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REVIEW

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Fingolimod (FTY720): A Recently Approved Multiple Sclerosis Drug Based on a Fungal Secondary Metabolite

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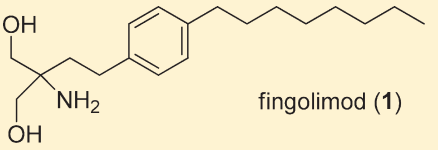
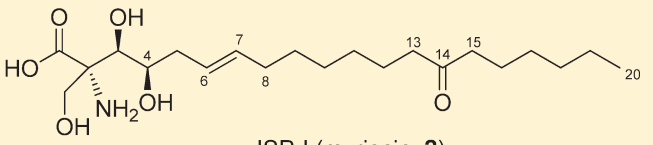
ABSTRACT: Fingolimod (Gilenya; FTY720), a synthetic compound based on the fungal secondary metabolite myriocin (ISP-I), is a potent immunosuppressant that was approved (September 2010) by the U.S. FDA as a new treatment for multiple sclerosis (MS). Fingolimod was synthesized by the research group of Tetsuro Fujita at Kyoto University in 1992 while investigating structure−activity relationships of derivatives of the fungal metabolite ISP-I, isolated from *Isaria sinclairii*. Fingolimod becomes active *in vivo* following phosphorylation by sphingosine kinase 2 to form fingolimod-phosphate, which binds to extracellular G protein-coupled receptors, sphingosine 1-phosphates, and prevents the release of lymphocytes from lymphoid tissue. Fingolimod is orally active, which is unique among current first-line MS therapies, and it has the potential to be used in the treatment of organ transplants and cancer. This review highlights the discovery and development of fingolimod, from an isolated lead natural product, through synthetic analogues, to an approved drug.



fingolimod (**1**)



ISP-I (myriocin; **2**)

MULTIPLE SCLEROSIS: THE DISEASE AND ITS CURRENT TREATMENT

Over 2.5 million people worldwide suffer with the debilitating disease multiple sclerosis (MS). MS is a neurodegenerative disorder of the central nervous system that is estimated to affect twice as many women as men.[1] The disease causes irreversible nerve damage, resulting in a wide range of symptoms, including fatigue, depression, pain, motor weakness, visual disturbances, and vertigo.[1−3] The onset of symptoms is usually rapid and sudden, commonly appearing around age 30.[1] The clinical course of MS varies greatly, from quickly progressive, resulting in clinical disability and possibly death, usually within 25 years,[4,5] to recurring symptoms that reduce a patient's quality of life, but without resulting in decreased life span. If untreated, approximately 50% of patients with MS are incapable of walking unaided within 15 years of disease onset.[6,7]

The clinical course of MS is best predicted by the specific disease classification, either relapsing-remitting (RR), primary-progressive (PP), secondary-progressive (SP), or progressive-relapsing (PR).[8] Approximately 85−90% of patients are diagnosed with RR-MS,[9,10] defined by an ongoing and unpredictable cycle of acute episodes of symptoms followed by a distinguishable recovery period.[8] The disease eventually becomes progressive in 30−40% of those diagnosed with RR-MS, changing their classification to SP-MS.[6,10] For this, the relapse−recovery cycle may or may not continue, but the symptoms progressively worsen, which is atypical of RR-MS.[8] With PP-MS, the disease continually, albeit gradually, progresses from onset.[8] PR-MS is similar to PP-MS in that the disease continually progresses in severity from onset, but with distinguishable relapses and possible recovery time in between episodes.[8]

Autoimmune disorders, such as MS, are marked by defective immune system responses. MS occurs when T- and B-lymphocytes cross the blood−brain barrier, progress into the central nervous system, and attack healthy cells.[11,12] Specifically, they destroy the myelin sheath and damage axons, resulting in inflammation.[11] This in turn causes irreversible nerve and tissue damage and accounts for the wide range of symptoms observed in MS.[11−13]

The cause of MS is unknown, but is believed to result from genetic predisposition in combination with environmental factors, which may include some type of infection.[12] However, to date, none of these factors have been proven to cause MS.[12] Diagnosis is often based solely on the presence of the attributed disease symptoms and patterns.[14] If a definitive diagnosis cannot be made from observable symptoms, magnetic resonance imaging (MRI) can aid in diagnosis.[14] MRI scans of patients with MS show distinct plaques in the brain and spinal cord,[12] usually measuring at least 3 mm in diameter.[14]

Current medicinal treatment options for patients with MS include immunomodulators, immunosuppressants, and anti-inflammatory agents.[15] Four different immunomodulators are approved by the U.S. Food and Drug Administration (FDA)

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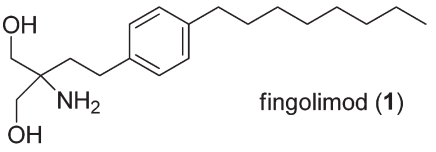
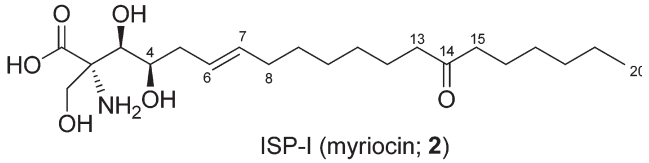
for treatment of relapsing MS.[16] Three are interferon betas (IFNβ) and include IFNβ-1b (Betaseron, Bayer HealthCare Pharmaceuticals) approved in 1993, IFNβ-1a intramuscular once weekly (Avonex, Biogen Idec) approved in 1996, and IFNβ-1a subcutaneously thrice weekly (Rebif, EMD Serono, Inc. and Pfizer Inc.) approved in 2002. The fourth immunomodulator, glatiramer acetate (GA) (Copaxone, Teva Pharmaceutical Industries, Ltd.), was approved in 1997. These four treatments, administered via either subcutaneous or intramuscular injection, only modestly affect disease progression, with a 30% reduction in disease relapse rates being reported.[15] The most commonly reported side effects include injection site reactions/necrosis and influenza-like symptoms.[17−19] Immunosuppressants are also used for treatment, with cyclosporin A[20] (Neoral and Sandimmune, Novartis Pharmaceuticals), approved in 1983, and tacrolimus, also known as FK506[21,22] (Prograf, Astellas Pharma US, Inc.), approved in 1994, being the two most common. However, these agents cause complete suppression of the immune system, leaving the patient susceptible to life-threatening secondary infections.[23] They also have toxic side effects at high doses, including renal impairment and kidney damage.[24,25] In 2002 the FDA approved mitoxantrone (Novantrone, EMD Serono, Inc.) for treatment of worsening MS. Mitoxantrone has the properties of both immunosuppressants and immunomodulators, although its use is limited by cardiotoxicity.[16]

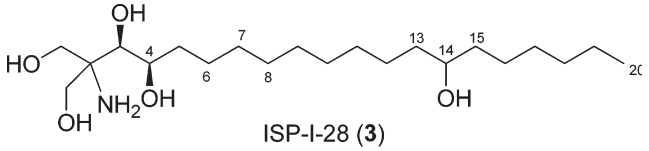
In short, MS is a debilitating disease that often progresses, resulting in clinical disability. The current treatment options, while offering some benefits, are not ideal for myriad reasons. However, new treatment strategies may emerge due to the recent approval of fingolimod (**1**), given the trade name Gilenya by Novartis Pharma. The progenitor to fingolimod, myriocin (**2**, ISP-I), was discovered from a fungus and reported in 1972.[26] To date, fingolimod has shown unprecedented efficacy for reducing annual relapse rates and symptoms,[27−29] and combined with its oral bioavailability, it may become an important component in the arsenal to combat MS.



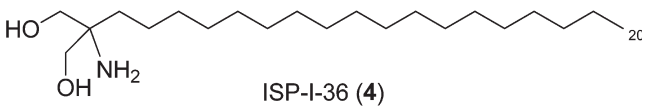
fingolimod (**1**)



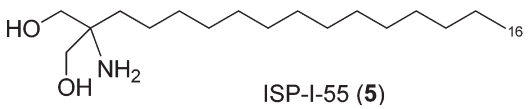
ISP-I (myriocin; **2**)



ISP-I-28 (**3**)



ISP-I-36 (**4**)



ISP-I-55 (**5**)

DISCOVERY

Cyclosporin A and FK506 were discovered initially as antifungal agents, and currently, both are clinically important immunosuppressants.[20−22] Cyclosporin A was reported in 1976 from the fungus *Trichoderma polysporum*, which was later reidentified as *Tolypocladium inflatum*.[20] FK506 was isolated from the bacterium *Streptomyces tsukubaensis* in 1987.[21,22] The discovery and development of these compounds supported and validated the screening of fungi and other microorganisms in pursuit of new immunosuppressants.[30]

During the late 1980s and early 1990s, Tetsuro Fujita and coworkers were also studying the fungus *Tolypocladium inflatum*.[31] In these studies they isolated a cyclic depsipeptide that was an active antibiotic, which was reported previously from the fungus *Isaria sinclairii*.[31] Fujita and colleagues thereafter focused their efforts on extracts of *Isaria sinclairii*.[31]

Isaria sinclairii is native to Asia, mainly China, Korea, and Japan, and is classified as an entomopathogenic fungus.[32] It is the imperfect stage of *Cordyceps sinclairii* (Clavicipitaceae) and is closely related to *Cordyceps sinensis* Sacc., whose Chinese name, Dong Chong Xia Cao, means "winter worm, summer grass";[32] this species was reclassified recently to *Ophiocordyceps sinensis*.[33] Fungal spores infect the larvae of suitable insect hosts, including members of the order Hymenoptera and Lepidoptera; the fungus is parasitic, growing within the host and resulting in death of the insect.[32] The fungus completely colonizes the insect cadaver, and in the spring and summer white fruiting bodies appear as stalks up to 6 cm in height.[34] Fungi at this stage of development are regarded as mysterious and mystical in some Asian cultures and have been used for thousands of years in traditional Chinese medicine, as they are believed to impart eternal youth.[32]

Fujita and colleagues[31] utilized two assays, one *in vitro* and one *in vivo*, to evaluate the fungus and its metabolites. To screen for immunosuppressive activity, they used a mouse allogeneic mixed lymphocyte reaction (MLR) assay. In this *in vitro* assay, spleen cells from two different strains of mice (BALB/c and C57BL/6) are cocultured and alloantigen is added to stimulate T-cell proliferation.[31] Samples were evaluated for inhibition of the proliferation of T-cells, with results reported as an IC_{50} value. The *in vivo* assay was performed by transplanting the dorsal skin of one rat (strain LEW) to the lateral thorax of a second rat (strain F344).[35] Test compounds were administered intraperitoneally daily until the skin grafts were rejected, as evidenced by 90% necrosis.[35] Compounds were scored on the basis of their ability to prolong rat skin graft survival. Using these assays in concert was key to the eventual development of fingolimod.

This evaluation process guided the isolation of a compound with immunosuppressant activity, which Fujita et al.[31] termed ISP-I (**2**). Upon structure elucidation, they found ISP-I was identical to myriocin[26] and thermozymocidine,[36] which were isolated previously from *Myriococcum albomyces* and *Mycelia sterilia*, respectively, via screening programs for antifungal agents.[26,36] ISP-I was shown by Fujita et al. to be 5- to 10-fold more potent than cyclosporin A in the MLR assay,[31] and at a dose of 0.1 mg/kg, ISP-I prolonged rat skin graft survival time by 2 days when compared to cyclosporin A at a dose of 1.0 mg/kg.[35] However, ISP-I had some unfavorable properties, being toxic to rats at a dose of 1.0 mg/kg, compared to 100 mg/kg for cyclosporin A, and by poor solublity.[35]

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Table 1. Compiled Assay Results of Compounds of Interest in the Development of Fingolimod (1)

	Assays Examined[a]				
compound	MLR assay IC_{50} value (nM)[35,39,41,42]	toxicity[b] *in vivo*[35,39,41,44]	rat skin graft survival time (days) at 1.0 mg/kg[35,41,44]	rat skin graft survival time (days) at 3.0 mg/kg[35,39,41,44]	active in SPT assay[35,39,41,42,44]
cyclosporin A	14	100	7.3	10.8	yes
ISP-I (**2**)	3 to 8	1.0	toxic		yes
ISP-I-28 (**3**)	1630	100	9.2	11.0	NR[c]
ISP-I-36 (**4**)	12	10	14.8	17.6	NR
ISP-I-55 (**5**)	5.9	10	37.3	45.5	no
fingolimod (**1**)	6.1	C[d]	39.5	52.0	no

[a] Numerical values are as reported in the primary literature. [b] Concentration (mg/kg) at which animals died in the *in vivo* rat skin graft assay. [c] Not reported. [d] Contradictory; the published toxicity data on fingolimod are inconsistent at 10 mg/kg in this assay, with one paper reporting it as nontoxic[39] and a latter paper reporting it as toxic.[44]

Researchers began to study ISP-I, with the goal of both simplifying the structure and improving the biological properties.[37,38] Between 1995 and 1998, results from the evaluation of upward of 50 analogues were reported.[35,37−42] In the published results from both the *in vitro* and *in vivo* assays, the activities of the analogues of ISP-I were compared to the activity of cyclosporin A. Structure−activity relationship studies guided the synthesis of compounds that had simplified structures, improved physical characteristics (i.e., solubility), and more potent activity (Table 1).[31,39]

The first analogue of interest was ISP-I-28 (**3**).[41] ISP-I-28 contained the following changes from ISP-I: reduction of the 6−7 double bond and reduction of the carboxylic acid and the 14-ketone[43] to alcohols. ISP-I-28 was less toxic than ISP-I (100 mg/kg compared to 1 mg/kg, respectively).[35] ISP-I-28 also prolonged rat skin graft survival time by 2 days, compared to cyclosporin A, both at a dose of 1.0 mg/kg, and ISP-I-28 was more soluble than ISP-I.[35] However, ISP-I-28 was less potent than ISP-I in the MLR assay, with an IC_{50} value of 1630 nM compared to 3 to 8 nM, respectively (Table 1).[35,42] ISP-I-28 was simplified further by removing three hydroxy groups, leaving an 18-carbon alkyl chain and resulting in the compound ISP-I-36 (**4**), which had an improved IC_{50} value (12 nM) in the MLR assay (Table 1).[41] ISP-I-36 also had improved activity in the rat skin graft assay, increasing the survival time by 5 days, compared to ISP-I-28, at a dose of 1.0 mg/kg. By shortening the alkyl chain of ISP-I-36 from 18 to 14 carbons, researchers generated ISP-I-55 (**5**), which was a more potent immunosuppressant in both the *in vivo* and *in vitro* assays. ISP-I-55 had an IC_{50} value of 5.9 nM (MLR assay) and more than doubled the survival time observed with ISP-I-36 in the rat skin graft assay (37.3 days with a 1.0 mg/kg dose; Table 1).[35]

The final modification was the introduction of an aromatic moiety, which researchers believed would improve activity by restricting conformation,[44] thereby leading to fingolimod (**1**). Positioning of the aromatic unit was critical, as its placement was shown to either decrease or increase immunosuppressant activity.[39] By moving it one carbon position in either direction, there was greater than 10-fold loss of potency in the MLR assay.[39] Moreover, the absorbance of the aromatic moiety in fingolimod was easy to detect analytically, a point that became beneficial in preclinical development studies.[30] More importantly, when compared to ISP-I, fingolimod had improved activity, a more favorable toxicity profile, and more desirable physical properties, including increased solubility (Table 1).[39,44]

A critical point in the discovery of fingolimod (**1**) was the use of the MLR assay to evaluate immunosuppressant activity.[30] Alternatively, researchers could have used the serine palmitoyltransferase (SPT) inhibition assay, which evaluates immunosuppressant activity based on a compound's ability to inhibit the enzyme serine palmitoyltransferase. Cyclosporin A, FK506, and ISP-I (**2**) are active in both the SPT and MLR assays.[35,42] However, the analogues ISP-I-55 (**5**) and fingolimod (**1**) show activity only in the MLR assay, suggesting that they operate via a different mechanism of action.[35,42] If the SPT assay would have been used to evaluate the immunosuppressant capabilities of these compounds, both ISP-I-55 (**5**) and fingolimod (**1**) would have shown no activity[35,42] and may not have been pursued further.[30] The reported bioassay results for compounds **1**−**5** and cyclosporin A are compiled in Table 1.

SYNTHESIS

Fingolimod (**1**) was derived from ISP-I (**2**) (discussed above) but contains various synthetic alterations.[37] At least 13 methods for the synthesis of fingolimod (**1**) and fingolimod-phosphate (**6**) have been developed. A detailed analysis of all of these is beyond the scope of this review. However, Table 2 provides a synopsis of the various synthetic strategies that have been published to date, and brief descriptions of a few are provided below.

In 2004 Seidel et al.[45] published an eight-step method utilizing iron-catalyzed cross-coupling reactions, starting from 2-(4-hydroxyphenyl)ethanol. They did not publish the overall yield of **1**, but syntheses with similar methods have resulted in overall yields of 6−24%.[39,44] In 2005 a shorter synthetic method was published by Sugiyama et al.[46] that required only five steps. The method was based on the Petasis reaction, which couples boronic acids, amines, and carbonyls to give amino alcohols. The resulting overall yield of **1** was 28%.[46] A seven-step approach was published subsequently by Kim et al.[47] that started with tris(hydroxymethyl)aminomethane (TRIS), which was converted to an aldehyde and then an alkyne. The alkyne was coupled to an aryl iodide via a Sonogashira reaction, hydrogenated, treated with acid, and purified. This methodology was practical, inexpensive, and resulted in a 64% overall yield of fingolimod.[47]

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Table 2. Highlights of Methods Used to Synthesize Fingolimod (1) and Fingolimod-P (6)

year	highlights of method
1995	First published method for the synthesis of fingolimod[39]
2000	Synthesis of fingolimod and analogues to evaluate immunosuppressive and lymphocyte-decreasing activity; method begins with Friedel−Crafts acylation of phenylalkyl acetates[44]
2000	Efficient 5-step method beginning with Friedel−Crafts acylation of 1-phenyloctane; 13% yield[78]
2001	Method to synthesize fingolimod using $MgSO_4$/MeOH/$NaNO_2$ to regioselectively open the ring of an epoxide[79]
2004	Synthesis of both enantiomers of fingolimod-P; used L-serine-derived oxazolizine to synthesize optically active intermediate, which was used to determine the absolute configuration of both enantiomers[80]
2004	Practical and scalable 8-step method based on iron-catalyzed cross-coupling, starting with 2-(4-hydroxyphenyl)ethanol;[45] similar methods have reported yields of 6−24%[39,44]
2005	Convenient 5-step method based on Petasis reaction, using dihydroxyacetone, benzylamine, and 2-(*p*-octylphenyl)vinylboronic acid; 28% yield[46]
2005	Practical asymmetric synthesis of both enantiamers of fingolimod-P based on lipase-catalyzed acylation, starting with *N*-acectylated fingolimod[81]
2005	Efficient and practical method to synthesize fingolimod-P based on monophosphorylation, using silver(I) oxide, tetrabenzyl pyrophosphate (TBPP), and tetrahexylammonium iodide[82]
2005	Determined that fingolimod is phosphorylated *in vivo* to form only the *S*-stereoisomer; performed efficient synthesis of both enantiomers of fingolimod-P in optically pure form, starting with fingolimod[54]
2006	Concise and practical 7-step method using palladium-catalyzed Sonogashira cross-coupling reaction; 64% yield[47]
2006	Convenient synthesis of both enantiamers of fingolimod-P from *p*-bromobenzaldehyde, using asymmetric Sharpless epoxidation[83]
2008	Convenient synthesis of immediate precursor of fingolimod, improving 1995 method;[39] precursor synthesized in 3 steps (vs 6); yield of precursor 41% (vs 18%)[84]

MECHANISM OF ACTION

A recent review by Brinkmann et al.[48] summarizes nicely the details of the mechanism of action of fingolimod. Briefly, the activation cascade of T- and B-cells begins with the phosphorylation of the sphingolipid sphingosine (**7**) by sphingosine kinase 2, to form sphingosine 1-phosphate (S1P) (**8**; Figure 1).[49] S1P is an activator of five different cell surface G-protein-coupled receptors (GPCRs), referred to as receptors $S1P_{1-5}$, which regulate a variety of cellular processes.[50] $S1P_{1-3}$ are expressed primarily on cells of the cardiovascular, immune, and central nervous systems, $S1P_4$ is expressed primarily in lymphoid tissue, and $S1P_5$ is expressed primarily in the spleen and central nervous system.[50] Activation of these GPCRs is necessary for the body's release of lymphocytes from the lymph nodes to the blood.[51]

The improper response of lymphocytes, as occurs with MS, causes internal inflammation, cell apoptosis, improper neuron firing, and severe pain.[15] Current medical treatments for MS, including cyclosporin A and FK506, act by inhibiting the enzyme serine palmitoyltransferase. Serine palmitoyltransferase is responsible for catalyzing the first step in sphingosine biosynthesis.[23] Inhibition of this enzyme results in the body's inability to produce sphingosine, preventing any activation of the GPCRs $S1P_{1-5}$. Ultimately this inhibits the release of T- and B-cells, rendering the body incapable of generating an immune response to any stimuli.[23]

Fingolimod (**1**) has a unique and novel mechanism of action. Once ingested, it is rapidly phosphorylated by sphingosine kinase 2 to form fingolimod-P (**6**; Figure 1).[49,52] Fingolimod-P resembles the ligand S1P and competes with it to bind to four of the five S1P receptors.[51] Fingolimod-P has the highest binding affinity for $S1P_1$, binding to $S1P_{3-5}$ with slightly lower affinity, and has no affinity for $S1P_2$.[53−55] Blood samples show that after **1** has circulated throughout the body the concentration of fingolimod-P is up to four times that of the parent.[55] This is essential for biological activity, as fingolimod itself has no binding affinity to any of the S1P receptors.[53−55]

A study was conducted to compare the pharmacokinetics of oral versus i.v. administration of fingolimod.[56] After i.v. administration, fingolimod (**1**) was present in the patient's blood, but fingolimod-P (**6**) was not.[56] Alternatively, during oral administration, presystemic phosphorylation of fingolimod to fingolimod-P may be a key, owing to the higher level of fingolimod-P measured after administration via this route.[56] These observations suggest that sphingosine kinase 2, which phosphorylates fingolimod, is active during either (or both) first pass metabolism through the liver and/or the absorption processes;[56] it is known that sphingosine kinase 2 is highly expressed in the liver.[57,58]

Ultimately fingolimod is metabolized in the liver, specifically by the cytochrome P450 enzyme CYP4F, with a half-life of 5−6 days.[59] It is metabolized primarily by oxidation of the hydroxy moieties into carboxylic acid derivatives and excreted in the urine.[60] Fingolimod undergoes a unique metabolic process, in which it is almost completely absorbed, is slowly excreted, and clearly favors oral administration of the drug. At present researchers are working to understand fingolimod's metabolic process more fully.[59,61]

Methods have been developed to synthesize fingolimod-P (**6**; Table 2); however, exploiting the body's natural phosphorylation processes for converting **1** to **6** may be the best option clinically. For instance, when **1** is phosphorylated *in vitro*, both the *S*- and *R*-enantiomers of fingolimod-P are produced. However, only the *S*-isomer has binding affinity to the S1P receptors.[40,53−55] Fortunately, phosphorylation of fingolimod (**1**) *in vivo* results in only the biologically active *S*-configuration being formed.[54]

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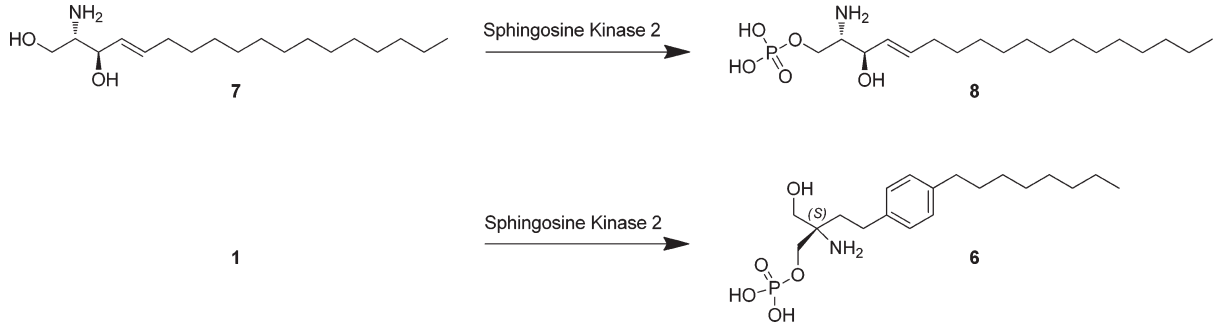

Figure 1. Mechanism of phosphorylation via sphingosine kinase 2 (adapted from Chun et al.[50]). The top shows the natural conversion of sphingosine (**7**) to sphingosine 1-phosphate (**8**), while the bottom shows the formation of fingolimod-phosphate (**6**) from fingolimod (**1**).

The novelty of the mechanism of action of fingolimod-P lies in its ability to redistribute the type of lymphocytes circulating in the blood, without reducing total lymphocytes. Central memory T-cells, which circulate regularly through lymph nodes,[62] are believed to be the subtype that are autoaggressive in MS patients.[50] Fingolimod-P (**6**) causes the lymphoid tissue to retain central memory T-cells, preventing them from entering into the blood.[63] Concomitantly, **6** also causes the concentration of effector memory T-cells in the blood to increase.[63] These latter T-cells do not circulate regularly through lymph nodes, and they are responsible for containing local pathogens and managing immune response memory.[62] This unique redistribution is crucial, as it prevents neurological damage by central memory T-cells, while preserving many necessary functions of the immune system carried out by other lymphocytes.

Fingolimod does not cause the destruction of any lymphocytes. Studies have shown that the overall count of lymphocytes circulating in the blood is reduced by approximately 70% during fingolimod treatment, compared with lymphocyte levels prior to treatment.[64] This decrease is due to the retention of lymphocytes in the lymphoid tissue. Once this redistribution occurs, lymphocyte counts in the blood remain stable throughout treatment and return to normal levels within 4 to 8 weeks after treatment discontinuation.[64]

Although fingolimod does not cause the destruction of lymphocytes, it does lead to degradation of the $S1P_{1,3-5}$ receptors.[65] Binding of either S1P or fingolimod-P to $S1P_{1,3-5}$ receptors causes internalization of the receptor, moving it from the plasma membrane into the cell.[66,67] Internalization of the receptor by S1P results in the receptor being recycled to the cell surface within approximately 2 h.[65] However, internalization caused by the binding of fingolimod-P (**6**) blocks the receptor recycling pathway and leads to receptor degradation.[65,67,68] It takes 2 to 8 days after exposure to fingolimod-P for cells to recover normal expression of $S1P_{1,3-5}$ receptors.[67] $S1P_{1,3-5}$ receptor degradation likely contributes to the prolonged immunosuppressant activity observed with fingolimod.[67,69,70] This provides a benefit in that fingolimod's activity is not dependent on the long-term stability of the compound *in vivo*, as short exposure time to fingolimod results in prolonged immunosuppressant activity.[67]

PHARMACOLOGY

Fingolimod, given the trade name Gilenya by Novartis Pharma, is the first orally active treatment for MS.[27] Two study groups, FREEDOMS (FTY720 Research Evaluating Effects of Daily Oral therapy in Multiple Sclerosis)[29] and TRANSFORMS (Trial Assessing Injectable Interferon versus FTY720 Oral in Relapsing-Remitting Multiple Sclerosis),[28] published results of phase III clinical trials in February 2010. All of the clinical studies on fingolimod to date have been on those patients with RR-MS.

The FREEDOMS trial ran for 24 months and included a total of 1272 patients, approximately 70% of whom were female.[29] All patients were 18 to 55 years of age and had relapsing-remitting MS (RR-MS). They were split into three treatment groups and administered daily either 0.50 mg of fingolimod (**1**), 1.25 mg of **1**, or placebo. The primary end point of the study was annualized relapse rates. The secondary end points were the time to disability progression and the growth or generation of lesions, as shown by MRI.[29] In this study the annualized relapse rate decreased 60% with a daily dose of 1.25 mg of **1** and 54% with a daily dose of 0.5 mg of **1**, relative to placebo.[29] The probability of disability progression, confirmed after six months, was 12.5% for 0.5 mg of **1**, 11.5% for 1.25 mg of **1**, and 19.0% for placebo.[29] Similar proportions of patients reported adverse events in all three treatment groups.[29] A few adverse events were reported more commonly with **1** treatment, including lower respiratory tract infections, macular edema, and elevated liver-enzyme levels.[29]

The TRANSFORMS study was conducted for 12 months and included 1292 patients between the ages of 18 and 55 who had RR-MS, approximately 67% of whom were female.[28] Patients were split into three treatment groups and received a daily dose of fingolimod (**1**) of either 0.50 or 1.25 mg or a weekly subcutaneous 30 μg dose of IFNβ-1a. This study[28] used the same end points as the FREEDOMS study.[29] Progression of the disease to disability was infrequent in all three treatment groups, as would be expected given the 12-month timeline.[28] Relapse rates were reduced by 38−52% in the fingolimod treatment groups versus the interferon IFNβ-1a treatment group.[28] There were also significantly fewer new lesions and less lesion growth in the fingolimod groups versus the interferon IFNβ-1a group.[28] Reported adverse events were similar to those reported in the FREEDOMS study, with the addition of localized skin cancer occurring in 10 patients, eight of whom were undergoing treatment with fingolimod.[28] The 10 identified skin cancers were all excised successfully and did not appear to be related to the dose of fingolimod; the data were not extensive enough to determine causality.[28]

Fingolimod has been shown in both phase III clinical trials,[28,29] as well as in other clinical studies,[56] to lower the patient's heart rate by approximately 10% upon treatment initiation.[60] Heart rate usually returns to its pretreatment rate within 7 to 14 days after the first dose of fingolimod and does not change again during continued treatment.[60]

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OTHER PROSPECTIVE USES

Organ Transplant. Fingolimod has the potential to be used in organ transplant. Researchers have suggested that it can prevent strong lymphocyte-mediated immune reactions in response to the implantation of new organs.[51] Additionally, it has been suggested that fingolimod strengthens endothelial cells and preserves their function, although this has been shown only at concentrations that are, at a minimum, 5-fold higher than the dose administered normally.[51] Regardless, these are promising concepts, as it implies that fingolimod may have other *in vivo* effects apart from the regulation of lymphocyte circulation.[51]

Cancer. Fingolimod (**1**) has been evaluated *in vitro* against three breast cancer cell lines (MCF-7, MDA-MC-231, and Sk-Br-3),[71,72] two colorectal cancer cell lines (HCT-116 and SW620),[71] and one prostate cancer cell line (LNCaP-AI).[72] Fingolimod inhibited the growth of these cell lines at IC_{50} values in the range 5−20 μM.[71,72] However, treatment of the three breast and two colorectal cancer cell lines with fingolimod-P (**6**) did not inhibit their growth.[71] A new analogue, (*S*)-fingolimod vinylphosphonate, was evaluated in MCF-7 and LNCaP-AI cell lines and showed similar results to those of fingolimod.[72] This suggests that fingolimod's mechanism of action in cancer cell proliferation may not be the same as in MS, as only fingolimod-P is active in MS treatment. Fingolimod has also been evaluated *in vivo* in tumors derived from the MGC803 (gastric adenocarcinoma) cell line in nude mice.[73] Mice were treated with 10 mg/kg fingolimod daily and observed for 20 days.[73] Fingolimod inhibited tumor growth and did not cause any notable side effects.[73] The potential of fingolimod to treat various types of cancer is an ongoing area of investigation.

CONCLUSION

The discovery of fingolimod (**1**) was due largely to the persistence of the research group of Tetsuro Fujita. The lead natural product, ISP-I (**2**), which was isolated from *Isaria sinclairii*,[31] gave them a framework to explore, particularly coupled with their clever and productive bioassay methodologies.[31,35] Many analogues of ISP-I were tested before fingolimod was discovered with improved physical properties,[37] a short synthetic method,[47] and a novel mechanism of action.[48,50] Fingolimod (**1**), under the trade name Gilenya, was first approved for use in Russia on September 10, 2010, and subsequently received U.S. FDA approval 12 days later. As Gilenya is the only current FDA-approved first-line treatment for MS that is orally available, it has the potential to revolutionize the therapy of this debilitating disease,[27−29] and it is already being lauded by some as one of the top 10 medical inventions of the year.[74] Given this impact, it was estimated that Novartis may profit upward of $1 billion annually from sales of Gilenya,[75] adding it to the long list of natural product inspired blockbuster drugs.[76,77] Future research on **1** will likely focus on more detailed analysis of its mechanism of action, while continuing to test it for the treatment of other diseases. Moreover, there are certainly other research groups looking to develop second- and third-generation analogues that further enhance potency and/or minimize side effects.

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