI3K/Akt, TSC1/TSC2, and AMPK, and acts through downstream effectors S6K and E4BP1 to exert its effects.

hypoxia, also act via the TSC to cause an inhibition of mTOR signaling. The nutrient status of the cell, for example, amino-acid supply, influences the activity of mTOR. Although the mechanisms by which this occurs are less well characterized, it is known that the pathway is active when nutrients are readily available and downregulated in states of starvation.[3]

A number of other pathways can interact with the signaling described thus far. For example, phosphatase and tensin homolog (PTEN) is a tumor suppressor that counteracts the effects of PI3K by dephosphorylating phosphatidylinositol (3,4,5)-trisphosphate and therefore prevents activation of Akt.[4] Neurofibromin 1 (NF1) inhibits Ras, which can activate the mTOR pathway via PI3K/Akt.[5] Moreover, the mTOR/rictor complex can phosphorylate Akt, which therefore results in feedback and further signaling through the pathway.[2]

Downstream of mTOR are two main effectors, S6K1 and 4E-BP1, both of which control the translation of specific mRNAs and the synthesis of particular proteins. Phosphorylation of 4E-BP1 by mTOR ultimately results in the initiation of translation of certain mRNAs that have regulatory subunits in the 5′-untranslated terminal regions, including those that are needed for cell cycle progression and are involved in cell cycle regulation.[6] Phosphorylation and activation of S6K1 are also involved in cell growth and proliferation, possibly via translation of mRNAs that have a terminal 5′-oligopyrimidine tract such as those that encode ribosomal proteins and elongation factors. However, because translation of these latter genes was shown to be intact in $S6K1^{-/-}/S6K2^{-/-}$ mice, other mechanisms are probably involved.[7] Importantly, S6K1 also has an inhibitory feedback function on the pathway by phosphorylating and inhibiting insulin receptor substrate-1, thus reducing growth factor–stimulated signaling through PI3K/Akt/mTOR.[8] It also phosphorylates and inactivates BCL2 antagonist of cell death (BAD), a proapoptotic molecule.[6] The numerous proteins whose translation is regulated through mTOR and its downstream targets include cyclin D1, MYC avian myelocytomatosis viral oncogene homolog (c-MYC), and hypoxia-inducible factor-lα (HIF-1α).[6]

INHIBITORS OF THE mTOR PATHWAY

Mechanisms of action and mechanisms of resistance

Inhibitors of the mTOR pathway that are actively being studied include sirolimus, temsirolimus, everolimus, and AP23573 (now called deferolimus) (**Figure 2**). Sirolimus and the rapalogs exert their effects by the same mechanism. Each drug binds to the intracellular binding protein FK506-binding protein (FKBP12) to form a complex, which then binds to mTOR at the FKBP12–rapamycin binding domain, interfering with its ability to signal adequately to its downstream effectors. Exactly how the sirolimus–FKBP12 complex interrupts mTOR signaling is not known, but it may involve a destabilization of the interaction between mTOR and raptor.[1]

Given the complexity of the mTOR pathway, there are many potential sites of resistance. Defects in the binding of sirolimus to FKBP12 because of mutations in FKBP12 and mutations in the FKBP12–rapamycin binding domain both confer resistance by interfering with the binding of the sirolimus–FKBP12 complex to mTOR.[9] Other potential mechanisms of resistance include decreased levels of 4E-BP1 and mutations in S6K1.[10] The complexity of the pathway and feedback loops within it may also contribute to resistance. For example, it is possible that inhibiting the negative feedback of S6K1 on insulin receptor substrate-1 may contribute to resistance to the antiproliferative effects of mTOR inhibition.[9]

Sirolimus. In the early 1970s, sirolimus, the first inhibitor of mTOR, was discovered as part of a screening program for new antifungal agents and was first named rapamycin because it was isolated from a soil sample from Rapa Nui.[11] Not long after, the inhibitory effect on the immune system was recognized in rats,[12] but it was not until the late 1980s[13] and early 1990s[14] that it was developed as an immunosuppressant. Both the antifungal and immunosuppressive activities are the result of the drug's ability to

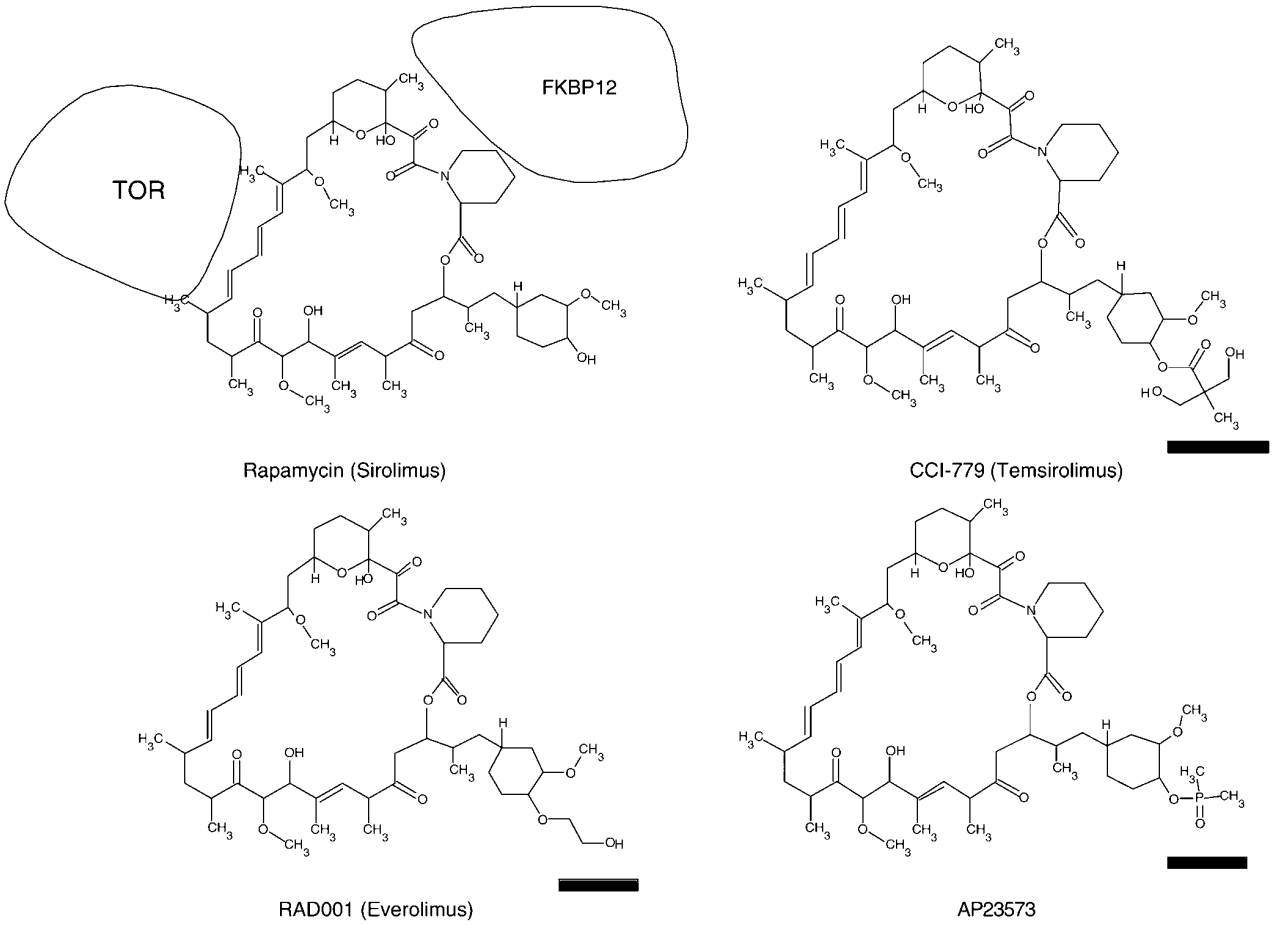

Figure 2 Inhibitors of the mammalian target of rapamycin (mTOR) pathway that are actively being investigated. The chemical structure of sirolimus and the newer analogues are similar. The bars indicate sites of structural differences among the agents.

interrupt the complex intracellular signaling cascade of the mTOR pathway, which is relatively conserved from yeasts to humans.[15] As described, this ultimately causes a decrease in protein synthesis. In yeast and molds, the interruption of synthesis of proteins involved in cell cycle progression interferes with growth of the microorganisms.[16] In T lymphocytes, this interferes with the ability of the cell to respond to cytokines and therefore blocks their proliferation and differentiation,[17] leading to immunosuppression. As sirolimus had been used for the prevention of allograft rejection, most of the pharmacology data come from solid organ transplant patients.

Sirolimus is a macrocyclic lactone produced by *Streptomyces hygroscopicus.*[18] It is poorly soluble in water and therefore can only be given orally. It is available in both liquid and tablet formulations. A study of stable renal allograft recipients in which patients were converted from liquid sirolimus to the tablet form demonstrated relative bioequivalent pharmacokinetics for the two formulations. Although the tablet formulation resulted in a lower maximum concentration (C_{max}), the area under the concentration–time curves (AUCs) of the two formulations were similar.[19] Absorption of sirolimus is rapid with peak concentrations attained in about 2 h, but bioavailability is low (~15%)[20] and exhibits wide interpatient variability. This variability has been largely attributed to the effects of intestinal cytochrome *P*450 3A enzymes (CYP3A) and P-glycoprotein activity on sirolimus absorption.[21] Studies on renal transplant patients have shown that the coadministration of cyclosporine affects the bioavailability of sirolimus and that when the drugs are administered concomitantly, both the C_{max} and the AUC of sirolimus are increased, possibly because of inhibition of CYP3A4 and P-glycoprotein by cyclosporine.[22] The administration of a high-fat meal will also affect absorption. In a study of healthy volunteers, coadministration of sirolimus with a high-fat meal resulted in a slower absorption but a 35% increase in AUC.[23]

The volume of distribution of sirolimus is large (~12 l/kg), indicating wide distribution into tissues and necessitating, in many instances, a loading dose. Most of the drug partitions into red blood cells (~95%), with small amounts in lymphocytes and granulocytes (~1% each).[24] Of the 3% in plasma, only 2.5% is free and the remainder is protein-bound.[24]

The metabolism of sirolimus is mainly via hepatic CYP3A enzymes. In a study of 18 adults with mild to moderate hepatic impairment, AUC and half-life of sirolimus were significantly increased and weight-normalized apparent oral clearance was significantly decreased, as compared with healthy controls,[25] suggesting that dose adjustments may be needed for patients with hepatic impairment. Sirolimus has multiple metabolites, all with low immunosuppressive activity (<10% relative to sirolimus).[26] The excretion is primarily fecal. The clearance is widely variable (1.45–6.93 ml/min/kg) and the half-life is long (~62 h),[20] allowing for once-daily dosing.

The interaction of sirolimus with CYPA34 and P-glycoprotein not only leads to wide interpatient variability in absorption and metabolism but also to the potential for a number of drug interactions. Commonly encountered inhibitors of CYP3A4 and P-glycoprotein include ketoconazole, cyclosporine, erythromycin, and ritonavir, along with grapefruit juice; inducers of CYP3A4 include dexamethasone, phenytoin, carbamazepine, rifampin, and phenobarbital, to name a few. These medications should be used cautiously and avoided if possible in patients treated with sirolimus.

When used as an immunosuppressant for the prevention of solid organ graft rejection, therapeutic drug monitoring has been an important issue. A study of 150 renal transplant patients showed a correlation between trough drug levels (which correlate well with sirolimus AUC) and both incidence of adverse side effects (for levels >15 ng/ml) and acute graft rejection (for levels <5 ng/ml).[27] One approach that has been recommended is to monitor all patients during the initial phase of treatment (for ~2 months) and thereafter based on individual patient characteristics.[28]

Rapalogs. Temsirolimus, everolimus, and deferolimus (**Figure 2**) are all structurally similar to sirolimus, differing mainly at a single position of the lactone ring (C-40) unrelated to both the mTOR- and FKBP12-binding sites. Temsirolimus is a water-soluble dihydroxymethyl propionic acid ester prodrug of sirolimus. Both intravenous and oral formulations are available, although recent development has focused on the intravenous formulation. To date, temsirolimus has been mainly developed as an anticancer agent, and it was approved by the FDA on 30 May 2007 for the treatment of advanced renal cell carcinoma. Everolimus is an oral 40-*O*-(2-hydroxyethyl) derivative (not prodrug) of sirolimus approved in Europe as an immunosuppressant for the prevention of cardiac and renal allograft rejection in adults.[29] More recently, it has been studied as an anticancer agent. Deferolimus is the newest addition to the rapalogs. It contains a phosphine oxide substitute on the lactone ring and is available in both oral and intravenous formulations. It is currently being investigated for the treatment of a number of different malignancies.

As mentioned, sirolimus and the rapalogs inhibit mTOR by forming a complex with FKBP12, which then binds to mTOR. Few data are available regarding differences in the ability of the drugs to inhibit mTOR. One study showed that the binding of everolimus to FKBP12 was approximately threefold weaker than that of sirolimus *in vitro*.[30] However, further *in vivo* studies on rats showed similar efficacy of the two agents in terms of immunosuppressive activity. The mTOR inhibitors are very specific in their action, and there is no evidence to date of any effects other than those on mTOR. All are known to result in a decrease in the phosphorylation of the downstream effectors 4E-BP1 and S6K1[31–34] and the degree of this effect is being studied as a potential biomarker of mTOR inhibition.

An often stated reason for the development of new mTOR inhibitors is to improve the pharmacokinetic properties of sirolimus, mainly the poor bioavailability and insolubility in water. The chemical modifications of temsirolimus and deferolimus have resulted in water-soluble formulations that are currently being studied as intravenous agents. Few data are available regarding the oral formulations of these rapalogs and, to date, the main oral alternative to sirolimus is everolimus. Although everolimus exhibits greater polarity than sirolimus, the bioavailability is only slightly improved and is still relatively low (~16%).[35] Similar to sirolimus, CYP3A4 and P-glycoprotein affect its absorption and contribute to wide interpatient variability.[36]

The rapalogs share many characteristics with sirolimus, including extensive partitioning into red blood cells,[36,37] metabolism by hepatic CYP3A enzymes,[38,39] and primarily fecal excretion.[35] In a study of patients with liver cirrhosis, the clearance of everolimus was reduced by 53% and the half-life prolonged 84%, showing the need for dose reductions in patients with liver impairment.[40] Although similar studies of temsirolimus have not been published, given the extensive hepatic metabolism, it is possible that this agent may also require dose adjustment in patients with liver dysfunction. As the rapalogs are substrates of CYP3A4 and P-glycoprotein, potential drug interactions are also a concern, as with sirolimus. There are differences in the half-lives, potentially affecting the optimal dosing schedules. The half-life of everolimus in stable renal transplant patients was 24–35 h[41] and 13–25[42,43] and 45–74 h,[37] respectively, for the intravenous formulations of temsirolimus and deferolimus.

Temsirolimus is the only rapalog that is a prodrug. It quickly undergoes hydrolysis to sirolimus after intravenous administration. Sirolimus can be seen as early as 15 min after the start of temsirolimus infusion, reaches peak concentration at 0.5–2.0 h, and then decreases monoexponentially.[43] After a dose of temsirolimus, the exposure to sirolimus exceeds that of parent drug because of the differences in half-lives, with the mean sirolimus/temsirolimus ratio being 2.5–3.5.[43] Although temsirolimus exhibits inhibitory activity against mTOR, most of its clinical effects are probably due to the sirolimus metabolite.[44,45]

Tolerability of the mTOR inhibitors

The four mTOR inhibitors exhibit remarkably similar side effect profiles and are in general well tolerated by patients,

Table 1 Multiple applications of mTOR inhibitors that have been studied, both clinically and preclinically, along with the rationale for possible efficacy of mTOR inhibition

Application	Rationale	Comments	Clinical data	Preclinical data
Immune				
Graft rejection GVHD Autoimmune diseases ALPS Asthma	Inhibition of proliferation of T cells in response to growth-promoting cytokines (IL-2) Inhibition of proliferation of B cells and mast cells Suppression of B-cell immunoglobulin production in response to certain stimuli	Effects of mTOR inhibitors on T-cell subsets and function: Increase in the suppressor function of CD4+ T cells Thymic atrophy and a reduction in CD4+CD8+ thymocytes secondary to increased apoptosis without affecting total numbers of T and B cells in the periphery An increase in the percent of CD4+CD25+ regulatory T cells An increase in the numbers of alloreactive CD103+CD8+ regulatory T cells	Refs. 51–61	Refs. 17, 62–71
Cancer				
Malignant tumors Benign hamartomas Radiation and chemosensitization	Antiproliferative effect by inhibiting cell cycle progression and causing G_1 arrest Proapoptotic effect by interfering with phosphorylation of BAD Antiangiogenic effect by interfering with HIF-1α Many proteins involved in hamartoma syndromes are linked to the mTOR pathway Radiation can induce signaling of Akt/mTOR, which can be attenuated by mTOR inhibition	Many different tumor types have shown responsiveness to mTOR inhibition, including both solid tumors and hematologic malignancies At doses used for anticancer treatment, mTOR inhibitors do not appear to be immunosuppressive TSC1/TSC2 (tuberous sclerosis), PTEN (cowden disease), LKB1 (Peutz–Jeghers syndrome), NF1 (neurofibromatosis) are hamartomas syndromes linked to the mTOR pathway Other potential applications of the antiangiogenic effect of mTOR inhibitors include diseases characterized by neovascularization, such as endometriosis and keratitis	Refs. 72–75	Refs. 5, 67, 76–89
Benign diseases characterized by abnormal proliferation				
Cardiac stents Hypertrophic myocarditis Pulmonary fibrosis Hepatic fibrosis ADPKD	By blocking progression through the cell cycle and causing G_1 arrest, mTOR inhibitors have an antiproliferative effect that may be beneficial for a number of different benign diseases	Sirolimus-eluting stents were approved in 2003 for angioplasty to open clogged coronary arteries	Refs. 90–92	Refs. 93–98
Neurodegenerative disorders				
Huntington disease	Huntington disease is characterized by accumulation of intraneuronal proteins that interfere with cellular processes. Increased autophagy, through inhibition of mTOR, may increase the degradation of the intracellular proteins that characterize Huntington disease			Refs. 99, 100
Infectious diseases				
Fungal infections HIV	The mTOR pathway is relatively conserved from yeasts to humans. Blocking the pathway in yeasts and fungi interferes with their growth and proliferation Infection with HIV-1 requires expression of the viral coreceptor CCR5 on the cell surface. In T cells, this expression depends on signaling through IL-2, which is blocked by mTOR inhibition	Early studies demonstrated potent activity against *Candida albicans* and *Aspergillus*, among other species. However, because of the immunosuppressive effects, these are not typically used for treatment of fungal infections		Refs. 16, 18, 101, 102
Metabolic disorders				
Type II diabetes Obesity	Via intracellular connection with the insulin receptor/IRS-1/PI3K/Akt pathway and its role in cellular response to nutrients, mTOR may be important in the development of insulin resistance and obesity			Refs. 103, 104

ADPKD, autosomal dominant polycystic kidney disease; ALPS, autoimmune lymphoproliferative syndrome; CCR5, CC chemokine receptor 5; GVHD, graft-versus-host disease; HIV, human immunodeficiency virus; IL-2, interleukin-2; IRS-1, insulin receptor substrate-1; mTOR, molecular target of rapamycin; PI3K, phosphoinositide-3 kinase; TSC, tuberous sclerosis complex.

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