The introduction of oral disease modifying therapies has transformed the treatment landscape for patients with multiple sclerosis (MS). Fingolimod (Gilenya®, Novartis, Basel, Switzerland), the first oral therapy to be approved, has demonstrated clinical efficacy as a result of modulation of subtype 1 sphingosine-1-phosphate (S1P1) receptors. This leads to retention of lymphocytes in the lymph nodes, preventing their entry into the central nervous system. However, fingolimod can cause adverse effects as a result of its interaction with other S1P receptor subtypes, which are expressed in numerous tissues, including cardiac myocytes. More selective S1P receptor agents are currently in phase II and II clinical development. Siponimod, ozanimod, ponesimod and amiselimod have demonstrated efficacy with improved safety profiles compared with fingolimod. While more long-term data are needed, these selective S1P receptor modulators appear to be promising options for the treatment of MS and other disorders associated with autoimmunity and inflammation.

In the past decade, a number of novel therapies targeting specific molecules involved in the inflammatory or immune system activation cascades have become available, improving the management of multiple sclerosis (MS). However, most new therapies are biological drugs, which need to be injected and are therefore associated with reduced convenience, compliance and injection- or infusion-related adverse effects (AEs). Most current disease modifying therapies (DMTs) for MS primarily target the immunological inflammatory component of the disease without acting directly on the central nervous system (CNS) and have been shown to be only partially effective. In addition, chronic immunosuppression is associated with mainly opportunistic infections.

Another drawback of current DMTs is that, to date, most have shown limited efficacy against secondary progressive MS (SPMS), although ocrelizumab has been approved for progressive MS after showing activity in patients with primary progressive MS. A new class of oral targeted therapies for MS has the potential to overcome these limitations. This review aims to discuss the clinical development of sphingosine-1-phosphate (S1P1) receptor modulators for the treatment of patients with relapsing forms of MS (RMS) or SPMS.

**Mechanism of action of sphingosine-1-phosphate receptor modulators**

Sphingosine-1-phosphate is an active phospholipid that is produced by the phosphorylation of sphingolipids, present in the cell membrane, by sphingosine kinase-1 or -2 (SphK1/2; Figure 1). It regulates numerous biological processes including immunity, inflammatory, angiogenesis, heart rate, smooth muscle tone, cell differentiation, cell migration and survival, calcium homeostasis and endothelium integrity, and is found in high concentrations in erythrocytes, brain, spleen and eyes. Its effects are mediated by S1P receptors, which have seven transmembrane segments and are coupled to G-proteins. There are five known subtypes: S1P1–S5, which are broadly distributed in tissues; S1P1, which is expressed mostly in lymphoid tissue and the lungs; and S1P3, which is found in the spleen, skin and oligodendrocytes. Therefore, S1P receptors are found in multiple organ systems including the immune, cardiovascular, and respiratory systems, as well as in the CNS. In the CNS, expression of S1P receptors has been reported on oligodendrocytes, astrocytes, neurons and microglia in experimental conditions. Both B- and T-lymphocytes express S1P1, and, to a lesser extent, S1P3 and S1P5.

Sphingosine-1-phosphate signalling plays an important role in lymphocyte trafficking, particularly egress from lymph nodes and migration into the blood and target tissues (Figure 1). In patients with MS, S1P receptors reduce the release of lymphocytes from secondary lymphoid tissues, thymus and bone marrow, resulting in lymphopaenia. The binding of S1P receptor modulators to S1P1 on central memory T-cells (TCM) causes these cells to internalise their own S1P1, resulting in TCM that no longer respond to S1P signals. Any new S1P receptors produced inside the cell remain in an inactive state until S1P receptor modulation is removed.
Therefore, TCM do not leave the lymph node in response to S1P signals. As a result, fewer circulating lymphocytes are available to infiltrate the CNS and mount an autoimmune reaction on the axon myelin sheath.\(^8,12,13\)

Modulators of the S1P receptor prevent these autoreactive cells from migrating into the CNS. By contrast, the levels of peripheral effector memory T-cells (TEM) are mostly unaffected by S1P receptor modulators, preserving immunosurveillance and the ability to respond to and contain locally invading pathogens.\(^11\)

Fingolimod (Gilenya®, Novartis, Basel, Switzerland) is a lipophilic sphingosine-like agent that is phosphorylated by SphK1/2 to become fingolimod-P, an S1P analogue. Fingolimod-P binds to the S1P<sub>1</sub> receptor and is internalised in the same way as S1P, but the receptor is then degraded, preventing cell surface signalling.\(^8\)

Fingolimod is an agonist of four S1P receptor subtypes (S1P<sub>1</sub>, S1P<sub>3</sub>, S1P<sub>4</sub> and S1P<sub>5</sub>)\(^14\) and induces immunosuppression through inhibition of recirculation of naïve T-cells and the release of antigen-activated T-cells from the draining lymph nodes to lymph and to the blood compartment.\(^15\) It crosses the blood-brain barrier and may have direct CNS effects, distinguishing it from immunologically targeted MS therapies, although this has not been demonstrated in humans.\(^13\)

Fingolimod has also been found to attenuate neuroinflammation in rats by regulating the activation and neuroprotective effects of microglia, mainly via S1P<sub>1</sub>.\(^16,17\) Fingolimod also has direct CNS effects via suppression of pathogenic astrocyte...
Sphingosine-1-phosphate (S1P) receptors are G protein-coupled receptors (GPCRs) that play a crucial role in the immune system. These receptors are activated by the lipid mediator S1P, which is involved in the regulation of lymphocyte trafficking, vascular permeability, and inflammation. Fingolimod, an oral selective S1P1 receptor modulator, has demonstrated efficacy and safety in the treatment of multiple sclerosis (MS) and other autoimmune diseases.

**Clinical efficacy and safety of fingolimod**

Fingolimod has shown efficacy in reducing the frequency of MS relapses and improving disability progression compared to placebo in phase III clinical trials. The therapeutic effects of fingolimod are caused by rapid internalisation, degradation, and functional antagonism of S1P1 receptors, leading to lymphocyte sequestration in the lymph nodes.

Despite a growing body of clinical evidence supporting the efficacy of fingolimod, its clinical use has been limited by safety concerns with respect to cardiac effects, infections, and macular oedema. However, cardiac symptoms, including bradycardia and atrioventricular conduction block on drug initiation, are transient. A number of factors should be considered before initiation of fingolimod or require monitoring while on treatment, including first-dose monitoring, pregnancy, diabetes mellitus, posterior reversible encephalopathy syndrome, basal cell carcinoma, infections such as varicella, opportunistic cryptococcal infections and progressive multifocal leukoencephalopathy (PML). These cardiac effects are due to activation of S1P1 on cardiac myocytes, which subsequently disappears by downregulation of S1P1 receptors.

**Selective sphingosine-1-phosphate receptor modulators**

Following the approval of fingolimod, a number of selective S1P receptor modulators entered clinical development (Table 1) and several are currently being evaluated in phase III clinical studies (Table 2). These agents differ in their selectivity and activation potency (EC50). The therapeutic effects of these compounds are caused by rapid internalisation, degradation, and functional antagonism of S1P1 receptors, leading to lymphocyte sequestration in the lymph nodes. Since they do not affect S1P3 receptors, they are expected to be associated with fewer AEs, including those occurring after the first dose. In contrast to the long half-life and slow elimination of fingolimod, all of the selective S1P receptor modulators in clinical development (apart from amiselimod) have a shorter half-life and show a reduced time to lymphocyte recovery after treatment discontinuation compared with fingolimod, which is an important consideration for patients who need to interrupt medication.

**Ponesimod**

Ponesimod (Actelion, Basel, Switzerland) is an orally active selective S1P1 and S1P2 receptor that is eliminated within 1 week after discontinuation and its pharmacological effects are rapidly reversible.

### Table 1: Summary of sphingosine-1-phosphate receptor modulator pharmacokinetics and pharmacodynamics

<table>
<thead>
<tr>
<th>Receptor selectivity</th>
<th>Pro-drug (requires phosphorylation in vivo)</th>
<th>Tmax (h)</th>
<th>Time to lymphocyte count reduction (h)</th>
<th>Lymphocyte decrease from baseline (%)</th>
<th>T1/2 (h)</th>
<th>Time to lymphocyte count recovery after treatment discontinuation (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fingolimod</td>
<td>S1P1, S1P3, S1P4, S1P5</td>
<td>Yes</td>
<td>12–16</td>
<td>4–6</td>
<td>70</td>
<td>144–216</td>
</tr>
<tr>
<td>Ponesimod</td>
<td>S1P3</td>
<td>No</td>
<td>2–4</td>
<td>6</td>
<td>50–70</td>
<td>30</td>
</tr>
<tr>
<td>Siponimod</td>
<td>S1P5</td>
<td>No</td>
<td>3.0–4.5</td>
<td>4–6</td>
<td>33–76</td>
<td>30</td>
</tr>
<tr>
<td>Ozanimod</td>
<td>S1P1, S1P3</td>
<td>No</td>
<td>6–8</td>
<td>6–12</td>
<td>34–68</td>
<td>17–21</td>
</tr>
<tr>
<td>Amiselimod</td>
<td>S1P1, S1P3</td>
<td>Yes</td>
<td>12–16</td>
<td>No data</td>
<td>60–66</td>
<td>380–420</td>
</tr>
</tbody>
</table>

h = hour; S1P1–5 = sphingosine-1-phosphate receptor subtypes 1–5; T1/2 = elimination half-life; Tmax = time to maximum plasma concentration.
In a double-blind, placebo-controlled, dose-finding phase IIb study (n=464), once-daily treatment with ponesimod 10, 20 or 40 mg significantly reduced the number of new T1 Gd+ lesions and ARR, as well as increasing the time to first confirmed relapse compared with placebo (6.2). Mean ARR was 53% lower with 40 mg ponesimod versus placebo (0.25 versus 0.53; p=0.0363). Time to first confirmed relapse increased with ponesimod compared with placebo.

In all ponesimod groups, the majority of AEs were mild or moderate in intensity and the proportions of patients who had one or more AE during the treatment period were similar across all ponesimod and placebo groups. Frequently reported treatment emergent AEs (TEAEs) with a higher incidence in the ponesimod groups compared with placebo were anxiety, dizziness, dyspnoea, increased ALT, influenza, insomnia and peripheral oedema. Incidences of dyspnoea and peripheral oedema appeared to be dose related, with substantially more cases reported in the ponesimod 40 mg group compared with the ponesimod 10 and 20 mg groups. During the treatment period, a total of 27 serious AEs (excluding hospitalisations for MS relapse) were reported. These included two malignancies: one in the ponesimod 10 mg group and one in the placebo group. Cardiac AEs associated with ponesimod treatment initiation included first-degree and second-degree atrioventricular block (AVB) and bradycardia. All AEs related to heart rate and rhythm occurred on day 1; there was no need for intervention and no recurrence of these AEs later during treatment. Among patients receiving ponesimod who discontinued due to cardiac AEs, 2.6% required treatment compared with none in the placebo group. The proportion of patients with one or more infection-associated AE was similar across the four groups. There were no treatment discontinuations due to lymphopaenia. The proportion of patients with one or more respiratory AE was higher in the ponesimod groups compared with placebo (73.9–77.2%) and placebo (74.4%). Frequent TEAEs with a higher incidence in ponesimod groups were anxiety, dizziness, dyspnoea, increased ALT, influenza, insomnia and peripheral oedema. Some cardiac and respiratory TEAEs, but all resolved on treatment discontinuation.
than in the placebo group (ponesimod 10 mg, 9.3%; ponesimod 20 mg, 16.7%; ponesimod 40 mg, 31.9%; placebo, 6.6%), leading to premature discontinuation of ponesimod in seven patients. The onset of dyspnoea usually occurred within the first month of treatment; all cases resolved. A dose-dependent decrease in forced expiratory volume in 1 second (FEV1) was also observed with ponesimod treatment.41

In a small study (n=16) in healthy subjects, ponesimod treatment led to a marked reduction in overall T- and B-cells, with a dramatic reduction in the number of CD4+ cells, whereas CD8+ and natural killer (NK) cells were less affected.42 A phase III study is currently ongoing to investigate the efficacy and safety of ponesimod in 1,100 patients with RMS (NCT02425644). The primary objective of the trial is to assess whether ponesimod is superior to teriflunomide in reducing the ARR over 108 weeks. This study will be the first to compare the efficacy and safety of two oral treatments in RMS patients. Another ongoing phase III study, the Clinical Study to Compare the Efficacy and Safety of Ponesimod to Placebo in Subjects With Active Relapsing Multiple Sclerosis Who Are Treated With Dimethyl Fumarate (Tecfidera®) (POINT) is evaluating ponesimod as add-on therapy with dimethylfumarate (DMF) in patients in patients who have received DMF for at least 6 months prior to commencing the study (NCT02907177).

Ozanimod
Ozanimod (formerly RPC1063, Celgene) is an orally active selective S1P1, and S1P5, modulator that induces lymphopenia and regulates immune response.36,43 It was evaluated in a phase II/III randomised, multicentre trial (n=258), Efficacy and Safety Study of RPC1063 in Relapsing Multiple Sclerosis Patients (RADIANCE). The number of Gd+ enhancing lesions were significantly lower with ozanimod compared with placebo.43

Two recent phase III studies have evaluated two doses of oral ozanimod compared with IFNβ-1a in people with relapsing-remitting MS (RRMS). Two-year findings showed significant reduction in ARR for ozanimod 1.0 mg (ARR=0.28).44 There was also a significant reduction in β-1a (ARR=0.17, p<0.0001) and 0.5 mg (ARR=0.22, p=0.0168) compared with IFNβ-1a in 1,313 patients with RMS. The number of new or enlarging T2 lesions compared with IFNβ-1a in ARR. The number of new or enlarging T2 lesions and the adjusted mean number of Gd+ at month 12 demonstrated a significant reduction for both ozanimod groups compared with IFN β-1a. The rate of discontinuation due to AEs was also low and similar across treatment groups. No first dose, clinically relevant cases of bradycardia and no AVB of second degree or higher were reported.

Amiselimod
Amiselimod (formerly MT-1303, Mitsubishi Tanabe Pharma, Japan) is a potent S1P1, modulator that also shows high selectivity for S1P1, receptors. In a phase II trial of patients (n=415) with active RRMS, amiselimod 0.2 mg and 0.4 mg significantly reduced the total number of Gd+ T1-weighted lesions after 24 weeks of treatment (patients treated with 0.1 mg amiselimod had a similar number of these lesions compared with the placebo group). ARRs were lower with amiselimod 0.2 and 0.4 mg than with placebo, although the difference was significant only in the 0.4 mg group (n=104). Brain volume loss was similar in the amiselimod and placebo groups, although reductions in grey matter volume were significantly smaller with all amiselimod doses than with placebo (n=103).45 The incidence of TEAEs, including infections and cardiac disorders, were similar in the amiselimod treatment groups (56% of the 0.1 mg group, 67% of the 0.2 mg group, and 56% of the 0.4 mg group) to the incidence in the placebo group (64%); the most common TEAEs were headache and nasopharyngitis. No serious TEAE was reported for more than one patient in any group and no clinically significant heart rate reduction was observed at any amiselimod dose.46 A recent study found that amiselimod showed high potency with minimal cardiac effects at the anticipated clinical dose and is unlikely to require dose titration.47

Siponimod
Siponimod (Novartis, Basel, Switzerland) is a novel alkoxymino derivative that binds to both S1P1 and S1P5, its half-life is relatively short, allowing for fast immune reconstitution.48 Its efficacy and safety was investigated versus placebo in the phase III Exploring the Efficacy and Safety of Siponimod in Patients With Secondary Progressive Multiple Sclerosis (EXPAND) study in 1,651 patients with SPMS, a condition for which treatment options are limited. Siponimod reduced the risk of 3-month confirmed disability progression (CDP) by 21% versus placebo (hazard ratio [HR]: 0.79; p=0.013). Siponimod also reduced the risk of 6-month CDP by 26% (p<0.001). Siponimod 10 mg reduced the risk of T2 lesion number by 56% (p<0.0001) and new T2 lesion number by 42% (p=0.002).49 At least one TEAE was reported in 88.7% in the siponimod group and 83% taking IFNβ-1a. The majority were mild; the most common AEs across all treatment groups were nasopharyngitis, headache, increased ALT, influenza-like illness, hypertension, increased gamma-glutamyl transferase, pharyngitis and urticaria tract infection. Incidences of ALT increase were low, transient and generally resolved without study drug discontinuation. The overall incidence of serious AEs was low and similar across treatment arms. Discontinuation of study drug due to AEs occurred in 4.3% of the ozanimod 1.0 mg group, 10% of the ozanimod 0.5 mg group, 7.3% of ozanimod 0.2 mg group, and 3.1% of ozanimod 0.1 mg group. No second degree or higher AVBs were observed. Serious cardiac AEs occurred in 0.0% for ozanimod 1.0 mg, 0.7% for ozanimod 0.5 mg and 0.5% for IFN β-1a groups. Infection rates were similar across treatment arms; serious infection rates were low and similar across treatment arms, with no serious opportunistic infections.

Recently, positive results were announced from the phase III Study of RPC1063 in Relapsing MS (SUNBEAM, n=1,346).45 Both ozanimod 0.5 and 1.0 mg treatment groups demonstrated statistically significant reductions compared with IFN β-1a in ARR. The number of new or enlarging T2 lesions and the adjusted mean number of Gd+ at month 12 demonstrated a significant reduction for both ozanimod groups compared with IFN β-1a. The rate of discontinuation due to AEs was also low and similar across treatment groups. No first dose, clinically relevant cases of bradycardia and no AVB of second degree or higher were reported.

Discontinued sphingosine-1-phosphate 1 agents
Although successful in phase II clinical trials, clinical development of the S1P, modulator ceralifimod (Merck KGaA, Darmstadt, Germany) was halted after the premature discontinuation of a phase III study. Clinical development of CS-07771 and GSK2018682 for MS reached phase I stage but appears to have been discontinued since.50 Possible reasons are competition from emerging therapies.

Summary and concluding remarks
The discovery of the ability of S1P receptors and their modulators to block immune cell trafficking led to the regulatory approval of fingolimod, the first orally active drug treating RMS. This has stimulated research into
more selective S1P receptors, which have improved pharmacodynamics and are less likely to cause off-target AEs than fingolimod. These include selective S1P1, modulator (ponesimod) and dual agonists on S1P1 and S1P5 (siponimod, ozanimod, amiselimod). Selective S1P receptor agonists offer a convenient alternative to other MS drugs that are associated with broad immune suppression, as well as the potential for benefit in a number of autoimmune and inflammatory conditions such as psoriasis, Crohn’s disease, ulcerative colitis, polyarthritis, dermatomyositis, liver failure, renal disease, acute stroke and transplant rejection. While long-term safety data of selective S1P receptor agonists are needed, the growing body of such data on the efficacy and safety of fingolimod is reassuring. It is likely that, in the near future, more S1P receptor modulators will be approved for the treatment of MS and other disorders associated with autoimmunity and inflammation.


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