Mechanisms of Fingolimod's Efficacy and Adverse Effects in Multiple Sclerosis

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Until recently, all approved multiple sclerosis (MS) disease treatments were administered parenterally. Oral fingolimod was approved in September 2010 by the US Food and Drug Administration to reduce relapses and disability progression in relapsing forms of MS. In the clinical trials that led to approval, fingolimod reduced not only acute relapses and magnetic resonance imaging lesion activity but also disability progression and brain volume loss, suggesting preservation of tissue. Fingolimod's mechanism of action in MS is not known with certainty. Its active form, fingolimod-phosphate (fingolimod-P), is a sphingosine 1-phosphate receptor (S1PR) modulator that inhibits egress of lymphocytes from lymph nodes and their recirculation, potentially reducing trafficking of pathogenic cells into the central nervous system (CNS). Fingolimod also readily penetrates the CNS, and fingolimod-P formed in situ may have direct effects on neural cells. Fingolimod potently inhibits the MS animal model, experimental autoimmune encephalomyelitis, but is ineffective in mice with selective deficiency of the $S1P_1$ S1PR subtype on astrocytes despite normal expression in the immune compartment. These findings suggest that S1PR modulation by fingolimod in both the immune system and CNS, producing a combination of beneficial anti-inflammatory and possibly neuroprotective/reparative effects, may contribute to its efficacy in MS. In clinical trials, fingolimod was generally safe and well tolerated. Its interaction with S1PRs in a variety of tissues largely accounts for the reported adverse effects, which were seen more frequently with doses 2.5 to 10× the approved 0.5mg dose. Fingolimod's unique mechanism of action distinguishes it from all other currently approved MS therapies.

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Until recently, a key limitation of all approved therapies to treat relapsing–remitting multiple sclerosis (RRMS) was their parenteral administration route. Fingolimod (FTY720, Gilenya, Novartis AG, Basel, Switzerland), approved by the US Food and Drug Administration (FDA) in September 2010 to reduce relapses and accumulation of disability in patients with relapsing forms of multiple sclerosis (MS), is the first oral disease-modifying therapy. This review summarizes the biologic effects of fingolimod potentially responsible for its efficacy and adverse effects (AEs).

MS Pathogenesis

MS pathogenesis is multifactorial, producing multifocal central nervous system (CNS) lesions with perivenular inflammation, demyelination, axonal transection, neuronal degeneration, and gliosis in both white and gray matter.[1] Proinflammatory $CD4^+$ and $CD8^+$ effector T cells reactive to CNS myelin antigens are postulated to mediate the initial phases of lesion formation. More recently, other T-cell subsets, B cells, monocyte-macrophages, and natural killer cells have been implicated in both effector and regulatory mechanisms. Inflammatory processes predominate in early disease (reflected most directly in relapses and magnetic resonance imaging [MRI] lesion activity), but progression may reflect neurodegeneration. Intrinsic repair processes fail to compensate for ongoing damage in most patients. Although oligodendrocyte precursors and premyelinating oligodendrocytes extending processes to demyelinated axons exist in chronic lesions,[2] remyelination is incomplete and variable between lesions[2] and patients.[3] Animal studies suggest that remyelination may be neuroprotective, but only before persistent neuronal damage occurs.[4] The implication is that comprehensive MS treatment strategies must address several pathogenic mechanisms to limit not only ongoing inflammatory tissue damage but also degeneration, and augment remyelination and axonal regeneration.

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Lymphocyte Recirculation

Adaptive immunity requires recirculation of T cells and B cells between secondary lymphoid organs and tissues to monitor for antigens. Although it is estimated that 73% of the body's lymphocytes are in lymphoid tissue and 2% in the blood, $\sim 500 \times 10^9$ (equal to the total body number) traffic between blood and lymphoid tissues daily.[5] Naive T cells move from blood into lymph nodes (LNs) in search of antigen presented by dendritic cells. If activated, they proliferate, differentiate to effector cells, and migrate to B-cell areas in the LN or exit the LN to travel to inflamed tissues. A fraction of primed T cells become long-lived memory cells that, upon rechallenge, generate an accelerated and enhanced immune response. Several functional subsets of memory T cells are distinguished.[6] Central memory T cells (T_{CM}), like naive T cells, recirculate through secondary lymphoid tissues. Upon secondary antigenic challenge, they provide B-cell help and generate a new wave of effector T cells. In contrast, effector memory T cells (T_{EM}) reside in the tissues to provide an immediate response to pathogens, and do not recirculate through LNs. It is presumed there is a comparable dependence on autoreactive T-cell recirculation between blood, CNS, and LNs to perpetuate the abnormal inflammatory response in MS.[7]

Biology of Sphingosine 1-Phosphate

Sources of Sphingosine 1-Phosphate

Sphingosine 1-phosphate (S1P) is a bioactive lysophospholipid that mediates diverse physiological functions. It is generated from sphingomyelin by sequential reactions catalyzed by sphingomyelinase, ceramidase, and sphingosine kinase (SphK). There are 2 SphK isozymes, SphK1 and SphK2, with different kinetic properties, tissue distribution, developmental expression pattern, and regulation.[8,9] Erythrocytes are a main source of plasma S1P, which is also produced by platelets during activation and thrombotic processes. Other sources include mast cells, vascular and lymphatic endothelial cells, and fibroblasts as well as CNS sources (see below).

S1P regulates diverse cellular responses, including proliferation, differentiation, survival, cytoskeletal reorganization, process extension, chemoattraction and motility, and cell–cell adherence and tight junction formation. As a result, S1P is involved in numerous physiologic processes, including immunity; vascular and pulmonary smooth muscle tone; endothelial barrier function; and morphogenesis and function of the cardiac, vascular, and nervous systems.

Tissue S1P levels are tightly regulated by a balance among synthesis, release, and degradation. Concentrations approximate 0.5 to 6pmol/mg wet weight,[10] with the lowest levels in heart and testes, and higher levels in brain, spleen, and eye. The concentration of S1P is also relatively high in blood and lymph, but low in LNs. This concentration gradient plays an important role in lymphocyte trafficking (see below).

S1P Receptors

Extracellular S1P functions in both a paracrine and autocrine fashion by binding to 5 S1P receptors (S1PRs) that constitute a widely expressed, developmentally regulated family of G protein-coupled receptors characterized by 7 transmembrane domains.[8,11–14] Subtypes $S1P_1$, $S1P_2$, and $S1P_3$ are ubiquitously expressed. $S1P_4$ is primarily expressed by lymphoid cells. $S1P_5$ is primarily expressed in spleen and CNS white matter (oligodendrocytes). Differential cell-specific S1PR expression, changes related to cellular history and exposure to other mediators, differential coupling to G proteins and downstream signaling pathways, and cross-talk with other receptors provide for a wide dynamic range of S1P/S1PR-mediated actions. Signaling can be terminated by cell surface phosphohydrolase-mediated dephosphorylation of S1P to sphingosine and by receptor phosphorylation, uncoupling from G proteins, and internalization (Fig 1).

S1PR Expression by Immune Cells

Resting T cells and B cells express $S1P_1$ and lower levels of $S1P_4$ and $S1P_3$.[15,16] The S1PR profile is similar for $CD4^+$, $CD8^+$, and $CD4^+CD25^+$ T cells. The latter comprises the regulatory T cell subset that inhibits the activation and proliferation of other immune cells and is thought to be important in the control of autoimmunity. S1P–$S1P_1$ interaction plays a key role in lymphocyte trafficking, particularly egress from LNs. During lymphocyte recirculation, there is cyclical expression of $S1P_1$ by lymphocytes.[17] S1PRs are normally downregulated on circulating T cells in blood and lymph, where the concentration of S1P is relatively high. Conversely, after a few days in the LNs, where the S1P concentration is low, T cells re-express S1PRs. If after entering the LN, T cells fail to encounter their cognate antigen in the appropriate context that leads to activation, they exit through the efferent lymphatics in response to an S1P concentration gradient.[18] Antigen-induced activation leads to downregulation of $S1P_1$ expression and initial retention of activated T cells. After proliferation and differentiation, $S1P_1$ upregulation reestablishes responsiveness to the LN-lymphatic S1P gradient, thereby allowing egress.

Pharmacology of Fingolimod

Fingolimod—2-amino-2-(2-[4-octylphenyl]ethyl)-1,3-propanediol hydrochloride—was identified in the early 1990s from an extensive chemical derivatization program

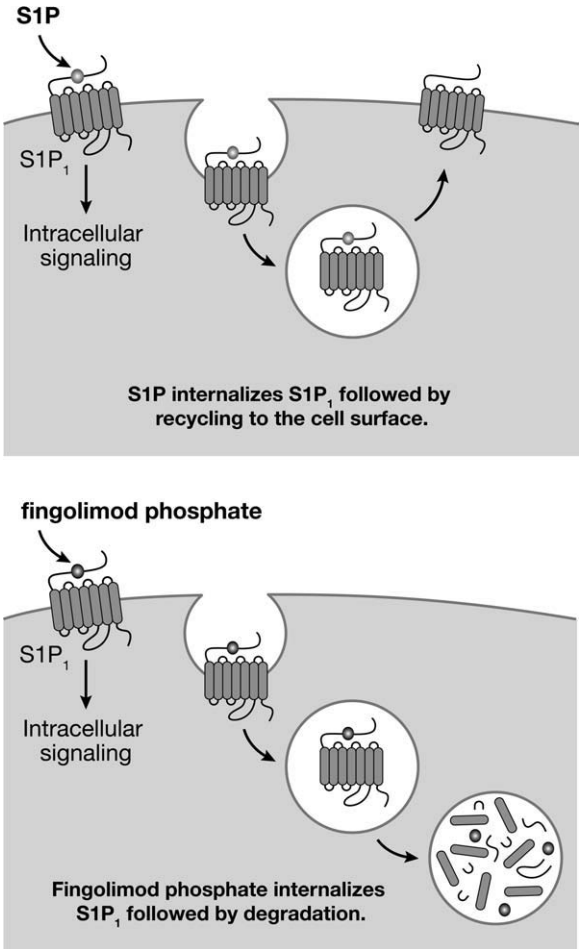

FIGURE 1: Comparison of the interactions of sphingosine 1-phosphate (S1P) and fingolimod-phosphate with the $S1P_1$ receptor subtype.

of myriocin (ISP-1, thermozymocidin), an immunosuppressant isolated from the entomopathogenic fungus *Isaria sinclairii*.[19] Bioavailability after oral administration is >90%.[20,21] Blood levels are nearly linearly dose-related in the range of 0.125 to 5mg/day with low interindividual variability.[20,22–25] Fingolimod is >99% protein bound in blood. Consistent with the molecule's amphipathic characteristics, it has a large volume of distribution and is extensively distributed to tissues, including brain.[21,25–27]

Fingolimod is a prodrug and is reversibly phosphorylated to fingolimod-P, the active moiety,[28,29] predominantly by SphK2 rather than SphK1.[30–34] It is presumed that because fingolimod-P is polar, it does not readily penetrate the blood–brain barrier (BBB). Rather, fingolimod crosses the BBB and is phosphorylated by endogenous SphKs in the CNS.[27,30] Fingolimod-P is dephosphorylated back to fingolimod by sphingosine phosphatase and irreversibly metabolized by cytochrome P450 enzymes, primarily CYP4F2 with minor contributions from CYP2D6, 2E1, 3A4, and 4F12, to inactive carboxylic acid metabolites, then excreted in urine.

Fingolimod-P binds with high affinity to 4 of 5 S1PR subtypes: $S1P_1$, $S1P_3$, $S1P_4$, and $S1P_5$ but not $S1P_2$.[28] As shown in Figure 1, binding to $S1P_1$ initially causes agonist effects, which are followed by aberrant receptor phosphorylation, long-lasting internalization, ubiquitination, and proteosomal receptor degradation, leading to a pharmacologic null state (functional antagonism).[35] Following fingolimod-P binding, internalized $S1P_1$ receptors may also maintain an active conformational state for a period of time with persistent signaling via adenylyl cyclase inhibition and extracellular-signal regulated kinase (ERK) phosphorylation, and resultant cellular responses.[36] S1P does not have this action. Thus, the functional consequences of fingolimod-P interaction with $S1P_1$ are a complex mixture of agonistic and functional antagonistic effects, at least within the immune system.

Down-modulation of $S1P_1$ expression on lymphocytes by fingolimod renders them unresponsive to the LN-efferent lymphatic S1P gradient required for egress, rapidly reducing lymphocyte counts in thoracic duct, peripheral blood, and spleen.[28,37,38] Redistribution of lymphocytes from blood to LNs does not produce lymphadenopathy, however, because the lymphocytes in blood represent only about 2% of the total lymphocyte count in the body.[5]

Fingolimod Efficacy in MS Clinical Trials

Fingolimod's efficacy in RRMS is supported by a 6-month, placebo-controlled phase II study[39]; a 2-year, placebo-controlled phase III study (FTY720 Research Evaluating Effects of Daily Oral Therapy in Multiple Sclerosis [FREEDOMS][40]); a 1-year phase III study (Trial Assessing Injectable Interferon versus FTY720 Oral in Relapsing–Remitting Multiple Sclerosis [TRANSFORMS])[41] with an active comparator (interferon beta-1a [IFNβ-1a]); and a >4 year phase II extension[42] (Table 1). These studies all demonstrated benefit of fingolimod on relapses and MRI lesion activity. FREEDOMS showed slowing of disability progression, and both phase III studies showed a reduction in brain volume loss. Of note, fingolimod did not produce pseudoatrophy, the transient acceleration of brain volume loss seen with initiation of high-dose corticosteroids, IFNβ,[43,44] and natalizumab.[45] There was no clear-cut dose effect for clinical or MRI outcomes comparing 1.25mg with 5mg in the phase II study or 0.5mg with 1.25mg in FREEDOMS and TRANSFORMS.

Potential Mechanisms of Fingolimod's Efficacy in MS

Fingolimod's mechanism of action in MS is not known with certainty. The predominant view is that immunologic effects, specifically inhibition of lymphocyte egress

TABLE 1: Clinical Trials of Fingolimod in MS

Study	Treatment	Patient Population	Status and Results
Phase II study[39,42]	Fingolimod 5.0mg or 1.25mg vs placebo	Relapsing MS	Status: core study (6 months) completed; long-term extension ongoing
			Results—month 6 analysis (n = 281):
			ARR: 0.35–0.36 (vs 0.77; $p \leq 0.01$ for each dose vs placebo)
			Relapse free: 86% of patients (vs 66%; $p < 0.01$ for each dose vs placebo)
			Free from Gd-enhancing lesions: 77–82% of patients (vs 47%; $p < 0.001$ for each dose vs placebo)
			Percentage brain volume change: −0.22 to −0.40 (vs −0.31; p = NS for each dose vs placebo)
			Month 48 analysis (n = 155):
			ARR: 0.18–0.20 (continuous fingolimod)
			Relapse free: 63–70% of patients (continuous fingolimod)
			Free from Gd-enhancing lesions: >95% of patients (continuous fingolimod)
FREEDOMS (phase III)[40]	Fingolimod 0.5mg or 1.25mg vs placebo	RRMS	Status: core study (24 months) completed; long-term extension ongoing
			Results—month 24 analysis (n = 1,272):
			ARR: 0.16–0.18 (vs 0.40; $p < 0.001$ for each dose vs placebo)
			Relapse free: 70–75% of patients (vs 46%; $p < 0.001$ for each dose vs placebo)
			Free from new/enlarged T2 lesions: 51–52% of patients (vs 21%; $p < 0.001$ for each dose vs placebo)
			Free from Gd-enhancing lesions: 90% of patients (vs 65%; $p < 0.001$ for each dose vs placebo)
			Percentage brain volume change: −0.84 to −0.89 (vs −1.31; $p < 0.001$ for each dose vs placebo)
TRANSFORMS (phase III)[41,142]	Fingolimod 0.5mg or 1.25mg vs IM IFNβ-1a	RRMS	Status: core study (12 months) completed; long-term extension ongoing)
			Results—month 12 analysis (n = 1,292):
			ARR: 0.16–0.20 (vs 0.33; $p < 0.001$ for each dose vs IFNβ-1a)
			Relapse free: 80–83% of patients (vs 69%; $p < 0.001$ for each dose vs IFNβ-1a)

TABLE 1 (Continued)

Study	Treatment	Patient Population	Status and Results
			Free from new/enlarged T2 lesions: 48–55% of patients (vs 46%; $p = 0.01$ for 0.5mg dose vs IFNβ-1a)
			Free from Gd-enhancing lesions: 90–91% of patients (vs 81%; $p < 0.001$ for each dose vs IFNβ-1a)
			Percentage brain volume change: −0.30 to −0.31 (vs −0.45; $p < 0.001$ for each dose vs IFNβ-1a)
			Month 24 analysis (n = 1,027):
			ARR: 0.18–0.20 (vs 0.33; $p < 0.001$ for each dose vs IM IFNβ-1a/fingolimod)
			Relapse free: 71–73% of patients (vs 60%; $p < 0.001$ for each dose vs IFNβ-1a/fingolimod)
			Free from new/enlarged T2 lesions: 34–42% of patients (vs 33%; $p < 0.05$ for 0.5mg dose vs IFNβ-1a/fingolimod)
			Free from Gd-enhancing lesions: 86% of patients (vs 77%; $p < 0.05$ for each dose vs IFNβ-1a/fingolimod)
			Percentage brain volume change: −0.61 to −0.66 (vs −0.67; p = NS for each dose vs IFNβ-1a/fingolimod)
FREEDOMS II (phase III)[143,144]	Fingolimod 0.5mg or 1.25mg vs placebo	RRMS	Status: ongoing (24-month trial + extension)
INFORMS (phase III)[141]	Fingolimod 0.5mg or 1.25mg vs placebo	PPMS	Status: ongoing (36-month trial)
Japanese study (phase II)[145]	Fingolimod 0.5mg or 1.25mg vs placebo	Relapsing MS	Status: ongoing (6-month trial)

ARR = annualized relapse rate; Gd = gadolinium; IFNβ-1a = interferon beta-1a; IM = intramuscular; MS = multiple sclerosis; NS = not significant; PPMS = primary progressive MS; RRMS = relapsing-remitting MS.

from LNs and interruption of recirculation to the CNS, account for the benefit on MS features that most directly reflect infiltration of blood-borne inflammatory cells into the CNS—relapses and MRI lesion activity. Slowed disability progression and brain volume loss indicate tissue preservation, but it is not yet clear whether this represents an indirect effect of reduced inflammatory damage, a direct neuroprotective effect, augmented repair, or a combination. Several observations, discussed below and summarized in Table 2 and Figure 2, suggest that direct CNS effects may contribute. It is noteworthy that in renal transplantation trials, fingolimod showed only modest efficacy, even as an adjunctive therapy,[46–48] suggesting that it does not have potent immunosuppressant effects in humans.

Inhibition of Lymphocyte Recirculation

In phase II and phase III MS studies, fingolimod decreased peripheral blood lymphocyte counts starting within hours of the first dose, reaching 20 to 30% of baseline (mean, 500–600/mm^3) within several weeks.[39–41] The degree of lymphopenia and its persistence after drug discontinuation were dose dependent, although the relationships were not linear.[22–24,39–41,49] In FREEDOMS, fingolimod 0.5mg reduced the mean ± standard deviation lymphocyte count

TABLE 2: Observations That Suggest Mechanisms Other Than Interference with Lymphocyte Recirculation May Be Involved in Fingolimod's Efficacy in MS

Between 0.5 and 5mg, there is a dose effect on peripheral blood lymphocyte level but lack of consistent dose effect on clinical or MRI efficacy measures.
Slowing of disability progression and brain volume loss in MS indicates preservation of CNS tissue.
Fingolimod readily enters the CNS and is phosphorylated in situ.
S1P is produced in the CNS.
S1PRs are expressed by neural cells.
S1P and fingolimod have multiple effects on neural cell growth and function in vitro.
Benefit of fingolimod has been shown in animal models in which peripheral immune and direct CNS effects can be distinguished.
Deletion of $S1P_1$ from CNS cells, particularly astrocytes, reduces EAE severity and fingolimod efficacy.

CNS = central nervous system; EAE = experimental autoimmune encephalomyelitis; MRI = magnetic resonance imaging; MS = multiple sclerosis; S1P = sphingosine 1-phosphate; $S1P_1$ = S1PR subtype 1; S1PR = S1P receptor.

from $1.84 \pm 0.62 \times 10^9$/l at baseline to $0.49 \pm 0.34 \times 10^9$/l at month 24,[40] corresponding to a mean of 27.2% of baseline with a range of 6.4 to 135.4% (Novartis, data on file). Lymphopenia persisted at a stable reduced level with continued treatment.[40,41] Because fingolimod causes lymphocyte redistribution rather than depletion, the lymphopenia is reversible. In FREEDOMS when fingolimod was discontinued, mean lymphocyte counts rose within several days and reached the normal range (0.8×10^9/l) within 6 weeks.[50] By 3 months, mean lymphocyte count was 80% of baseline (vs 94% in the placebo group). Johnson et al reported 2 patients with sustained lymphopenia, for 9 and 34 months, after fingolimod discontinuation.[51] Thus, prolonged lymphopenia rarely may occur following fingolimod therapy. The functional consequences of this response remain uncertain.

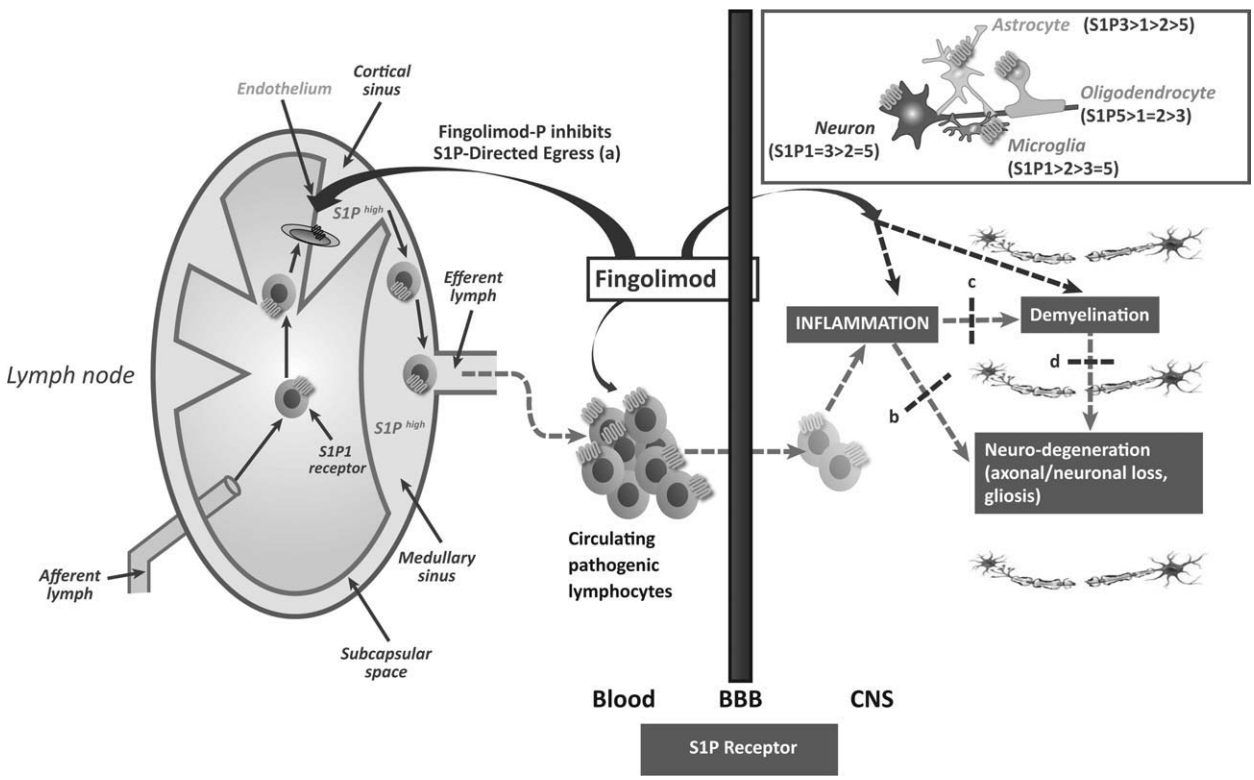

FIGURE 2: Potential effects of fingolimod on the pathogenesis of multiple sclerosis. BBB = blood–brain barrier; CNS = central nervous system; S1P = sphingosine 1-phosphate.

Fingolimod affects both T cells and B cells. Effects on circulating granulocytes, monocytes, eosinophils, erythrocytes, and platelets are modest or absent.[22,23] T cells are affected more than B cells.[22,23] $CD4^+$ T cells are affected more than $CD8^+$ T cells, decreasing the blood CD4/CD8 ratio.[22,52] Fingolimod preferentially impairs recirculation of T cells expressing the LN homing receptors CCR7 and CD62L (naive and T_{CM}).[52–54] The latter population includes interleukin (IL)-17 producing T cells (T_h17 cells),[55] which have been implicated in MS pathogenesis and response to IFNβ therapy.[56]

In humans, approximately 30% of circulating T cells are resistant to the LN trapping effect of fingolimod over the dose range tested in MS clinical trials.[39–41] It is likely that this population contains $CD8^+$ T_{EM} cells,[57,58] which lack expression of the LN homing receptors and, therefore, do not regularly recirculate through LNs. These long-lived cells persist in tissues and may provide at least partial immunologic memory and protection against pathogenic infections.

Fingolimod also inhibits B-cell trafficking. Mice treated with fingolimod have decreased immunoglobulin G (IgG) plasma cell and germinal center responses because of decreased egress from spleen, with reduced cell numbers in bone marrow and blood.[59] However, in mice treated with fingolimod or that lacked $S1P_1$ in B cells, IgG-secreting cells could still be induced and localized normally in secondary lymphoid organs.[59] Thus, analogous to fingolimod's effects on T cells, interference with B-cell recirculation might contribute to its efficacy in MS, although this hypothesis is less well studied, particularly in humans, and must account for maintained B-cell functions.

Overall, many studies indicate that fingolimod at therapeutically relevant concentrations modulates T-cell and B-cell trafficking rather than function. The expression levels of a variety of surface markers, including chemokine receptors and adhesion molecules, are unaltered.[52] Fingolimod does not inhibit T-cell activation, proliferation, differentiation to an effector phenotype, or cytokine production, or antibody production by B cells.[60–62]

Neurobiology of S1P and Potential Direct CNS Effects of Fingolimod

Production of S1P in the CNS

Although S1P is present at significant levels within the CNS, its physiologic role remains to be defined. Key synthetic enzymes, SphK1 or SphK2, are expressed in the CNS. However, it is notable that single deletions of SphK1 or SphK2 do not produce obvious CNS defects,[63] illustrating the uncertainty of non-in vivo approaches that may not accurately reflect the redundant roles for SphKs in the CNS. Expression of S1PRs in both the immune system and CNS in embryogenesis and adulthood suggests roles in development, neuroinflammation, and neurodegeneration (Table 3).[64]

Cells of neuronal lineage, encompassing varied developmental stages and subtypes, are a potential source of S1P in the CNS. In vivo, S1P has been reported to be preferentially detectable in neurons in normal spinal cord.[65] Rat cerebellar cortical granule cells in culture release S1P.[66] Cells of neuronal lineage can produce S1P in response to a number of factors, including nerve growth factor, fibroblast growth factor (FGF), phorbol esters, dibutyryl cyclic adenosine monophosphate, and forskolin.[10,67] Cultured astrocytes also secrete S1P when stimulated by phorbol esters, FGF, and tumor necrosis factor-alpha (TNFα).[66,68,69] S1P levels increase in spinal cord following traumatic injury[65] and in association with inflammation in experimental autoimmune encephalomyelitis (EAE).[70] The cellular source of S1P in these pathologic conditions is unknown.

Astrocytes

A central role for astrocytes in MS pathogenesis has been postulated.[71,72] Astrocytes are the most abundant cells in the CNS and in MS lesions. Potential ways astrocytes might contribute to MS lesion pathogenesis include matrix metalloproteinase secretion and BBB breakdown; adhesion molecule expression and chemokine secretion, facilitating inflammatory cell entry; and secretion of TNFα and lymphotoxin-α, causing oligodendrocyte death and axonal damage. Finally, the astrogliosis and gliotic scar formation that characterize chronic MS lesions might interfere with precursor cell migration into the lesion, remyelination, or axonal regeneration.

$S1P_1$ can be expressed widely in many lineages within the CNS under varied conditions. However, recent in situ hybridization data combined with conditional knockout mice for $S1P_1$ indicated that most of the specific signal in the normal CNS comes from astrocytes.[70] Astrocytes mainly express $S1P_1$ and $S1P_3$ along with other subtypes at low levels.[73–78] Immunohistochemical studies demonstrated a marked increase in $S1P_1$ and $S1P_3$ expression by reactive astrocytes in active and chronic MS lesions.[79] Several lines of evidence also suggest that an S1P-FGF autocrine loop mechanism might influences astrocyte proliferation.[69,70,73,75,80,81] In vivo, intracranial injection of S1P in mice induces astrogliosis.[75] Treatment of cultured human astrocytes with fingolimod-P inhibits production of inflammatory cytokines.[79]

TABLE 3: S1P Biology-Targeted KO Animals and siRNA-Treated Cell Lines Relevant to the CNS Degenerative and Inflammatory Diseases

Model	Details	Results
SphK2 KO mice[34]	Targeted in-bred Balb/c mouse KO model; homozygous $SphK2^{-/-}$ compared to $SphK2^{+/+}$ WT littermate controls	Fingolimod induced lymphopenia in WT but not in KO mice.
		Fingolimod phosphate induced a transient lymphopenia in KO mice.
		Results indicate that SphK2 is required for the phosphorylation of fingolimod and to maintain fingolimod phosphate levels in vivo.
$Hexb^{-/-}$, $SphK1^{-/-}$, or $S1P_3^{-/-}$ double null mice[32,64]	Sandhoff disease mouse model of neurodegeneration deficient in SphK1 or $S1P_3$	Deletion of SphK1 resulted in milder Sandhoff-like disease course, with reduced glial cell proliferation and less severe astrogliosis.
		Similar results were found with deletion of the gene for the $S1P_3$ receptor.
		Results suggest a functional role of S1P synthesis and receptor expression in astrocyte proliferation and that the SphK1/S1PR signaling axis may be important in the pathogenesis of neurodegenerative diseases.
		Administration of fingolimod to SphK1 KO mice resulted in lymphopenia, suggesting that SphK1 is not required for activation of fingolimod in vivo.
S1P lyase KO[146]	Targeted KO; homozygous and heterozygous inbred Balb/c mouse model	Both heterozygous and homozygous mice with decreased S1P lyase activity demonstrated marked lymphopenia, with accumulation of mature T cells in the thymus and LNs.
		Homozygous S1P lyase KO was either lethal or had reduced lifespan, possibly associated with aberrant sphingolipid storage.
		These findings suggest that lymphocyte trafficking is sensitive to S1P lyase activity.
siRNA-mediated downregulation of S1PR gene expression[147]	HUVEC treated with S1P and siRNA vs S1PRs	Treatment with siRNA vs $S1P_1$ and $S1P_3$ resulted in downregulation of IL-8 and MCP-1 gene expression.
		THP-1 cell chemotaxis was reduced toward the S1P-treated HUVEC-conditioned medium relative to control.
		These results indicate a role for $S1P_1$ and $S1P_3$ receptors in S1P-associated inflammatory response.
siRNA-mediated downregulation of SphK1 gene expression[148]	Primary cultures of rat oligodendrocyte precursors	SphK1 downregulation abolished NT-3–mediated survival of oligodendritic precursors.
		These data suggest a functional link with SphK1 as a modulator of NT-3 support of oligodendrocyte development.

CNS = central nervous system; HUVEC = human umbilical vein endothelial cells; IL-8 = interleukin-8; KO = knockout; LN = lymph node; MCP-1 = monocyte chemotactic protein-1; NT-3 = neurotrophin-3; S1P = sphingosine 1-phosphate; S1PR = S1P receptor siRNA = small interfering RNA; SphK1 = sphingosine kinase type 1; SphK2 = sphingosine kinase type 2; THP-1 = human acute monocytic leukemia cell line; WT = wild type.

Overall, these data suggest that fingolimod could have direct effects on astrocytes relevant to MS.

Oligodendrocytes

$S1P_5$ mRNA and protein are abundantly expressed in the CNS, predominantly by oligodendrocytes.[82–86] Cultured progenitor cells and mature oligodendrocytes also express $S1P_1$ and, in some studies, lower levels of $S1P_3$ and $S1P_2$.[85–92] Platelet-derived growth factor treatment of rat oligodendrocyte precursor cells upregulated $S1P_1$ and downregulated $S1P_5$.[88]

S1P has a number of effects on cells of oligodendrocyte lineages, including differentiation, migration, and survival, depending on the assessed developmental stage.[86,88] Similarly, a variety of fingolimod-P effects at concentrations attained in brain and cerebrospinal fluid of treated animals[27] have been reported on cultured cells of oligodendrocyte lineages, which also represent a range of different developmental stages. Fingolimod-P stimulated the differentiation of oligodendrocyte precursor cells into oligodendrocytes at low concentrations,[88] but high concentrations inhibited progenitor migration and differentiation.[88,89,91] Fingolimod-P protected oligodendrocyte progenitor cells from apoptosis induced by growth factor deprivation, inflammatory cytokines, or microglial activation.[91,93] Fingolimod-P also improved survival of cultured oligodendrocytes, inhibiting apoptosis during serum withdrawal and glucose deprivation.[88,90] In progenitors but not mature oligodendrocytes, this effect was mimicked by the selective $S1P_1$ agonist SEW2871. Fingolimod-P also stimulated membrane elaboration and process extension by mature oligodendrocytes cultured from adult human brain in a time- and dose-dependent manner.[90] Overall, these studies suggest fingolimod treatment could directly affect oligodendrocytes in MS.

Neurons

There is evidence that sphingolipids, including ceramide, sphingosine, and S1P, play important roles in the regulation of neuronal growth, differentiation, survival, and function.[94] Neural progenitor cells and neurons can express $S1P_1$, $S1P_3$, and to a lesser extent $S1P_2$, depending on the cell culture conditions[82,95,96] Genetic deletion of $S1P_1$ or combined deletion of SphK1 and SphK2 in mice severely disrupts neurogenesis, with increased apoptosis and decreased proliferation of neuroblasts, ultimately leading to neural tube defects,[63] suggesting that S1P signaling is important during embryonic CNS development and growth. Studies of cultured neurons and neuronlike cell lines identified a number of S1P effects, including cytoskeletal reorganization and morphological changes,[97–99] cytoprotection,[100,101] and electrophysiologic changes.[102] $S1P/S1P_1$ may mediate migration of neural stem cells to sites of spinal cord injury.[65] S1P has also been reported to stimulate neural stem cell proliferation and morphological changes.[95]

There have been relatively few studies of direct neuronal effects of fingolimod. Fingolimod-P treatment of primary cortical neuron cultures and embryonic stem cell-derived neuronlike cells resulted in a dose-dependent increase in phosphorylation of ERK1/2 and transcription of the CREB transcription factor followed by increased brain-derived neurotrophic factor mRNA.[103] At present, it remains possible but uncertain whether fingolimod treatment of MS has relevant direct effects on neurons.

Fingolimod Activity in EAE

EAE is a well-studied animal model of MS, involving inflammatory CNS demyelination and later stage neurodegeneration induced in susceptible laboratory animal strains by immunization with a variety of CNS antigen preparations. Fingolimod has been studied in a number of EAE variants in both mice and rats (summarized in Table 4), where it prevented development of clinical and histological disease when given prophylactically[104–107] and reversed manifestations when given therapeutically after disease onset.[104–108] Clinical benefit was accompanied by decreases in electrophysiological abnormalities,[105] demyelination,[107,109] axonal loss,[107,109] synaptic dysfunction, and dendritic damage.[110]

Fingolimod's effects on lymphopenia in EAE is dose dependent. However, its therapeutic effects only somewhat correlate, while also showing non–dose-dependent effects,[108] supporting the existence of distinct mechanisms that could involve direct CNS actions. Observations that support direct CNS effects include the following. First, intraventricular fingolimod administration 2 weeks after disease onset in acute EAE in dark agouti rats lessened clinical features, demyelination, and axonal damage without producing lymphopenia.[111] Second, and most critically, recent studies of EAE in CNS cell-specific conditional (loxP) $S1P_1$ knockout mice strongly support a role for $S1P_1$ signaling in astrocytes that promotes EAE pathogenesis as well as fingolimod efficacy distinct from effects on peripheral blood lymphocyte levels.[70] Conditional deletion of $S1P_1$ in neuronal cell lineages (via synapsin-cre) had no effect on EAE severity or fingolimod efficacy. By contrast, mice with panneural $S1P_1$ deletion that produced loss in all CNS cell types including astrocytes (using nestin-cre) resulted in EAE that was reduced in severity and abrogated fingolimod efficacy. Considering the in situ hybridization results that identified astrocytes as the predominant cell

TABLE 4: Efficacy of Fingolimod in CNS Injury Animal Models

Experimental Model	Timing of Fingolimod Treatment	Results and Comments
PLP-induced relapsing EAE in SJL/J mice[108]	Therapeutic	Fingolimod initiated at the peak of the initial acute relapse resulted in rapid improvement in clinical status and reversal of changes in the expression of mRNA encoding some myelin proteins and inflammatory mediators in the brain.
MBP-induced acute EAE in Lewis rats[104]	Prophylactic	Complete inhibition of EAE clinical and histological manifestations.
	Therapeutic	Significant inhibition of the progression of EAE clinical manifestations and infiltration of inflammatory cells into the spinal cord. The number of peripheral lymphocytes was decreased.
PLP-induced relapsing EAE in SJL/J mice[104]	Prophylactic	Complete inhibition of EAE clinical and histological manifestations. Decrease of T and B cells in peripheral blood.
	Therapeutic	Inhibition of clinical relapses and reduction in EAE-associated clinical manifestations.
MOG-induced EAE in DA rats[105]	Prophylactic	Protection against the emergence of EAE symptoms, neuropathology, and visual and somatosensory evoked potential abnormalities.
	Therapeutic	Reversal of paralysis and normalization of electrophysiological disturbances, which correlated with decreased brain and spinal cord demyelination.
Spinal cord homogenate-induced EAE in ABH mice[106]	Prophylactic	Complete inhibition of disease development.
	Therapeutic	Inhibition of subsequent relapses and slowed development of disability.
DA rat EAE model[109]	Therapeutic	Rescue therapy with fingolimod up to 1 month after onset of EAE reversed clinical manifestations, blood–brain barrier disruption, demyelination, and axonal loss.
MOG-induced EAE in DA rats[107]	Prophylactic	Protection against the development of clinical disease.
	Therapeutic	Reduction in clinical scores and attenuation of CNS inflammation, demyelination, and axonal loss.
MOG-induced chronic relapsing EAE in C57BL/6 mice[110]	Prophylactic	Prevention of synaptic abnormalities manifested as loss in sensitivity to the cannabinoid CB1 receptor agonist HU210 in single cell recordings of striatal neurons in brain slices. Prevention of loss of dendritic spines on striatal neurons.
MOG-induced monophasic EAE in C57BL/6 mice with conditional deletion of $S1P_1$[70]	Therapeutic	EAE severity was reduced and fingolimod efficacy was eliminated in mutants lacking $S1P_1$ on CNS cells, particularly astrocytes. Immune function was preserved in CNS mutants based on normal fingolimod effects on lymphocyte trafficking and adoptive transfer experiments.

TABLE 4 (Continued)

Experimental Model	Timing of Fingolimod Treatment	Results and Comments
Lewis rat traumatic brain injury[113]	Started immediately after injury	Decreased accumulation of macrophages and microglia.
Lewis rat traumatic spinal cord injury model[65]		Levels of S1P increased 7 days after spinal cord contusion, produced by astrocytes and microglia. S1P was chemoattractant for neural stem/progenitor cells via $S1P_1$.
Sprague-Dawley rat traumatic spinal cord injury[114]	Started immediately after injury	Improved functional recovery, higher somatosensory evoked response amplitude and reduced latency, and milder pathological changes.

ABH = antibody high; CNS = central nervous system; DA = dark agouti; EAE = experimental autoimmune encephalomyelitis; MBP = myelin basic protein; MOG = myelin oligodendrocyte glycoprotein; PLP = proteolipid protein; S1P = sphingosine 1-phosphate.

type expressing $S1P_1$, this result supported astrocyte involvement in both processes. Consistent with this interpretation, similar results were obtained using an independent driver to produce selective deletion of $S1P_1$ in astrocytes (GFAP-cre). Astrogliosis was also reduced in both nestin-cre and GFAP-cre conditional mutant mice, as it was with fingolimod treatment in wild-type mice. Strikingly, the immunologic effects of fingolimod remained intact in all CNS mutants based on both normal lymphocyte trafficking responses as well as adoptive transfer experiments.[70] Conversely, mice with deletion of $S1P_1$ from T cells exhibited similar EAE induction and therapeutic response to fingolimod compared to controls. These data implicate astrocytic $S1P_1$ in the pathogenesis of EAE and as a therapeutic target of fingolimod.

Fingolimod Activity in Other Animal Models of CNS Pathology

To determine whether fingolimod can effectively treat a delayed-type hypersensitivity (DTH) inflammatory response within the CNS behind an intact BBB, Lewis rats were injected stereotactically in the striatum with heat-killed bacillus Calmette-Guérin (BCG).[112] Four weeks later, after the initial inflammatory response had resolved, intradermal injection of BCG produced a focal DTH lesion in the CNS with self-limited BBB disruption. Fingolimod treatment 19 to 31 days after intradermal injection, after the transient BBB disruption had resolved, reduced the CNS inflammatory response and resultant demyelination.

In a rat traumatic brain injury model, fingolimod treatment reduced infiltration of macrophages and microglia.[113] Similarly, in rat spinal cord injury, fingolimod treatment improved functional recovery.[114]

Safety and Tolerability of Fingolimod

General Points

In published trials, fingolimod was generally well tolerated. The overall safety profile was better for 0.5mg, the approved dose, than 1.25mg. Given that the efficacy advantages of the 2 fingolimod doses over placebo and IFNβ-1a were similar, 0.5mg appeared to have a better benefit-to-risk profile.

The overall MS safety experience comprises 2,615 patients and approximately 4,583 patient-years of exposure.[115] The proportions of patients discontinuing medication or study participation due to an AE, laboratory abnormality, or abnormal test result was low (4–10%) in fingolimod groups in the phase III trials.[40,41] In FREEDOMS, the risks of any AE, serious AE, or AE leading to drug discontinuation were similar between fingolimod 0.5mg and placebo.[40] Five deaths occurred during FREEDOMS and TRANSFORMS: 2 in the placebo arms and 3 in the fingolimod 1.25mg arms, with no deaths in the fingolimod 0.5mg group of either trial.[40,41] Specific AEs associated with fingolimod included headache, influenza, diarrhea, back pain, cough, dyspnea, lower respiratory tract infection, elevation of liver enzymes, transient bradycardia, and slowed atrioventricular (AV) conduction on treatment initiation, blood pressure effects, and macular edema.[39–41,115,116] Known pharmacodynamic effects of fingolimod mediated by S1PRs account for many of the observed AEs. For others, the mechanism is uncertain. FDA recommendations related to fingolimod use are summarized in Table 5.[116] In addition, to better clarify

TABLE 5: US Food and Drug Administration Recommendations Related to Use of Fingolimod[116]

Administration	The approved dose is 0.5mg by mouth once per day.
	Bioavailability after oral administration is unaffected by food, so it can be taken without regard to meals.[20,21]
	The elimination half-life averages 8.8 days.[20,24] Steady state levels are reached after 4–8 weeks.[25,49] Pharmacokinetics are not affected by ethnicity, gender, and mild to moderate hepatic or renal impairment.[20]
Drug–drug interactions	Fingolimod does not interact significantly with other drugs used to treat MS, including fluoxetine, paroxetine, carbamazepine, baclofen, gabapentin, oxybutynin, amantadine, modafinil, amitriptyline, pregabalin, and corticosteroids.
	Ketoconazole, a potent inhibitor of CYP3A and CYP4F, increases fingolimod and fingolimod-phosphate exposure up to 70%.
	At present, there are no data concerning the safety and utility of combining fingolimod with other immunomodulatory or immunosuppressive medications.
	Patients on class Ia or class III antiarrhythmic drugs, beta blockers, or calcium channel blockers should be monitored for accentuated cardiac effects at initiation of fingolimod therapy.
Immunizations	Live attenuated vaccines should be avoided during and for 2 months after stopping fingolimod therapy.
Hepatic abnormalities	Elevations of liver enzymes may occur in patients receiving fingolimod, and patients with pre-existing liver disease may be at increased risk.
	Recent transaminase and bilirubin levels should be checked prior to treatment.
	No specific monitoring schedule is indicated once fingolimod is initiated, but hepatic function tests should be assessed in patients who develop symptoms suggestive of hepatic dysfunction.
	Fingolimod should be discontinued in patients who develop significant liver injury.
	Fingolimod exposure is increased with severe hepatic impairment and should be used with caution in this setting.
Cardiac effects	Patients receiving class Ia (eg, quinidine, procainamide) or class III (eg, amiodarone, sotalol) antiarrhythmic drugs, beta blockers, and calcium channel blockers; with a baseline low heart rate; or with a history of syncope, sick sinus syndrome, second-degree or higher AV conduction block, ischemic heart disease, or congestive heart failure may be at increased risk.
	Patients should have an electrocardiogram prior to treatment.
	All patients should be monitored for signs and symptoms of bradycardia for 6 hours after the first dose of fingolimod. Bradycardia or AV conduction slowing may require treatment with isoproterenol or atropine.
	If fingolimod is discontinued for >2 weeks, the effects on heart rate and AV conduction may recur on reintroduction, so the same precautions apply.
Macular edema	Ophthalmological exam should be performed before starting fingolimod and 3–4 months after treatment initiation.
	Visual symptoms and acuity should be monitored at routine evaluations. If a patient reports visual disturbance at any time during treatment, additional ophthalmological evaluation should be undertaken.
	Patients with diabetes mellitus and uveitis are at increased risk of macular edema and should have regular ophthalmologic evaluations.

TABLE 5 (Continued)

	In patients who develop macular edema, the risk of continuation of fingolimod or rechallenge is uncertain.
Blood pressure	Blood pressure should be monitored during fingolimod treatment.
Pulmonary effects	Routine pulmonary function testing is not needed prior to or during fingolimod treatment, but should be considered if clinically indicated.
Infection	Patients should have a recent complete blood count prior to initiation of fingolimod.
	Patients without a history of chicken pox or varicella-zoster virus vaccination should undergo serologic testing for varicella antibodies. Vaccination of antibody-negative patients should be considered prior to initiation of therapy, and therapy should be postponed for 1 month.
	Fingolimod therapy should not be started in patients with acute or chronic infections.
	Patients should be monitored for signs and symptoms of infection during fingolimod therapy and for 2 months after discontinuation.
	Consider suspending fingolimod treatment if a patient develops a serious infection.
	Concomitant use of fingolimod with antineoplastic, immunosuppressive, and immunomodulatory agents would be expected to increase the risk of immunosuppression.
Malignancy	No special monitoring for cancer during fingolimod treatment is recommended.

AV = atrioventricular; MS = multiple sclerosis.

the safety of fingolimod in clinical practice, the FDA required a prospective postmarketing safety study. These ongoing studies will better elucidate fingolimod's safety profile.

Hepatic Effects

After lymphopenia, increased alanine aminotransferase (ALT) was the most common laboratory abnormality. Increases in aspartate transaminase or bilirubin were uncommon. The abnormalities generally were mild and asymptomatic, with no cases of symptomatic liver injury or a pattern/severity indicative of significant hepatocellular damage. The abnormalities were reversible, returning to normal with discontinuation of treatment. Like other AEs, risk of hepatic abnormalities was dose dependent. In an integrated analysis of all patients in MS trials, ALT $\geq 3\times$ upper limit of normal (ULN) occurred in 94 of 1,172 (8.0%) patients treated with fingolimod 0.5mg, and elevation $\geq 10\times$ ULN occurred in 2 of 1,172 (0.2%) patients.[115] After fingolimod discontinuation, median time to recovery of ALT to >ULN but $\leq 2\times$ ULN was 64 days.[115]

Cardiac Effects

S1P regulates heart rate and conduction.[117] $S1P_1$, $S1P_2$, and $S1P_3$ are the dominant receptors in the cardiovascular system,[118] including atrial myocytes.[119] Fingolimod binding to S1PRs in atrial myocytes initially leads to activation of G protein-gated cholinergic potassium channels (I_{KACh}) eliciting an inward rectifying potassium current, membrane hyperpolarization, reduced cell excitability, and decreased firing rate.[120] Receptor desensitization makes this effect self-limited. This phenomenon is mediated by $S1P_3$ in rodents and rabbits[38,121,122] but by $S1P_1$ in humans.[122]

In clinical trials, fingolimod induced a transient, dose-dependent, usually mild negative chronotropic effect, reaching a maximum 4 to 5 hours after the first dose and attenuating over time despite continued dosing and increasing blood levels.[123] In a pooled analysis of FREEDOMS and TRANSFORMS, there were mean reductions of ~8bpm at nadir with the 0.5mg dose and ~11bpm with 1.25mg.[124] The decrease in heart rate usually was asymptomatic; in the phase III trials, dizziness, fatigue, chest discomfort, and palpitations were reported in <1% of fingolimod-treated patients, and there were no cases of syncope. No cases of symptomatic bradycardia developed beyond 24 hours. The heart rate effect attenuated with chronic treatment and returned to baseline by 1 month.[124]

Fingolimod also can cause dose-dependent slowing of AV conduction. In a pooled analysis of FREEDOMS and TRANSFORMS,[124] first-degree AV block was the

most common abnormality, with mean P-R prolongation of 4.5 milliseconds with 0.5mg and 11.3 milliseconds with 1.25mg. Second-degree block (Mobitz type I and type 2:1) was rare and also more frequent with 1.25mg. Mobitz type II and higher degree of block were not seen. The incidence of electrocardiographic abnormalities was comparable across treatment groups at 1 month.

Vascular Effects

S1P and fingolimod have complex effects on endothelial barrier function, vascular tone, blood flow, and blood pressure.[117] Vascular and lymphatic endothelial cells express high levels of $S1P_1$ and lower levels of $S1P_2$ and $S1P_3$.[125–127] The effects of S1P and fingolimod on endothelial cells are heterogeneous, augmenting tight junction and barrier function in some vascular beds and increasing permeability in other tissues.[128–131] The direct effects of S1P on vascular smooth muscle cells are mainly via $S1P_3$, which tends to cause vasoconstriction.[132] However, S1P and fingolimod induce endothelial nitric oxide synthase expression and nitric oxide production by endothelial cells via $S1P_3$, indirectly producing vasodilation.[133,134]

MACULAR EDEMA. In MS clinical trials, macular edema occurred in 0.3% of patients treated with fingolimod 0.5mg and 1.1% of patients on 1.25mg.[115] Most cases developed in the first 3 to 4 months of treatment. Approximately half were symptomatic; the remaining cases were identified by ophthalmological exam. Most cases improved or resolved with fingolimod discontinuation. The pathogenesis of fingolimod-related macular edema is unknown but may relate to effects on endothelial barrier function.

BLOOD PRESSURE. In phase III MS trials, patients treated with fingolimod 0.5mg had a mild increase in blood pressure (~2mmHg increase in systolic blood pressure and ~1mmHg increase in diastolic blood pressure) over the first 6 months of treatment, which persisted but did not increase further with continued treatment.[40,135] Blood pressure elevation may relate to effects on vascular smooth muscle.

MISCELLANEOUS VASCULAR EVENTS. Rare or single cases of ischemic and hemorrhagic stroke, peripheral arterial occlusive disease, and posterior reversible encephalopathy syndrome were reported in patients treated with fingolimod 1.25 or 5mg but not 0.5mg.[39–41] It is possible these vascular phenomena relate to effects on vascular endothelial or smooth muscle cells.

Pulmonary Effects

S1PRs are expressed by airway smooth muscle cells, and S1P may mediate airway hyper-responsiveness in some pathologic conditions.[136–138] Alveolar epithelium expresses $S1P_3$, and S1P administered in the airways disrupts alveolar epithelial barrier function.[130]

In the phase III MS trials, cough was reported as an AE in 5 to 10% of fingolimod-treated patients versus 4 to 8% of control patients, and dyspnea was reported as an AE in 2 to 7% of fingolimod-treated patients versus 2 to 5% of controls.[40,41] Several patients discontinued fingolimod because of unexplained dyspnea. In a combined analysis of FREEDOMS and TRANSFORMS,[115] minor fingolimod dose-dependent decreases in forced expiratory volume at 1 second (FEV_1) and diffusing capacity for carbon monoxide (D_LCO) were seen at month 1 and were stable thereafter. At month 24 in FREEDOMS, the mean reduction from baseline in percentage of predicted FEV_1 was 3.1% for fingolimod 0.5mg and 2.0% for placebo. Reductions from baseline in D_LCO were 3.8% with fingolimod 0.5mg and 2.7% with placebo. FEV_1 effects reversed following fingolimod discontinuation. At present there are insufficient data to determine the reversibility of decreased D_LCO or whether asthma, chronic obstructive pulmonary disease, or pulmonary hypertension increase the risk of fingolimod-related pulmonary AEs.

Infection

Because fingolimod is a potent immunomodulator, increased susceptibility to infection, including opportunistic infections, would not be unexpected. However, several factors may mitigate this risk. Fingolimod-induced lymphopenia reflects redistribution to LNs rather than depletion. Fingolimod appears to specifically retain those T cells that regularly recirculate through LNs—that is, naive T cells and T_{CM} (including T_h17 T cells), but not effector T cells and T_{EM}—that are important for immune surveillance and memory immune responses in the peripheral tissues.[55,57] Many aspects of immune function are preserved with fingolimod therapy, including lymphocyte numbers in LNs and tissues, function of LN and circulating lymphocytes, ability to generate antibodies, and innate immune mechanisms. However, the preferential trafficking effects on naive T cells and T_{CM} still potentially might affect local immune responses.[139] Normal volunteers treated with fingolimod for 1 month could mount IgG responses to both T cell-dependent (keyhole limpet hemocyanin) and T cell-independent (pneumococcal polysaccharide vaccine, PPV-23) novel antigens, although the response was somewhat reduced and delayed.[140]

The proportions of patients with infection AEs, severe infections, and serious infections were similar in the treatment groups in FREEDOMS and in an integrated analysis of all MS studies, aside from increased lower respiratory tract infections (mainly bronchitis) across treatment groups.[115] Overall, herpes virus infections were diagnosed in 2 to 9% of patients. In TRANSFORMS, they occurred in 5.5% of patients in the fingolimod 1.25mg group compared to 2.1% in the 0.5mg group and 2.8% with IFNβ-1a.[41] The incidence was similar across treatments in FREEDOMS[40] and the integrated analysis.[115] Most herpes infections were mild. A total of 11 herpes virus infection-related serious AEs were seen, including 1 case of fatal disseminated primary varicella zoster and 1 case of fatal herpes simplex encephalitis in TRANSFORMS. Both cases had complicating factors, but a role for fingolimod cannot be ruled out. There have been no cases of progressive multifocal leukoencephalopathy with fingolimod.

There was no clear-cut relation between the level of lymphopenia and infection risk in a pooled analysis of FREEDOMS and TRANSFORMS.[50] When fingolimod-treated patients were grouped based on nadir lymphocyte count, 156 of 206 (76%) patients with a nadir of $<0.2 \times 10^9$/l had an infection of any type compared to 344 of 475 (72%) with nadir 0.2 to 0.4×10^9/l, 97 of 168 (58%) with nadir $>0.4 \times 10^9$/l, and 301 of 418 (72%) placebo-treated patients. There was no clear-cut relationship between lymphocyte count and rates of any infection per patient-year, lower respiratory tract infection, or herpes infection.

Malignancy

Like infection, because of fingolimod's immunomodulatory and cell growth effects, there is a potential for increased risk of malignancy. In TRANFORMS there were 3 cases of melanoma in the fingolimod 0.5mg group and none in the other arms.[41] However, in FREEDOMS 1 case of melanoma was observed in each of the 1.25mg and placebo groups.[40] Thus, there was no clear-cut association of melanoma or other malignancies with fingolimod in the integrated safety analysis.[115]

Target Population for Fingolimod Therapy

Both FREEDOMS and TRANSFORMS showed that fingolimod is efficacious in both treatment-naive and previously treated patients.[40,41] For patients with an inadequate response to previously available agents and/or intolerable side effects, fingolimod is a reasonable alternative. The observations that IL-17 production is elevated in some IFNβ nonresponders[56] and that fingolimod reduces circulating IL-17–producing T_h17 cells[55] suggest that fingolimod may specifically be effective in patients with continued activity during IFNβ therapy, as was observed in TRANSFORMS.[41] For patients not currently on treatment, fingolimod was approved by the FDA as a first-line agent, that is, patients are not required to fail other agents prior to initiating fingolimod. For patients currently receiving an approved MS treatment with effective disease control and good tolerability, although the oral route of administration is understandably attractive, it seems prudent not to switch therapy routinely until there is greater long-term experience with fingolimod in routine practice. There are no published data concerning the safety and efficacy of fingolimod as combination therapy in MS.

Completed clinical trials of fingolimod in MS were restricted to patients with a relapsing course, the type of MS for which it was FDA approved. There are no published data concerning use in progressive MS or neuromyelitis optica. A 3-year phase III trial in primary progressive MS is ongoing.[141] The phase II study enrolled patients aged 18 to 60 years,[39] and the phase III studies enrolled patients aged 18 to 55 years.[40,41] Thus, the safety and efficacy of fingolimod in pediatric and elderly patients are not established. There have been no controlled studies of safety in pregnant women. Because studies in rats and rabbits demonstrated fetal development toxicity, including teratogenicity and embryo lethality,[116] fingolimod is pregnancy category C, and women of childbearing potential should use effective contraception during and for 2 months after fingolimod treatment. Fingolimod is excreted in the milk of rats. It is not known if it is excreted in milk in humans.[116]

Conclusions

A phase II and 2 phase III MS trials demonstrated fingolimod's benefit on relapses, disability progression, MRI lesion activity, and brain volume loss. Its safety profile and tolerability, including oral route of administration, make fingolimod an attractive treatment option for patients with relapsing forms of MS. Interaction with S1PRs on T cells and B cells, inhibition of egress from LNs, and reduced recirculation of inflammatory cells to the CNS are the currently accepted mechanism of efficacy in EAE and MS. However, direct effects in the CNS may also contribute to its efficacy, including potential neuroprotective and/or reparative actions. As there are no currently available treatments for MS demonstrated to limit damage directly or improve repair, there is a major unmet medical need in this regard, particularly

for purely progressive forms of MS. Further studies are needed to determine whether fingolimod meets this need. Interaction of fingolimod with S1PRs in a variety of tissues accounts for many of its off-target AEs. Ongoing studies will better define the S1PR mechanisms accounting for both its beneficial immunomodulatory and neuroprotective actions and AEs when used to treat MS.

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Potential Conflicts of Interest

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References

1. Frohman EM, Racke MK, Raine CS. Multiple sclerosis—the plaque and its pathogenesis. N Engl J Med 2006;354:942–955.
2. Chang A, Tourtellotte WW, Rudick RA, Trapp BD. Premyelinating oligodendrocytes in chronic lesions of multiple sclerosis. N Engl J Med 2002;346:165–173.
3. Patrikios P, Stadelmann C, Kutzelnigg A, et al. Remyelination is extensive in a subset of multiple sclerosis patients. Brain 2006; 129:3165–3172.
4. Stangel M. Neuroprotection and neuroregeneration in multiple sclerosis. J Neurol 2008;255(suppl 6):77–81.
5. Westermann J, Pabst R. Distribution of lymphocyte subsets and natural killer cells in the human body. Clin Invest 1992;70: 539–544.
6. Sallusto F, Geginat J, Lanzavecchia A. Central memory and effector memory T cell subsets: function, generation, and maintenance. Annu Rev Immunol 2004;22:745–763.
7. Massberg S, Von Andrian UH. Fingolimod and sphingosine-1-phosphate—modifiers of lymphocyte migration. N Engl J Med 2006;355:1088–1091.
8. Spiegel S, Milstien S. Sphingosine-1-phosphate: an enigmatic signalling lipid. Nat Rev Mol Cell Biol 2003;4:397–407.
9. Le Stunff HL, Milstien S, Spiegel S. Generation and metabolism of bioactive sphingosine-1-phosphate. J Cell Biochem 2004;92:882–899.
10. Edsall LC, Spiegel S. Enzymatic measurement of sphingosine 1-phosphate. Anal Biochem 1999;272:80–86.
11. Fukushima N, Ishii I, Contos JJA, et al. Lysophospholipid receptors. Annu Rev Pharmacol Toxicol 2001;41:507–534.
12. Hla T. Signaling and biological actions of sphingosine-1-phosphate. Pharmacol Res 2003;47:401–407.
13. Ishii I, Fukushima N, Ye X, Chun J. Lysophospholipid receptors: signaling and biology. Annu Rev Biochem 2004;73:321–354.
14. Chun J, Hla T, Lynch KR, et al. International union of basic and clinical pharmacology. LXXVIII. Lysophospholipid receptor nomenclature. Pharmacol Rev 2010;2010:579–587.
15. Graeler M, Goetzl EJ. Activation-regulated expression and chemotactic function of sphingosine 1-phosphate receptors in mouse splenic T cells. FASEB J 2002;16:1874–1878.
16. Graler MH, Goetzl EJ. The immunosuppressant FTY720 downregulates sphingosine 1-phosphate G-protein-coupled receptors. FASEB J 2004;18:551–553.
17. Lo CG, Xu Y, Proia RL, Cyster JG. Cyclical modulation of sphingosine-1-phosphate receptor 1 surface expression during lymphocyte recirculation and relationship to lymphoid organ transit. J Exp Med 2005;201:291–301.
18. Schwab SR, Pereira JP, Matloubian M, et al. Lymphocyte sequestration through S1P lyase inhibition and disruption of S1P gradients. Science 2005;309:1735–1739.
19. Im D-S. Linking Chinese medicine and G-protein-coupled receptors. Trends Pharmacol Sci 2003;24:2–4.
20. Kovarik JM, Schmouder R, Barilla D, et al. Single-dose FTY720 pharmacokinetics, food effect, and pharmacological responses in healthy subjects. Br J Clin Pharmacol 2004;57:586–591.
21. Kovarik JM, Hartmann S, Bartlett M, et al. Oral-intravenous crossover study of fingolimod pharmacokinetics, lymphocyte responses and cardiac effects. Biopharm Drug Dispos 2007;28: 97–104.
22. Budde K, Schmouder R, Nashan B, et al. Pharmacodynamics of single doses of the novel immunosuppressant FTY720 in stable renal transplant patients. Am J Transplant 2003;3:846–854.
23. Kahan BD, Karlix JL, Ferguson RM, et al. Pharmacodynamics, pharmacokinetics, and safety of multiple doses of FTY720 in stable renal transplant patients: a multicenter, randomized, placebo-controlled, phase I study. Transplantation 2003;76: 1079–1084.
24. Kovarik JM, Schmouder R, Barilla D, et al. Multiple-dose FTY720: tolerability, pharmacokinetics, and lymphocyte responses in healthy subjects. J Clin Pharmacol 2004;44:532–537.
25. Skerjanec A, Tedesco H, Neumayer HH, et al. FTY720, a novel immunomodulator in de novo kidney transplant patients: pharmacokinetics and exposure-response relationship. J Clin Pharmacol 2005;45:1268–1278.
26. Meno-Tetang GML, Li H, Mis S, et al. Physiologically based pharmacokinetic modeling of FTY720 (2-amino-2[2-(-4-octylphenyl)ethyl]propane-1,3-diol hydrochloride) in rats after oral and intravenous doses. Drug Metab Dispos 2006;34:1480–1487.
27. Foster CA, Howard LM, Schweitzer A, et al. Brain penetration of the oral immunomodulatory drug FTY720 and its phosphorylation in the central nervous system during experimental autoimmune encephalomyelitis: consequences for mode of action in multiple sclerosis. J Pharmacol Exp Ther 2007;323:469–475.
28. Brinkmann V, Davis MD, Heise CE, et al. The immune modulator FTY720 targets sphingosine 1-phosphate receptors. J Biol Chem 2002;277:21453–21457.
29. Mandala S, Hajdu R, Bergstrom J, et al. Alteration of lymphocyte trafficking by sphingosine-1-phosphate receptor agonists. Science 2002;296:346–349.
30. Billich A, Bonrnancin F, Devay P, et al. Phosphorylation of the immunomodulatory drug FTY720 by sphingosine kinases. J Biol Chem 2003;278:47408–47415.
31. Paugh SW, Payne SG, Barbour SE, et al. The immunosuppressant FTY720 is phosphorylated by sphingosine kinase type 2. FEBS Lett 2003;554:189–193.
32. Allende ML, Sasaki T, Kawai H, et al. Mice deficient in sphingosine kinase 1 are rendered lymphopenic by FTY720. J Biol Chem 2004;279:52487–52492.
33. Kharel Y, Lee S, Snyder AH, et al. Sphingosine kinase 2 is required for modulation of lymphocyte traffic by FTY720. J Biol Chem 2005;280:36865–36872.

34. Zemann B, Kinzel B, Muller M, et al. Sphingosine kinase type 2 is essential for lymphopenia induced by the immunomodulatory drug FTY720. Blood 2006;107:1454–1458.

35. Oo ML, Thangada S, Wu M-T, et al. Immunosuppressive and anti-angiogenic sphingosine 1-phosphate receptor-1 agonists induce ubiquitinylation and proteosomal degradation of the receptor. J Biol Chem 2007;282:9082–9089.

36. Mullershausen F, Zecri F, Cetin C, et al. Persistent signaling induced by FTY720-phosphate is mediated by internalized S1P1 receptors. Nat Chem Biol 2009;5:428–434.

37. Matloubian M, Lo CG, Cinamon G, et al. Lymphocyte egress from thymus and peripheral lymphoid organs is dependent on S1P receptor 1. Nature 2004;427:355–360.

38. Sanna MG, Liao J, Jo E, et al. Sphingosine 1-phosphate (S1P) receptor subtypes S1P1 and S1P3, respectively, regulate lymphocyte recirculation and heart rate. J Biol Chem 2004;279:13839–13848.

39. Kappos L, Antel J, Comi G, et al. Oral fingolimod (FTY720) for relapsing multiple sclerosis. N Engl J Med 2006;355:1124–1140.

40. Kappos L, Radue E-W, O'Connor P, et al. A placebo-controlled trial of oral fingolimod in relapsing multiple sclerosis. N Eng J Med 2010;362:387–401.

41. Cohen JA, Barkhof F, Comi G, et al. Oral fingolimod or intramuscular interferon for relapsing multiple sclerosis. N Eng J Med 2010;362:402–415.

42. Montalban X, O'Connor P, Antel J, et al. Oral fingolimod (FTY720) shows sustained low rates of clinical and MRI disease activity in patients with relapsing multiple sclerosis: four-year results from a phase II extension (P06.128) [abstract]. Neurology 2009;72(suppl 3):A313.

43. Rudick RA, Fisher E, Lee J-C, et al. Use of the brain parenchymal fraction to measure whole brain atrophy in relapsing-remitting MS. Neurology 1999;53:1698–1704.

44. Hardmeier M, Wagenpfeil S, Freitag P, et al. Rate of brain atrophy in relapsing MS decreases during treatment with IFNb-1a. Neurology 2005;64:236–240.

45. Miller DH, Soon D, Fernando KT, et al. MRI outcomes in a placebo-controlled trial of natalizumab in relapsing MS. Neurology 2007;68:1390–1401.

46. Tedesco-Silva H, Mourad G, Kahan BD, et al. FTY720, a novel immunomodulator: efficacy and safety results from the first phase 2A study in de novo renal transplantation. Transplantation 2005; 79:1553–1560.

47. Salvadori M, Budde K, Charpentier B, et al. FTY720 versus MMP with cyclosporine in de novo renal tansplantation: a 1-year, randomized controlled trial in Europe and Australasia. Am J Transplant 2006;6:2912–2921.

48. Tedesco-Silva H, Pescovitz MD, Cibrik D, et al. Randomized controlled trial of FTY720 versus MMF in de novo renal transplantation. Transplantation 2006;82:1689–1697.

49. Park SI, Felipe CR, Machado PG, et al. Pharmacokinetic/pharmacodynamic relationships of FTY720 in kidney transplant recipients. Braz J Med Biol Res 2005;38:683–694.

50. Francis G, Kappos L, O'Connor P, et al. Lymphocytes and fingolimod—temporal pattern and relationship with infections (P442). Mult Scler 2010;16(suppl 10):S146–S147.

51. Johnson TA, Shames I, Keezer M, et al. Reconstitution of circulating lymphocyte counts in FTY720-treated MS patients. Clin Immunol 2010;137:15–20.

52. Bohler T, Waiser J, Schuetz M, et al. FTY720 exerts differential effects on CD4+ and CD8+ T-lymphocyte subpopulations expressing chemokine and adhesion receptors. Nephrol Dial Transplant 2004;19:702–713.

53. Yanagawa Y, Masubuchi Y, Chiba K. FTY720, a novel immunosuppressant, induces sequestration of circulating mature lymphocytes by acceleration of lymphocyte homing in rats, III. Increase in frequency of CD62L-positive T cells in Peyer's patches by FTY720-induced lymphocyte homing. Immunology 1998;95: 591–594.

54. Hofmann M, Brinkmann V, Zerwes H-G. FTY720 preferentially depletes naive T cells from peripheral and lymphoid organs. Int Immunopharmacol 2006;6:1902–1910.

55. Mehling M, Lindberg R, Raulf F, et al. Th17 central memory T cells are reduced by FTY720 in patients with multiple sclerosis. Neurology 2010;75:403–410.

56. Axtell RC, de Jong BA, Boniface K, et al. T helper type 1 and 17 cells determine efficacy of interferon-b in multiple sclerosis and experimental encephalomyelitis. Nat Med 2010;16:406–413.

57. Mehling M, Brinkmann V, Antel J, et al. FTY720 therapy exerts differential effects on T cell subsets in multiple sclerosis. Neurology 2008;71:1261–1267.

58. Brinkmann V. FTY720 (fingolimod) in multiple sclerosis: therapeutic effects in the immune and the central nervous system. Br J Pharmacol 2009;158:1173–1182.

59. Kabashima K, Haynes NM, Xu Y, et al. Plasma cell S1P1 expression determines secondary lymphoid organ retention versus bone marrow tropism. J Exp Med 2006;203:2683–2690.

60. Chiba K, Yanagawa Y, Masubuchi Y, et al. FTY720, a novel immunosuppressant, induces sequestration of circulating mature lymphocytes by acceleration of lymphocyte homing in rats. I. FTY720 selectively decreases the number of circulating mature lymphocytes by acceleration of lymphocyte homing. J Immunol 1998;160:5037–5044.

61. Brinkmann V, Chen S, Feng L, et al. FTY720 alters lymphocyte homing and protects allografts without inducing general immunosuppression. Transplant Proc 2001;33:530–531.

62. Xie JH, Nomura N, Koprak SL, et al. Sphoingosine-1-phosphate receptor agonism impairs the efficiency of the local immune response by altering trafficking of naive and antigen-activated CD4+ T cells. J Immunol 2003;170:3662–3670.

63. Mizugishi K, Yamashita T, Olivera A, et al. Essential role for sphingosine kinases in neural and vascular development. Mol Cell Biol 2005;25:11113–11121.

64. Wu Y-P, Mizugishi K, Bektas M, et al. Sphingosine kinase 1/S1P receptor signaling axis controls glial proliferation in mice with Sandhoff disease. Hum Mol Genet 2008;17:2257–2264.

65. Kimura A, Ohmori T, Ohkawa R, et al. Essential roles of sphingosine 1-phosphate/S1P1 receptor axis in the migration of neural stem cells toward a site of spinal cord injury. Stem Cells 2007;25: 115–124.

66. Anelli V, Bassi R, Tettamanti G, et al. Extracellular release of newly synthesized sphingosine-1-phosphate by cerebellar granule cells and astrocytes. J Neurochem 2005;92:1204–1215.

67. Rius RA, Edsall LC, Spiegel S. Activation of sphingosine kinase in pheochromocytoma PC12 neuronal cells in response to trophic factors. FEBS Lett 1997;417:173–176.

68. Riboni L, Viani P, Bassi R, et al. Cultured granule cells and astrocytes from cerebellum differ in metabolizing sphingosine. J Neurochem 2000;75:503–510.

69. Bassi R, Anelli V, Giussani P, et al. Sphingosine-1-phosphate is released by cerebellar astrocytes in response to bFGF and induces astrocyte proliferation through G1-protein-coupled receptors. Glia 2006;53:621–630.

70. Choi JW, Gardell SE, Herr DR, et al. FTY720 (fingolimod) efficacy in an animal model of multiple sclerosis requires astrocyte sphingosine 1-phosphate receptor (S1P1) modulation. Proc Natl Acad Sci U S A 2011;108:751–756.

71. Williams AC, Piaton G, Lubetzki C. Astrocytes—friends or foes in multiple sclerosis? Glia 2007;55:1300–1312.

72. Nair A, Frederick TJ, Miller SD. Astrocytes in multiple sclerosis: a product of their environment. Cell Mol Life Sci 2008;65: 2702–2720.

73. Pebay A, Toutant M, Premont J, et al. Antiproliferative properties of sphingosine-1-phosphate in human hepatic myofibroblasts. Eur J Neurosci 2001;13:2067–2076.

74. Malchinkhuu E, Sato K, Muraki T, et al. Assessment of the role of sphingosine 1-phosphate and its receptors in high-density lipoprotein-induced stimulation of astroglial function. Biochem J 2003;370:817–827.

75. Sorensen SD, Nicole O, Peavy RD, et al. Common signaling pathways link activation of murine PAR-1, LPA, and S1P receptors to proliferation of astrocytes. Mol Pharmacol 2003;64: 1199–1209.

76. Rao TS, Lariosa-Willingham KD, Lin F-F, Palfreyman EL. Pharmacological characterization of lysophospholipid receptor signal transduction pathways in rat cerebrocortical astrocytes. Brain Res 2003;990:182–194.

77. Rao TS, Lariosa-Willingham KD, Lin F-F, et al. Growth factor pretreatment differentially regulates phosphoinositide turnover downstream of lysophospholipid receptor and metabotropic glutamate receptors in cultured rat cerebrocortical astrocytes. Int J Dev Neurosci 2004;22:131–135.

78. Mullershausen F, Craveiro LM, Shin Y, et al. Phosphorylated FTY702 promotes astrocyte migration through sphingosine-1-phosphate receptors. J Neurochem 2007;102:1151–1161.

79. van Doorn R, van Horssen J, Verziji D, et al. Sphingosine 1-phosphate receptors 1 and 3 are upregulated in multiple sclerosis lesions (P662). Mult Scler 2010;16(suppl 10):S228.

80. Sato K, Ishikawa K, Ui M, Okajima F. Sphingosine 1-phosphate induces expression of early growth response-1 and fibroblast growth factor-2 through mechanism involving extracellular signal-regulated kinase in astroglial cells. Mol Brain Res 1999;74:182–189.

81. Riboni L, Viani P, Bassi R, et al. Basic fibroblast growth factor-induced proliferation of primary astrocytes. J Biol Chem 2001; 276:12797–12804.

82. Glickman M, Malek RL, Kwitek-Black AE, et al. Molecular cloning, tissue-specific expression, and chromosomal localization of a novel nerve growth factor-regulated G-protein-coupled receptor, nrg-1. Mol Cell Neurosci 1999;14:141–152.

83. Im D-S, Heise CE, Ancellin N, et al. Characterization of a novel sphingosine 1-phosphate receptor, Edg-8. J Biol Chem 2000; 275:14281–14286.

84. Dev KK, Mullershausen F, Mattes H, et al. Brain sphingosine-1-phosphate receptors: implications for FTY720 in the treatment of multiple sclerosis. Pharmacol Ther 2008;117:77–93.

85. Terai K, Soga T, Takahashi M, et al. Edg-8 receptors are preferentially expressed in oligodendrocyte lineage cells of the rat CNS. Neuroscience 2003;116:1053–1062.

86. Jaillard C, Harrison S, Stankoff B, et al. Edg8/S1P5: an oligodendroglial receptor with dual function on process retraction and cell survival. J Neurosci 2005;25:1459–1469.

87. Yu N, Lariosa-Willingham KD, Lin F-F, et al. Characterization of the lysophosphatidic acid and sphingosine-1-phosphate-mediated signal transduction in rat cortical oligodendrocytes. Glia 2004;45:17–27.

88. Jung CG, Kim HJ, Miron VE, et al. Functional consequences of S1P receptor modulation in rat oligodendroglial lineage cells. Glia 2007;55:1656–1667.

89. Novgorodov AS, El-Alwani M, Bielawski J, et al. Activation of sphingosine-1-phosphate receptor S1P5 inhibits oligodendrocyte progenitor migration. FASEB J 2007;21:1503–1514.

90. Miron VE, Hall JA, Kennedy TE, et al. Cyclical and dose-dependent responses of adult human mature oligodendrocytes to fingolimod. Am J Pathol 2008;173:1143–1152.

91. Miron VE, Jung CG, Kim HJ, et al. FTY720 modulates human oligodendrocyte progenitor process extension and survival. Ann Neurol 2008;63:61–71.

92. Miron VE, Schubart A, Antel JP. Central nervous system-directed effects of FTY720 (fingolimod). J Neurol Sci 2008;274:13–17.

93. Coelho RP, Payne SG, Bittman R, et al. The immunomodulator FTY720 has a direct cytoprotective effect in oligodendrocyte progenitors. J Pharmacol Exp Ther 2007;323:626–635.

94. Buccoliero R, Futerman AH. The roles of ceramide and complex sphingolipids in neuronal function. Pharmacol Res 2003;47:409–419.

95. Harada J, Foley M, Moskowitz MA, Waeber C. Sphingosine-1-phosphate induces proliferation and morphological changes of neural progenitor cells. J Neurochem 2004;88:1026–1039.

96. McGiffert C, Contos JJA, Friedman B, Chun J. Embryonic brain expression analysis of lysophospholipid receptor genes suggest roles for s1p1 in neurogenesis and s1p1-3 in angiogenesis. FEBS Lett 2002;531:103–108.

97. Postma FR, Jalink K, Hengeveld T, Moolenaar WH. Sphingosine-1-phosphate rapidly induces rho-dependent neurite retraction: action through a specific cell surface receptor. EMBO J 1996;15:2388–2395.

98. Sato K, Tomura H, Igarashi Y, et al. Exogenous sphingosine 1-phosphate induces neurite retraction possibly through a cell surface receptor in PC12 cells. Biochem Biophys Res Commun 1997;240:329–334.

99. Van Brocklyn JR, Tu Z, Edsall LC, et al. Sphingosine 1-phosphate-induced cell rounding and neurite retraction are mediated by the G protein-coupled receptor H218. J Biol Chem 1999;274: 4626–4632.

100. Edsall LC, Pirianov GG, Spiegel S. Involvement of sphingosine 1-phosphate in nerve growth factor-mediated neuronal survival and differentiation. J Neurosci 1997;17:6952–6960.

101. Culmsee C, Gerling N, Lehmann M, et al. Nerve growth factor survival signaling in cultured hippocampal neurons is mediated through TRKA and requires the common neurotrophin receptor P75. Neuroscience 2002;115:1089–1108.

102. Kajimoto T, Okada T, Yu H, et al. Involvement of sphingosine-1-phosphate in glutamate secretion in hippocampal neurons. Mol Cell Biol 2007;27:3429–3440.

103. Deogracias R, Klein C, Matsumoto T, et al. Expression of brain-derived neurotrophic factor is regulated by fingolimod (FTY720) in cultured neurons (P728). Mult Scler 2008;14(suppl 1):S243.

104. Kataoka H, Sugahara K, Shimano K, et al. FTY720, sphingosine 1-phosphate receptor modulator, ameliorates experimental autoimmune encephalomyelitis by inhibition of T cell infiltration. Cell Mol Immunol 2005;2:439–448.

105. Balatoni B, Storch MK, Swoboda E-M, et al. FTY720 sustains and restores neuronal function in the DA rat model of MOG-induced experimental autoimmune encephalomyelitis. Brain Res Bull 2007;74:307–316.

106. Pryce G, Al-Izki S, Baker D, Giovannoni G. Control of chronic relapsing progressive EAE with fingolimod (P01.092). Neurology 2008;70(suppl 1):A29.

107. Papadopoulos D, Rundle J, Patel R, et al. FTY720 ameliorates MOG-induced experimental autoimmune encephalomyelitis by suppressing both cellular and humoral immune responses. J Neurosci Res 2010;88:346–359.

108. Webb M, Tham C-S, Lin F-F, et al. Sphingosine 1-phosphate receptor agonists attenuate relapsing-remitting experimental autoimmune encephalitis in SJL mice. J Neuroimmunol 2004;153:108–121.

109. Schubart AS, Howard L, Seabrook T, et al. FTY720 suppresses ongoing EAE and promotes a remyelinating environment preventing axonal degeneration within the CNS (P06.166). Neurology 2008;70(suppl 1):A339.

110. Rossi S, De Chiara V, Motta C, et al. Fingolimod treatment prevents the clinical, synaptic and dendritic abnormalities of

experimental autoimmune encephalomyelitis (P880). Mult Scler 2010;16(suppl 10):S309–S310.

111. Schubart A, Seabrook T, Rausch M, et al. CNS mediated effects of FTY720 (fingolimod) (P07.101). Neurology 2007;68(suppl 1):A315.

112. Anthony DC, Sibson NR, Leppert D, Piani Meier D. Fingolimod (FTY720) therapy reduces demyelination and microglial activation in a focal delayed-type hypersensitivity model of multiple sclerosis during the remission phase (P814). Mult Scler 2010;16(suppl 10):S283–S284.

113. Zhang Z, Zhang Z, Fauser U, et al. FTY720 attenuates accumulation of EMAP-II+ and MHC-II+ monocytes in early lesions of rat traumatic brain injury. J Cell Mol Med 2007;11:307–314.

114. Zhang J, Zhang A, Sun Y, et al. Treatment with immunosuppressants FTY720 and tacrolimus promotes functional recovery after spinal cord injury in rats. Tohoku J Exp Med 2009;219:295–302.

115. Collins W, Cohen J, O'Connor P, et al. Long-term safety of oral fingolimod (FTY720) in relapsing multiple sclerosis: integrated analyses of phase 1 and 3 studies (P843). Mult Scler 2010; 16(suppl 10):S295.

116. NDA 02257—FDA approved labeling text for Gilenya (fingolimod) capsules. September 21, 2010. Available at: http://www.accessdata.fda.gov/drugsatfda_docs/label/2010/022527s000lbl.pdf. Accessed March 23, 2011.

117. Peters SLM, Alewijnse AE. Sphingosine-1-phosphate signaling in the cardiovascular system. Curr Opin Pharmacol 2007;7:186–192.

118. Mazurais D, Roberts P, Gout B, et al. Cell type-specific localization of human cardiac S1P receptors. J Histochem Cytochem 2002;50:661–669.

119. Liliom K, Sun G, Bunemann M, et al. Sphingosylphosphocholine is a naturally occurring lipid mediator in blood plasma: a possible role in regulating cardiac function via sphingolipid receptors. Biochem J 2001;355:189–197.

120. Koyrakh L, Roman MI, Brinkmann V, Wickman K. The heart rate decrease caused by acute FTY720 administration is mediated by the G protein-gated potassium channel IKACh. Am J Transplant 2005;5:529–536.

121. Forrest M, Sun S-Y, Hajdu R, et al. Immune cell regulation and cardiovascular effects of sphingosine 1-phosphate receptor agonists in rodents are mediated by distinct receptor subtypes. J Pharmacol Exp Ther 2004;309:758–768.

122. Gergely P, Wallstrom E, Nuesslein-Hildesheim B, et al. Phase I study with the selective S1P1/S1P5 receptor modulator BAF312 indicates that S1P1 rather than S1P3 mediates transient heart rate reduction in humans (P437). Mult Scler 2009;15(suppl 2):S125–S126.

123. Schmouder R, Serra D, Wang Y, et al. FTY720: placebo-controlled study of the effect on cardiac rate and rhythm in healthy subjects. J Clin Pharmacol 2006;46:895–904.

124. DiMarco JP, O'Connor P, Cohen JA, et al. First-dose effect of fingolimod: pooled safety data from two phase 3 studies (TRANSFORMS and FREEDOMS) (P830). Mult Scler 2010; 16(suppl 10):S290.

125. Liu Y, Wada R, Yamashita T, et al. Edg-1, the G protein-coupled receptor for sphingosine-1-phosphate, is essential for vascular maturation. J Clin Invest 2000;106:951–961.

126. Chae S-S, Proia RL, Hla T. Constitutive expression of the S1P1 receptor in adult tissues. Prostaglandins Other Lipid Mediat 2004; 73:141–150.

127. Singer II, Tian M, Wickham LA, et al. Sphingosine-1-phosphate agonists increase macrophage homing, lymphocyte contacts, and endothelial junctional complex formation in murine lymph nodes. J Immunol 2005;175:7151–7161.

128. Brinkmann V, Cyster JG, Hla T. FTY720: sphingosine 1-phosphate receptor-1 in the control of lymphocyte egress and endothelial barrier function. Am J Transplant 2004;4:1019–1025.

129. McVerry BJ, Garcia GN. Endothelial cell barrier regulation by sphingosine 1-phosphate. J Cell Biochem 2004;92:1075–1085.

130. Brinkmann V, Baumruker T. Pulmonary and vascular pharmacology of sphingosine 1-phosphate. Curr Opin Pharmacol 2006;6:244–250.

131. Marsolais D, Rosen H. Chemical modulators of sphingosine-1-phosphate receptors as barrier-oriented therapeutic molecules. Nat Rev Drug Discov 2009;8:297–307.

132. Salomone S, Yoshimura S-i, Reuter U, et al. S1P3 receptors mediate the potent constriction of cerebral arteries by sphingosine 1-phosphate. Eur J Pharmacol 2003;469:125–134.

133. Rosen H, Goetzl EJ. Sphingosine 1-phosphate and its receptors: an autocrine and paracrine network. Nat Rev Immunol 2005;5:560–570.

134. Tolle M, Levkau B, Keul P, et al. Immunomodulator FTY720 induces eNOS-dependent arterial vasodilatation via the lysophospholipid receptor S1P3. Circ Res 2005;96:913–920.

135. Cohen JA. Online supplement to: Oral fingolimod or intramuscular interferon for relapsing multiple sclerosis. N Engl J Med 2010;362:402–415.

136. Pfaff M, Powaga N, Akinci S, et al. Activation of the SPHK/S1P signalling pathway is coupled to muscarinic receptor-mediated regulation of peripheral airways. Respir Res 2005;6:48.

137. Kume H, Takeda N, Oguma T, et al. Sphingosine 1-phosphate causes airway hyper-reactivity by rho-mediated myosin phosphatase inactivation. J Pharmacol Exp Ther 2007;320:766–773.

138. Roviezzo F, Di Lorenzo A, Bucci M, et al. Sphingosine-1-phosphate/sphingosine kinase pathway is involved in mouse airway hyperresponsiveness. Am J Respir Cell Mol Biol 2007;36:757–762.

139. Pinschewer DD, Ochsenbein AF, Odermatt B, et al. FTY720 immunosuppression impairs effector T cell peripheral homing without affecting induction, expansion, and memory. J Immunol 2000;164:5761–5770.

140. Schmouder R, Boulton C, Wang N, David OJ. Effects of fingolimod on antibody response following steady-state dosing in healthy volunteers: a 4-week randomised, placebo-controlled study (P412). Mult Scler 2010;16(suppl 10):S135.

141. Novartis. FTY720 in patients with primary progressive multiple sclerosis (INFORMS). ClinicalTrials.gov Identifier: NCT00731692. Available at: http://clinicaltrials.gov/ct2/show/NCT00731692. Accessed March 23, 2011.

142. Khatri B, Barkhof F, Comi G, et al. 24-month efficacy and safety from the TRANSFORMS extension study of oral fingolimod (FTY720) in patients with relapsing-remitting multiple sclerosis(P03.125). Neurology 2010;74(suppl 2):A239.

143. Calabresi PA, Goodin D, Jeffery D, et al. Oral fingolimod (FTY720) in relapsing-remitting multiple sclerosis: baseline patient demographics and disease characteristics from a 2-year phase III trial (FREEDOMS II) (P05.038). Neurology 2010;74(suppl 2):A416–A417.

144. Novartis. Efficacy and safety of fingolimod (FTY720) in patients with relapsing-remitting multiple sclerosis (FREEDOMS II). ClinicalTrials.gov Identifier: NCT00355134. Available at: http://clinicaltrials.gov/ct2/show/NCT00355134. Accessed March 23, 2011.

145. Novartis and Mitsubishi Tanabe Pharma. Efficacy and safety of FTY720 in patients with relapsing multiple sclerosis (MS). ClinicalTrials.gov Identifier: NCT00537082. Available at: http://clinicaltrials.gov/ct2/show/NCT00537082. Accessed March 23, 2011.

146. Vogel P, Donoviel MS, Read R, et al. Incomplete inhibition of sphingosine 1-phosphate lyase modulates immune system function yet prevents early lethality and non-lymphoid lesions. PLoS ONE 2009;4:e4112.

147. Lin CI, Chen CN, Lin PW, Lee H. Sphingosine 1-phosphate regulates inflammation-related genes in human endothelial cells through S1P1 and S1P3. Biochem Biophys Res Commun 2007; 355:895–901.

148. Saini HS, Coelho RP, Goparaju SK, et al. Novel role of sphingosine kinase 1 as a mediator of neurotrophin-3 action in oligodendrocyte progenitors. J Neurochem 2005;95:1298–1310.