Fingolimod  
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iDrugs 2005 8(3):236-253  © The Thomson Corporation ISSN 1369-7056

Mitsubishi Pharma Corp and Novartis AG are developing fingolimod, an orally active immunosuppressant affecting lymphocyte re-circulation, for the potential prevention of transplant rejection and the treatment of autoimmune diseases, including multiple sclerosis. Fingolimod is a synthetic sphingosine analog that becomes phosphorylated in vivo and acts as a sphingosine-1-phosphate receptor agonist.

Introduction  
Contemporary immunosuppressive therapies are largely unsatisfactory, which is in part due to the low therapeutic index of the two current mainstay immunosuppressants cyclosporin A (CsA) and tacrolimus. These drugs potently block lymphokine production by inhibiting calcineurin function during T-cell activation, but exert serious mechanism-based toxicity [371251], [402151], [505878]. Significant adverse side effects also limit the utility of other drugs that suppress lymphocyte activation or proliferation at different levels, such as sirolimus, everolimus, leflunomide and mycophenolate mofetil (MMF) [371251], [483827]. The latter agents are useful in multi-drug regimens mitigating their own toxicity and that of calcineurin inhibitors or corticosteroids. However, there is a pressing need for immunosuppressants with novel modes of action and improved safety to provide for better prophylaxis of transplant rejection and more effective treatment of chronic autoimmune/inflammatory diseases (such as rheumatoid arthritis and multiple sclerosis) [505878].

Fingolimod (FTY-720), discovered by researchers at Yoshitomi Pharmaceutical Industries Ltd (now Mitsubishi Pharma Corp) [176944], [225279], may go some way toward fulfilling this need. While still not entirely elucidated, the mechanism of action of fingolimod appears to be quite unique since it reflects an alteration of the trafficking of lymphocytes rather than of their activation or proliferation [371332], [558907], [558908]. Numerous studies have demonstrated the ability of fingolimod to prolong allograft survival in rodents, dogs and non-human primates and to act synergistically with inhibitors of calcineurin or proliferation, without exerting major toxic effects [558907]. The immunosuppressive activity of fingolimod was also established in various rodent models of autoimmune diseases [558907]. These encouraging preclinical data prompted the development of fingolimod, by Mitsubishi and Novartis AG, for potential use in transplant rejection and autoimmune diseases [522816], [538366]. Over recent years, phase I and II clinical trials in renal transplantation have provided preliminary evidence that fingolimod is well tolerated and efficacious when administered in combination with CsA [558910], [558912]. The preclinical and clinical studies described below were all carried out using oral delivery, unless otherwise specified.

Synthesis and SAR  
Fingolimod is a synthetic sphingosine analog initially generated by the chemical modification of myriocin (ISP-1), a natural product from the ascomycete Isaria sinclairii [371535]. Myriocin, first described as an antifungal antibiotic in 1972 [371531], was re-discovered more than 20 years later as an immunosuppressive metabolite. Although potently immunosuppressive in vivo, myriocin caused fatal gastrointestinal toxicity [371535], and various synthetic derivatives were thus tested to identify a safer compound. Simplified 2-alkyl-2-amino-1,3-propanediol structures demonstrated reduced toxicity while retaining immunosuppressive activity [176999], [186975], [225279], [226420]. Insertion of a phenyl ring into the alkyl side chain led to the development of fingolimod, which exhibited improved immunosuppressive activity and safety [176944], [225279]. Synthesis of additional analogs demonstrated that while the length of the hydrophobic alkyl chain is not critical, the position of the phenyl ring is highly important for activity, with the optimum length between the phenyl ring and the quaternary carbon being two carbon atoms [371349], [377090]. None of these analogs proved pharmacologically superior to fingolimod [371349], [377090]. Of the two hydroxymethyl groups present in the hydrophilic portion of fingolimod, only the pro-S hydroxymethyl group appeared essential for immunosuppressive activity [371349], [377090]. Moreover, only the R-enantiomer configuration at the chiral carbon of a fingolimod analog was immunosuppressive [371349], [377090]. Procedures for the synthesis of fingolimod and its
chiral analogs and corresponding phosphates have been described [378422], [477725], [530769], [558913], [558914], [559658], [579915].

Preclinical Development

Although fingolimod was initially reported to potently inhibit mouse T-cell proliferation in mixed-lymphocyte cultures [176944], this was not confirmed in subsequent studies [558965]. At concentrations up to 1 µM, fingolimod did not substantially affect either proliferation or interleukin (IL)-2 production of antigen- or mitogen-stimulated rat [371352], [371354] or human T-cells [371356], nor did it inhibit IL-2-driven T-cell growth [371352]. In this respect, the action of fingolimod clearly differed from CsA and tacrolimus (which inhibit IL-2 production) [371338] and sirolimus (which inhibits IL-2-dependent proliferation) [371358]. Sub-micromolar concentrations of fingolimod nevertheless exerted a synergistic effect with CsA and sirolimus in suppressing T-cell proliferation in vitro [371356]. At higher concentrations (> 4 to 5 µM), fingolimod alone induced apoptosis of mature T-cells [371360], [371362], thymocytes [371363] and non-lymphoid cells [371363], [371364], [558969], [558970]. Fingolimod also promoted apoptosis of lymphocytes [371365] and enhanced superantigen-mediated T-cell deletion in vivo [371368], but, as discussed below, it is highly improbable that such effects contribute to the mechanism of immunosuppressive action of fingolimod.

Despite its weak immunosuppressive activity in vitro, fingolimod proved to be a potent immunosuppressant in rodent models of graft rejection. Administration of the compound at > 0.1 mg/kg dose-dependently prolonged the survival of skin [212189], [242515], heart [212190] and liver [371356] allografts in rats. Fingolimod was efficacious when administered at 0.5 or 1 mg/kg for 2 to 5 weeks in rats with small bowel allotransplant known to elicit a strong rejection response [371371], [558982], [558983]. At higher doses (3 mg/kg), fingolimod significantly augmented limb [371369] and joint [371370] allograft survival in rats. Immunosuppression with fingolimod also protected corneal allograft from rejection [558984], [558985], and promoted long-term pancreatic islet allograft survival and function [371506], [477727], [477833] in mice and rats. In cardiac transplantation models, fingolimod not only prolonged the survival of the allograft [371372], but also reduced the development of graft atherosclerosis associated with chronic rejection [371372], [558986]. In these studies, fingolimod treatment was usually initiated the day before transplantation and continued for several weeks thereafter. In some instances fingolimod was also effective when administered only for 2 days, either from the time of transplantation of heart [371352] or liver [371377] allograft, or before transplantation of kidney allograft [371378]. Moreover, fingolimod prolonged liver allograft survival in rats if given at 5 mg/kg only on days 3 and 4 post-transplantation [371377]. This dose also reversed ongoing acute rejection of cardiac allograft if administered on day 3 to 7 post-transplantation in mice [558987]. Fingolimod treatment (0.5 mg/kg/day), delayed to 20 weeks after transplantation, ameliorated chronic allograft nephropathy induced by CsA (1.5 mg/kg/day) in a renal transplantation model [558988]. However, fingolimod failed to prolong skin allograft survival in rats when administered from day 4 post-transplantation [378421], and overall, fingolimod proved more potent if given before transplantation, rather than post-operatively only. Importantly, fingolimod was also active in transplantation models in larger species. At a dose of 5 mg/kg, fingolimod, administered for only 2 days prior to transplantation, delayed the rejection of renal allograft in dogs [371377]. Chronic treatment at lower doses also prolonged the survival of renal and liver allografts in dogs [233473], [371379], [371380], [584577]. Furthermore, once-daily administration of fingolimod at a dose of 3 mg/kg/day, initiated at least 2 days before transplantation and continued thereafter, extended renal allograft survival by 33 to 85 days in cynomolgus monkeys [477762].

In addition to benefits derived from lone administration, fingolimod displayed strong synergy with other immunosuppressive agents in various transplantation models. This was first demonstrated for skin allograft in rats, and cardiac allograft in rats and dogs, in which low doses of fingolimod potentiated the effect of sub-therapeutic doses of CsA [212189], [212190], [212191]. Subsequent studies replicated this observation in rat models of skin, cardiac, small bowel or liver transplantation [371356], [371390], [371391], [371394], [477824] and in mouse models of cardiac transplantation [371372], [477825]. The combination of fingolimod with CsA proved highly effective in rat models of small bowel transplantation, where it prevented graft rejection and graft-versus-host reaction [371371], as well as cardiac transplantation, where it abrogated chronic rejection [558986]. Fingolimod plus CsA treatment prevented graft vessel disease in a rat carotid artery transplantation model [477821], [477831]. Moreover, fingolimod (1 or 3 mg/kg) administered every day or every other day in combination with CsA (15 mg/kg) inhibited the rejection of porcine islet xenografts in rats, while treatment with either drug alone was ineffective [558992]. Synergistic effects between fingolimod and CsA were further documented in canine models of kidney [371377], [371392], [371396] and liver [371380] allotransplantation. Similarly, fingolimod (0.1 to 0.3 mg/kg/day) given intravenously or orally synergized with sub-therapeutic doses of CsA (10 to 30 mg/kg/day) to markedly prolong renal allograft survival in cynomolgus monkeys [371394], [477762]. Rejection-free graft survival was extended to between 32 and 101 days with this low-dose, combined-treatment regimen [477762]. Fingolimod was also demonstrated to synergize with low doses of tacrolimus in preventing rejection of skin [371398], heart [371398], [371401] and liver [371403] allografts in rats. Furthermore, the combination of fingolimod (5 mg/kg) with tacrolimus (1 mg/kg) significantly improved survival of rat-to-hamster skin xenografts [371404]. However, in a liver transplantation model in dogs, the combination of fingolimod (0.1 mg/kg) with tacrolimus (0.5 mg/kg) was less effective than tacrolimus alone and caused mortality from infectious complication due to over-immunosuppression [371380]. This suggested that careful dose adjustment would be needed if fingolimod and tacrolimus were to be used together in clinical regimens. Fingolimod was further demonstrated to exert synergistic effects when administered with immunosuppressants other than calcineurin inhibitors. For example, the combination of fingolimod with sirolimus or everolimus resulted in potent
suppression of allograft rejection in rats [371356], [396003], mice [477828] and monkeys [477762], [558993]. In the latter model, the triple-daily combination of fingolimod (0.1 mg/kg)/CsA (10 mg/kg)/everolimus (0.25 mg/kg) resulted in further increased graft survival (from 47 to >100 days) compared with either drug given alone or in double combination [477762]. The co-administration of a low, nontoxic dose of mycophenolate sodium (10 mg/kg/day) with low doses of fingolimod (0.03 or 0.1 mg/kg/day) was also synergistic and prolonged heart allograft survival in rats [477726], [477728]. Moreover, fingolimod synergized with the blockade of CD28-mediated T-cell co-stimulation by CTLA-4-immunoglobulin (Ig) to prevent cardiac allograft rejection [559003] or obliterative bronchiolitis in tracheal transplantation [559005].

Fingolimod facilitated the induction of tolerance to allografts in experimental systems involving the administration of allochimeric class I major histocompatibility complex (MHC) antigen [371409], or intrathymic injection of donor splenic cells [371505], in rats. In contrast, fingolimod prevented tolerance induction by donor-specific blood transfusion in intestinal transplantation [396001], [559007] or by an anti-CD4 mAb in a rat kidney transplantation model [559008]. However, establishment of transplant tolerance was not influenced by fingolimod co-treatment in other models [559007], [559009].

Several studies revealed that fingolimod may help alleviate grafted organ damage due to ischemia-reperfusion (IR) injury, a significant problem in clinical transplantation. This was observed in rat models involving cold preservation of kidney graft in which fingolimod treatment of recipients, either immediately (1 mg/kg iv) [559019] or 24 h (0.5 mg/kg po) [559026] prior to reperfusion ameliorated the morphological and functional consequences of post-transplant IR injury. A protective role of fingolimod (1 mg/kg iv) was also suggested in renal IR injury models in mice [409000], [559032]. Similarly, fingolimod pretreatment diminished the biochemical and histological manifestations of tissue injury in rat models of warm hepatic IR [477817], [559036], although an earlier study reported that such a treatment may aggravate IR-induced liver injury [371381]. More recently, in a rat model of segmental hepatic ischemia, fingolimod (1 mg/kg iv) prevented hepatocyte apoptosis and decreased the acute phase inflammatory response in both normal and cirrhotic livers when administered 20 min before ischemia and 10 min before reperfusion [584578].

Fingolimod inhibited various other T-cell-mediated immune responses in rodents, in addition to transplant rejection. These included graft-versus-host reactions [212192], [474285], contact allergy [477810], delayed-type hypersensitivity [371388], acute viral myocarditis [559576] and airway inflammation induced by adoptive transfer of Th1 or Th2 cells [530768]. Fingolimod prevented the spontaneous development of dermatitis in NC/Nga mice, a model for human atopic dermatitis [559047]. Importantly, fingolimod proved efficacious at suppressing several experimentally induced autoimmune diseases in mice or rats, including myocarditis [371433], experimental autoimmune uveoretinitis (EAU) [371444], thyroiditis [371438], collagen- and adjuvant-induced arthritis [371538], encephalomyelitis (EAE) [477724], [559040], [559041], [559042] and type 1 diabetes [371388], [371411], [371433], [492753], [559045]. Chronic fingolimod administration prevented the spontaneous development of autoimmune diabetes in NOD mice [559046], [559047] and slowed the progression of systemic lupus erythematosus-like syndrome in MRL-lpr/lpr mice [559048]. It must be noted that fingolimod treatment generally needed to be initiated before, or at the time of disease induction, to be effective in these models. However, the pathology of EAU could still be significantly reduced when the drug was administered after disease onset [371444], and in the case of thyroiditis induced by neonatal thyrmectomy and irradiation, fingolimod significantly reversed ongoing autoimmune disease [371438]. Furthermore, administration of fingolimod (3 mg/kg/day ip) to SJL mice with established relapsing-remitting EAE, a chronic disease that mimics the predominant form of human MS, resulted in a rapid and sustained improvement in the clinical status of the mice, which was maintained as long as dosing was continued [559042]. Similarly, in IL-10 gene knockout mice, a model of inflammatory bowel disease, fingolimod significantly decreased the severity of colitis when administered for 4 weeks after disease onset [559051]. While such data suggest a role for fingolimod monotherapy in the treatment of autoimmunity, it appears probable that, as for transplantation, the drug may be of greater utility when combined with other immunosuppressive agents. This possibility has not been explored in the studies published so far, but is suggested in patent application WO-2004028521 (described below).

A striking feature of the in vivo action of fingolimod, invariably observed in the aforementioned studies, was the induction (at immunosuppressive doses) of a marked decrease in the number of peripheral blood lymphocytes (PBLs). For example, a single-dose administration of fingolimod (0.1 mg/kg) in rats reduced PBL counts by >90% between 3 and 24 h, with a return to baseline level within a week [371354], [396003]. In baboons or cynomolgus monkeys receiving fingolimod (0.1 or 0.3 mg/kg/day), peripheral lymphopenia occurred as soon as 4 h after treatment, reaching 60 to 80% by 24 to 48 h [371447], [417322]. This effect was somewhat more rapid and pronounced on T-cells than B-cells, with CD4+ cells being more greatly reduced than CD8+ cells [371354], [371447], [417322]. In cynomolgus monkeys that were chronically treated with fingolimod, PBL counts decreased to ~30 and 14% of pretreatment values at doses of 0.03 and 3.0 mg/kg/day, respectively and only ~4% of peripheral CD4+ T-cells were refractory to depletion by the drug, compared with ~30% for CD8+ T-cells [477762]. As a correlate of this peripheral lymphopenia, fingolimod reduced the infiltration of allografts by T-cells [371483], [371489], [558982], [558985], especially if the drug was administered before this infiltration occurred [378421]. Significantly diminished T-cell infiltration of autoimmune disease target organs was similarly documented along with PBL depletion in animal models of autoimmunity, following treatment with fingolimod [371388], [559040], [559047]. Interestingly, in the transplantation studies, the few T-cells present in the grafts of fingolimod-treated animals expressed IFN-γ mRNA [371483], whereas these cytokines were
markedly suppressed in CsA- or tacrolimus-treated recipients [371403], [371483]. The combination of fingolimod with either CsA or tacrolimus abrogated both the T-cell infiltration and cytokine mRNA expression in the graft [371403], [371483], which may account for the synergism in the graft protection mentioned above. Furthermore, studies with fingolimod analogs revealed that their ability to cause lymphopenia correlated well with their efficacy in promoting rat skin allograft survival [377090]. There was also a close correlation between the degree of circulating lymphocyte depletion and heart allograft survival in rats treated with low doses of fingolimod (0.01 to 0.1 mg/kg) in conjunction with everolimus [396003]. This further suggests that lymphopenia and the associated reduction of graft infiltrating lymphocytes play a crucial role in the immunosuppressive effect of fingolimod.

It was initially proposed that the lymphopenia caused by fingolimod reflected apoptotic cell death [371360], [371452], [371507]. However, the blood concentrations of immunosuppressive doses of fingolimod proved to be > 2-fold lower than those required to induce apoptosis [371354]. In addition, apoptotic cells could not be detected in the PBL of baboons following treatment with low doses of fingolimod [371447]. Similarly, apoptosis rates were not increased in the PBL of patients receiving fingolimod [438642]. In mice, dye-labeled lymphocytes that had been depleted from the blood after fingolimod treatment (0.3 mg/kg) reappeared after cessation of treatment [371454], and there was no evidence for deletion of antiviral memory cells by the drug [371388]. Moreover, an S-enantiomer analog of fingolimod, which did not induce lymphopenia, proved as potent as the lymphopenia-inducing R-enantiomer analog in causing lymphocyte apoptosis in vitro [477818]. Therefore, lymphocyte apoptosis is unlikely to play a significant role in fingolimod-induced lymphopenia.

An alternative and more plausible explanation for the lymphopenic effect of fingolimod was provided by the finding that, concomitant with a reduction of PBL numbers, the drug increased lymphocyte numbers in the peripheral and mesenteric lymph nodes (LN) and in Peyer’s patches (PP), but not in spleen [371354], [371455], [371473]. This suggested that fingolimod altered the trafficking of lymphocytes such that they became sequestered in LN and PP. One possibility could be that fingolimod accelerated the homing of lymphocytes to these tissues, a process known to involve both specialized adhesion molecules and chemokines [371457], [559052]. While antibodies directed against lymphocyte homing molecules such as CD62L, CD49d and CD11a interfered with fingolimod-induced lymphocyte sequestration, expression of these molecules was not altered by the drug [371354], [371462]. Furthermore, evidence was obtained that fingolimod can act independently of CD62L [559481]. An augmentation of lymphocyte responses to homing chemokines was also postulated to mediate fingolimod-induced lymphocyte sequestration in LN and PP [371332], [477822]. This hypothesis was based on the observation that nanomolar concentrations of the drug stimulated T-cell chemotaxis to certain chemokines in vitro [559484]. However, fingolimod proved capable of producing lymphocyte sequestration in mice lacking the chemokine receptors CCR7 and CXCR5 [559497], [559499], which are known to play prominent roles in lymphocyte homing to secondary lymphoid organs [559052]. Although other chemokine receptors, such as CCR2 and CXCR4, may participate in the action of fingolimod [431191], [559052], [559577], further observations indicated that fingolimod-induced lymphocyte sequestration resulted from an inhibition of lymphocyte emigration from LN and PP rather than from an enhanced attraction to these organs [558908]. In this respect, it is worth noting that lymphocyte re-circulation to the blood requires lymphocytes to enter the thoracic duct lymph (TDL) after their transit through secondary lymphoid organs [371457], [559052]. Interestingly, fingolimod treatment decreased lymphocyte counts in the TDL to a greater extent than in the blood [371354]. Histological analyses of LN from fingolimod-treated mice revealed an accumulation of lymphocytes on the abluminal side of the lymphatic endothelium, along with an emptying of lymphatic sinuses, indicating that lymphocyte egress into lymph was blocked [558906], [559053]. This may result in an inhibition of both the re-circulation of naïve T-cells and the release of antigen-activated T-cells from the draining lymph node to lymph and to the blood compartment [558905]. In addition, fingolimod inhibited the passage of mature T-cells from the thymus into blood [371477], [559504].

A breakthrough in understanding the pharmacological effects of fingolimod came from the discovery that the drug is rapidly phosphorylated in vitro. Furthermore, the resulting phosphorylated fingolimod (fingolimod-P) inhibited lymphocyte re-circulation and acted as a potent agonist on several members of the sphingosine-1-phosphate (SIP) receptor family, namely SIP1, SIP3, SIP4 and SIP5 [477724], [559503]. Upon binding their natural sphingolipid ligands, such cell-surface G protein-coupled receptors have been reported to elicit a variety of responses in diverse cell types [559054], [559057], SIP1 receptors, which are expressed on lymphocytes and endothelial cells [559058], appear to be the most important SIP receptors with regard to the immunosuppressive action of fingolimod. This was first suggested by structure-activity analyses of semi-selective SIP receptor agonists [559059], [559060]. Moreover, a potent SIP1-selective agonist, structurally unrelated to SIP and fingolimod-P, induced peripheral lymphopenia by preventing the entry of lymphocytes into lymph, in a manner similar to fingolimod [559511]. A role for SIP1 in lymphocyte trafficking was further demonstrated by the observation that mice lacking this receptor on their lymphocytes exhibited an almost complete absence of T-cells, and severe deficiency of B-cells, in their blood [559513]. Moreover, SIP1-negative T- and B-cells, transferred to a normal host, accumulated in secondary lymphoid organs from which they failed to exit [559513]. A study conducted with knockout mice revealed that selective deletion of SIP1 in T-cells produced a block in the egress of mature T-cells from the thymus into the periphery [559515]. Most importantly, exposure of normal lymphocytes to fingolimod-P in vivo or in vitro downregulated expression of SIP1 through internalization and rapid degradation, thereby inducing an SIP1-negative phenotype [559515], [559502]. This implied that fingolimod-P behaves as a partial SIP1 agonist in lymphocytes, rather than a full agonist as originally thought [477724], [559503]. Therefore, expression...
of functional SIP1 by lymphocytes appears to be required for their egress from the thymus and secondary lymphoid organs. The egress-blocking effects of fingolimod and consequent depletion of T- and B-cells from the circulation may thus result from the inactivation of this receptor by the fingolimod-P metabolite. In addition, the downregulation of SIP1 by fingolimod on marginal zone B-cells, a unique subset of sessile B-cells with a partially activated phenotype, was shown to cause their rapid relocation to lymphoid follicles [559540]. This indicated that the action of the drug may extend beyond a blockade of lymphocyte egress and affect lymphoid tissue compartmentalization. Fingolimod was further demonstrated to suppress the antibody response to a T-dependent antigen by inhibiting the formation of germinal centers in peripheral lymphoid tissues of mice [559543].

Evidence was obtained that fingolimod may also alter the barrier function of the vascular endothelium. Fingolimod-P (10 nM) induced calcium mobilization and MAP kinase activation, and promoted survival and adherens junction assembly in endothelial cells in vitro [515624], [530766], [559544]. Consistent with these observations in vitro, the enhanced vascular permeability for macromolecules induced by vascular endothelial growth factor (VEGF) in vivo was inhibited by fingolimod treatment (10 µg) in mice [530766]. Whether such an effect contributes to the aforementioned ability of the drug to attenuate graft vessel disease [477831] and IR injury [559036] remains to be investigated.

Fingolimod was also demonstrated to exert antitumor effects, albeit through still poorly defined mechanisms. At micromolar concentrations in vitro, the drug inhibited proliferation and promoted the apoptosis of various human or mouse tumor cell lines, including glioma [559869], bladder cancer [559870] breast cancer [452318], [585263], prostate cancer [585260], [585264], hepatoma [514900], [515624], [585261] and myeloma [574214], while affecting normal cell counterparts to a lesser extent. Tumor cell death induced by fingolimod may be due to activation of pro-apoptotic signaling pathways [585263], [585264], and/or inhibition of anti-apoptotic pathways, such as the phosphoinositide 3-kinase/Akt pathway [585261], but there is no evidence that this could be mediated via S1P receptors. An intriguing possibility, which remains to be confirmed, is that high concentrations of fingolimod may inhibit sphingosine kinase activity, thereby resulting in intracellular accumulation of sphingosine [585298], an apoptosis inducer [585262]. Selective cancer cell apoptosis also appeared to underlie the ability of fingolimod (5 or 10 mg/kg ip) to suppress the growth of human tumor cells xenografted in nude mice [452318], [492802], [515620], [515624], [559870]. However, additional antitumor actions of fingolimod in vivo may relate to a modulation of angiogenesis [515620], [530766] and a prevention of metastasis [452318].

Metabolism and Pharmacokinetics
Owing to its amphipathic character, fingolimod demonstrated good oral bioavailability (80% in rats, 60% in dogs and 40% in monkeys), and was highly distributed in the cellular blood components [371259]. As mentioned previously, fingolimod was rapidly phosphorylated in vivo in rats, monkeys and humans, giving rise to the biologically active fingolimod-P metabolite [477724], [559503]. Phosphorylation of fingolimod also occurred in cells incubated with the compound [559503], and the MDR-1 multidrug transporter appeared to mediate the efflux of fingolimod-P from cells [559545]. Moreover, fingolimod-P was detected in plasma following intravenous administration of fingolimod in rats, and of the two chiral forms of a fingolimod analog, the R-enantiomer was readily phosphorylated, whereas the S-enantiomer exhibited only trace phosphorylation in rat blood [477724], [559503], which was consistent with the R-enantiomer being the immunosuppressive isomer [377090], [477818]. Following oral administration of fingolimod, the blood level of fingolimod-P exceeded that of the parent compound by up to 4-fold [477724]. Fingolimod was phosphorylated in vitro by both sphingosine kinase type 1 (SPHK1) and type 2 (SPHK2), but SPHK2 was more effective, suggesting that it may be the relevant enzyme in vivo [530765], [559546], [585265]. While phosphorylation of fingolimod appeared reversible in vivo [477724], the compound was irreversibly metabolized by hepatic oxidation to carboxylic acid derivatives devoid of immunosuppressive activity that were excreted in urine and feces [371344], [413804]. A study using human liver microsomes suggested that CYP4F3, or another closely related form of P450 enzyme, was the primary catalyst of fingolimod oxidation, which resulted in the formation of two metabolic products [371490]. Since none of the major drug-metabolizing P450 enzymes appear to be involved in this metabolism, interactions of fingolimod with potential co-medications such as CsA, tacrolimus or sirolimus are unlikely. This is consistent with earlier findings in dogs [371377], and with data from human studies that demonstrated the lack of pharmacokinetic or pharmacodynamic cross-interference between fingolimod and CsA [559547], [559548]. However, a potentiation of fingolimod exposure by CsA co-administration was noted in cynomolgus monkeys [417322].

A pharmacokinetic study in baboons revealed that when administered as a single oral dose, fingolimod (0.3 mg/kg) displayed a Cmax of 2.16 ng/ml, an AUC0-12h of 77.9 ng.h/ml and a t1/2 of 36 ± 12 h. Upon repeated dosing at 0.03 mg/kg, the compound accumulated over time to reach a stable blood concentration (0.72 ng/ml) by days 7 to 9 [371447]. In cynomolgus monkeys treated with fingolimod as a single dose either orally (0.1 or 1 mg/kg) or intravenously (0.1 mg/kg), a linear three-compartment model characterized the time course of fingolimod concentrations with a t1/2 of ~ 31 h, a CI of ~ 0.53 1/h/kg and a bioavailability of ~ 38% [448400]. This long terminal t1/2 most likely reflected extensive tissue distribution and binding of the compound, particularly as the steady state Vd of the drug was also notably large (Vdss = 15 l/kg) [448400].

The pharmacokinetics of fingolimod (0.25 to 3.5 mg) were analyzed following the administration of single doses in renal transplant patients (n = 20) [477737]. Fingolimod displayed a prolonged absorption phase with a Tmax of 12 to 24 h and an elimination t1/2, ranging from 89 to 157 h, which was dose-independent. The Cmax (0.16 to 2.8 ng/ml) and AUC (28 to 434 ng.h/ml) were proportional to the dose, with low interpatient variability. Moreover, fingolimod...
possessed an unusually high apparent Vd (median = 1407 l), suggesting widespread tissue distribution and a relatively low apparent oral Cl (median = 158 ml/min) [477737]. A study in healthy volunteers further revealed that after a single 1-mg dose of fingolimod, neither the Cmax nor AUC differed significantly between fasting and fed states [559550]. Pharmacokinetic parameters consistent with the single-dose data were obtained in a multiple-dose study of fingolimod in renal transplant patients, in which the compound was administered once-daily, at doses of 0.125 to 5.0 mg for 28 days [559547]. There was an approximately 10-fold drug accumulation over the dosing period before steady-state levels were reached by day 28. At steady-state, the median Tmax (~ 8 h) and the mean t1/2 (~ 200 h) were dose-independent, levels were reached by day 28. At steady-state, the median accumulation over the dosing period before steady-state renal transplant patients, in which the compound was [559550]. Pharmacokinetic and pharmacodynamic modeling in renal transplant patients revealed that the EC50 value for lymphopenia was achieved at a 0.5-mg dose of fingolimod and blood concentrations of 0.6 ng/ml. Since effective doses of fingolimod were reported at 2.5 to 5 mg/day, this suggested that the immunosuppressive effect of fingolimod may depend upon the induction of a high degree of lymphopenia (~ 80%) [579948]. Furthermore, analysis of the effects of single doses of ≤ 40 mg in healthy volunteers indicated that the duration of lymphopenia correlated with the extent of drug exposure, therefore, high-dose regimens of fingolimod may have clinical potential [579944].

Pharmacokinetic and pharmacodynamic modeling in renal transplant patients (n = 65) maintained on CsA plus corticosteroids, to assess the effects of multiple doses of fingolimod [559547]. A randomized, double-blind, placebo-controlled, time-lagged phase I clinical trial was conducted to evaluate single ascending doses of fingolimod (0.25 to 3.5 mg) in stable renal transplant patients (n = 32) receiving a CsA (Neoral)-based immunosuppressive regimen [477737], [559555], [559556], [559557]. Transient, asymptomatic bradycardia was observed after fingolimod administration, but overall the drug was well tolerated with no serious adverse events. The treatment significantly and dose-dependently reduced peripheral lymphocyte count by 30 to 70%. Lymphocyte depletion was noticeable within 4 h, reached a nadir at 6 to 12 h and receded by 24 to 72 h post-treatment, except at the highest fingolimod dose where it persisted for > 96 h. In contrast, the numbers of blood natural killer (NK) cells, granulocytes and monocytes were not altered by fingolimod [559556]. Although fingolimod reduced all lymphocyte subsets, T-cells were depleted more than B-cells and CD4+ cells were depleted more than CD8+ cells [559556]. Further analysis revealed that within the peripheral blood CD3+ lymphocyte population, CD62L+ cells decreased by the greatest extent (~ 57%), whereas CCR5+ cells declined only marginally (~ 10%) [559557].

A second randomized, double-blind, placebo-controlled phase I clinical trial was conducted in stable renal transplant patients (n = 65) maintained on CsA plus corticosteroids, to assess the effects of multiple doses of fingolimod [559547]. Patients received once-daily doses of fingolimod (0.125, 0.25,
Fingolimod was investigated in combination with everolimus in de novo renal transplant patients at increased risk of delayed graft function, a known detrimental factor for graft survival [559567]. In this prospective, open-label clinical trial, patients (n = 52) received a loading dose of 5 mg of fingolimod and 4 mg of everolimus at least 2 h before transplantation, followed by post-transplant maintenance treatment with 2.5 mg of fingolimod, 4 mg of everolimus and corticosteroids. At 12 months, 44% BCAR, 25% graft loss and 7.7% death were reported, which compared favorably with the efficacy of conventional regimens in similar patient populations [559567].

Phase II clinical trials of fingolimod in autoimmune diseases were reported to have commenced in Europe and the US by early 2000 [368251]. In November 2003, fingolimod was in phase II trials for MS [513950]. However, data from these trials have yet to be released.

Phase III
Fingolimod is under investigation for the prevention of acute rejection and graft loss in kidney transplantation patients in ongoing phase III clinical trials [513830], [523073]. A randomized, open-label, active control, parallel-assignment, safety and efficacy phase III clinical trial was initiated in April 2004, the primary outcomes of which were to compare the efficacy of fingolimod in combination with two other drugs versus a marketed drug to prevent rejection. A second clinical trial, initiated in December 2004, is examining the efficacy and safety of fingolimod in patients undergoing their first kidney transplant (www.clinicaltrials.gov).

Side Effects and Contraindications
Fingolimod has exhibited a good tolerability profile in the clinical trials conducted so far [477737], [559547], [559560], [559561], [559567]. As noted above, the most common adverse event observed in patients receiving higher doses of the drug was reversible bradycardia [559560]. Heart rates decreased to a nadir by approximately 4 to 8 h after fingolimod dosing and recovered after 24 h. This transient
bradycardia at treatment initiation was asymptomatic, with normal blood pressure at the time of nadir, and required no clinical intervention. Although all patients recovered without sequelae in these studies, pronounced baseline bradycardia prior to fingolimod treatment might be considered as a contraindication to the drug. Nevertheless, recently released data from an open-label clinical trial in renal transplant patients who had received at least one year of treatment with either fingolimod (2.5 or 5 mg/day; n = 94) or MMF (n = 327) revealed no significant difference in cardiac function, including heart rate and the incidence of bradycardia, between treatment arms [570796]. Furthermore, no additional organ toxicity has been observed, and no clinical signs or symptoms of pulmonary dysfunction have been identified in patients receiving fingolimod therapy [559560]. Overall, there has been no significant difference between the incidences of adverse events in fingolimod-treated patients compared with patients receiving placebo. Similarly, the incidences and types of infections (bacterial, viral and fungal) were comparable among patients treated with multiple doses of fingolimod or placebo [559560]. Indeed, the incidence of cytomegalovirus infections in kidney graft recipients receiving fingolimod was slightly lower than in those treated with MMF [558912]. Furthermore, there was no emergence of malignancy during treatment or follow-up phases of the studies. It is also important to note that regarding combined treatment studies, fingolimod did not appear to aggravate any of the adverse side effects of co-administered immunosuppressants, including CsA [559548], [559560] and everolimus [559567].

Patent Summary

Fingolimod is described along with other 2-amino-1,3-propanediol compounds in the 1994 patent application WO-09408943, assigned to Taito Co Ltd and Yoshitomi Pharmaceutical (now Mitsubishi Pharma). This patent application reports the immunosuppressive activity of fingolimod in rat models of skin allograft and adjuvant arthritis. Accordingly, fingolimod is claimed as an immunosuppressant useful for inhibiting rejection in organ or bone marrow transplantation and as a preventive or remedy for autoimmune diseases. The Novartis patent application WO-02076995, published in October 2002, claims 2-amino-propanol derivatives, including the phosphorylated form of fingolimod, for their immunosuppressive property mediated via peripheral lymphocyte depletion and their ability to bind to S1P receptors. Similar claims are made in a prior patent application, WO-00218395, assigned to Merck & Co Ltd and published in July 2002. This application describes phosphate derivatives of fingolimod as immunoregulatory compounds useful for treating immune-mediated diseases and conditions such as bone marrow, organ and tissue transplant rejection. It is reported that incubation of fingolimod in whole blood from mice gives rise to the phosphorylated fingolimod metabolite that exhibits immunosuppressive activity in vivo. More recently, in October 2004, Novartis and Mitsubishi published WO-2004089341, which claims the manufacture of a solid formulation of fingolimod for the treatment of autoimmune disease, transplant rejection, inflammation and viral myocarditis.

A number of additional patent applications cover the use of fingolimod administered alone, or in combination with other immunosuppressive agents. WO-00101978, assigned to Welfide Corp (previously Yoshitomi Pharmaceutical), claims the utility of fingolimod for the treatment of viral myocarditis and other viral diseases causing inflammatory cell injuries in various organs. The data supporting this claim were published by Miyamoto et al [559576]. The use of fingolimod for preventing renal transplant rejection in patients at risk of delayed graft function is claimed in WO-02067915, published in September 2002 and assigned to Novartis and Welfide. The combination of the drug with everolimus is suggested to be most efficient for that purpose. The Novartis patent application WO-02100148, published in December 2002, claims fingolimod as a component of a multi-drug regimen, for the prevention or treatment of rejection of pancreatic islet cell allograft or xenografts. The combined treatment with fingolimod, everolimus and basiliximab is reported to be useful for preventing islet allograft rejection. WO-03035068, also assigned to Novartis, discloses the combination of fingolimod with the macrolide pimecrolimus, an analog of tacrolimus, for immunosuppressive therapy. Treatment with the two agents in combination, but not alone, for 4 weeks starting at disease induction is demonstrated to prevent development of experimental autoimmune uveitis in rats. One particularly interesting patent application is WO-2004028521, recently published by Novartis, which claims the use of fingolimod given alone or in combination with another immunomodulator, for the potential treatment of demyelinating diseases, such as multiple sclerosis or Guillain-Barré syndrome. Supporting data obtained in a SJL mouse model of chronic progressive EAE demonstrated that treatment with fingolimod in combination with IFNβ, when disease is fully established, prevented disease progression for 1 month, whereas IFNβ alone only marginally inhibited disease progression for ~ 1 week. Combined treatment with fingolimod and everolimus was also noted to curtail development of optic neuritis and neurological symptoms in a rat EAE model.

Several Novartis patent applications further claim that fingolimod may be of therapeutic value in non-immune diseases. A method for the treatment of solid tumors and other cell proliferative disorders using SIP receptor agonists, such as fingolimod-P, is proposed in WO-03097028. It is disclosed that fingolimod-P acts as an agonist of angiogenesis on its own, but surprisingly, as an antagonist of S1P-mediated angiogenesis, and can modulate angiogenesis-dependent tumor growth. WO-2004010987 proposes the use of SIP receptor agonists, including fingolimod-P, to treat cardiovascular disease such as congestive heart failure. This is based on the negative chronotropic effect of fingolimod observed in vivo. It is of note, however, that since this effect is mediated via S1P3 [559509], compounds with greater affinity than fingolimod-P for this receptor would be more appropriate for this indication, a point not clearly stated in the patent application. The use of fingolimod and its analogs as antifungal agents is claimed in WO-03009836. The data presented in this patent application states that fingolimod accelerated endocytosis and degradation of ubiquitin.
pathway-dependent nutrient transporter(s), thereby causing growth inhibition of yeast by starvation of nutrients such as leucine and tryptophan. A recent publication reported that such effects of fingolimod mimicked those of the natural yeast sphingolipid phytosphingosine [578613]. In addition, two patent applications published in December 2004 claim the use of 2-amino-1-3-propanediol derivatives, including fingolimod, for the treatment of pain. These patent applications, namely WO-2004110421 and WO-2004105773, are assigned to Aventis Research & Technologies and present a novel potential use for the compound.

Current Opinion
Fingolimod has emerged as one of the most innovative immunosuppressants to be identified over the past decade. In contrast to conventional immunosuppressive agents, it does not impair T- and B-cell activation, proliferation and effector function, but interferes with the re-circulation of lymphocytes between lymphoid organs and blood. By inducing the sequestration of lymphocytes in LN and PP, fingolimod impedes their recruitment and the ensuing pathogenic damages within peripheral inflammatory tissues and graft sites. Evidence was obtained indicating that fingolimod is actually a prodrug, in that it needs to be phosphorylated in order to exert its pharmacological effects [559503]. The fingolimod-P metabolite thus generated acts as a high-affinity ligand of the S1P1 receptor on lymphocytes, inducing its aberrant internalization and rendering lymphocytes unresponsive to the physiological lipid mediator S1P, which normally provides an obligatory signal for the egress of lymphocytes from lymphoid organs [558908]. In addition to disrupting lymphocyte homing patterns, the alteration of S1P receptor signaling by fingolimod-P may also affect the vascular endothelium, which suggests further complexity in the effects of the compound. Therefore, the progress achieved to date in understanding the molecular mode of action of fingolimod has uncovered unexpected aspects of the regulation of lymphocyte trafficking and has established S1P receptors as key targets for future drug development [477764], [559574].

Preclinical studies indicated that, besides dampening acute allograft rejection, fingolimod also appears to be capable of inhibiting graft vascular disease, a hallmark of chronic rejection that is poorly controlled by currently available immunosuppressive drugs. In addition, fingolimod may mitigate ischemia-reperfusion injury, known to impair long-term graft survival. Data from autoimmune disease models also suggest that fingolimod treatment may reverse an established pathogenic immune response, although this requires substantiating by further investigation. Importantly, both the preclinical and clinical studies revealed that fingolimod has a favorable safety profile. Apart from inducing transient bradycardia, a clinically manageable side effect, the compound was generally well tolerated in patients. In particular, fingolimod did not display the nephrotoxicity, diabetogenicity and neurotoxicity of calcineurin inhibitors, the myelotoxicity of anti-metabolites, or the lipid altering effects of sirolimus-type drugs. Furthermore, experimental studies demonstrated that unlike other immunosuppressive agents fingolimod does not cause global immunosuppression and may spare elements of non-specific and memory lymphocyte components of host resistance to infections. Fingolimod also offers the advantages of a simple chemical structure, facilitating large-scale production, and water solubility, such that it can be easily formulated for oral administration with good bioavailability. Moreover, while the peripheral lymphopenia response may provide a convenient pharmacodynamic parameter for therapeutic monitoring of fingolimod, the linear pharmacokinetics of the compound appear conducive to standardized dosing recommendations, with minimal interpatient and intrapatient variations.

Collectively these properties make fingolimod an obvious candidate for clinical development. At present, there are only a few issues that might encumber this development. One relates to the possible immunological consequences of lymphocyte trafficking alterations produced by long-term treatment with fingolimod, such as a disturbance of peripheral tolerance mechanisms [559007], [559008], [559009]. Another issue relates to whether prolonged occupancy and/or modulation of S1P receptors by fingolimod-P would produce as yet unknown side effects, particularly when considering the potentially widespread physiological roles of these receptors [559505]. Notwithstanding these concerns, the well-documented ability of fingolimod to strongly synergize with immunosuppressants that block lymphocyte activation or proliferation remains a highly valuable property. In this respect, the pharmacological spectrum of fingolimod fully satisfies the criteria of a multi-drug therapy paradigm, in which combination of agents with distinct modes of action and without overlapping toxicities represent a powerful approach to optimize safety and efficacy [371251]. The preclinical studies provided convincing evidence that low-dose treatment with fingolimod in conjunction with sub-therapeutic doses of CsA, tacrolimus, sirolimus or everolimus enabled potent immunosuppression in the absence of serious side effects. Data from the phase IIb clinical trial in renal transplant patients similarly indicated that fingolimod might permit the use of reduced doses of CsA to provide an improved safety/efficacy margin compared with standard immunosuppression [558912].

Although it is difficult to predict the true medical impact of fingolimod before completion of the ongoing clinical trials, the data accumulated so far suggest that this drug has the potential to adequately complement the existing immunosuppressant arsenal. If approved for use in renal transplantation the possible indications of the drug may be extended to the treatment of a broad spectrum of immunopathological conditions, encompassing not only graft rejection, but also autoimmune diseases and chronic inflammation.

Commercial Opinion
In January 2005, analysts at Deutsche Bank predicted that, on the back of the promising phase II clinical data, annual sales for fingolimod could potentially reach US $1 billion, although this will depend on results from ongoing phase III clinical trials [583902].
Licensing

**Novartis AG**
In September 1997, Novartis licensed fingolimod for exclusive development worldwide, except in Japan, for transplantation and autoimmune diseases. In Japan, the compound would be co-developed and co-marketed with Mitsubishi (formerly Yoshitomi) [266426].

Development history

By August 2004, fingolimod had received Orphan Drug Status in Japan for renal transplant rejection [558345].

<table>
<thead>
<tr>
<th>Developer</th>
<th>Country</th>
<th>Status</th>
<th>Indication</th>
<th>Date</th>
<th>Reference</th>
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<td>Novartis AG</td>
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<td>Phase III</td>
<td>Transplant rejection</td>
<td>19-NOV-03</td>
<td>513830</td>
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<td>Novartis AG</td>
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<td>Transplant rejection</td>
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<td>Mitsubishi Pharma Corp</td>
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<td>Transplant rejection</td>
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<td>538366</td>
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<tr>
<td>Novartis AG</td>
<td>Switzerland</td>
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<td>Multiple sclerosis</td>
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<tr>
<td>Novartis AG</td>
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<td>Autoimmune disease</td>
<td>03-JUL-00</td>
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<td>Novartis AG</td>
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<td>Mitsubishi Pharma Corp</td>
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<td>Mitsubishi Pharma Corp</td>
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<td>Autoimmune disease</td>
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<td>Novartis AG</td>
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<td>Discovery</td>
<td>Inflammatory bowel disease</td>
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<td>Novartis AG</td>
<td>Switzerland</td>
<td>Discovery</td>
<td>Autoimmune disease</td>
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<td>Mitsubishi Pharma Corp</td>
<td>Japan</td>
<td>Discovery</td>
<td>Myocarditis</td>
<td>13-NOV-00</td>
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Literature classifications

Key references relating to the drug are classified according to a set of standard headings to provide a quick guide to the bibliography. These headings are as follows:

**Chemistry**: References that discuss synthesis and structure-activity relationships.

**Biology**: References that disclose aspects of the pharmacology of the drug in animal models.

**Metabolism**: References that discuss metabolism, pharmacokinetics and toxicity.

**Clinical**: Reports of clinical phase studies in volunteers providing, where available, data on the following: whether the experiment is placebo-controlled or double- or single-blind; number of patients; dosage.

**Chemistry**

<table>
<thead>
<tr>
<th>Study Type</th>
<th>Result</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>SAR.</td>
<td>Synthesis of fingolimod analogs demonstrated that the optimal length between the phenyl ring and the quaternary carbon was two carbon atoms. Of the two hydroxymethyl groups present in the hydrophilic portion of fingolimod, only the pro-S hydroxymethyl group is essential for immunosuppressive activity. Only the R-enantiomer configuration at the chiral carbon of fingolimod analogs displayed biological activity <em>in vivo</em>.</td>
<td>377090</td>
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**Biology**

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<th>Study Type</th>
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<th>Experimental Model</th>
<th>Result</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td><em>In vivo</em></td>
<td>Suppression of skin allograft rejection.</td>
<td>Rats treated with either fingolimod or a combination of fingolimod and CsA.</td>
<td>Fingolimod strongly inhibited intragraft T-cell infiltration but not IL-2 and IFNγ mRNA expression, while CsA had opposite effects. Combination of the two drugs resulted in inhibition of both T-cell infiltration and cytokine expression.</td>
<td>371483</td>
</tr>
<tr>
<td><em>In vivo</em></td>
<td>Suppression of renal allograft rejection.</td>
<td>Fingolimod (0.1 to 3 mg/kg/day) administered either alone or in combination with CsA (10 to 30 mg/kg/day) or everolimus (0.25 to 0.50 mg/kg/day) to cynomolgus monkeys who had undergone kidney allotransplantation.</td>
<td>Fingolimod alone prolonged allograft survival at 3.0 mg/kg/day, but not at a 0.3 mg/kg/day. Efficacious regimens were established by combining fingolimod doses of 0.1 to 0.3 mg/kg/day with subtherapeutic doses of CsA or everolimus. Fingolimod caused a decrease of peripheral lymphocyte counts to ~ 30 and 14% of pretreatment values at doses of 0.03 and 3.0 mg/kg/day, respectively.</td>
<td>477762</td>
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<tr>
<td><em>In vivo</em></td>
<td>Suppression of EAE.</td>
<td>SJL mice with established relapsing-remitting EAE administered once-daily doses of fingolimod (3 mg/kg ip).</td>
<td>Fingolimod resulted in a rapid and sustained clinical improvement, which was maintained while dosing continued.</td>
<td>559042</td>
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### Metabolism

<table>
<thead>
<tr>
<th>Study Type</th>
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</thead>
<tbody>
<tr>
<td>In vitro</td>
<td>Metabolism.</td>
<td>Human liver microsomes and cDNA-expressed cytochrome P450 enzymes.</td>
<td>Fingolimod was metabolized to two products by a CYP4F member of the P450 enzyme family. Since none of the major P450s were involved, drug-drug interactions of fingolimod with CsA, tacrolimus or sirolimus are unlikely.</td>
<td>371490</td>
</tr>
<tr>
<td>In vivo/ in vitro</td>
<td>Metabolism.</td>
<td>Pharmacokinetic analysis of fingolimod in rats.</td>
<td>Fingolimod-P was detected in plasma after intravenous administration of fingolimod and in cells incubated with the compound. An R-enantiomer analog of fingolimod was readily phosphorylated, whereas the S-enantiomer exhibited only trace phosphorylation in rat blood.</td>
<td>559503</td>
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<tr>
<td>In vivo</td>
<td>Pharmacokinetics.</td>
<td>Baboons administered single doses of fingolimod (0.3 mg/kg/day) over a period of several days.</td>
<td>Fingolimod resulted in a peak blood concentration of 2.16 ng/ml, an AUC of 77.9 ng.h/ml and a t1/2 of 36 h. Repeated daily dosing at 0.03 mg/kg yielded a maximum blood level of 0.72 ng/ml after 7 days.</td>
<td>371447</td>
</tr>
<tr>
<td>In vivo</td>
<td>Pharmacokinetics.</td>
<td>A randomized, double-blind, placebo-controlled, time-lagged phase I clinical trial of single-ascending doses of fingolimod (0.25 to 3.5 mg) in stable renal transplant patients (n = 32) receiving a CsA-based regimen.</td>
<td>Fingolimod prolonged the absorption phase, with an elimination t1/2 ranging from 89 to 157 h that was independent of dose. The Cmax and AUC were proportional to the dose, with low intersubject variability. Fingolimod demonstrated a high apparent Vd.</td>
<td>477737</td>
</tr>
<tr>
<td>In vivo</td>
<td>Pharmacokinetics.</td>
<td>A randomized, double-blind, placebo-controlled phase I clinical trial of multiple doses of fingolimod (0.125 to 5 mg/day) in stable renal transplant patients (n = 65) receiving a CsA-based regimen.</td>
<td>Fingolimod accumulated ~ 10-fold over the dosing period before reaching steady-state levels by day 28. At that time, the median Tmax and the mean t1/2 were dose-independent, while the Cmax and AUC were proportional to dose, with 21 to 52% interindividual coefficients of variation for the latter parameter. There was no evidence of metabolic interaction between fingolimod and CsA.</td>
<td>559547</td>
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### Clinical

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<th>Effect Studied</th>
<th>Model Used</th>
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<tr>
<td>Safety and efficacy.</td>
<td>A randomized, double-blind, placebo-controlled, time-lagged phase I clinical trial of single-ascending doses of fingolimod (0.25 to 3.5 mg) in stable renal transplant patients (n = 32) receiving a CsA-based regimen.</td>
<td>Fingolimod was associated with transient bradycardia, but otherwise was well tolerated. Fingolimod induced a reversible peripheral lymphopenia that reached a nadir by 8 to 12 h post-treatment and receded within 24 to 72 h, except at the highest dose, where it persisted for more than 96 h.</td>
<td>477737</td>
</tr>
<tr>
<td>Safety and efficacy.</td>
<td>A randomized, double-blind, placebo-controlled phase I clinical trial of multiple doses of fingolimod (0.125 to 5 mg/day) in stable renal transplant patients (n = 65) receiving a CsA-based regimen.</td>
<td>Fingolimod did not cause any significant increase in adverse events or change in renal function. A dose-dependent peripheral lymphopenia was observed throughout the drug treatment period. Lymphocyte counts started to recover within 3 days in all dose groups following the discontinuation of fingolimod treatment.</td>
<td>559547</td>
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<tr>
<td>Safety and efficacy.</td>
<td>A multicenter, randomized, open-label, dose-finding phase IIa clinical trial comparing fingolimod (0.25 to 2.5 mg/day) with MMF (2 g/day) administered with a standard CsA-based regimen in de novo renal transplant patients (n = 208).</td>
<td>Fingolimod was associated with transient bradycardia, however, the frequency of adverse events did not differ among treatment groups. There was no evidence of exacerbation of CsA-related toxicity by fingolimod. At 3 months post-transplant, the incidence of BCAR was lowest with fingolimod at 2.5 mg (9.8%) compared with MMF (17.1%).</td>
<td>559560</td>
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<tr>
<td>Safety and efficacy.</td>
<td>Multicenter, randomized, partially blinded phase IIb clinical trial in de novo kidney transplant patients (n = 258). Patients were given fingolimod either at 2.5 mg/day with a full or reduced CsA dose, or at 5 mg/day with a reduced CsA dose. A fourth group of patients received MMF (2 to 3 g/day) plus full-dose CsA regimen.</td>
<td>Fingolimod (2.5 mg) demonstrated a 15.8% incidence of BCAR at 12 months post-transplant in patients administered full-dose CsA compared with an incidence of BCAR of 21.1% for MMF. Reducing CsA exposure by ≤ 50% increased the incidence of rejection with fingolimod at 2.5 mg, but provided effective graft rejection prophylaxis with a fingolimod dose of 5 mg.</td>
<td>559561</td>
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<td>Safety and efficacy.</td>
<td>Proof-of-concept, open-label clinical trial in de novo renal transplant patients (n = 52) at increased risk of delayed graft function. Patients received 5 mg of fingolimod and 4 mg of everolimus before transplantation, followed by 2.5 mg/day of fingolimod and 4 mg/day of everolimus plus corticosteroids post-transplantation.</td>
<td>At 12 months after transplantation, 44% BCAR, 25% graft loss and 7.7% death was reported across the treatment group. These figures compared favorably with the efficacy of conventional regimens in similar patient populations.</td>
<td>559567</td>
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Fingolimod

Dumont 247

Associated patent

Title 2-Amino-1,3-propanediol compound and immunosuppressant.

Assignee Taito Co Ltd/Yoshitomi Pharmaceutical Industries Ltd

Publication WO-09408943 28-APR-94


Inventors Fujita T, Sasaki S, Yoneta M, Mishina T, Adachi K, Chiba K.

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•• of outstanding interest
• of special interest


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371358 Mechanism of action of the immunosuppressant, rapamycin. Dumont FJ, Su Q LIFE SCI 1995 58 373-395


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• In this model, fingolimod augmented the mean survival time of heart allografts and inhibited the progression of graft atherosclerosis in mice.


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** This study demonstrates that in mice infected with lymphocytic choriomeningitis virus, fingolimod effectively reduced the re-circulation of CD8+ effector T-cells and their recruitment to peripheral lesions without affecting the induction and expansion of antiviral immune responses in secondary lymphoid organs.

371390 Synergistic interaction of FTY720 with cyclosporine or sirolimus to prolong heart allograft survival. Stepkowski SM, Wang M, Qu X, Yu J, Okamoto M, Tegajal N, Kahan BD TRANSPLANT PROC 1998 30 5 2214-2216


371398 FTY720, a novel immunosuppressant, shows a synergistic effect in combination with FK506 in rat allograft models. Hoshino Y, Yangawa Y, Ohtsuki M, Nakayama S, Hashimoto T, Chiba K TRANSPLANT PROC 1999 31 1-2 1224-1226


• Per-transplant administration of fingolimod on days 1 and 0 prolonged cardiac allograft survival in rats, and this effect was potentiated by post-transplant administration of tacrolimus.


371444 Effects of FTY720, a novel immunosuppressant, on experimental autoimmune uveoretinitis in rats. Kurose S, Ikeda E, Tokiwa M, Hikita NB, Mochizuki M EXP EYE RES 2000 70 1 7-15


• Oral treatment with fingolimod (0.03 to 0.3 mg/kg) caused peripheral lymphopenia affecting CD4+ T-cells more than CD8+ T-cells and B-cells, and was well tolerated in baboons.

371452 Evidence that FTY720 induces T cell apoptosis in vivo. Nagahara Y, Enosawa S, Ikekita M, Suzuki S, Shiniomiya T IMMUNOPHARMACOLOGY 2000 48 1 75-78


371462 FTY720, a novel immunosuppressant, induces sequestration of circulating mature lymphocytes by acceleration of lymphocyte homing in rats. III. Increase in frequency of CD62L positive T cells in Peyer’s patches by FTY720 induced lymphocyte homing. Yanagawa Y, Masubuchi Y, Chiba K IMMUNOLOGY 1999 95 4 591-594

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- This study provided the first evidence that fingolimod inhibits the egress of mature T-cells from the thymus.


- This seminal study shows that fingolimod decreased the infiltration of skin allografts by host T-cells without markedly suppressing intra-graft cytokine production and that CsA had opposite effects. The synergistic action of fingolimod combined with CsA on prolongation of allograft survival may thus reflect the respective inhibitions of T-cell infiltration and cytokine production in grafts.


371490 Role of CYP4F in the metabolic clearance of FTY720 - prediction of low drug to drug interaction potential. Zimmerlin AG, Patten CJ. TRANSPLANTATION 2000 69 S191


371538 Effect of FTY720, a novel immunosuppressant, on adjuvant and collagen induced arthritis in rats. Matsura M, Imayoshi T, Okumoto T INT J IMMUNOPHARM 2000 22 323-331


- Provides comprehensive SAR analysis of fingolimod analogs showing that the position of the phenyl ring is critical for activity and that only the R-enantiomer displays potent T-cell-decreasing and immunosuppressive activity.

378421 The significance of timing of FTY720 administration on the immunosuppressive effect to prolong rat skin allograft survival. Yanagawa Y, Hoshino Y, Chiba K, NIK J IMMUNOPHARM 2000 22 8 597-602

- This study suggests that, to inhibit skin allograft acute rejection, fingolimod should be administered before the occurrence of increased T-cell infiltration into the grafts.


396003 The peripheral lymphocyte count predicts graft survival in DA to Lewis heterotopic heart transplantation treated with FTY720 and SDZ RAD. Nikolova Z, Hof A, Bauml yin Y, Hof RP. TRANSPLANT IMMUNOL 2000 8 2 115-124

402151 Treatment of transplant rejection: Are the traditional immunosuppressants good enough? Dumont FJ CURR OPIN INVEST DRUGS 2001 2 3 357-363


413804 FTY720 metabolism in humans. Schmouder R, Dannecker R, Choudhury S, Barilla D, Sablinski T AM J TRANSPLANTATION 2001 1 1 Abs 1338

417322 The novel immunosuppressant FTY720 induces peripheral lymphodepletion of both T- and B-cell counts by up to 80 to 90% in cynomolgus monkeys. The degree of peripheral lymphopenia was not directly proportional to fingolimod blood levels.

423717 Mitsubishi Pharma Corp. COMPANY WORLD WIDE WEB SITE 2001 October 01


- Demonstrates that fingolimod is phosphorylated in vivo, thereby giving rise to its immunosuppressive entity, fingolimod-P, which acts as a potent agonist of four sphingosine-1-phosphate receptors to modulate lymphocyte trafficking.

477725 First asymmetric synthesis of chiral analogs of the novel immunosuppressant FTY720. Hinterding K, Albert R, Cottens S TETRAHEDRON LETT 2002 43 45 8089-8097

477726 Effects of mycophenolate sodium with or without FTY720 in a DA-to-Lewis rat heart transplantation model. Matsumoto Y, Hof RP. TRANSPLANT PROC 2002 34 7 2891-2892


• In this phase I study of single oral doses of fingolimod in renal transplant patients on a CsA-based regimen, the investigational drug was well tolerated, displayed dose-proportional pharmacokinetics and caused a reversible peripheral lymphopenia within 6 h.

47776 Oral efficacy of the new immunomodulator FTY720 in cynomolgus monkey allotransplantation, given alone or in combination with cyclosporine or RAD. Schuurman HJ, Menninger K, Audet M, Kunkler A, Maurer C, Vedrine C, Bernhard M, Gaschen L, Brinkmann V, Quesniaux V TRANSPLANTATION 2002 74 7 951-960

• Fingolimod was effective alone and synergized with CsA and/or everolimus in preventing acute kidney allograft rejection in cynomolgus monkeys.


477818 FTY720: Dissection of membrane receptor-operated, stereospecific effects on cell migration from receptor-independent antiproliferative and apoptotic effects. Brinkmann V, Wilt C, Kristofic C, Nikolova Z, Hof RP TRANSPLANT PROC 2001 33 7-8 797-3008

• This study demonstrates that the R-enantiomer, but not the S-enantiomer of a fingolimod analog is immunosuppressive in vivo. In addition, both enantiomers are shown to induce lymphocyte apoptosis at micromolar concentrations, indicating that the immunosuppressive effect of the drug is not associated with apoptosis induction.

477821 Neointima suppression in rat carotid allografts by FTY720 combined with a small fixed cyclosporine dose is related to a diminution of the peripheral lymphocyte count. Nikolaova Z, Hof RP TRANSPLANT PROC 2001 33 3 2184-2185

477822 FTY720: Altered lymphocyte traffic results in allograft protection. Brinkmann V, Pirschewer DD, Feng L, Chen S TRANSPLANTATION 2001 72 5 764-769

477823 FTY720 alters lymphocyte homing and protects allografts without inducing general immunosuppression. Brinkmann V, Chen S, Feng L, Pirschewer D, Nikolaova Z, Hof R TRANSPLANT PROC 2001 33 1-2 530-531

477824 Combined FTY720/cyclosporine A treatment promotes graft survival and lowers the peripheral lymphocyte count in DA to Lewis heart and skin transplantation models. Nikolaova Z, Hof A, Baumlín Y, Hof RP TRANSPLANT IMMUNOL 2001 8 4 267-277

477825 Combined FTY720/cyclosporine treatment promotes graft survival and lowers the peripheral lymphocyte count in a murine cardiac allotransplantation model. Nikolaova Z, Hof A, Baumlín Y, Hof RP TRANSPLANTATION 2001 72 1 168-171

477828 Efficacy of SDZ RAD compared with CsA monotherapy and combined RAD/FTY720 treatment in a murine cardiac allotransplantation model. Nikolaova Z, Hof A, Baumlín Y, Hof RP TRANSPLANT IMMUNOL 2001 9 1 43-49


49275 FTY720, a novel immunomodulator, prevents autoimmune diabetes in a DRBB rat. Popovic J, Claxton DB, Beiser KJ, Tong PY, Kovarik JM, Moore WV DIABETES 2003 52 Suppl 6 Abs 1187-P

492802 American Urological Association - 98th Annual Meeting, Chicago, IL, USA. Jamison J IDDB MEETING REPORT 2003 April 26-01 May

500183 Inflammation 2003 - Sixth World Congress (Part III), Vancouver, Canada. Evans R IDDB MEETING REPORT 2003 August 02-06

505878 Immunosuppressants in advanced clinical development for organ transplantation and selected autoimmune diseases. Kovarik JM, Burtin P EXPERT OPIN EMERG DRUGS 2003 1 47-62

51380 Industry leading pipeline to bring novel treatments to patients and sustain above-market growth at Novartis. Novartis AG PRESS RELEASE 2003 November 19


523073 Taking stock of the future: Phase III pipelines heading into 2004. FDC REPORTS PINK SHEET 2004 65 52 24-26


• The results of this study show that fingolimod can be phosphorylated by both human sphingosine kinase-1 and -2, but the latter is ~ 30-fold more efficient compared with sphingosine kinase-1.


• This study demonstrates that fingolimod can affect vascular cells as the compound was phosphorylated by endothelial cells in vitro to promote signaling, adherens junction assembly and cell survival. Furthermore, the drug administration potently blocked VEGF-induced vascular permeability in mice.


• This study shows that fingolimod could suppress airway inflammation induced by adoptively transferred antigen-specific Th1 or Th2 cells in mice. In addition, the compound inhibited airway inflammation, induction of bronchial hyper-responsiveness, and goblet cell hyperplasia in an actively antigen-sensitized murine asthma model.


538366 Overview of pipeline projects (as of May 12, 2004). Mitsubishi Pharma Corp COMPANY WORLD WIDE WEB SITE 2004 May 12

558345 Approval of 4 NCEs including Hespera recommended. PHARMA JPN 2004 August 30

558906 FTY720: Sphingosine 1-phosphate receptor-1 in the control of lymphocyte egress and endothelial barrier function. Brinkmann V, Cyster JG, Hla T AM J TRANSPLANTATION 2004 4 7 1019-1025
• Provides an excellent review of the molecular mechanism(s) underlying the immunosuppressive action of fingolimod.


558912 FTY720 immunomodulation: Optimism for improved transplant regimens. Ferguson R TRANSPL PROC 2004 36 2 Suppl 549-553


558914 Synthesis, stereochemical determination and biochemical charac-


558920 FTY720-induced lymphocyte homing modulates post-
• Fingolimod treatment of rats 4 h before transplantation of renal grafts that had been cold-preserved for 4 h reduced graft acute tubular damage, IL-1 production and neutrophil infiltration.

558921 FTY720 impacts necrosis development after ischemia-

558922 FTY720 pretreatment reduces warm hepatic ischemia
• Results of this study demonstrate that pretreatment of rats with fingolimod in combination with siRNAs or antisense oligonucleotides abrogated graft arteriosclerosis.

• The combination of basiliximab, for induction, and of everolimus and fingolimod, for maintenance immunosuppression, produced prolonged survival of islet allografts in cynomolgous monkeys.


• Delayed treatment with either fingolimod or fingolimod-P initiated at the peak of the first phase of EAE, caused an immediate and rapid improvement of disease that lasted for as long as dosing was continued in this model.
The effect of immunomodulators on prevention of autoimmune diabetes is stage dependent: FTY720 prevents diabetes at three different stages in the diabetes-resistant biobreeding rat. Popovic J, Kover KL, Moore WV. PEDIATR DIABETES 2004 5 1-3-9

This study demonstrated that fingolimod treatment prevented autoimmune diabetes in this model if administered before and/or during stimulation and expansion of the autoreactive T-cells, but was increasingly less effective if delayed until the later stages of the disease.


In this report fingolimod treatment of IL-10-/- mice with established colitis, was shown to ameliorate the clinical and histological manifestations of colitis, apparently by decreasing lymphocyte numbers and IFN-γ production in the colonic mucosa.

Homing and cellular traffic in lymph nodes. Von Andrian UH, Mempel TR. NAT REV IMMUNOL 2003 3 11 867-878


FTY720-enhanced T cell homing is dependent on CCR2, CCR5, and CXCR4: Evidence for distinct chemokine compartments. Yopp KC, Fu S, Hong SM, Randolph GJ, Ding Y, Krieger NP, Bromberg JS. J IMMUNOL 2004 173 2 855-865


This ground-breaking paper demonstrates that fingolimod is phosphorylated in vivo, thereby giving rise to its immunosuppressive entity, fingolimod-P, which acts as a potent agonist of four sphingosine 1-phosphate receptors to modulate lymphocyte trafficking.


Constitutive expression of the S1P1 receptor in adult tissues. Chae SS, Proia RL. HLA T PROTAGLANDINS OTHER LIPID MEDIATORS 2004 73 1-2 141-150


Clinical and histological manifestations of colitis, apparently by decreasing lymphocyte numbers and IFN-γ production in the colonic mucosa.


Lymphocyte egress from thymus and peripheral lymphoid organs is dependent on S1P receptor 1. Matouliam B, Lo CG, Cinamon G, Lesnenski MJ, Xu Y, Brinkmann V, Allende ML, Proia RL. Cyster-JG. J BIOL CHEM 2004 247 3572-3576

Expression of the sphingosine 1-phosphate receptor, S1P1, on T-cells controls thymic emigration. Allende ML, Dreier JL, Mandala S, Proia RL. J BIOL CHEM 2004 279 15 15396-15401


This study demonstrates that fingolimod induces a prolonged internalization of S1P1 in transfected hepatoma cells and inhibits S1P-mediated chemotaxis in lymphocytes.


FTY720 stimulates multidrug transporter- and cysteinyl leukotriene-dependent T cell chemotaxis to lymph nodes. Hong SM, Fu S, Mao X, Yopp A, Gunn MD, Randolph GJ. Bromberg JS. J CLIN INVEST 2003 111 5 627-637

The immunosuppressant FTY720 is phosphorylated by sphingosine kinase type 2. Paugh SW, Payne SG, Barbour SE, Milstien S, Spiegel EJ. FEBS LETT 2003 584 1-2 189-193

The results of this study show that sphingosine kinase-2 is much more effective than sphingosine kinase-1 in phosphorylating fingolimod in vitro.


Treatment with fingolimod at doses up to 5.0 mg/day for 28 days in stable renal transplant patients receiving CSA and prednisone was well tolerated and produced a dose-dependent reversible peripheral lymphopenia.


The pharmacokinetics of single-dose fingolimod and steady-state CsA were not altered during co-administration in subjects with psoriasis.

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578613 Genetic, biochemical, and transcriptional responses of Saccharomyces cerevisiae to the novel immunomodulator FTY720 largely mimic those of the natural sphingolipid phytosphingosine. Welsch CA, Roth LW, Goetschy JF, Movva NR J BIOL CHEM 2004 279 35 36720-36731


579440 FTY720 high dose: Lymphocyte response at single doses up to 40 mg. Slade A, Kovarik JM, Hunt TL, Schmouder R AM J TRANSPLANT 2004 4 Suppl 10 Abs 293


583902 Novartis: Forecasts trimmed, mixed R&D news. DEUTSCHE BANK AG 2005 January 21


585262 Sphingosine-dependent apoptosis: A unified concept based on multiple mechanisms operating in concert. Suzuki E, Handa K, Toledo MS, Hakomori S PROC NATL ACAD SCI USA 2004 101 41 14788-14793


• Fingolimod was phosphorylated and elicited lymphopenia in mice lacking SPHK1, indicating that this enzyme is not required for the functional activation of fingolimod.

570796 American Society of Nephrology - 37th Annual Meeting and Renal Week 2004, St Louis, MO, USA. Del Vecchio L IDDB MEETING REPORT 2004 October 29