CASE HISTORY

Fingolimod (FTY720): discovery and development of an oral drug to treat multiple sclerosis

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Abstract | The discovery of fingolimod (FTY720/Gilenya; Novartis), an orally active immunomodulatory drug, has opened up new approaches to the treatment of multiple sclerosis, the most common inflammatory disorder of the central nervous system. Elucidation of the effects of fingolimod — mediated by the modulation of sphingosine 1-phosphate (S1P) receptors — has indicated that its therapeutic activity could be due to regulation of the migration of selected lymphocyte subsets into the central nervous system and direct effects on neural cells, particularly astrocytes. An improved understanding of the biology of S1P receptors has also been gained. This article describes the discovery and development of fingolimod, which was approved by the US Food and Drug Administration in September 2010 as a first-line treatment for relapsing forms of multiple sclerosis, thereby becoming the first oral disease-modifying therapy to be approved for multiple sclerosis in the United States.

Demyelination
Damage of the myelin sheath of axons. A demyelinating disease is any disease of the nervous system in which the myelin sheath is damaged. This impairs the conduction of signals in the affected nerves.

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Multiple sclerosis (MS) is a chronic autoimmune disorder of the central nervous system (CNS) that is characterized by inflammation leading to astrogliosis, demyelination, and loss of oligodendrocytes and neurons[1]. MS is the leading cause of neurological disability in young and middle-aged adults, affecting an estimated 2.5 million individuals worldwide[2]. The prevalence is greatest in Caucasians, with high prevalence rates reported in Europe, Canada, USA, Australia, New Zealand and northern Asia[3,4].

Most patients are diagnosed between the ages of 20 and 40 years (in a 2:1 female to male ratio)[1]. At diagnosis, ~85% of patients have relapsing–remitting MS (RRMS), which is characterized by recurrent acute exacerbations (relapses) of neurological dysfunction, followed by recovery. A substantial proportion (42–57%) of relapses may result in incomplete recovery of function and lead to permanent disability and impairment[5]. Within 6–10 years of disease onset, 30–40% of patients with RRMS have progressed to secondary progressive MS[6], in which a less inflammatory and more neurodegenerative course of disease seems to take precedence. Secondary progressive MS presents with steady progression in disability, with or without superimposed relapses. A summary of the pathophysiology of MS is given in FIG. 1.

Treatment strategies for MS usually involve the management of symptoms and the use of disease-modifying drugs to reduce the frequency of relapses and to slow the progression of disability. Established first-line therapies — interferon-β (IFN-β) products and glatiramer acetate (Copaxone; Teva) — provide ~30–35% reduction in the relapse rate compared with placebo over 2 years[7–10] (TABLE 1). IFN-β1a has also been shown to reduce the progression of disability in patients with RRMS[11]. These agents are administered by injections (with dosing schedules ranging from daily subcutaneous injections to weekly intramuscular injections), and may affect the immune system on several levels. More frequent side effects include influenza-like symptoms and injection-site reactions, which can affect tolerability and compliance[12]. Less commonly reported adverse events for IFN-β therapies include liver dysfunction and cytopaenias[13].

A more recently approved therapy, natalizumab (Tysabri; Elan/Biogen-Idec), is a humanized monoclonal antibody specific for the α4 subunit of the integrin α4β1 (also known as very late antigen 4) on lymphocytes[14,15]. It is administered through intravenous infusions every 4 weeks and seems to offer enhanced efficacy compared with other approved products[15]. However, natalizumab has been associated with hypersensitivity reactions and

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with progressive multifocal leukoencephalopathy, a rare but seriously disabling or fatal infectious demyelinating disease of the brain[14]. Another product, the cytostatic agent mitoxantrone (for which the cellular target has not been identified), is approved for use in severe forms of relapsing MS. However, cumulative dose-related cardiac toxicity and a risk of secondary leukaemia limit the total amount that can be administered[16]. Because of their safety profiles, natalizumab and mitoxantrone are currently used only as second- and third-line treatments.

Drugs under development for MS include the monoclonal antibodies rituximab, ocrelizumab and ofatumumab, which target CD20 to deplete B cells, as well as alemtuzumab (Campath-1H), which targets CD52 to deplete T and B cells and some monocyte-derived dendritic cells[17]. Also in development are small molecules, including the oral agents cladribine (a cytotoxic adenosine deaminase-resistant purine nucleoside), fumarate (an activator of the nuclear factor E2-related factor 2 transcriptional pathway), laquinimod (the cellular target of which has not been identified), and teriflunomide (a cytostatic inhibitor of dihydroorotate dehydrogenase, which catalyses the rate-limiting step in the *de novo* synthesis of pyrimidines). All these agents target lymphocytes as well as other cells with the aim of inhibiting the immune-system-mediated attack on the CNS[18].

Given the limitations of currently available therapies, the development of oral MS treatments that might offer more effective and more convenient treatment has been the focus of considerable drug discovery and development efforts in recent years. Here, we describe the research that led to the approval of the novel oral sphingosine 1-phosphate (S1P) receptor modulator fingolimod (FTY720/Gilenya; Novartis) by the US Food and Drug Administration as the first oral, first-line treatment for relapsing MS, discussing the mechanistic aspects of S1P receptors in MS and highlighting key results from the Phase III studies.

Discovery and characterization of fingolimod

Initial investigations. The structure of fingolimod was first described in 1995–1996, following a chemical derivatization programme based on the fungal metabolite myriocin (also known as ISP-1)[19,20] (FIG. 2). Early studies in animals showed that fingolimod synergized with calcineurin inhibitors, which inhibit the proliferation of T cells and prolong organ graft survival[21–24]. Compared with myriocin, fingolimod had no activity against serine palmitoyltransferase[25,26] and did not inhibit the activation and proliferation of T and B cells or the production of cytokines and antibodies[27,28]. In a clinically relevant model of bone marrow transplantation, fingolimod prevented the development of graft-versus-host disease, whereas in the same animals, the drug had no effect on the donor T cell-dependent graft-versus-leukaemia reaction[29]. Together, these data indicated that fingolimod might be acting differently to classical immunosuppressants (such as cyclosporine, tacrolimus, corticosteroids, methotrexate, mitoxantrone or lymphocyte-depleting antibodies) — an intriguing observation that prompted extensive follow up.

In animal models and in humans, fingolimod reduced peripheral blood lymphocyte counts, affecting $CD4^+$ T cells, $CD8^+$ T cells and B cells[22,30,31], and it was speculated that the drug might accelerate the homing of lymphocytes into lymph nodes[22,24,32]. *In vitro* studies initially suggested that fingolimod may promote T cell apoptosis[33] or inhibit cytosolic phospholipase A2 (REF. 34) and S1P lyase[35]; however, these effects occurred at drug concentrations more than 100-fold in excess of the low nanomolar exposures required for *in vivo* activity[23,36]. These hypotheses had to be discarded when it was shown that fingolimod's mode of action is linked to G protein-coupled receptors (GPCRs) for S1P[36,37] and altered lymphocyte trafficking[38,39].

Fingolimod targets S1P receptors. Early studies had raised the possibility that fingolimod might interfere directly with the trafficking of T cells rather than with their activation[28,40]. Furthermore, GPCRs were involved, as pretreatment of lymphocytes with *Pertussis* toxin (PTX) to block GPCR-Gαi signalling prevented the retention of T cells in lymph nodes by fingolimod[28,41]. The close structural homology of fingolimod with sphingosine (FIG. 2), a metabolite of the cell-membrane constituent sphingomyelin, prompted investigation of whether fingolimod affects intracellular sphingolipid metabolism. This led to the discovery that, like sphingosine, fingolimod is a substrate of sphingosine kinases (SPHKs) and that the fingolimod phosphate (fingolimod-P) thus generated (FIG. 2) acted at a new class of GPCRs, which were termed S1P receptors[36,37].

Bioassays for receptor binding and function revealed that fingolimod-P (but not parent fingolimod) functions as an agonist at $S1P_1$, $S1P_4$ and $S1P_5$ receptors (EC_{50} values of ~0.3–0.6 nM) and at 10-fold higher concentrations at $S1P_3$ receptors (EC_{50} values of ~3 nM) *in vitro*, but it had no activity at $S1P_2$ receptors[36,37]. Analysis of chiral analogues of fingolimod showed that fingolimod-P was the biologically active form *in vivo*[36] and that fingolimod was primarily phosphorylated by SPHK2 (REF. 42) to produce (*S*)-fingolimod-P, whereas the (*R*)-enantiomer was not found *in vivo*[43] (FIG. 2).

The key structural features of fingolimod are first, the aminodiol polar head group, which is phosphorylated by SPHK2; second, a 1,4 disubstituted phenyl ring, which acts as a rigid linker group; and, third, the lipophilic tail, which is important for interacting with

Oligodendrocytes
Brain cells protecting the axons (the long projection of nerve cells) in the central nervous system (the brain and spinal cord) of higher vertebrates. A single oligodendrocyte can extend its processes to more than 50 axons, wrapping around ~1 mm of myelin sheath around each axon.

Calcineurin inhibitors
Inhibitors (including cyclosporine and tacrolimus) that bind to the cytosolic protein cyclophilin of immunocompetent lymphocytes, especially T cells, to inhibit the phosphatase calcineurin and, thus, interleukin-2 (IL-2) production and IL-2-dependent proliferation.

Lymph nodes
Small, spherical organs of the immune system that are distributed widely throughout the body and linked by lymphatic vessels. Lymph nodes are garrisons of T, B and other immune cells and act as filters for foreign particles and antigens. These foreign antigens are then presented to T cells by professional antigen-presenting cells (called dendritic cells), and the activated T cells recirculate to blood and initiate protective immunity against infection.

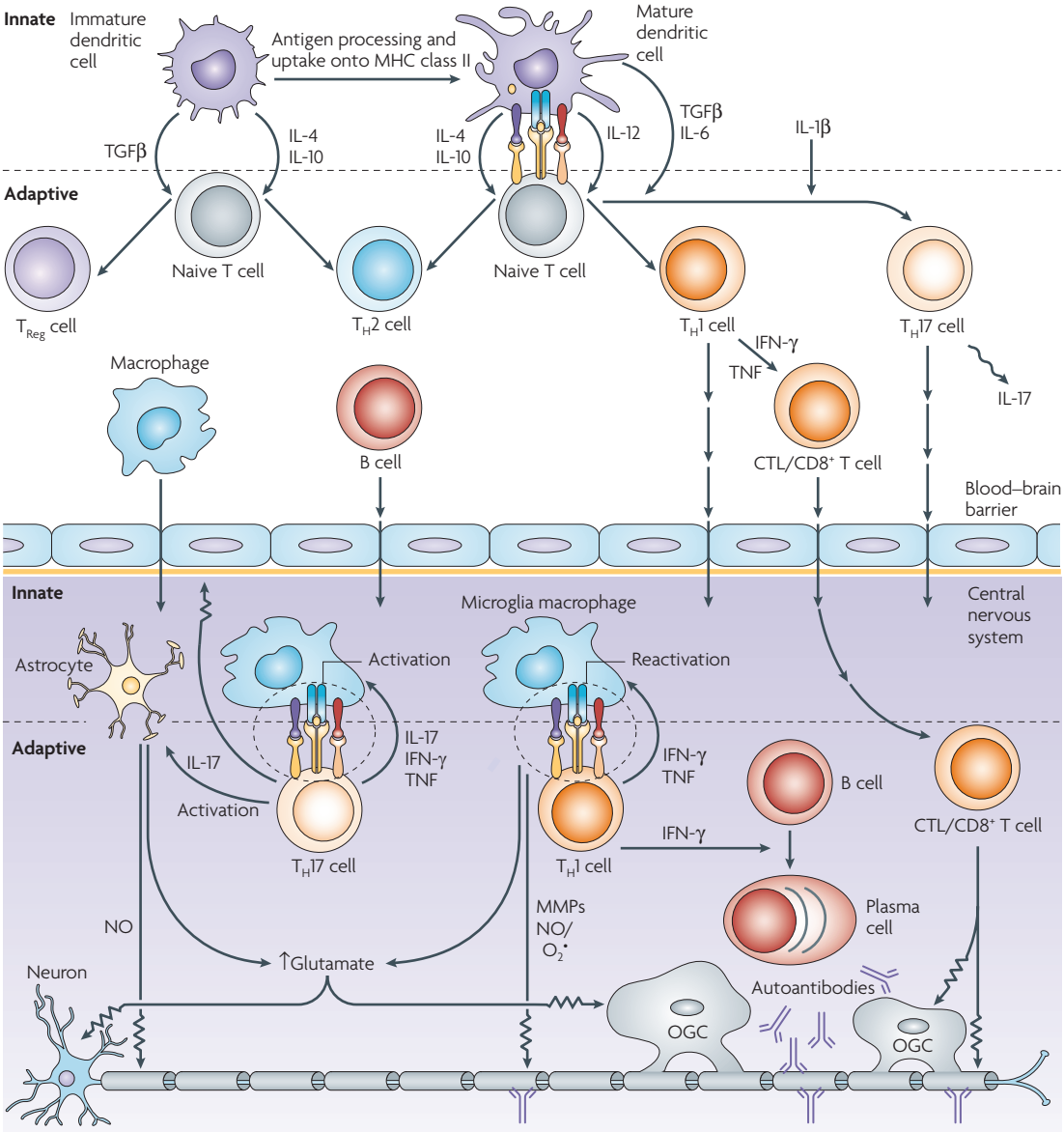


Figure 1 | **A simplified view of the pathophysiology of multiple sclerosis and therapeutic targets.** Immature dendritic cells are central players in innate immune responses and are involved in the maintenance of peripheral tolerance, presumably by promoting regulatory T cell responses. Abnormally activated (mature) antigen-presenting dendritic cells can be found in patients with multiple sclerosis (MS), and effective regulation and immune tolerance is partially lost[1–6,151]. This favours the clonal expansion of self-cross-reactive T cells in lymph nodes. The nature of the antigen, the co-stimulatory signals and the cytokine environment in the lymph nodes directs the differentiation of cells towards $CD4^+$ T helper 1 (T_H1), T_H2, T_H17 or regulatory T (T_{Reg}) cells and $CD8^+$ cytotoxic T lymphocytes (CTLs). Primed autoreactive cells recirculate to blood and, after reactivation by phagocytes at leptomeningeal vessels[76], penetrate the blood–brain barrier to invade the central nervous system (CNS), where they are reactivated, clonally expanded and terminally differentiated by self antigen presented on dendritic cells[74,78]. The presence of autoreactive T_H1 and T_H17 cells, CTLs and plasma B cells in the CNS, together with abnormally activated astrocytes and microglia, lead to an increased production of inflammatory cytokines, reactive oxygen species, excitotoxicity, autoantibody production and direct cytotoxicity, which are all involved in demyelination and axonal, neuronal and blood–brain barrier damage that is present in patients with MS. Fingolimod targets sphingosine 1-phosphate (S1P) receptors, of which the mRNA is widely expressed in immune, vascular and neural cells. The expression levels of the five S1P receptor subtypes in cell membranes versus cytoplasmic compartments may vary considerably depending on the activation and differentiation stage of the cells and the tissue levels of the ligand S1P[48]. Relevant targets of fingolimod may include cell membrane-expressed $S1P_1$ on T and B cells[38,55], $S1P_1$ and $S1P_3$ on astrocytes[114,115] and, perhaps, $S1P_1$ on neurons[102], $S1P_1$ and $S1P_5$ on oligodendrocytes (OGCs)[101,109], and $S1P_1$ on endothelial cells of the blood–brain barrier[48]. S1P receptors modulate the actin cytoskeleton of cells, thereby affecting pseudopodia formation and migration of lymphocytes, process outgrowth and retraction in neural cells, junctional communication between those cells, endothelial cell migration, and angiogenesis and endothelial permeability barriers[48]. IFN, interferon; IL, interleukin; MHC class II, major histocompatibility complex class II; MMPs, matrix metalloproteinases; NO, nitric oxide; TGFβ, transforming growth factor-β; TNF, tumour necrosis factor. Figure adapted from *Nature Reviews Drug Discovery* REF. 151

Table 1 | **Selected disease-modifying drugs in relapsing multiple sclerosis**

Agent (route and frequency of administration)	Trade name (company)	Impact on disease activity relative to placebo			
		Relapse rate	MRI active lesions*	Confirmed EDSS progression‡	Change in T2 lesion volume
Interferon-β1b (subcutaneous injection every other day)[8,152]	Betaferon (Schering); Betaseron (Bayer); Extavia (Novartis)	Annualized: −34% ($p = 0.0001$)	−83% ($p < 0.009$)	−29% (not significant)	−17.3% ($p = 0.001$)
Interferon-β1a (intramuscular injection once a week)[9]	Avonex (Biogen Idec)	Annualized: −18%/−32%§ ($p \leq 0.04$)	−36% ($p = 0.05$)	−37% ($p = 0.02$)	−4% (not significant)
Interferon-β1a (subcutaneous injection three times a week)[7]	Rebif (EMD Serono/ Pfizer)	Mean: −32% ($p < 0.005$)	−78% ($p < 0.0001$)	−30% ($p < 0.05$)	−14.7% ($p < 0.0001$)
Glatiramer acetate (subcutaneous injection, daily)[10,153]	Copaxone (Teva Pharmaceuticals)	Mean: −29% ($p = 0.007$)	−35% ($p < 0.001$)‖	−12% (not significant)	−8.3% ($p = 0.0011$)‖
Natalizumab (intravenous infusion every 4 weeks)[154,155]	Tysabri (Biogen Idec/Elan Pharmaceuticals)	Annualized: −68% ($p < 0.001$)	−83% ($p < 0.0001$)	−42% ($p < 0.001$)	−18% ($p < 0.001$)

EDSS, expanded disability status scale; MRI, magnetic resonance imaging.*Definitions for MRI active lesions varied slightly across studies. ‡Definitions for EDSS progression varied slightly across studies. §–18% reflects analysis of intent-to-treat population, –32% reflects 2-year completer analysis. ‖MRI not assessed in pivotal trial, separate MRI study.

the hydrophobic binding pocket of the S1P receptors[44,45]. The phosphorylation is a stereospecific process with only the (pro-*S*) hydroxymethyl group being efficiently phosphorylated[36,43]. Derivatives that lack a (pro-*S*) hydroxymethyl group are not phosphorylated and do not show immunomodulatory activity *in vivo*. Limited substitution of the central phenyl ring is permitted without loss of activity.

Daily doses of fingolimod at 0.1–0.3 mg per kg were highly effective at inhibiting the development of experimental autoimmune encephalomyelitis (EAE), an animal model of human MS[36,46], whereas 10–100-fold higher doses or combination with classical immunosuppressants were required to prolong organ graft survival in animals[19,23,24]. Phase II and Phase III clinical trials for renal transplantation ultimately showed that fingolimod, even at doses 10-fold above those currently used in MS, did not provide sufficient immunosuppressive potential to allow reduction of concomitant cyclosporine treatment or to provide a greater benefit than standard care[47]. Collectively, these data suggested that fingolimod may provide more benefit in the treatment of autoimmune and neurodegenerative diseases, thus turning the focus of clinical development from organ transplantation to MS.

Biology of S1P and S1P receptors. Many studies have examined the immunomodulatory actions of S1P, and REFS 48–52 provide comprehensive reviews of S1P biology. S1P concentrations are high in plasma but low in tissues, and excessive production of the pleiotropic mediator at inflammatory sites may participate in various pathological conditions. A detailed description of S1P receptor function is beyond the scope of this review and the reader is referred to REF. 48 for details; a brief summary is given below.

The $S1P_1$ receptor is predominantly expressed by immune cells, neural cells, endothelial cells and smooth muscle cells, and it is found with a gradient of brain > lung = spleen > heart and vasculature > kidney. Genetic deletion of the $S1P_1$ receptor in mice suggests that it has a key role in angiogenesis and neurogenesis, as well as in the regulation of immune cell trafficking, endothelial barrier function and vascular tone. The $S1P_2$ receptor also shows widespread expression, and its loss leads to a large increase in the excitability of neocortical pyramidal neurons, demonstrating a role in the development and/or mediation of neuronal excitability. Furthermore, the $S1P_2$ receptor is essential for the proper functioning of the auditory and vestibular systems, and $S1P_2$ deficiency results in deafness. The $S1P_3$ receptor is expressed in the heart, lung, spleen, kidney, intestine, diaphragm and at certain cartilaginous regions, but genetic deletion of the $S1P_3$ receptor does not result in an obvious phenotype. However, the receptor may fine-tune some cardiovascular functions, including the regulation of heart rate, but the role of the $S1P_1$ receptor relative to the $S1P_3$ receptor in heart rate regulation in humans is still debated. The $S1P_4$ receptor has a more restricted expression pattern and is detectable predominantly within immune compartments and on leukocytes, but it is also found on human airway smooth muscle cells. $S1P_4$-deficient mice have not been described, and its functional role remains elusive. The $S1P_5$ receptor is identical to the rat nerve growth factor-regulated GPCR neuregulin 1, and is predominantly expressed in oligodendrocytes in the white matter tracts of the CNS. However, no deficits in myelination are observed in $S1P_5$-deficient mice and, thus, its precise role in oligodendrocyte function remains to be determined. Below, we outline how fingolimod acts on $S1P_1$ receptors to exert its effects relevant to MS.

Experimental autoimmune encephalomyelitis
(EAE). An animal model of inflammatory demyelinating diseases of the central nervous system, including multiple sclerosis and acute disseminated encephalomyelitis. The most commonly used antigens in rodents are spinal cord homogenate, purified myelin, myelin protein such as myelin basic protein, proteolipid protein and myelin oligodendrocyte glycoprotein, or peptides of these proteins, all resulting in distinct models with different disease characteristics regarding both immunology and pathology.

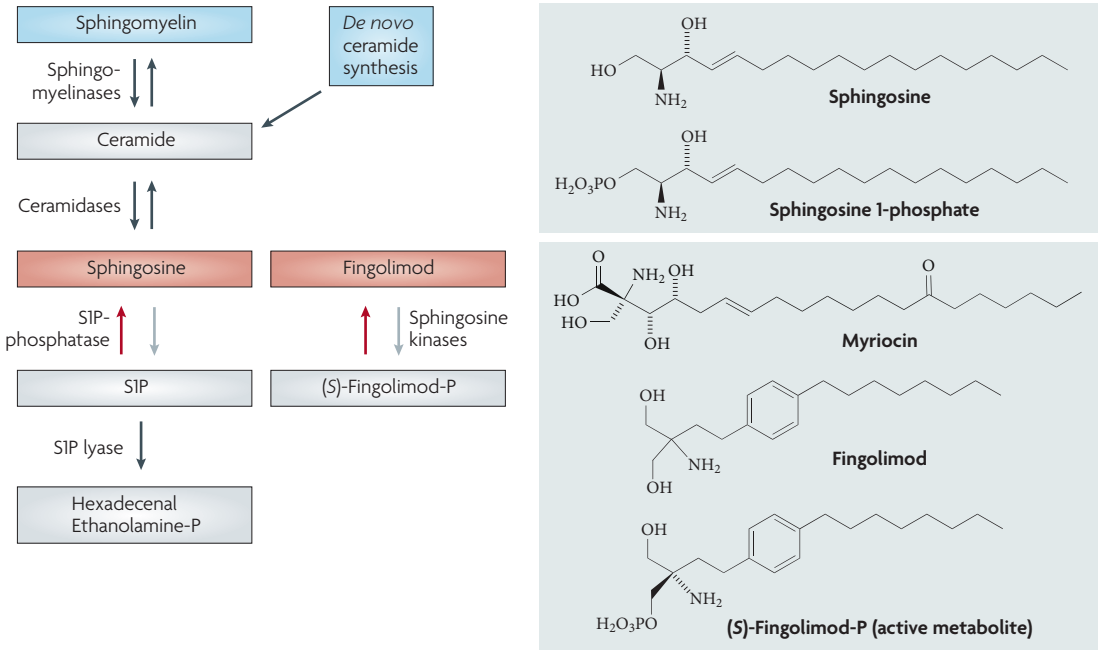


Figure 2 | **Fingolimod and sphingolipid metabolism.** Fingolimod and fingolimod-phosphate (fingolimod-P) are structural analogues of sphingosine and sphingosine 1-phosphate (S1P), respectively. S1P is generated by the intracellular ceramide pathway, and ceramide is formed through *de novo* biosynthesis or through degradation of the cell membrane constituent sphingomyelin. Ceramide is *N*-deacetylated to yield sphingosine, and both sphingosine and fingolimod are phosphorylated by sphingosine kinases to yield S1P and (*S*)-fingolimod-P, respectively, whereas (*R*)-fingolimod-P is not found *in vivo*. The (*S*)-fingolimod-P is the biologically active principle of the drug in animal models of autoimmune disease[36,43].

Fingolimod inhibits the function of S1P$_1$ receptors to favour CC-chemokine receptor 7-mediated retention of lymphocytes in lymph nodes. Studies in transfected cell lines indicated that fingolimod caused S1P receptors to internalize from cell membranes[53]. This raised the possibility that the drug might act as a 'functional antagonist' at these receptors to inhibit S1P-mediated migration. That is, although fingolimod-P acts initially as an agonist at S1P receptors, its effects are inhibitory in the longer term on S1P receptor function. The reduction of blood lymphocyte counts associated with fingolimod treatment could be mimicked by conditional deletion of S1P$_1$ receptors from haematopoietic cells or T cells[38,39,54], and treatment of wild-type mice with fingolimod caused internalization of cell-membrane-expressed S1P$_1$ receptors in lymph node T cells[55]. The consequent decrease of S1P$_1$ receptor activity correlated with accumulation of lymphocytes at the lymphatic endothelial barriers in the lymph nodes[37,56], suggesting that the drug may inhibit egress of lymphocytes from these organs into cortical sinuses and efferent lymph.

This hypothesis was verified using conditional S1P$_1$-deficient and SPHK-deficient mice, in which egress from lymph nodes was shown to require lymphocytic S1P$_1$ receptors[38,39,55] and the local production of S1P by lymphatic endothelial cells expressing the lymphatic vessel endothelial hyaluronan receptor 1 (LYVE1)[56,57]. Imaging studies showed that S1P$_1$ receptors are dispensable for the initial attachment and rolling of the T cells on lymphatic endothelium, but they are key for T cell migration across the endothelial barriers[58]. Specifically, the S1P$_1$ signal was needed to overcome GPCR-Gαi-mediated retention signals involving the lymph node-homing receptor CC-chemokine receptor 7 (CCR7)[55]. Early studies showed that treatment of lymphocytes with PTX prevented the retention of T cells by fingolimod in lymph nodes[28]. It was then found that S1P$_1$-deficient T cells and CCR7-overexpressing T cells were retained in lymph nodes for longer, whereas CCR7-deficient T cells left lymph nodes more rapidly than wild-type cells[55]; this suggested antagonistic roles for the S1P$_1$ receptor and CCR7. PTX treatment restored egress competence even to S1P$_1$-deficient lymphocytes[55], suggesting that they could egress independently of S1P$_1$ receptor signalling. A similar regulation may occur in B cells, in which the loss of CCR7 in plasmablasts coincided with accelerated egress from lymph nodes[59].

A recent study used a knock-in mouse (*S1p1r*$^{S5A/S5A}$) in which the carboxy-terminal serine-rich S1P$_1$ motif, which is important for S1P$_1$ receptor internalization but dispensable for S1P$_1$ receptor signalling, is mutated. T cells expressing the mutant S1P$_1$ receptor were not retained in lymph nodes by fingolimod, providing further evidence that internalization and functional antagonism of lymphocytic S1P$_1$ receptors are required to inhibit lymphocyte egress by the drug[60]. So, the inability of some new competitive S1P$_1$ receptor inhibitors to inhibit lymphocyte egress[61,62] may relate to suboptimal pharmacokinetic properties of the compounds[57].

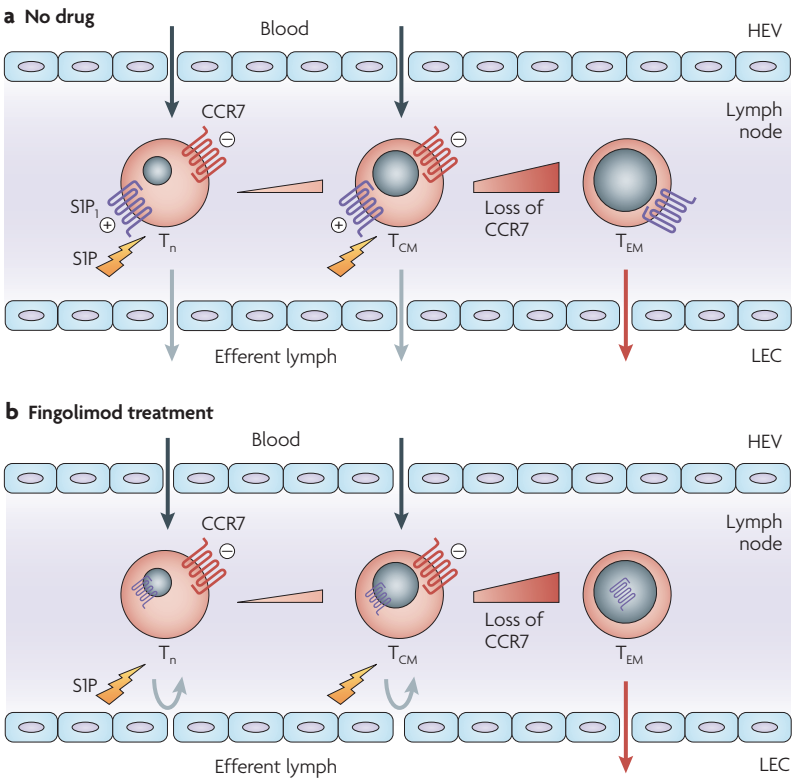
Box 1 | **Proposed model of T cell retention by fingolimod in lymph nodes**

According to the signal strength model of T cell differentiation (see part **a** of the figure), the generation of protective immune responses requires high intensity antigenic stimulation of naive T cells (T_n) in lymph nodes; this generates CC-chemokine receptor 7 ($CCR7^-$) effector memory T cells (T_{EM}), which recirculate to infected tissues and display effector function[63]. By contrast, antigenic stimuli of weaker intensity predominantly generate $CCR7^+$ central memory T cells (T_{CM}), which home to T cell areas of secondary lymphoid organs, and have little or no effector function but await antigenic restimulation to proliferate and differentiate into T_{EM} cells (the thickness of the wedges between the cell subsets indicates the duration and intensity of the antigenic stimulation and the resulting T cell proliferation).

T_n and T_{CM} cells recirculate between blood and secondary lymphoid organs and home to lymph nodes through high endothelial venules (HEVs) in a CCR7-dependent manner (black arrows). To egress from lymph nodes, they require activation of the sphingosine 1-phosphate receptor 1 ($S1P_1$) by lymphatic endothelial cell (LEC)-derived S1P, and this overrides CCR7-mediated retention in lymph nodes[55] (light grey arrows). Fully differentiated T_{EM} cells have irreversibly lost CCR7 (REF. 63) and related retention signals and, as a consequence, are less dependent on $S1P_1$ for egress[55] (red arrows, see also BOX 2). Accordingly, treatment with *Pertussis* toxin, which blocks CCR7–Gαi-signalling, restored egress competence to lymphocytes[28,55] and to $S1P_1$-deficient T cells[55]. These data suggest a model whereby T cells that have divided several times switch to a state favouring egress over retention[31,55,73].

Fingolimod, after phosphorylation, binds to $S1P_1$ receptors[36,37] on T cells and causes aberrant internalization of the receptor[55] (see part **b** of the figure). This reduces the responsiveness of T cells to the egress signal S1P and favours CCR7-mediated retention in lymph nodes[55]. Fingolimod was shown to predominantly retain $CD62L^{hi}CD44^{low}$ T_n cells and $CD62L^{hi}CD44^{hi}$ T_{CM} cells in lymph nodes of mice, causing a relative increase in $CD62L^{low}CD44^{hi}$IFN-γ (interferon-γ)^{hi} T_{EM} cells in blood[72] (not shown). A similar subset selectivity was observed in fingolimod-treated transplant patients[39] and patients with multiple sclerosis: fingolimod retained $CCR7^+CD45RA^+$ T_n cells and $CCR7^+CD45RA^-$ T_{CM} cells in lymph nodes, but spared $CCR7^-CD45RA^-$ and $CCR7^-CD45RA^+$ T_{EM} cell subsets[31,73]. In humans, a preferential retention of $CD4^+$ compared to $CD8^+$ T cells occurred[31], and this related to the higher content of $CCR7^+$ naive cells in the $CD4^+$ compartment[31,63].

These data are consistent with a model whereby down-modulation of lymphocytic $S1P_1$ receptors by fingolimod favours CCR7-mediated retention of T_n and T_{CM} cells, but not T_{EM} cells, in lymph nodes[31,55].



Collectively, the above described findings support the hypothesis that $S1P_1$ signalling in lymphocytes overrides CCR7-mediated retention to promote their egress from lymph nodes, and that functional antagonism of $S1P_1$ by fingolimod favours lymphocyte retention in lymph nodes. Egress of terminally differentiated $CCR7^-$ effector T cells and effector memory T cells[63] may be less dependent on $S1P_1$ receptor signalling and, as a consequence, be less affected by fingolimod.

Fingolimod selectively retains CCR7$^+$ central memory T cells: the relevance to MS. Immunological memory is the hallmark of the adaptive immune system and results from the clonal expansion and differentiation of antigen-specific lymphocytes that ultimately persist for a lifetime[63,64]. In adult humans, more than 60% of blood T cells express antigen-experienced effector and memory phenotypes[63,64]. According to the 'signal-strength model' of T cell activation, the strength of the signals delivered by the T cell receptor and by co-stimulatory receptors drives T cells through hierarchical thresholds of differentiation[63,64]. Formation of protective memory in humans requires a high-intensity antigenic stimulation that promotes the generation of $CCR7^-CD45RA^-$ effector memory T (T_{EM}) cells that migrate readily to inflamed peripheral tissues and display immediate effector function. By contrast, antigenic stimuli of low intensity and/or short duration preferentially generate $CCR7^+CD45RA^-$ central memory T (T_{CM}) cells that home to T cell areas of secondary lymphoid organs, and have little or no effector function but await antigenic restimulation to proliferate and differentiate into T_{EM} cells[63–71]. The nature of the antigen, the co-stimulatory signals and the cytokine environment present during the activation of T cells in lymph nodes directs the development of functional T helper (T_H), regulatory and cytolytic phenotypes within T_{CM} and T_{EM} cells, with full polarization achieved at the terminal T_{EM} state[63].

Phenotypic and functional analysis of blood lymphocytes from fingolimod-treated mice[72], from patients following renal transplant[39] and from patients with MS[31,73] provided evidence that the drug preferentially reduced $CCR7^+$ naive T cells and T_{CM} cells in blood, but it spared $CCR7^-$ T_{EM} cells (BOX 1; FIG. 3), which is consistent with $S1P_1$- and CCR7-dependent regulation of egress from lymph nodes. It is therefore likely that fingolimod may retain all T_{CM} cell subsets in lymph nodes, irrespective of T_H1, T_H2, T_H17 cell or cytolytic commitment, but may spare all these subsets at the T_{EM} stage. According to their T_{EM} phenotype, blood T cells from fingolimod-treated patients secreted less interleukin-2 (IL-2) but more IFN-γ on a per cell basis following *ex vivo* restimulation by superantigens[31].

The retention of T_{CM} cells might be sufficient for the therapeutic effect of fingolimod, because in MS more than 90% of T cells that accumulate in the cerebrospinal fluid (CSF) express a T_{CM} phenotype, and the T cell infiltrate does not contain naive T cells and is relatively depleted of T_{EM} cells[74]. The clonal expansion of disease-relevant T_{CM} cells may occur first in CNS-draining lymph nodes following low-intensity cross-reaction on self antigen[75]. These cells could then recirculate and could be restimulated at

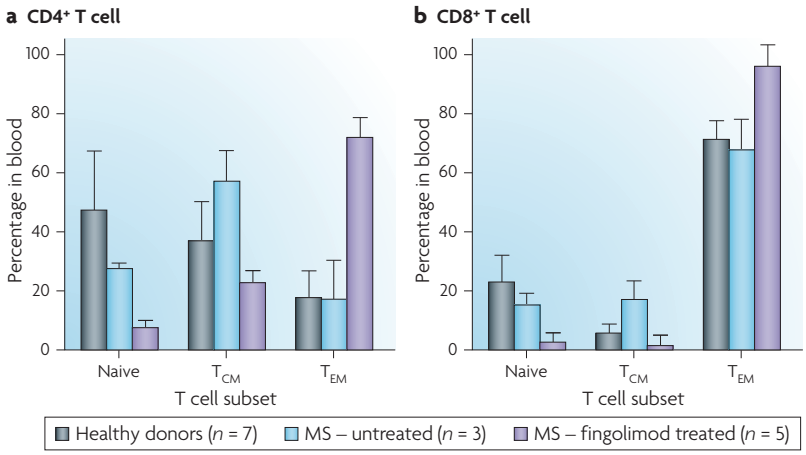


Figure 3 | **T cell subsets in blood of fingolimod-treated patients with multiple sclerosis.** Human peripheral blood $CD4^+$ and $CD8^+$ T cells were characterized for expression of naive and memory phenotypes using multicolour flow cytometry. Naive T cells (CC-chemokine receptor 7 ($CCR7^+CD45RA^+$); central memory T cells (T_{CM}) ($CCR7^+CD45RA^-$); effector memory T cells (T_{EM}) (containing $CCR7^-CD45RA^-$ 'T_{EM}' and $CCR7^-CD45RA^+$ 'T_{EMRA}' effector memory subsets). Data are presented as means ± SD[31,73].

leptomeningeal vessels by antigen-presenting phagocytes, to invade CNS tissues[76,77], and by local dendritic cells, to proliferate and differentiate into $CCR7^-$ effectors and T_{EM} cells[74,78]. Fingolimod treatment strikingly reduced the inflammatory T cell infiltrate in the CNS and ameliorated EAE disease[79], despite a relative increase in the T_{EM} cell counts in blood[31,72], suggesting that selective retention of T_{CM} cells in lymph nodes is sufficient to control central inflammation. These data also suggest that, in MS, the number of self-reactive T cells in the circulating T_{EM} cell pool might be low and that the bulk of T_{EM} cells may be generated locally from T_{CM} cells within the CNS.

T_{CM} cells may contain considerable numbers of pre-committed pro-inflammatory T_H17 cells, because fingolimod caused a substantial reduction of blood lymphocytes carrying the T_H17 marker IL-17 and the transcription factor retinoic acid-related orphan receptor C transcript variant 2 (RORC2), the human orthologue of rodent RORγt[73,80]. Fingolimod prevented the accumulation of T_H17 cells in the sciatic nerves of animals with experimental autoimmune neuritis[81,82], suggesting retention of T_H17 cells in lymph nodes.

Collectively, these data suggest that fingolimod may interrupt MS pathology by selectively retaining T_{CM} cells, including pre-committed T_H17 cells, in lymph nodes. This prevents the local generation of terminally differentiated effector T cells and T_{EM} cells in the CNS, and it abrogates T cell-mediated astrogliosis and disruption of the blood–brain barrier (BBB)[83], killing of neural cells[84,85] and recruitment of additional T cells[84] and macrophages[86] to the CNS. At the same time, fingolimod might largely spare peripheral T_{EM} cells involved in defence against infection (see BOX 2 for a discussion of the properties of fingolimod and relevance to risk of infection).

Conditional deletion of $S1P_1$ receptors from haematopoietic cells in mice also reduced the egress of mature thymocytes from the thymus[38,54,87]. However, long-term (16 week) treatment of mice with clinically relevant doses of fingolimod did not prevent (but only slowed) egress of mature thymocytes from the thymus, allowing the development of full haematopoietic chimerism and mature T cells after bone marrow transplantation[72]. These data suggest that the therapeutic effects of fingolimod primarily relate to the retention of T and B cells in lymph nodes, rather than to reduced T cell maturation. The more notable reduction of blood T cells in immunologically naive mice (up to 98%) compared to immune-experienced humans (~70%) by fingolimod may relate to the larger proportion of naive $CCR7^+$ T cells in the experimental animals.

Fingolimod and endothelial barriers. An increased expression of vascular endothelial growth factor (VEGF) is associated with demyelinated lesions in both MS and EAE, implicating changes in vasculature as a potential component of CNS plaque formation[77]. VEGF may directly promote the formation of new (leaky) microvascular structures, thereby impairing BBB function[88]. Endothelial barriers can be regulated by S1P receptors[89,90], and it has been speculated that fingolimod may interfere with this. Fingolimod was shown to restore permeability barriers in mouse models of VEGF-[91] and lipopolysaccharide-induced[92] leakage and in EAE[93,94]. Moreover, the drug reduced excessive *de novo* angiogenesis in tumour models[95] and in the lumbar spinal cord of EAE-diseased mice[96].

So, it is possible that, as in T cells, fingolimod-P might down-modulate $S1P_1$ receptors on the membranes of migrating endothelial cells to reduce S1P-directed chemotaxis[97], a process crucial to angiogenesis[98]. At the same time, agonistic $S1P_1$ receptor signalling by fingolimod-P and/or S1P might often continue in the intact vascular endothelium, even after receptor internalization[99], and this could make endothelial barriers less permeable, particularly in inflammatory situations[91] in which $S1P_1$ receptor expression in endothelial cells is upregulated[100]. On the other hand, barrier function might be reduced if endothelial $S1P_1$ receptors are functionally antagonized by the drug[90]. More data are needed to allow a definitive conclusion regarding any direct effects of fingolimod on BBB function.

Fingolimod and S1P-dependent neuroinflammation. Administration of fingolimod to rats at doses that show therapeutic effects in EAE models (0.3 mg per kg) resulted in high picomolar levels of free fingolimod-P in the CSF[46], suggesting that the drug could also modulate S1P receptors expressed in oligodendrocytes[101], neurons[102], astrocytes[103,104] and non-neurally derived microglia[102]. *In vitro* studies with S1P and fingolimod-P suggested roles for S1P receptors in astrocyte migration[105–107] and in oligodendrocyte process extension and retraction[108]. In addition, treatment of organotypic cerebellar slices with fingolimod subsequent to lysolecithin-induced demyelination enhanced process extension by oligodendrocytes and supported remyelination[109]. The early increase of glial fibrillary acidic protein staining (a marker of astrocyte activation) in the slice cultures may relate to a transient activation of S1P receptors by

T_H17 cell
(T_H helper 17 cell). A subset of T_H cells that produce interleukin-17. They are considered developmentally distinct from T_H1 and T_H2 cells and are thought to have a key role in autoimmune diseases such as multiple sclerosis, psoriasis, autoimmune uveitis, juvenile diabetes and rheumatoid arthritis.

Astrocytes
Star-shaped glial cells in the brain and spinal cord that provide biochemical support to endothelial cells from the blood–brain barrier and that also provide nutrients to the nervous tissue.

the drug, before receptor internalization and functional antagonism and suppression of astrogliosis (see below). Together, these data suggest the possibility that fingolimod may directly affect neural cells.

Box 2 | **Sparing of CCR7$^-$ effector memory T cells by fingolimod**

Current data suggest that, in multiple sclerosis (MS), peripheral central memory T cells (T_{CM}) may contain the bulk of the autoreactive T cells, which invade the central nervous system[31,74,80]. By contrast, the frequency of MS-pathogenic T cells within the peripheral effector memory T cell (T_{EM}) pool may be low[31,74], but T_{EM} cells are pivotal in the defence against infection[63]. The figure shows phenotypic and functional associations within virus-specific $CD8^+$ T_{CM} cells and T_{EM} cells in humans, indicating the expression and fate of the co-stimulatory receptor CD28, the lymph node homing receptors CC-chemokine receptor 7 (CCR7) and CD62L (also known as L-selectin), the effector molecules perforin and interferon-γ (IFN-γ), and the growth factor interleukin-2 (IL-2) in a resting state of the T cell subsets[146]. The relative induction of virus-specific T_{CM} versus T_{EM} subsets is indicated after clearance of the influenza virus or during latent infection with hepatitis C virus (HCV), Epstein–Barr virus (EBV), human immunodeficiency virus (HIV), and cytomegalovirus (CMV).

Studies in models of lymphocytic choriomeningitis virus and vesicular stomatitis virus infection did not indicate suppressive effects of fingolimod on antigen presentation or on T cell and B cell activation, proliferation, differentiation and effector function *in vivo*[40]. Current data from humans and animals suggest that CCR7$^-$ T_{EM} cells generated in the lymph nodes in response to infection or vaccination would recirculate largely independently of sphingosine 1-phosphate receptor 1 ($S1P_1$) (BOX 1) and, thus, may not be affected by fingolimod. Indeed, a recently completed open-label, observational, prospective clinical study showed that fingolimod-treated individuals mount an influenza virus vaccine-specific humoral and cellular immune response that is comparable to drug-untreated controls[147].

In mice, incomplete retention of antigen-activated T cells by fingolimod has also been observed after primary infection with *Listeria monocytogenes* [148] and after adoptive transfer of *ex vivo* primed T cells[149]. In *L. monocytogenes*-[148] and *Mycobacterium bovis*[150]-immune mice, cellular and humoral recall immunity was not impaired by the drug, allowing clearance of the infection.

Collectively, these data might provide a rationale for why the overall incidence of infection observed in clinical trials was similar between the fingolimod-treated patients and control groups[125,127], despite a reduced blood lymphocyte count (see main text). However, long-term follow up of fingolimod-treated patients is necessary to substantiate this possibility further.

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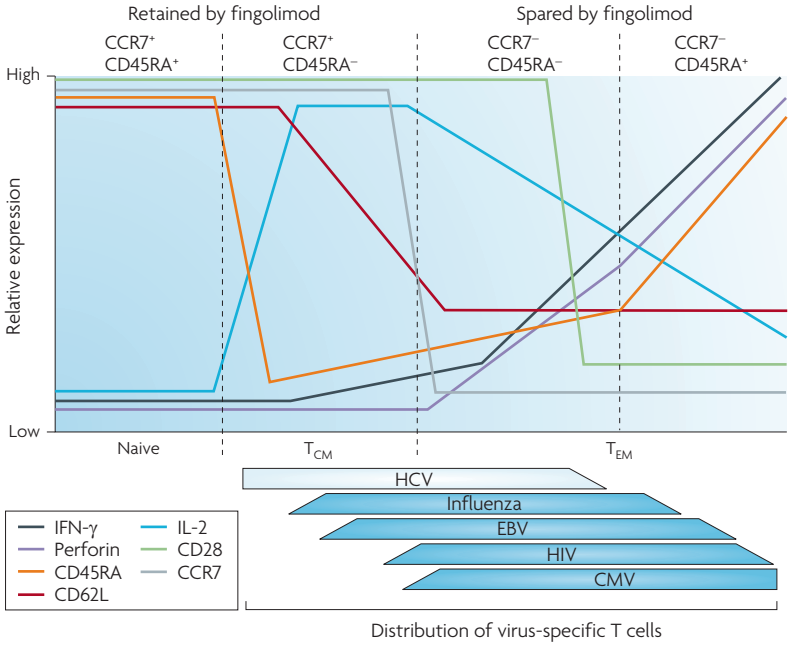


Several *in vivo* studies support a key role of S1P and $S1P_1$ receptors in the development of the nervous system. $S1P_1$ receptors are involved in the migration of neural stem cells and progenitor cells[102], and genetic deletion of either the $S1P_1$ receptor or SPHKs (to eliminate S1P) caused severe disturbance of neurogenesis and neural tube closure, resulting in increased apoptosis in the developing brain[110], which suggest cell-protective, pro-survival effects of S1P. In disease, however, excessive S1P production and cellular survival may sustain inflammation: various inflammatory cytokines including IL-1 and tumour necrosis factor activate SPHKs[111], and activation of the SPHK1–S1P pathway in microglia increased expression of pro-inflammatory cytokines and production of nitric oxide[112]. In patients with MS, increasing disability correlated with higher S1P levels in the CSF[113]. Likewise, S1P levels were increased in the spinal cord of mice after injury[102] and during EAE-related inflammation[114]; injection of S1P into the striata caused astrogliosis and central inflammation[104], perturbing gap junctional communication of astrocytes with other neural cells[115].

It is, therefore, plausible to assume that these pathogenic mechanisms are influenced beneficially by down-modulation of S1P responses. Recent data showed that IL-17 directly activates astrocytes[116] and that reactive astrocytes in active and chronic inactive MS lesions show dramatically increased expression of $S1P_1$ and $S1P_3$ receptors[117]. The $S1P_1$ receptor[55] has been shown to be internalized by fingolimod, and mice with conditional deletion of $S1P_1$ in neural cells, particularly astrocytes, showed reduced EAE disease severity and attenuated astrogliosis, demyelination and axonal damage compared with wild-type counterparts[114]. Furthermore, genetic deletion of either SPHK1 (to reduce S1P levels) or the $S1P_3$ receptor in mice reduced astrogliosis and improved motor function during the terminal stages of neuronopathic Sandhoff disease[103]. These data provide evidence to suggest that functional antagonism of S1P receptors in the CNS might reduce central inflammation in neurodegenerative disorders, including MS (BOX 3). The therapeutic effect might, at least in part, relate to a restoration of effective gap-junctional communication of astrocytes with other neural cells and endothelial cells in the BBB, as gap junction channels connect the cytoplasm of contacting cells and coordinate electric and metabolic activity[115]. Together, these processes could support repair mechanisms and remyelination and explain the therapeutic effects of fingolimod in animal models of MS.

Fingolimod in animal models of MS. Protective effects of fingolimod in animal models of MS were first described in 2002; in a Wistar rat model, administration of the drug at 0.3 mg per kg per day prevented the onset of EAE[36]. Protection required the conversion of fingolimod to its phosphate metabolite[36], which acted at S1P receptors on lymphocytes[36,38]. The protective effect correlated with reduced blood lymphocyte counts[36], which is consistent with the retention of T cells in lymph nodes[38,56,115].

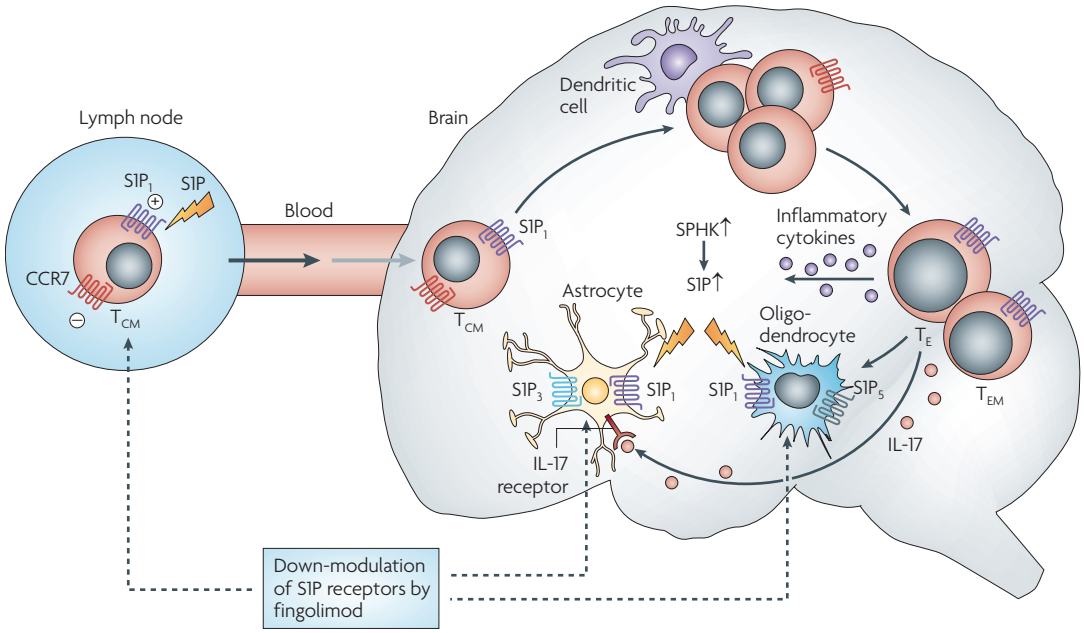
Follow-up studies confirmed prophylactic and therapeutic activities of fingolimod in other rat and mouse EAE models[79,118,119]. Protection was associated with a reduction

Box 3 | **Proposed model of the mode of action of fingolimod in multiple sclerosis**

In multiple sclerosis (MS), pathogenic central memory T (T_{CM}) cell clones committed to T helper 1 (T_H1) and T_H17 lineages are expanded following low-intensity cross-reaction on self antigens in lymph nodes that drain the antigen from the central nervous system (CNS). T_{CM} cells egress from lymph nodes and recirculate to blood in a sphingosine 1-phosphate receptor 1 ($S1P_1$)-dependent manner[38], and they invade the CNS following restimulation by self antigen presented on leptomeningeal phagocytes[76]. In the CNS, T_{CM} cells are reactivated by self antigen presented on microglia and/or dendritic cells and this causes proliferation and local differentiation of effector T cells (T_E) and, perhaps, effector memory T cells (T_{EM})[74]. T_E cells activate astrocytes through interleukin-17 (IL-17)[116], directly kill neural cells[84] and secrete inflammatory cytokines that activate sphingosine kinases (SPHKs) in many cell types[111]. This increases the production of S1P, which may signal S1P receptors on many cell types to enhance neuroinflammation and gliosis[104,111,112,115].

Down-modulation of lymphocytic $S1P_1$ receptors by fingolimod leads to retention of self-reactive T_{CM} cells in the lymph nodes and prevents their invasion into the CNS and their local clonal expansion and differentiation into T_E and T_{EM} cells[38,55,79]. It seems likely that fingolimod may retain all T_{CM} cell subsets in the lymph nodes, irrespective of T_H1, T_H2, T_H17 or cytolytic commitment, but it may spare all these subsets at the T_{EM} stage; this would not affect the therapeutic activity of fingolimod as terminal differentiation of T_{CM} cells into MS-pathogenic T_{EM} cells occurs at CNS sites rather than at peripheral lymphoid tissues[74,76,78].

In the CNS, down-modulation of S1P receptors on neural cells may reduce hyperactivation, particularly of astrocytes, by excess S1P[113,114,115].



in the numbers of T cells and macrophages in spinal cords, and this correlated with a reduction of mRNA encoding the inflammatory markers IFN-γ, IL-2, IL-6 and granulocyte–macrophage colony-stimulating factor[79,118,119]. Treatment with fingolimod started at the peak of the first relapse was more efficacious than IFN-β therapy[119], an observation that was also seen later in a clinical trial (see below).

In a dark agouti rat model, late-stage rescue treatment started 4 weeks after disease onset reversed paralysis in established EAE and normalized the electrophysiological responses, including visual and somatosensory evoked potentials, and this correlated with decreased demyelination in the brain and spinal cord[93,94]. Furthermore, BBB breakdown was reversed, as measured by immunoglobulin precipitation[93], and this was associated with a reduction in the levels of mRNA encoding the matrix metalloproteinase 9 gene (*Mmp9*) and an increase in the mRNA levels of its counter-regulator, tissue inhibitor of metalloproteinase 1 (*Timp1*), resulting in a proteolytic balance that favours the preservation of BBB integrity[93]. Collectively, these data suggest that fingolimod might also have therapeutic value beyond relapsing MS in the more chronic forms of the disease, and analysis in the respective models is ongoing.

Maximal efficacy of the drug was achieved with a dose of 0.3 mg per kg, resulting in fingolimod levels of 5.4 ng per ml in plasma and 0.07 ng per ml in CSF, and fingolimod-P levels of 7.4 ng per ml in plasma and 0.23 ng per ml in the CSF[46]. These data provided guidance towards the dose selection in humans, in which a similar blood exposure was achieved with a dosing of 1.25–0.5 mg fingolimod per patient per day.

Clinical studies of fingolimod

Clinical pharmacology. The clinical pharmacology of fingolimod has been investigated in more than 1,000 subjects in 30 studies. Fingolimod has a slow absorption period, with the time of maximal concentration at 12–24 hours post dose[120–122], when comparable concentrations of

Table 2 | **Efficacy of fingolimod versus placebo in the FREEDOMS study**

End points*	Fingolimod (0.5 mg)	Fingolimod (1.25 mg)	Placebo
Clinical			
Annualized relapse rate (primary endpoint)	• 0.18 • $p < 0.001$‡ • $n = 425$	• 0.16 • $p < 0.001$‡ • $n = 429$	• 0.40 • $n = 418$
Risk of disability progression (hazard ratio; 95% CI) 3-month confirmed	• 0.70 (0.52, 0.96) • $p = 0.024$‡ • $n = 425$	• 0.68 (0.50, 0.93) • $p = 0.017$‡ • $n = 429$	Not applicable
MRI			
Median (mean) number of new or enlarged T2 lesions over 24 months	• 0.0 (2.5) • $p < 0.001$‡ • $n = 370$	• 0.0 (2.5) • $p < 0.001$‡ • $n = 337$	• 5.0 (9.8) • $n = 339$
Median (mean) number of Gd-enhancing lesions at month 24	• 0.0 (0.2) • $p < 0.001$‡ at each time point • $n = 369$	• 0.0 (0.2) • $p < 0.001$‡ at each time point • $n = 343$	• 0.0 (1.1) • $n = 332$
Median (mean) percent change in brain volume over 24 months	• –0.7 (–0.8) • $p < 0.001$‡ • $n = 357$	• –0.7 (–0.9) • $p < 0.001$‡ • $n = 334$	• –1.0 (–1.3) • $n = 331$

CI, confidence interval; Gd, gadolinium. *All analyses of clinical end points used the intent-to-treat population. Magnetic resonance imaging (MRI) analyses used the evaluable data set. ‡Indicates statistical significance versus placebo at two-sided 0.05 level. Determination of *p* values: aggregate annualized relapse rate by negative binomial regression adjusting for treatment, pooled country, number of relapses in previous 2 years and baseline (EDSS) score; time to 3-month month confirmed disability progression by Cox's proportional expanded disability status scale hazards model adjusted for treatment, pooled country, baseline EDSS score and age; new or enlarged T2 lesions by negative binomial regression adjusted for treatment and pooled country; Gd-enhancing lesions by rank analysis of covariance (ANCOVA) adjusted for treatment, pooled country, and baseline number of Gd-enhancing lesions; and percentage change in lesion and brain volume by rank ANCOVA adjusted for treatment, pooled country, and corresponding baseline value.

fingolimod and fingolimod-P are detectable in the blood[120]. Owing to a high volume of distribution, fingolimod has a half-life of ~9–10 days[123]. With daily dosing of fingolimod, pharmacokinetic steady state is achieved after 1–2 months. The drug is cleared by a metabolic pathway that predominantly utilizes cytochrome P450 4F[124]. Given that this oxidative enzyme system is not known to contribute to the metabolism of any other drugs, there seems to be a low potential of drug–drug interactions.

Pharmacodynamic studies show that the drug has a rapid onset. Within hours of the first dose of fingolimod, a dose-dependent decrease in the peripheral lymphocyte count is apparent. With continued daily dosing, both a stable blood concentration and a stable reduction in the number of circulating blood lymphocytes are observed[124], with an average reduction of 77% with the 1.25 mg dose and 73% with the 0.5 mg dose; cell counts remained stable for the entire treatment period[125–127]. An increase of the peripheral blood lymphocyte count was evident within days of stopping fingolimod treatment, and it typically returned to normal range within 6 weeks[128].

Therapeutic effects in clinical studies. The first clinical evidence that fingolimod has therapeutic benefits in MS was provided in a 6-month, placebo-controlled Phase II trial involving 281 patients with relapsing MS[129]. Patients receiving 1.25 mg fingolimod or 5.0 mg fingolimod daily had a lower median total number of gadolinium-enhancing lesions (the primary end point) than those receiving placebo. The annualized relapse rate was 0.77 in the placebo group, compared with 0.35 in the 1.25 mg fingolimod group ($p = 0.009$) and 0.36 in the 5.0 mg fingolimod group ($p = 0.01$). When the 6-month placebo-controlled phase of the trial was completed, patients had the option to join a long-term extension in which all patients received fingolimod. During this extension phase, patients who switched from placebo to fingolimod also showed reductions in annualized relapse rates and lesion counts compared with the placebo phase[126,130].

Following on from this positive outcome, fingolimod was then evaluated in a 2-year, double-blind Phase III study (known as FREEDOMS), involving 1,272 patients with RRMS. The patients were randomized to receive a daily dose of 0.5 mg or 1.25 mg of fingolimod or placebo[127]. At baseline, patients had a mean of 1.4–1.5 relapses in the previous year and 2.1–2.2 relapses in the previous 2 years, and a mean expanded disability status scale (EDSS) score of 2.3–2.5. A total of 1,033 (81.2%) patients completed the study. The annualized relapse rate was 0.18 in the 0.5 mg fingolimod group, 0.16 in the 1.25 mg fingolimod group, and 0.40 in the placebo group ($p < 0.001$ for either dose versus placebo). At doses of 0.5 mg and 1.25 mg, fingolimod significantly reduced the risk of disability progression over the 24-month period. The cumulative probability of disability progression confirmed after 3 months was 17.7% with 0.5 mg fingolimod, 16.6% with 1.25 mg fingolimod, and 24.1% with placebo. Both fingolimod doses showed improved effects compared with placebo with regard to magnetic resonance imaging (MRI)-related measures (number of new or enlarged lesions on T2-weighted images, gadolinium-enhancing lesions, and brain-volume loss) (TABLE 2).

Fingolimod was also evaluated in a 1-year, double-blind, double-dummy Phase III study (known as TRANSFORMS) involving 1,292 patients with RRMS, comparing fingolimod with IFN-β1a, an established therapy for MS[125]. Patients were randomized to receive a daily dose of 1.25 or 0.5 mg fingolimod orally, or a weekly intramuscular injection of IFN-β1a. At baseline, patients had a mean of 1.5 relapses in the previous year and 2.2–2.3 relapses in the previous 2 years, and a mean EDSS score of 2.2. A total of 1,153 patients (89.2%) completed the study. The annualized relapse rate was significantly lower in both groups receiving fingolimod — 0.20 in the 1.25 mg group and 0.16 in the 0.5 mg group — than in the group receiving IFN-β1a (0.33). Disability progression was not different between the groups; however, the study duration was too short to adequately explore this end point. Both fingolimod doses showed improved effects compared with IFN-β1a with regard to MRI measures (number of new or enlarged lesions on T2-weighted images, gadolinium-enhancing lesions, and brain-volume loss at 12 months) (TABLE 3).

Overall, these Phase III trials demonstrated that oral fingolimod had greater efficacy compared with intramuscular IFN-β1a and placebo with regard to reducing the rates of relapse and MRI evidence of inflammatory

Gadolinium-enhanced lesions
Gadolinium is used as a contrast agent in magnetic resonance imaging (MRI). In multiple sclerosis, gadolinium causes areas of inflammation to be more pronounced than other areas of the brain. This can be seen on the MRI results and indicates where the disease is active.

Table 3 | **Efficacy of fingolimod versus IFN-β1a in the TRANSFORMS study**

End points*	Fingolimod (0.5 mg)	Fingolimod (1.25 mg)	Interferon-β1a (30 μg)
Clinical			
Annualized relapse rate (primary end point)	• 0.16 • $p < 0.001$‡ • $n = 429$	• 0.20 • $p < 0.001$‡ • $n = 420$	• 0.33 • $n = 431$
Risk of disability progression (hazard ratio; 95% CI) 3-month confirmed	• 0.71 (0.42, 1.21) • $p = 0.209$ • $n = 429$	• 0.85 (0.51, 1.42) • $p = 0.543$ • $n = 420$	Not applicable
MRI			
Median (mean) number of new or enlarged T2 lesions over 12 months	• 0.0 (1.7) • $p = 0.004$‡ • $n = 380$	• 1.0 (1.5) • $p < 0.001$‡ • $n = 356$	• 1.0 (2.6) • $n = 365$
Median (mean) number of Gd-enhancing lesions at 12 months	• 0.0 (0.2) • $p < 0.001$‡ • $n = 374$	• 0.0 (0.1) • $p < 0.001$‡ • $n = 352$	• 0.0 (0.5) • $n = 354$
Median (mean) percent change in brain volume over 12 months	• –0.2 (–0.3) • $p < 0.001$‡ • $n = 368$	• –0.2 (–0.3) • $p < 0.001$‡ • $n = 345$	• –0.4 (–0.5) • $n = 359$

CI, confidence interval; Gd, gadolinium. *All analyses of clinical end points used the intent-to-treat population. Magnetic resonance imaging (MRI) analyses used the evaluable data set. ‡Indicates statistical significance versus interferon (IFN)-β1a at two-sided 0.05 level. Determination of *p* values: aggregate annualized relapse rate by negative binomial regression adjusting for treatment, country, number of relapses in previous 2 years and baseline expanded disability status scale (EDSS) score; risk of disability progression by Cox's proportional hazards model adjusted for treatment, country, baseline EDSS score, and age; new or enlarged T2 lesions by negative binomial regression adjusted for treatment, country, number of relapses in previous 2 years and baseline EDSS score; Gd-enhancing lesions by rank analysis of covariance (ANCOVA) adjusted for treatment, country, and baseline number of Gd-enhancing lesions; and percentage change in brain volume by Wilcoxon's rank sum test.

lesion activity. The progression of clinical disability was statistically significantly reduced by fingolimod over 2 years compared with placebo. In addition, the convenience of the oral route of administration of fingolimod might lead to increased treatment adherence compared with injectable therapies, with a potential associated impact on effectiveness, but this remains to be studied.

Adverse events. In the completed fingolimod Phase II and III studies and their extensions, more than 2,600 patients with MS had been treated with fingolimod, representing approximately 4,500 patient-years of treatment exposure. In addition to the typical clinical and laboratory safety assessments, the two Phase III studies included a number of specialized cardiological, ophthalmological, pulmonary and dermatological assessments to support characterization of the potential clinical consequences of S1P receptor modulation.

In the 2-year FREEDOMS study[127], the overall incidence of serious adverse events was similar between all groups (10.1%–13.4%). In the 1-year TRANSFORMS study[125], the incidence of serious adverse events was higher in patients receiving 1.25 mg fingolimod (10.7%) than in those receiving 0.5 mg fingolimod (7%) or IFN-β1a (5.8%). In addition, the incidence of adverse events leading to drug discontinuation was similar between the 0.5 mg fingolimod and control groups (7.5–7.7% in the 2-year study; 3.7–5.6% in the 1-year study), but higher in the 1.25 mg fingolimod group (14.2% in the 2-year study and 10.0% in the 1-year study). Together, these data indicated that the 0.5 mg dose of fingolimod offered the most favourable benefit–risk profile.

Most, if not all, adverse reactions observed with fingolimod in these trials may relate to its mechanism of action; that is, as a modulator of $S1P_1$ receptors. The transient and generally asymptomatic reduction of heart rate and slowing of the atrioventricular conduction on starting fingolimod treatment (mainly after the first dose) could be because of a short-term, $S1P_1$–Gαi-dependent activation of the G protein-gated potassium channel IK_{ACh} in atrial myocytes, before internalization and/or desensitization of $S1P_1$ receptors[131,132]. This mechanism is similar to the heart rate reduction produced by acetylcholine acting at muscarinic receptors[133]. Accordingly, bradycardia was responsive to atropine[134] or isoproterenol, a β-adrenergic receptor agonist[135]. Animal data suggested that the transient bradycardia produced by fingolimod[136] and natural S1P[137] may involve predominantly $S1P_3$ receptors[136,138]; however, bradycardia in humans was also observed with the $S1P_1$–$S1P_5$-double agonist BAF312 (REF. 139), suggesting a causal role of $S1P_1$. The observed species differences may relate to the higher expression of the $S1P_1$ receptor in human ventricular, septal and atrial cardiomyocytes compared with the $S1P_3$ receptor[131].

The occurrence of macular oedema (in <1% of patients) and the mild increase in blood pressure (1–2 mm Hg on average) could relate to down-modulation of $S1P_1$–Gαi signalling in endothelial cells[97]. This may lead to an increased $S1P_2$- and/or $S1P_3$–$G_{12/13}$ signalling by endogenous plasma S1P and a reduced endothelial barrier function[89,90,140,141], and to diminished activation of vasodilatory, blood pressure regulating endothelial nitric oxide synthase (eNOS)[48,100,142]. Similar effects may occur with all functional antagonists of $S1P_1$ receptors, irrespective of their direct reactivity with the $S1P_2$ and/or $S1P_3$ receptors.

Isolated cases of peripheral vascular occlusion, posterior reversible encephalopathy syndrome and ischaemic stroke have been reported at doses higher than the recommended 0.5 mg dose, but the relationship between these effects and fingolimod is uncertain[129,143]. In animals, differential expression of S1P receptors and cross-regulation by other growth factors was observed in vascular beds under homeostatic versus inflammatory conditions[100,138,140], rendering definitive mechanistic conclusions difficult.

The combined data from the Phase III trials did not suggest an increased incidence of malignancies associated with fingolimod treatment[125,127]. The overall incidence of infections in fingolimod-treated patients was comparable to that of patients in control groups[125,127]. With regard to differences, in the placebo-controlled study, a slightly higher incidence of lower respiratory tract or lung infections was observed in patients receiving fingolimod (11.4% and 9.6% in the 1.25 mg and 0.5 mg groups,

Magnetic resonance imaging
(MRI). A non-invasive medical diagnostic technique in which the absorption and transmission of high-frequency radio waves are analysed as they irradiate the hydrogen atoms in water molecules and other tissue components placed in a strong magnetic field. This computerized analysis provides a powerful aid to the diagnosis and treatment planning of many diseases, including multiple sclerosis. Image contrast can be further enhanced by 'weighting' the image to capture more of either the longitudinal (termed T1) or transverse (termed T2) relaxation components.

T2-weighted images
The areas of abnormality on transverse (T2)-weighted magnetic resonance imaging (MRI) scans in patients with multiple sclerosis are pathologically nonspecific as they may represent areas of oedema, inflammation, demyelination, gliosis or tissue destruction. Such lesions are usually permanent, although they may decrease in size as acute lesions recover. Counting the number of new, or enlarging, T2 lesions over a period of time is an integral measure of MRI-detected disease activity over that time period.

respectively) compared with placebo (6.0%), whereas the incidence of urinary tract infections in patients receiving fingolimod (4.9% and 8.0% in the 1.25 mg and 0.5 mg groups, respectively) was lower than in those receiving placebo (11.2%)[127]. In the trial comparing fingolimod to IFN-β1a, two lethal herpes infections (one primary varicella zoster infection and one case of herpes simplex encephalitis) occurred in patients receiving 1.25 mg fingolimod[125]. These might have involved confounding factors related to the use of high-dose steroids to treat relapses in these patients. The primary varicella zoster infection occurred in a woman who was previously herpes zoster antibody negative, and who was exposed to a child with chickenpox during a course of steroids administered for a relapse, and the herpes simplex encephalitis occurred in a patient who received a course of steroids to treat a suspected relapse (see REF. 125 for details).

The mechanism behind the observed increase in liver enzymes[125,127] remains elusive; so far, there are no data from animal experiments indicating a direct effect on hepatocytes, vascular or biliary structures using doses that are several times higher than doses given to patients with MS[144]. Furthermore, fingolimod reportedly ameliorated the microcirculatory, biochemical and histological manifestations of hepatic ischaemia–reperfusion injury in rodents[145].

To date, the benefit–risk profile of fingolimod in MS has been characterized within the clinical programme for up to 2 years in controlled studies and up to 5 years in a long-term extension study. Fingolimod was shown to be effective and well tolerated, with a more favourable safety profile for the 0.5 mg dose than the 1.25 mg dose; identified drug-related adverse effects were generally reversible after drug discontinuation. Thorough observation and long-term follow up will continue to generate a more informed assessment of the benefits and risks of this new treatment option for relapsing MS.

Future development of fingolimod

In established EAE, fingolimod was effective as a rescue therapy, reversing paralysis, electrophysiological responses and BBB function[93,94]. These data suggest that the drug may find application beyond RRMS in primary progressive MS (PPMS) and more chronic forms of the disease. A Phase III study in patients with PPMS has now been initiated, to evaluate the efficacy and safety of the drug for up to 3–5 years, and the primary outcome is time to disability progression.

IL-17 secreted by T_H17 cells has been shown to be a key cytokine involved in the pathogenesis of autoimmune disease. In human MS and rodent experimental autoimmune neuritis, fingolimod reduced the numbers of circulating T_H17 cells[80–82], suggesting that the drug may find use in other autoimmune diseases involving the T_H17–IL-17 axis, including systemic lupus erythematosus, psoriasis, arthritis and diabetes (see REFS 23,48 for more details).

Apart from its immunomodulatory properties, fingolimod may act directly on neural cells, particularly astrocytes, reducing astrogliosis in models of MS[114]. Astrocytes are fundamental for brain homeostasis and are at the fulcrum of neurological diseases — including Alzheimer's disease, Huntington's disease, and Parkinson's disease — suggesting that such patients may also benefit from treatment with S1P receptor modulators.

Conclusion

The discovery and exploration of the new mode of action of fingolimod required extensive research over more than a decade and a complex clinical development programme that was challenging even for a large pharmaceutical organization. Fingolimod has shown improved efficacy compared with placebo and with a current first-line therapy in patients with RRMS. Additionally, fingolimod has shown an effect on disability and has demonstrated improved efficacy compared with one of the first-line IFN-β products in terms of relapses and MRI measurements over 12 months. Finally, fingolimod has prospectively shown a benefit in reducing brain atrophy compared with placebo and IFN-β on intent-to-treat analysis over the full duration of the studies[125–127]. Given the efficacy and tolerability seen in the clinical trials, and the convenience of once-daily oral administration, fingolimod might offer substantial benefits over current first-line therapies.

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Competing interests statement
The authors declare competing financial interests: see web version for details.