

# FTY720: Mechanism of Action and Potential Benefit in Organ Transplantation

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FTY720 is a novel immunomodulator that has proven effective in animal models of transplantation and autoimmunity, has achieved promising results in Phase I and Phase II studies of renal transplantation in humans, and is currently undergoing phase III studies. FTY720 acts as a high-affinity agonist at the sphingosine 1-phosphate receptor-1 (S1P1), where it internalises the receptor and causes alterations to the normal circulation of lymphocytes between the blood and lymphoid tissue. Unlike conventional immunosuppressants, FTY720 does not impair the activation, proliferation or effector functions of T- and B-cells. Further development of FTY720 is in progress, including trials in autoimmune disorders as well as transplantation. This review summarises the mechanism of action of FTY720, its effects in models of transplantation and autoimmunity, and results from clinical trials in humans.

**Key Words:** FTY720, sphingosine 1-phosphate, sphingosine 1-phosphate receptor agonist, lymphocyte sequestration

## INTRODUCTION

FTY720 is a novel immunomodulator that has proven effective in animal models of transplantation and autoimmunity,<sup>1</sup> has achieved promising results in Phase I and Phase II studies of renal transplantation in humans,<sup>2,4</sup> and is currently undergoing phase III studies. FTY720 acts as a high-affinity agonist at the sphingosine 1-phosphate receptor-1 (S1P1), where it internalises the receptor and causes alterations to the normal

circulation of lymphocytes between the blood and lymphoid tissue. As a result, lymphocytes are retained within lymph nodes and Peyer's patches and there is a marked reduction in circulating lymphocyte numbers.<sup>5</sup> Unlike conventional immunosuppressants, FTY720 does not impair the activation, proliferation or effector functions of T- and B-cells. Further development of FTY720 is in progress, including trials in autoimmune disorders as well as transplantation.

This review summarises the mechanism of action of FTY720, its effects in models of transplantation and autoimmunity, and results from clinical trials in humans.

## FTY720 AND THE S1P SYSTEM

### Sphingosine 1-phosphate

FTY720 was derived from ISP-1 (myriocin), a metabolite of the ascomycete fungus *Isaria sinclairii*.<sup>6</sup> FTY720 and ISP-1 are structurally similar to sphingosine, an amino alcohol that is phosphorylated by sphingosine kinase to form sphingosine 1-phosphate (S1P). S1P stimulates multiple signalling pathways, leading to a range of effects including calcium mobilisation from intracellular stores, polymerisation of actin, chemotaxis and cell migration, and escape of cells from apoptosis.<sup>7-10</sup> S1P is released by platelets during platelet activation and thrombotic processes,<sup>11</sup> by mast cells during inflammatory activation,<sup>12</sup> and by other non-haemopoietic cells such as endothelial cells,<sup>13</sup> and can be found in high nanomolar concentrations in blood.<sup>14</sup>

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### Phosphorylation of FTY720

Like sphingosine, FTY720 is phosphorylated by sphingosine kinase (SK) to form FTY720-P.<sup>15</sup> Phosphorylation has been confirmed by a number of research methodologies: FTY720 is a substrate for recombinant SK, it is phosphorylated *ex vivo* by rodent lymphoid tissues and whole blood, and it is rapidly phosphorylated *in vivo* in rats and monkeys. After oral dosing of FTY720 in rodents, the blood levels of FTY720-P were two to four times higher than the parent compound.<sup>15</sup> Investigation of two chiral analogues of FTY720 have shown the parent drug is not required for its biological activity<sup>15,16</sup> and that phosphorylation is essential for its effects.

### S1P receptors and lymphocyte migration

Five G-protein coupled receptors of S1P have been identified, labelled S1P<sub>1-5</sub>. S1P<sub>1</sub>, S1P<sub>2</sub> and S1P<sub>3</sub> are widely expressed. S1P<sub>1</sub> is the dominant receptor on lymphocytes, while S1P<sub>4</sub> is specifically expressed in lymphoid tissue and S1P<sub>5</sub> is expressed in the spleen and in white matter tracts of the central nervous system.<sup>7,8</sup> The receptors are known to regulate a variety of cellular functions that influence apoptosis, cytoskeletal rearrangements and cell motility.<sup>8</sup>

Phosphorylated FTY720 acts *in vitro* as an agonist, more potent than S1P itself, at the S1P<sub>1</sub>, S1P<sub>3</sub>, S1P<sub>4</sub> and S1P<sub>5</sub> receptors.<sup>15,16</sup> It is now thought that activity at the S1P<sub>1</sub> receptor is most important in its mechanism of action.

Circulation of naive T-cells occurs regularly between blood and lymphoid tissue. Both T- and B-cells require the S1P<sub>1</sub> receptor to allow their escape from peripheral lymphoid organs into the circulation, and that thymocytes require it to move out of the thymus.<sup>17</sup> Once FTY720 is phosphorylated, the compound acts as a high-affinity agonist at the G protein-coupled S1P<sub>1</sub> receptor on thymocytes and lymphocytes to cause an abnormal internalisation of the receptor from cell membranes and subsequent degradation. This interaction with the receptor differs markedly from the interaction with the normal ligand, S1P.<sup>17-19</sup> As a result the cells are unable to respond to an obligatory egress signal provided by circulating

S1P, and are therefore unable to move out of lymphoid organs. Although lymphocytes are unable to access peripheral inflammatory tissues and graft sites, lymphopenia occurs without causing generalised suppression of cell function.<sup>20,21</sup> The capacity of lymphocytes to exit lymphoid tissue is regained when S1P<sub>1</sub> receptors are re-expressed after FTY720 treatment ceases. FTY720 does not reduce antigen-driven T-cell activation and proliferation when it is used at clinically relevant concentrations.<sup>20,22</sup>

### Sequestration of lymphocytes in lymphoid tissue

FTY720 has been confirmed to cause accumulation of naive and activated CD4 and CD8 T-cells and B-cells in the lymph nodes and Peyer's patches,<sup>23,24</sup> with reduced numbers in the blood and spleen. It was initially thought that the effect may have resulted from increasing chemokine-dependent homing of cells into the lymphoid organs,<sup>23</sup> but the change in lymphocyte traffic is now known to be independent of homing receptors including CD62L,<sup>25</sup> CCR7<sup>26</sup> and CXCR5.<sup>27</sup> For example, treatment of mice with FTY720 restored homing of lymphocytes to lymph nodes after CD26L was blocked by anti-CD62L antibody.<sup>25</sup> FTY720 also antagonised the suppressive effects of anti-CD62L on the induction of allograft-specific tolerance.

### Apoptosis

FTY720 has been shown to cause apoptosis in T-cell lines at micromolar concentrations, raising the prospect that the drug may exert its effects by killing lymphocytes.<sup>28,29</sup> However, such a mechanism is highly unlikely, as only nanomolar blood levels are achieved by therapeutic dosing and even a high dose of 7.5 mg/kg/day resulted in sub-micromolar concentrations of the drug.<sup>15</sup> Apoptosis has been observed only with non-phosphorylated compounds.<sup>15,30</sup>

### Effect on heart rate

S1P receptor agonists have a negative chronotropic effect in perfused guinea pig hearts and in isolated sinoatrial node cells.<sup>31</sup> The effect coin-

cided with activation of a  $G\alpha_i$ -dependent, inward rectifying  $K^+$  channel that promoted membrane hyperpolarisation and thus reduced cellular excitability and pacemaker frequency. Studies with isolated guinea pig and rat atrial myocytes have confirmed that FTY720-P activates the same channel.

In rodents, the S1P<sub>3</sub> receptor is dominantly expressed in heart tissue.<sup>32,33</sup> FTY720 reduced heart rate in wild-type but not in S1P<sub>3</sub>-deficient mice. However, in humans there is high expression of S1P<sub>1</sub> receptors in the atrium and ventricle,<sup>34</sup> possibly indicating species differences in the distribution of receptor types and their involvement in the regulation of heart rate.

### FTY720 AND THE ENDOTHELIUM

FTY720 is thought to act on endothelial cells, where it enhances the adherens junction assembly, strengthens the endothelial barrier and assists in preserving the integrity of the vascular system. In animal models, FTY720 trough levels of about 10 ng/mL were associated with maximal lymphopenia,<sup>35</sup> but optimal efficacy in transplantation required concentrations that were at least 5-fold higher. The difference suggested that FTY720 may have additional beneficial effects unrelated to changes in lymphocyte circulation. The hypothesis is consistent with evidence that S1P<sub>1</sub> and/or S1P<sub>3</sub> receptors are involved in the embryonic development of blood vessels and the endothelium, the maintenance of haemostasis, the preservation of barrier integrity and cytoprotection.<sup>36-39</sup>

Both S1P and FTY720-P cause translocation of vascular endothelial cadherin to the focal contact sites between endothelial cells, and promote adherens junction assembly *in vitro*.<sup>37,40</sup> VEGF-induced vascular permeability for macromolecules has been blocked in mice by administration of FTY720<sup>40</sup> or S1P.<sup>38</sup> Because both compounds were effective it suggests that, unlike the situation in lymphocytes, the target receptors were not internalised by FTY720 treatment. It is possible that enhancement of endothelial barriers by FTY720 may help prevent vascular leakage that is an important feature of varied pathological processes including not chronic allograft rejection, athero-

genesis and ischaemia-reperfusion injury.

The full therapeutic potential of FTY720 might rely on agonism of a combination of S1P receptors on the surface of both lymphocytes and endothelial cells. In theory, such a combination of effects could lead both to the accumulation of lymphocytes within lymph nodes as well as changes to the endothelial cell barriers that also influence lymphocyte movement into and out of tissues.<sup>16,20,26,37,41</sup> Further research should provide additional information on the exact relationships between S1P receptor subtypes affected by FTY720 and individual disease states.

### EFFECTS OF FTY720 ON IMMUNE DEFENCES

FTY720 may differ from classical immunosuppressive therapy in respect to its effects on immune defences. For example, in a model of acute viral myocarditis FTY720 suppressed cellular infiltration and tissue necrosis while calcineurin inhibitors accelerated viral replication and worsened the pathology.<sup>42</sup> The drug did not impair humoral immunity in models of systemic infection using lymphocytic choriomeningitis virus or vesicular stomatitis virus.<sup>20</sup> It is not yet known whether FTY720 will be associated with a lower incidence of lymphoma and reactivation of latent virus infection after transplantation compared to conventional immunosuppressants.

### FTY720 IN MODELS OF TRANSPLANTATION

A large number of preclinical studies have demonstrated the efficacy of FTY720 in models of solid organ and pancreatic islet transplantation, as well as autoimmune disease (including multiple sclerosis and diabetes).<sup>5,22</sup> Allograft survival is prolonged by FTY720 alone or in combination with cyclosporin in grafts of the skin, heart and small bowel in rats, pancreatic islet cells in mice, liver and kidney in dogs, and kidney in monkeys.<sup>5</sup>

FTY720 is not an antiproliferative agent at therapeutic concentrations, but it synergises effectively with cyclosporin, FK506, RAD (everolimus) and rapamycin. When used with subtherapeutic

concentrations of cyclosporin, FTY720 protected allografted skin, heart, liver, kidney and small bowel<sup>22</sup> and prevented perivascular inflammation and graft arteriosclerosis<sup>43</sup> which are closely associated with chronic rejection. The regimens were well tolerated, did not increase the incidence of infection, and were not associated with renal, gastrointestinal, pancreatic or bone marrow toxicity.<sup>5</sup> Together, these results imply that FTY720 may be a valuable component of immunosuppressive regimens that prevent acute and chronic rejection episodes.

## EFFECTS ON RENAL AND HEPATIC FUNCTION

There has been no evidence from preclinical studies that FTY720 is associated with structural or functional changes in the liver or kidney.<sup>44</sup> For example, in animals treated with at a high oral dose of 5 mg/kg/day, histological examination at 3 weeks did not reveal any sclerosis, tubular changes, cellular infiltrates or fibrosis, and the hepatocyte, vascular and biliary structures were normal. Intravenous FTY720 at doses to 1 mg/kg did not alter renal cortical perfusion, renal artery flow or vascular resistance.

## USE IN HUMAN TRANSPLANT RECIPIENTS

Promising results have been obtained from clinical trials of FTY720 in humans. A randomised, multicentre, double-blind, placebo-controlled Phase I study included adults who had been maintained on cyclosporin microemulsion and prednisone (or equivalent) for at least 1 year after renal transplantation.<sup>3</sup> The study included 65 patients who received a total of 76 courses of treatment, at doses of 0.125, 0.25, 0.5, 1.0, 2.5 or 5.0 mg FTY720 or placebo for 28 days. FTY720 doses of 1.0 mg/day or more produced a significant reduction in the peripheral blood lymphocyte count by up to 85%, which reversed within 3 days after discontinuation of treatment. Compared with placebo-treated patients, FTY720 subjects did not experience a major increase in adverse events or change in renal function either during treatment or

throughout a follow-up period of 56 days.

A Phase II trial demonstrated the efficacy of FTY720 in preventing acute rejection in de novo renal transplant patients when used in addition to cyclosporin microemulsion and corticosteroids.<sup>4</sup> The clinical data to date suggest that FTY720 is safe at doses that synergise with antiproliferative agents. Ongoing phase III studies will define the clinical utility of the drug in transplantation.

Treatment with FTY720 at a dose of 5 mg appears to permit a 50% reduction in the dose of calcineurin inhibitors while effectively preventing graft rejection. FTY720 has no overlapping toxicity with classical immunosuppressants, and can be used safely with cyclosporin or everolimus in combination with corticosteroids.<sup>2</sup>

The pharmacokinetics of a single dose of FTY720 are characterised by linear dose-proportional exposure over a wide range of doses, only moderate interpatient variability, and a prolonged elimination half-life (89-157 hours). In steady state pharmacokinetics at a dose of 5 mg, the elimination half-life averaged 13 days. These factors suggest FTY720 can be administered with a simple once-daily schedule, without the need for blood-level monitoring or dose titration.<sup>45</sup>

A transient reduction in heart rate has been observed in transplant patients treated with FTY720. It is mild in most cases, but some patients have required treatment with atropine or a beta-agonist. As discussed above for animals, this effect may involve the action of FTY720-P at S1P3 and/or S1P1 on atrial myocytes, and mimic a transient activation of muscarinic receptors on atrial myocytes that contribute to vagally-mediated slowing of the pacemaker.<sup>46</sup>

## FTY720 IN AUTOIMMUNE DISEASE

There is considerable evidence that FTY720 influences the course of autoimmune diseases. For example, the drug inhibited the development of adjuvant- or collagen-induced arthritis in rats, with efficacy at least equal to optimal doses of mizoribine and prednisolone.<sup>47</sup> Similar benefits have been reported in models of autoimmune myocarditis,<sup>48</sup> uveoretinitis<sup>49</sup> and systemic lupus erythematosus.<sup>50</sup> FTY720 at a low dose of 0.3 mg/

kg/day suppressed acute<sup>15</sup> and chronic-relapsing forms of experimental autoimmune encephalitis, a model for human multiple sclerosis. Treatment has also been shown to reduce T-cell infiltration of pancreatic islets of autoimmune-induced diabetic NOD mice,<sup>51,52</sup> raising the prospect that FTY720 could alter the course of autoimmune type 1 diabetes.

## CONCLUSIONS

FTY720 is a potent modulator of S1P-related biological processes. It has proven to be effective in preserving allografts in animal models of transplantation and in human studies of renal transplantation, and is a promising option for use in combination with other classes of immunomodulators. Treatment has been well tolerated. There is evidence that FTY720 influences the course of autoimmune disorders, but clinical data in humans is not yet available.

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