



## The struggle to find the best combination of everolimus and cyclosporin



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The immunosuppressant everolimus was developed to improve on the pharmacokinetics, pharmacodynamics and oral bioavailability of sirolimus. Several studies have sought the best combination with cyclosporin. Recent results show that cyclosporin exposure can be reduced in combination with everolimus.

### Introduction

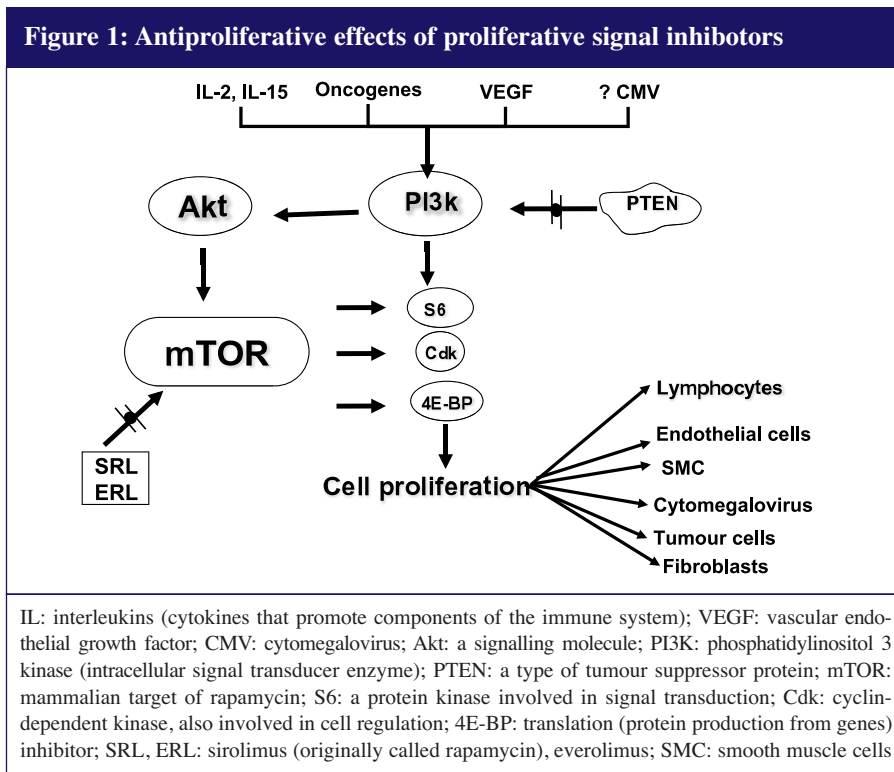
Everolimus belongs with sirolimus to the family of proliferation signal inhibitors. These are immunosuppressive agents that inhibit mammalian target of rapamycin (mTOR).

Rapamune (sirolimus), first discovered in soil samples from Easter Island (Polynesia) in 1964, is a macrocyclic antibacterial lactone isolated from the fungus *Streptomyces hygroscopicus* that was found to have potent immunosuppressive properties [1].

Everolimus, which was developed later, has greater polarity and is more hydrophilic. It was developed to improve on the pharmacokinetic and pharmacodynamic properties of sirolimus, especially oral bioavailability [2].

### Mechanism of action

In normal cells mTOR is an intracellular serine/threonine kinase that is a central controller of cell growth and proliferation. mTOR integrates signals from a variety of sources such as nutrients and growth factors [3]. Growth factors such as insulin growth factor, epidermal growth factor, platelet-derived growth factor and vascular endothelial growth factor (VEGF) bind to and activate receptors located on the cell surface. Receptors activate intracellular signalling cascades PI3K-Akt-mTOR (phosphatidylinositol 3 kinase - serine-threonine kinase - mTOR) leading to protein synthesis. mTOR regulation can affect angiogenesis, cell growth, nutrient uptake and use, and metabolism [4-5]. So when mTOR inhibitors are used as



immunosuppressants, the mTOR cascade is widespread and they may also act as cell growth blockers independently of their action as immunosuppressants, see Figure 1.

All the growth factors mentioned above act through tyrosine kinase receptors located at the cell surface. After binding to these receptors a lipid kinase (PI3K) is activated and phosphatidylinositol biphosphate (PIP2) is phosphorylated to phosphatidylinositol-3-phosphate (PIP3). This reaction may be reversed by a protein generated by tumour suppressor gene PTEN.

Once generated, PIP3 serves as a focal point for recruiting kinases including the Akt/protein kinase B family to the plasma membrane. Akts are a family of signalling molecules, including tuberlin (TSC2). mTOR lies downstream of the TSC2-TSC1-Rheb complex within the PI3K pathway, but also receives nutrient input signals. The ribosomal protein S6 kinase, the eukaryotic initiation factor 4E, and binding protein 1 are mTOR effector molecules that function as regulators of ribosome biogenesis and protein translation. mTOR function is inhibited by proliferative signal inhibitors [6]. Activation of mTOR function is linked to increased pro-

tein synthesis via a number of cellular processes, including growth, proliferation, angiogenesis and nutrient uptake.

Similarly the mTOR complex, which is located in lymphocytes, increases production of the hypoxia inducible factor-1 $\alpha$  protein, a transcriptional regulator of angiogenic growth factors, such as VEGF and PDGF. So when everolimus acts on mTOR it affects lymphocytes (anti-rejection activity), as well as on endothelial and smooth muscle cells (vascular remodelling), tumour cells (antineoplastic activity) and fibroblasts (anti-fibrotic activity).

## Clinical pharmacology of everolimus

### Pharmacokinetics

As stated above everolimus, which has greater polarity and is more hydrophilic, was developed to improve on the pharmacokinetic and pharmacodynamic properties of sirolimus, especially oral bioavailability. The chemical structures of everolimus and sirolimus differ by the presence of a 2-hydroxyethyl group at position 40.

Despite the similarities in chemical structure, there are important pharmacokinetic and pharmacodynamic differences between everolimus and sirolimus [7-9]. The pharmacokinetic differences are summarised in Table 1. The shorter half life and twice daily dosing for everolimus facilitates dose adjustment to achieve target levels. Everolimus also has a higher oral availability, a key objective in its development, and lower plasma protein binding. The major metabolites are negligible contributors to the biological effects of the drugs. Both sirolimus and everolimus are sub-

strates for hepatic and intestinal cytochrome P450 (CYP) 3A4 enzymes as well as for P-glycoprotein.

### Pharmacodynamics

The immunosuppressive potency of everolimus has been demonstrated in a variety of *in vivo* pre-clinical models; specifically, in mouse and rat cardiac and renal transplantation and in non-human primate renal and lung transplantation. The difference in potency between sirolimus and everolimus may explain the different target blood trough levels, which are 3–8 ng/mL for everolimus and 4–12 ng/mL for sirolimus. A wide range of presentations and formulations available for everolimus facilitates dose adjustment and ease of titration to reach target trough levels.

Everolimus is indicated for the prophylaxis of organ rejection in adult patients at low to moderate immunological risk who are given an allogeneic renal or cardiac transplant. Everolimus should be used in combination with cyclosporin microemulsion (CsA) and corticosteroids.

Sirolimus is indicated for the prophylaxis of organ rejection in adult patients at low to moderate immunological risk who receive a renal transplant. It should be used in combination with CsA and corticosteroids for two to three months.

### Differences between everolimus and sirolimus

Although everolimus and sirolimus belong to the same drug class, there are key differences in their clinical use. Twice daily dosing with Certican (everolimus) allows for ease of dose adjustment and it can be administered at the same time as CsA. No loading dose is required.

Everolimus and sirolimus should not be used long term in combination with a full dose calcineurin inhibitor (CNI). Sirolimus should be used initially with a CNI for the first two to three months post-transplant and can only be used as maintenance therapy if the CNI can be progressively discontinued.

CNI reduction in everolimus-treated renal transplant recipients should be started one month post-transplantation, while maintaining everolimus target trough levels. CNI dose reduction is also recommended for cardiac transplant recipients receiving everolimus. Everolimus has proved to be effective in two different doses, 1.5 and 3 mg/day, in preventing both acute and chronic heart allograft rejection. The B253 study documented everolimus' superiority over azathioprine [10].

### Studies in renal transplantation

The efficacy and safety of everolimus in renal transplantation were first documented by two phase III studies (B251-B201) [11-12]. Studies B251 and B201 were three-year, randomised, multicentre, parallel-group studies that investigated the efficacy and safety of everolimus, 1.5 mg or 3 mg/day, versus mycophenolate mofetil (MMF), 2 g/day, as part of a triple immunosuppressive therapy (concomitant cyclosporin and prednisone) in *de novo* renal transplant recipients. The studies were powered to demonstrate equivalence between everolimus and MMF. The primary objective for both studies was to compare the incidence of composite endpoints such as Biopsy-Proven Acute Rejection (BPAR) graft loss and death. A total of 583 patients were enrolled into study B251 from centres in North and South America, and 588 patients were enrolled for study B201 worldwide.

In both studies everolimus at doses of 1.5 mg and 3 mg/day + cyclosporin and steroids displayed equivalent immunosuppressive efficacy to MMF. In study B201 the incidence of cytomegalovirus (CMV) infection was significantly higher in the MMF group. However the cyclosporin/everolimus regimen was associated with dose-related increases in mean serum creatinine and decreases in

**Table 1: Pharmacokinetics of Certican (everolimus) and Rapamune (sirolimus)**

	Certican	Rapamune
Oral bioavailability	20%	14%
Time to t <sub>max</sub>	1–2 hours	1–2 hours
Elimination t <sub>1/2</sub>	28 hours	62 hours
Dosing internal	BID	OD
Time to steady state	4 days	5–7 days
Plasma protein binding	74%	92%

creatinine clearance compared with the cyclosporin/MMF regimen, which therefore interfered less with renal function. A further study (B156) indicated fewer renal side effects when the cyclosporin dose was reduced [13].

In the attempt to further improve the everolimus/cyclosporin combination, study A2307 [14] was started with two arms. Low doses of cyclosporin were combined with controlled doses of everolimus (C0 >3 ng/mL). One arm received basiliximab as induction, steroids, half dose cyclosporin and everolimus 1.5 mg/day, the other received basiliximab as induction, steroids, half dose cyclosporin and everolimus 3 mg/day. In both groups the everolimus dose was adjusted to obtain trough levels of 3–8 ng/mL. The conclusions of the study were that controlled dose everolimus with low-exposure cyclosporin was effective. Everolimus with low-exposure cyclosporin group showed better graft function (mean creatinine clearance, mean GFR) compared with historical full dose cyclosporin. Low incidence of CMV infection was confirmed.

Overall, studies A2306 and A2307 confirmed that in combination with low dose CsA, everolimus 1.5–3 mg/day resulted in a low rate of BPAR (12-month rate 13.7–15.8%) and graft loss (1.7–5.0%) and good renal function (12-month CrCl of 64 mL/min). Controlled dose everolimus with low-exposure cyclosporin is effective. The everolimus with low-exposure cyclosporin group showed better graft function (mean creatinine clearance, mean GFR) when comparing with the historical full cyclosporin dose and trough level (C0) monitoring.

In the Everest study [15] performed by our group, patients were given sufficient everolimus to obtain much higher trough levels of 8–12 ng/mL and very low dose cyclosporin (67% reduction of C2 levels). Cyclosporin monitoring using 2-hour post-dose samples (C2) is thought to be more efficacious than using pre-dose levels (C0). They were compared with patients on a standard everolimus regimen with low dose cyclosporin.

The conclusions of the study after one year are as follows:

- The combination of everolimus with very low dose CsA is effective and well tolerated.
- The non-inferiority of the upper everolimus/very low CsA arm was proven in terms of therapy failure.
- Creatinine clearance was higher in the upper everolimus group (treatment-compliant population) but not significantly.
- The upper everolimus/very low CsA group had a significantly better probability of graft survival.
- The upper everolimus/very low CsA group did not have a higher incidence of adverse events than the standard everolimus group.

The way the study was designed does not allow a direct comparison with the MMF/CsA combination.

In the supportive B156 study everolimus was associated with full dose or reduced dose cyclosporin. Both reduced and standard dose cyclosporin displayed similar efficacy. The incidence of serious infections and the occurrence of drug toxicity symptoms were higher in full dose cyclosporin. The mean creatinine value was higher and creatinine clearance was lower with full dose cyclosporin.

## Conclusion

Many transplantation studies are performed in an attempt to reduce the dose of calcineurin inhibitors, reduce toxicity and maintain renal function. Several regimens have been tested successfully, including several with the mTOR inhibitor everolimus at a dose of 1.5–3 mg/day. The recent Everest study showed it is possible to further reduce CsA exposure if everolimus trough levels are increased to 8–12 ng/mL.

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