

Sirolimus and FTY720: New Approaches to Transplant Immunosuppression

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DUE TO THE PLEIOTROPIC toxicities of presently available immunosuppressive agents (nucleoside synthesis blockers, calcineurin antagonists, and steroids), two new approaches to the transplant enterprise are being evaluated in clinical trials. These strategies are based upon the development of synergistic drug combinations that not only achieve greater efficacy but also permit marked reduction in the dose of each component drug. The new agents are primarily being developed to potentiate the actions of the calcineurin antagonists (cyclosporine [CsA] or tacrolimus) and thereby to minimize their nephrotoxicity, neurotoxicity, and other less common adverse events.¹

SIROLIMUS

Sirolimus (and its structural analog everolimus) acts as a proliferation signal inhibitor of a multifunctional kinase, mammalian target of rapamycin (mTOR). The enzyme catalyzes the activation of signal 2, CD28-induced transcrip-

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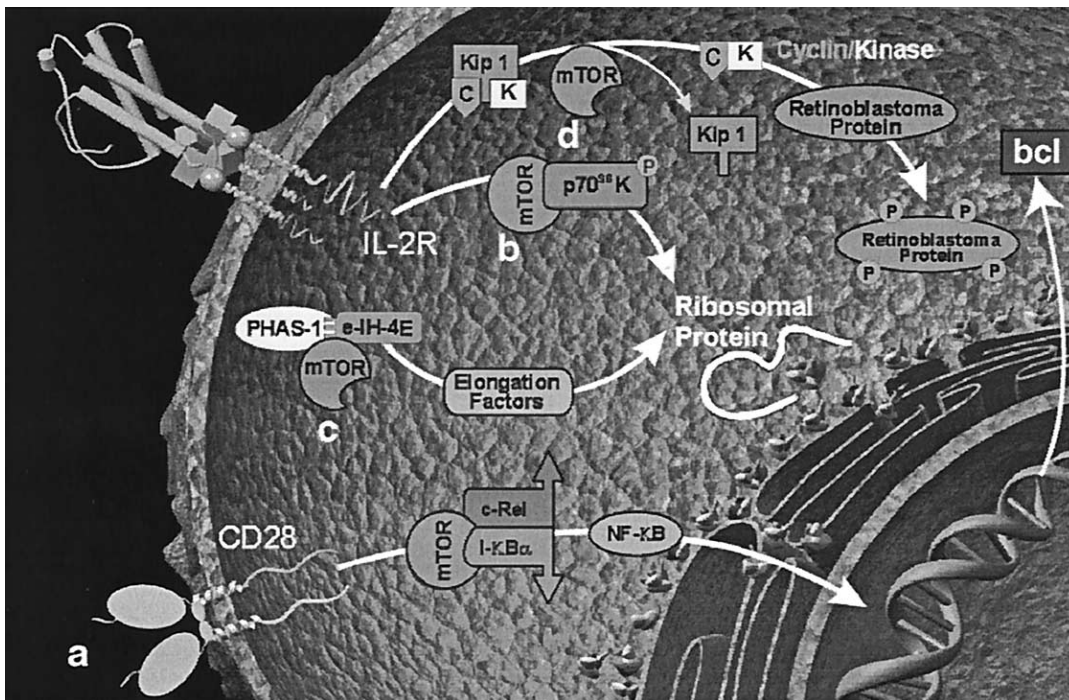


Fig 1. Actions of mTOR. **(a)** Transduction of DC28-mediated signal 2, leading to NF-κB. **(b)** Phosphorylation of P70S⁶ kinase, leading to S6 ribosomal protein. **(c)** Release of elongation factors for protein translation. **(d)** Dissociation of P27 Kipl, leading to activation of cyclin and cyclin-dependent kinase activities.

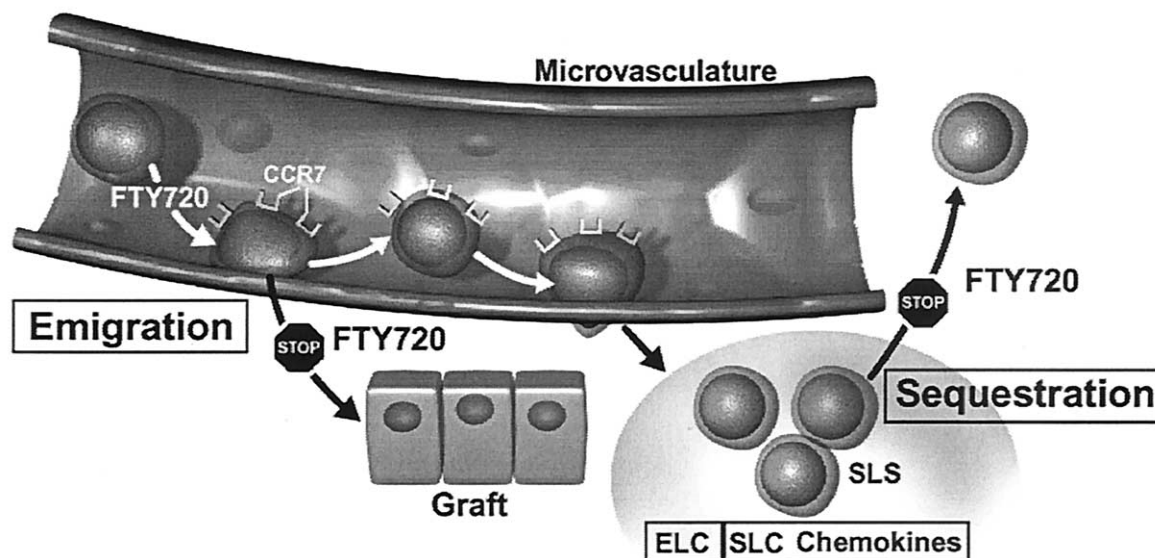


Fig 2. Schematic diagram of the effect of FTY720 to block emigration of lymphocytes into a graft with sequestration in secondary lymphoid structures (SLS). Up-regulation of chemokine receptor 7 binding to lymph node chemokines SLC and ELC may represent one component of drug action.

tion factors, including nuclear factor- κ B (NF- κ B); the dissociation of elongation factors for microsomal proteins translation; the synthesis of the S6 ribosomal protein; and the activation of cyclins and cyclin-dependent kinases (Fig 1). Based upon the exciting results of preclinical studies, sirolimus, a macrolide product of the actinomycete *Streptomyces hygroscopicus*, was evaluated in clinical trials.

Following a Phase I study of toxicity and a Phase I/II trial documenting efficacy, a multicenter Phase II trial revealed that treatment with sirolimus in combination with full or reduced exposures to CsA equally decreased the occurrence and severity of acute allograft rejection episodes among non-African American recipients. A median effect analysis of the large Phase III multicenter trials documented a synergistic interaction between sirolimus and CsA.² Subsequent experience in human renal transplantation has shown that compared to patients treated with full-dose CsA, recipients treated with a 60% reduced exposure to CsA with an almost 80% decrease in sirolimus display reductions in acute and chronic rejection as well as improvements in renal function. Despite the enhanced efficacy, patients in the sirolimus/CsA arms did not display an increased incidence of infectious or malignant complications. However, they did experience a range of non-immune toxicities, including exacerbation of the hypercholesterolemia associated with CsA, as well as myelosuppression, hypertriglyceridemia, and diarrhea due to sirolimus.

There are at least three circumstances in which the unique properties of sirolimus have been exploited in clinical renal transplantation. First, addition of sirolimus to a calcineurin antagonist-steroid regimen has been shown to reverse rejection episodes refractory to antilymphocyte

preparations. Second, because of the enhanced potency of the sirolimus-CsA combination, it has proved possible to withdraw steroids with little penalty and with possible benefits on the toxicities of hyperlipidemia, hypertension, and bone pain. Third, due to its lack of intrinsic nephrotoxicity in salt-depleted rats or in psoriatic patients, sirolimus offers unique advantages for maintenance immunosuppression, particularly in recipients bearing acutely or chronically injured renal allografts. In the setting of delayed graft function, induction therapy with sirolimus provides a window for freedom from calcineurin antagonists de novo.³ In the maintenance phase, the renal function of patients who display nephrotoxic effects of calcineurin antagonists may be improved by withdrawal of CsA or tacrolimus to a maintenance regimen of sirolimus/steroid treatment.

FTY720

Despite reductions in the exposures of CsA and sirolimus, the comorbidities of the regimen are substantial, a limitation that may be addressed through the introduction of a third synergistic agent that permits further decreases in the two baseline immunosuppressants. Preclinical studies have documented that administration of FTY720, a structural analog of myriocin, which is a biologic product of *Isaria sinclairii*, prolongs allograft survival in preclinical models. Experimental studies demonstrated that FTY720 produces synergistic interactions with CsA and/or sirolimus (SRL) in rats⁴ and in nonhuman primates.⁵

Upon oral administration, the drug acts to divert lymphocytes from the circulation and therefore from the inflamed graft. The cells are sequestered in secondary lymphoid

structures (SLS) (Fig 2). Studies in gene knockout animals bearing deletions or reductions in the expression of chemokine receptor 7 (CCR7) or the SLC and ELC chemokines suggest that this network may play a role, albeit not exclusive, in the drug's action. Chemokine receptor signals are transduced by G-proteins, some of which are also receptors for sphingosine-1-phosphate (S1P). By cross-reacting with S1P receptors, FTY-720-phosphate, which is generated by intracellular phosphorylation of the drug by sphingosine kinase, up-regulates actin polymerization and the cytoskeleton, thereby promoting cell mobility. FTY720 seems to enhance lymphocyte responsiveness to a variety of chemokines, not merely the SLC-ELC/CCR7 axis but also inflammatory mediators that would promote cell infiltration into the graft. Therefore our understanding of the molecular mechanism of drug action is incomplete.

Of great import to the use of FTY720, in addition to its unique mechanism of action, is the observation that the drug undergoes metabolism by the cytochrome P450 (CYP) 4F rather than CYP 3A4 system, rendering it free of pharmacokinetic interactions with calcineurin antagonists, sirolimus/everolimus, and a variety of other drugs used in the polypharmacy practice of transplantation. Furthermore, because FTY720 showed only modest interindividual variability, therapeutic drug monitoring is not likely to be useful.

A Phase I multiple-dose clinical study confirmed the findings in animal models: FTY720 produces a reversible, dose-dependent depletion of peripheral blood lymphocytes, but neither polymorphonuclear leukocytes nor monocytes, from the circulation.⁶ A multicenter, randomized, open-label early Phase II study showed that 2.5 mg/d doses of FTY720, when used in combination with CsA and steroids, was more effective for the prevention of acute rejection episodes than 2 g/d of mycophenolate mofetil. Another clinical study combined FTY720 (2.5 mg/d) and everolimus (doses targeted to achieve trough levels between 6 and 8 ng/mL) in a calcineurin antagonist-free regimen for patients at increased risk for delayed graft function (DGF). The regimen showed good tolerability, but in the absence of a control group, it was impossible to assess efficacy.

The primary side effect of FTY720 is bradycardia, which is at least partially dose-dependent, and is observed most commonly after administration of the loading dose. Based on the hypothesis of an interaction between FTY720-phosphate with S1P receptors including those mediating M2-muscarinic effects on atrial myocytes, bradycardia may ensue if FTY720 is administered concomitant with parasympathetic stimulation or with sympathetic beta-blockade.

CONCLUSION

Although the role of sirolimus has evolved from that of an adjunctive agent to the foundation of renal transplant immunosuppression, the side effect profile of the drug combination with CsA, particularly the hyperlipidemia and compromised renal function, produce appreciable comorbidity. FTY720, a sphingosine analog, the mechanism of action of which is uncertain, produces a reversible, dose-dependent depletion of the peripheral blood lymphocytes (but neither granulocytes nor monocytes). Addition of FTY720 to the regimen may permit further reduction in the doses of cyclosporine and of sirolimus/everolimus, an exciting new avenue for immunosuppressive development. Just as the action of sirolimus on signal 3, the cytokine-driven stimulus, proffered an attractive new mechanism of action 10 years ago, chemokines certainly represent a hot target for further drug development. In the interim, encouraging preclinical results with the use of FTY720 in combination with calcineurin antagonists and/or proliferation signal inhibitors (sirolimus or everolimus) suggest that regimens with low comorbidity may be within our grasp.

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