Fingolimod Mitsubishi Pharma/Novartis

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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 (MS) [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

Originator Mitsubishi Pharma Corp

Licensee Novartis AG

Status Phase III Clinical

Indications Autoimmune disease, Cancer, Inflammatory bowel disease, Insulin-dependent diabetes, Multiple sclerosis, Myocarditis, Transplant rejection

Actions Apoptosis inducer, Anticancer, Immunosuppressant, Lymphocyte trafficking modulator, Sphingosine kinase substrate, Sphingosine-1-phosphate receptor agonist

Technology Oral formulation

Synonym FTY-720
Registry Nos: 162359-56-0, 162359-55-9
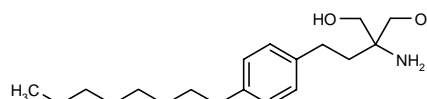
combination with CsA [558910], [558912]. The preclinical and clinical studies described below were all carried out using oral delivery, unless otherwise specified.

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],

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

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

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, non-toxic 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 immediatedly (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 [559575]. Importantly, fingolimod proved efficacious at suppressing several experimentally induced autoimmune diseases in mice or rats, including myocarditis [371443], experimental autoimmune uveoretinitis (EAU) [371444], thyroiditis

[477832], 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 thymectomy 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 IL-2 and interferon

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

[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], [559502], [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 [558965], [559503]. 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 [558965]. 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 vivo*. 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 (S1P) receptor family, namely S1P1, S1P3, S1P4 and S1P5 [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 [559505], [559507]. S1P1 receptors, which are expressed on lymphocytes and endothelial cells [559508], appear to be the most important S1P receptors with regard to the immunosuppressive action of fingolimod. This was first suggested by structure-activity analyses of semi-selective S1P receptor agonists [559509], [559510]. Moreover, a potent S1P1-selective agonist, structurally unrelated to S1P and fingolimod-P, induced peripheral lymphopenia by preventing the entry of lymphocytes into lymph, in a manner similar to fingolimod [559511]. A role for S1P1 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, S1P1-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 S1P1 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 S1P1 through internalization and rapid degradation, thereby inducing an S1P1-negative phenotype [559513], [559520]. This implied that fingolimod-P behaves as a partial S1P1 agonist in lymphocytes, rather than a full agonist, as

of functional S1P1 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 S1P1 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 relocalization 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 [558969], bladder cancer [558970] 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], [558970]. 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

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 C_{max} of 2.16 ng/ml, an $AUC_{(0-48 h)}$ of 77.9 ng.h/ml and a $t_{1/2}$ 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 $t_{1/2}$ of ~ 31 h, a Cl of ~ 0.53 l/h/kg and a bioavailability of ~ 38% [448400]. This long terminal $t_{1/2}$ most likely reflected extensive tissue distribution and binding of the compound, particularly as the steady state Vd of the drug was also notably large (Vd_{ss} = 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 T_{max} of 12 to 24 h and an elimination $t_{1/2}$ ranging from 89 to 157 h, which was dose-independent. The C_{max} (0.16 to 2.8 ng/ml) and AUC (28 to 434 ng.h/ml) were proportional to the dose

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