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An Update on Sphingosine-1-Phosphate Receptor 1 Modulators

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Abstract

Sphingolipids represent an essential class of lipids found in all eukaryotes, and strongly influence cellular signal transduction. Autoimmune diseases like asthma and multiple sclerosis (MS) are mediated by the sphingosine-1-phosphate receptor 1 (S1P₁) to express a variety of symptoms and disease patterns. Inspired by its natural substrate, an array of artificial sphingolipid derivatives has been developed to target this specific G protein-coupled receptor (GPCR) in an attempt to suppress autoimmune disorders. FTY720, also known as fingolimod, is the first oral disease-modifying therapy for MS on the market. In pursuit of improved stability, bioavailability, and efficiency, structural analogues of this initial prodrug have emerged over time. This review covers a brief introduction to the sphingolipid metabolism, the mechanism of action on S1P₁, and an updated overview of synthetic sphingosine S1P₁ agonists.

Graphical Abstract

Keywords

Sphingolipids; Sphingosine-1-phosphate; Sphingosine-1-phosphate receptor 1; Autoimmune modulators; S1P₁ agonists

Sphingolipids constitute a vast family of lipids present in all eukaryotic organisms, including plants, and are part of a dynamic homeostasis constantly maintaining an array of biologically active metabolites, responsible for regulating numerous cellular signaling pathways.^{1,2} Identified and isolated from bovine brain matter in 1884, its discoverer

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Thudichum named the archetypical lipid sphingosine after the Greek mythological creature Sphinx due to its “enigmatic nature”.³ Despite Klenk and Diebold finding the chain length of sphingosine to be a C18 chain in 1931, it was not until 1942 when Herbert Carter and coworkers determined the chemical structure of sphingosine by assigning the pattern of hydroxyl substituents on the linear carbon backbone.^{4,5}

Sphingosine is a key component of a complex lipid metabolism continuously forming and degrading bioactive metabolites and serves as the biosynthetic substrate for an array of diverse sphingolipids (Scheme 1).⁶ The dynamic balance between sphingolipid biosynthesis and degradation is determined by the concentrations of the lipids in distinct cellular compartments,⁷ and most of the enzymes responsible for the metabolism of these lipids are also membrane-bound. The concept of “membrane compartmentalization” is extremely important for a complete understanding of sphingosine biology. *De novo* sphingolipid biosynthesis starts on the cytoplasmic side of the endoplasmic reticulum (ER) where L-serine and palmitoyl-CoA are condensed by serine palmitoyltransferase to produce 3-ketosphinganine.^{8–10} Reduction of 3-ketosphinganine to dihydrosphingosine, a.k.a. sphinganine, is followed by *N*-acylation by one of six known ceramide synthases (CerS1–CerS6), providing dihydroceramides that differ in their acyl chain lengths, ranging from C14 to C26.^{1,11} If sphinganine is *N*-acylated with palmitic acid, dihydroceramide is formed. Oxidation of the C4 position by dihydroceramide desaturase introduces the double bond to produce ceramide, a key metabolite in sphingolipid homeostasis.¹¹ Depending on which polar head group is attached at the C1 hydroxyl position, a myriad of higher sphingolipids can be generated from ceramide in the Golgi apparatus—each stack of which may be regarded as a separate membrane compartment. Attachment of a phosphatidyl choline group at the primary hydroxyl group leads to sphingomyelin, whereas glycosylation leads to glucosylceramide, the simplest glycosphingolipid that can produce much more complex gangliosides by subsequent action of other glycosyltransferases.^{12,13} Reversible deacylation of ceramide by ceramidase is the only proven pathway for the biosynthesis of sphingosine, so far, and provides a dynamic balance point between sphingosine and ceramide.^{1,14} Sphingosine itself is phosphorylated by sphingosine kinases type 1 and 2 (SK1, SK2) to form sphingosine-1-phosphate (S1P), which can be dephosphorylated by the S1P-specific sphingosine-1-phosphate phosphatase type 1 and 2 (SPP1, SPP2) in the ER.^{1,15} Also located in the ER, the pyridoxal-dependent S1P lyase is responsible for the irreversible degradation of S1P into phosphoethanolamine and hexadecenal.^{16,17}

Ceramide as a regulatory nexus:

Ceramide, sphingosine, and S1P are all essential key substances for the regulation of cell growth and proliferation, and also provide for intimate regulation of vascular and epithelial integrity.^{7,18,19} S1P in particular strongly influences cell survival; it plays a significant role in chemotaxis, angiogenesis, vascular maturation, receptor-specific regulation endothelial barrier integrity and vascular permeability.^{20–22} S1P mobilizes internal calcium sources independently of inositol triphosphate, known to be essential for proliferation and to suppress apoptosis.^{23–26} The most intriguing aspect of S1P is its significance for innate and adaptive immunity which includes regulation of immune responses, immunosurveillance, and leukocyte differentiation and lymphocyte trafficking by binding to one of five known G

protein-coupled receptors (GPCRs) S1P₁₋₅.^{18,27,28} Agonism of the sphingosine-1-phosphate receptor 1 (S1P₁) poses a major gateway for the activation of the immune system by regulating differentiation, egress, and migration of a variety of immune cells, including macrophages, mast cells, natural killer cells, dendritic cells, neutrophils, and haematopoietic precursors.^{18,29} Differentiation, recirculation, and trafficking of T and B lymphocytes, in particular, plays a central role in the development of autoimmune diseases like asthma and multiple sclerosis and is mediated through S1P, making S1P₁ a pharmacological target of high interest.²⁹

Since three of the five known S1P receptor types are expressed ubiquitously, specificity of drugs targeting S1P receptors is crucial; the five S1P receptors display vastly different physiological effects upon activation.^{30,31} S1P₄ and S1P₅ occur only in distinct kinds of cells, however, S1P₁₋₃ (*a.k.a.* Endothelial Differentiation Genes EDG1, EDG2 and EDG3) exhibit omnipresent expression,³¹ and the role of these receptors have been extensively studied in the context of angiogenesis. Tight regulation of these three receptors is required for both angiogenesis and vascular homeostasis.²² Agonism of S1P₁ and S1P₃ by S1P produces different effects on endothelial cell barrier integrity with S1P₁ linked to Rac GTPase activation of the actin cytoskeleton and barrier enhancement.^{32,33} In contrast, ligation of S1P₃ is linked to Rho-dependent cytoskeletal rearrangement and barrier disruption.³²⁻³⁵ Both receptors trigger random spreading as well as chemotactic cell migration, and produce concentration-dependent effects. Activation of S1P₁ in particular aids the egress of T and B lymphocytes from lymphoid organs in the case of an immune response and controls migration of a variety of cell types, including endothelial and smooth muscle cells, which plays a central role in angiogenesis.^{29,36,37} Binding to S1P₂, however, abolishes cellular motility through downregulation of Rac activity and membrane ruffling, and favors retention of B leukocytes in germinal centers.³⁸

Synthetic sphingosine-1-phosphate analogues:

The majority of synthetic drugs are designed to be S1P₁ specific, as disease-induced agonism of S1P₁ is responsible for symptoms of the most prominent autoimmune diseases like asthma and MS.^{39,40} The most prominent drug for autoimmune disease treatment is FTY720, also known as fingolimod (Table 1); discovered by Yoshitomi Pharmaceutical Industries in 1992 and approved by the FDA in 2010, this derivative of the natural sphingolipid product myriocin (ISP-I) found in the fungus *Isaria sinclairii* acts as an immunosuppressant that binds competitively to S1P receptors, initiating the GPCR's internalization and subsequent ubiquitination, which leads to its degradation in a proteasome.⁴⁰⁻⁴² The decreasing number of S1P₁ receptors renders affected cells unresponsive to S1P, which in turn ceases differentiation and egress of lymphocytes that would otherwise target body's own tissues.⁴³⁻⁴⁵ In MS, this significantly reduces the frequency and severity of relapses.⁴⁶ In a clinical study on healthy human subjects a 1 mg dose of FTY720 decreased peripheral blood lymphocyte concentration by 38 ± 9% over the first 2 days before returning to baseline level over a week.⁴⁷ However, FTY720 displays various side effects in humans that correlate with its receptor promiscuity; the monophosphate acts as a substrate for all S1P receptors except S1P₂.^{43,48} Agonism of S1P₁ was reported to cause bradycardia, and activation of S1P₃ has been linked to the

development of hypertension.^{49,50} The original hypothesis was that bradycardia observed in humans with FTY720 was solely due to agonism of S1P₃, based on rat studies. Later clinical studies with S1P₃-sparing S1P₁ agonists such as siponimod or ponesimod also showed bradycardia, indicating that S1P₁ also plays a CV role in humans. Other common adverse effects experienced by patients treated with fingolimod are headache, fatigue, diarrhea, and nausea.⁵¹

Fingolimod is an orally-administered sphingosine analogue, and its biological activity at S1P₁ depends on *in vivo* phosphorylation to provide the agonist (*S*)-FTY720-monophosphate.^{41,52} This limits the effectiveness of the prodrug, as its phosphate is prone to the same phosphatases SPP1–2 that dephosphorylate S1P.⁵³ To overcome this weakness, Lu *et al.* developed non-hydrolyzable phosphonate and vinylphosphonate analogues in 2009.⁵⁴

Replacement of the phosphoester oxygen with a carbon atom and simultaneous introduction of the phosphorous-containing group renders the compound resistant to lipid phosphatases and grants it immediate bioavailability.⁵⁴ Introduction of the (vinyl-) phosphonate moiety introduces chirality into the compound, which proves to be crucial for its desired pharmacological effects; while the racemic ($\pm$) mixture of FTY720-phosphonate does display potent S1P₁ affinity that is comparable with the (*S*)-FTY720-phosphate agonist. Neither the pure (*R*) enantiomer of the unsaturated vinylphosphonate or the saturated phosphonate exhibit antiapoptotic effects on IEC-6 cells treated with the topoisomerase inhibitor CPT.^{55,56} The (*S*) isomers, however, show significant decrease of CPT-induced DNA fragmentation in treated cells, with the phosphonate being more potent (50% DNA fragmentation decrease) than the vinylphosphonate (21%). The EC₅₀ of FTY720-(*S*)-phosphonate is reported as 75 $\pm$ 21 nM.⁵⁴

The importance of the proper chiral form of constitutionally identical sphingosine-derived drugs is illustrated by the clinically relevant glycosphingolipid modulator and anti-tumor agent D-*threo*-PDMP (Figure 1).^{57,58} While D-*threo*-PDMP inhibits the formation of gangliosides by blocking glucosylceramide synthase, its enantiomer L-*threo*-PDMP causes precisely the opposite effect by increasing the biosynthesis of glycosphingolipids.^{59–61}

Synthesized in 1998 as a close derivative of FTY720, the chiral sphingosine analogue AAL-R also relies on *in vivo* phosphorylation in order to produce an antagonist for all S1P receptors except for S1P₂.^{63,64} The much more potent (*R*) enantiomer acts as an immunosuppressant by inhibiting virus-induced chemokine and cytokine production.^{64,65}

AAL-R displays its bioactivity through suppression of cytokines IL-1 α , IL-1 β , IL-6, IL-10, MCP1, and TNF α .^{66,67} Its stimulation of dendritic cell (DC) maturation and induction of T cell activation during lymphocytic choriomeningitis virus (LCMV) infection has also been reported.^{66,68} AAL-R proved to be particularly effective in the treatment of external infections like *Bordetella pertussis*.⁶⁹

Based on structure–activity relationship (SAR) studies performed on FTY720-phosphate, Clemens *et al.* and Davis *et al.* designed the fingolimod-phosphate derivatives VPC22277 and VPC22173 as well as its *meta* equivalent VPC23019.^{70,71} While VPC22277 showed clear S1P₁ and S1P₃ agonism by inhibiting T24 cell migration, VPC23019 displayed S1P_{1/3}

antagonism.⁷⁰ The *meta* analogue proved to be a potent competitive antagonist for S1P₁ and S1P₃, was unselective towards S1P₂, and acted as an agonist at S1P₄ and S1P₅.^{71,72} However, VPC23019 displayed a short half-life due to rapid dephosphorylation of the pre-installed phosphoester *in vivo*.⁷¹ The respective phosphonate analogue VPC44116 (Table 1) showed the expected longer cellular stability in *in vivo* experiments with rodents due to resistance towards SPP1–2.⁷³ In 2011, Kennedy *et al.* reported the cyclobutane derivative VPC03090.⁷⁴ The sphingosine analogue VPC03090 is phosphorylated by SK2 to produce the corresponding phosphate, which acts as a competitive antagonist for S1P₁ and S1P₃.⁷⁴ Shortening the lipophilic chain from C8 to C6 increases S1P₁ selectivity dramatically; compound W146 is a highly selective S1P₁ antagonist that does not interact with S1P₂, S1P₃, or S1P₅ (S1P₄ was not included in the essays), systematically blocking S1P₁ in the vascular system. This caused transient lymphopenia and simultaneous increases of CD4⁺ and CD8⁺ leukocyte populations in lymph nodes in rodent experiments.^{75,76} While being equipotent to its corresponding phosphate ester equivalent, the phosphonate W146 displays much greater *in vivo* stability with only the depicted (*R*) enantiomer being bioactive.^{71,75}

In 2005, Shimizu *et al.* investigated the effects of KRP-203 on immunomodulation in rodent allografts (Table 1). An analogue of FTY720, KRP-203 was found to enhance rat skin and heart allografts significantly; skin transplants of KRP-203–treated rats survived for approximately 10–20 % longer than subjects treated with FTY720; and KRP-203–treated mHC–disparate rat heart allografts were reported to experience no rejection at all.⁷⁷ Further findings include KRP-203–driven reduction of ET-1 and TGF- β ₁ secretion as macrophage mediators responsible for transplant arteriosclerosis; prevention of myocardial fibrosis after heart transplantation in rat models; as well as the *reduction* of CD4⁺ and CD8⁺ T lymphocyte concentrations in peripheral lymphoid organs.^{77–80}

Structural diversity of S1P₁ modulators:

S1P receptor interaction is not restricted to substrates strictly similar to S1P; agonism as well as antagonism are also accomplished with compounds that significantly differ in their structural composition from the natural substrate or its first artificial derivative fingolimod; NIBR-0213 marks a readily bioavailable compound with good bioavailability after oral doses and high selectivity for S1P₁ (Table 2).⁸¹ Potency, competition, and selectivity of NIBR-0213 for S1P₁ over S1P_{2–4} were confirmed by Quencard *et al.* in 2012 by Ca²⁺ mobilization assays.⁸¹ Unlike other S1P₁ antagonists like VPC11446 or W146, NIBR-0213 effectively reduces peripheral blood lymphocyte (PBL) counts as shown in experimental autoimmune encephalomyelitis (EAE), a common model for human MS.^{75,82–84} However, the therapeutic application of NIBR-0213 is limited, as it is less potent than FTY720: while oral administration of 0.1 mg/kg *q.d.* of fingolimod reduces PBL count by 80–86%, 30 mg/kg *b.i.d.* of orally administered NIBR-0213 gave a PBL count reduction of solely 75–85%.⁸⁵ In 2006, Pan *et al.* reported the discovery of an aminocarboxylate analogue of FTY720: AUY954 is highly receptor–selective with an EC₅₀ value of 1.2 nM exclusively at S1P₁.⁸⁶ AUY954 was found to induce significant and reversible reduction of peripheral circulating lymphocytes, as well as Erk and Akt kinase activation in Chinese hamster ovary (CHO) cells transfected with human S1P₁.⁸⁶ In 2011, further studies of AUY954 by Zhang *et al.* showed this compound is potent in the prevention of experimental autoimmune neuritis

(EAN) and in the inhibition of paraparesis.⁸⁷ Depression of lymphocyte and macrophage infiltration decreases local demyelination and suppresses expression of the cytokine Interleukin-17 (IL-17) as well as the matrix metalloproteinase MMP-9, which accounts for T lymphocyte blood nerve barrier (BNB) penetration during an inflammatory autoimmune response.^{87,88}

Pennington and Harrington *et al.* described a series of novel S1P₁ agonists based on the S1P receptor selective carbamoylnicotinamide in 2011 and 2012.^{89,90} The compound is characterized by its remarkable lack of a polar head group. Thus, it does not require bioactivation by phosphorylation, and yet is highly specific for S1P₁, with a >100-fold selectivity relative to S1P₂₋₅.^{65,89,91} The carbamoylnicotinamide synthesized by Pennington *et al.* causes substantial depletion of lymphocyte circulation (78–81% depletion in rats) 24 hrs after a single oral dose of 1 mg/kg, and has an EC₅₀ value of 35 nM with 96% efficacy.⁸⁹ Respective quinolinone derivatives were reported to inhibit lymphocyte recirculation and egress with equivalent S1P₁ selectivity.⁹⁰

The compound SEW2871 marks another highly selective S1P receptor substrate; with an EC₅₀ value of 13 ± 8 nM on S1P₁ and no S1P₂₋₅ interaction at a concentration of 10 μM in CHO cell lines, SEW2871 proved to be an exclusive S1P₁ agonist.⁸² The structurally S1P-deviant, highly nonpolar compound is reported to prolong murine heterotopic heart allograft survival through induction of lymphopenia and reduced allograft expression of inflammatory cytokines TNF-α, IFN-γ, and IL-2.^{82,92} Furthermore, SEW2871 is known to induce *in vitro* cellular migration through detectable cytoskeletal reorganization in S1P₁-transfected CHO cells and to reduce the rate of infection in C47BL/6 mice exposed to *Yersinia pestis*.^{82,93} However, clinical use of SEW2871 requires caution, as SEW2871 was reported to aggravate reperfusion arrhythmias. Tsukada *et al.* reported severe side effects in an *in vivo* rodent model with a risk for myocardial ischemia, resulting in hazards for potential patients. This may be due to SEW2871's distinct receptor specificity; extremely specific deactivation of solely a single S1P receptor type might lead macroscopically to a more severe collapse of a balanced signaling cascade between multiple S1P receptor types.⁹⁴

In 2011 Cee *et al.* reported the synthesis and activity of AMG 369, a compound with a carboxylic acid head group that does not require activation for bioavailability.⁹⁵ AMG 369 acts as a potent S1P₁ and S1P₅ agonist, showing low S1P₃ activity and being inactive towards S1P₂ and S1P₄.⁹⁵ The absence of cardiovascular effects even at concentrations as high as 10 mg/kg of AMG 369 in rats further indicate the correlation of bradycardia with S1P₃ agonism in the rodent model.^{82,95} Oral bioavailability was found to range between 34–64% in rat, dog, and non-human primate experiments, and a depletion of PDL counts 24 hrs after oral administration of 0.1 mg/kg led to a significant delay of EAE onset in the rat model.⁹⁵

The pursuit for greater S1P receptor specificity in order to minimize adverse effects in medical application resulted in the patented synthesis of a compound designated as BAF312 in 2004 by Pan *et al.*⁹⁶ Commercially named siponimod by Novartis and structurally very distant from FTY720, the compound displays potent agonism on S1P₁ and S1P₅ under complete S1P₃ sparing.⁹⁷ Similar to FTY720, BAF312 causes S1P₁ internalization, resulting

in the decrease of CD4⁺ T lymphocytes, naïve T cells, and B lymphocytes by 88% within 4–6 hrs after administration.⁹⁸ The effective dose for 50% depletion of original lymphocyte concentration after 6 hrs post administration is 0.14 mg/kg, with an 88% PDL count decrease 8 hrs after administration.⁹⁷ With its average half-life in human plasma being 56.6 hrs, siponimod has a shorter duration of action than fingolimod and its phosphate, which is reported to be 6–9 days.^{99,100} Hence, PBL counts are reported to return to original levels after 48h.⁹⁷ siponimod led to the finding that bradycardia is not an adverse effect exclusive to S1P₃ agonism: despite its total lack of affinity to S1P₃, BAF312 was found to induce transient bradycardia by activating G protein–coupled inwardly rectifying potassium (GIRK) channels in atrial myocytes.⁹⁸ Furthermore, siponimod easily trespasses the blood–brain barrier (BBB), entering the central nervous system (CNS) where it can act on S1P₁ of astrocytes and S1P₅ on oligodendrocytes.¹⁰¹ Siponimod passed phase II clinical studies on secondary progressive multiple sclerosis (SPMS) patients, where it proved to reduce new or newly enlarged T2 lesions up to 80% when administered orally on a daily 1.25–2 mg basis.^{99,102} Subsequent phase III clinical studies showed substantial reduction of disability progression in SPMS patients when compared to placebo, making siponimod an attractive novel autoimmune modulator for MS treatment.¹⁰³ Hence, Novartis currently seeks U.S. Food and Drug Administration (FDA) approval in the US for siponimod as a certified drug for SPMS treatment; application for EU approval by European Medicines Agency (EMA) is planned later in 2018.¹⁰⁴

Featuring close structural similarity with siponimod, ONO-4641 was another orally administered S1P receptor modulator and potential MS drug candidate for treatment of relapsing remitting multiple sclerosis (RRMS).^{105–107} Developed by Ono Pharmaceutical Co., the compound designated as ceralifimod features close structural similarity with siponimod, also acting specifically on S1P₁ and S1P₅.^{106,107} In an EAE model, ceralifimod caused retention of T and B lymphocytes in lymph nodes, leading to the decrease of lymphocytic infiltration of the CNS, which resulted in the retardation of the disease onset in rats.¹⁰⁸ In particular, depletion of CD3⁺, CD4⁺, CD8⁺, CD4⁺/CD25(high) lymphocytes is induced. Natural killer (NK) cell concentrations, however, are not affected by ONO-4641. The half-life of ceralifimod is reported to range between 81.9–89 hrs, being much more short-lived than the original fingolimod.¹⁰⁶ Ceralifimod did enter clinical phase II trials as a candidate for treatment of RRMS patients starting in 2010; the compound was found to significantly reduce MRI lesion formation and diminish the frequency of relapses in RRMS patients within a period of 6 months when administered in doses ranging from 0.05–0.15 mg per day.^{109,110} Despite promising preliminary data, Merck ceased further development ceralifimod with Ono Pharmaceutical Co. in 2014 due to “changes in market circumstances of MS treatment” and in anticipation of large phase III expenses.¹¹¹

Actelion Pharmaceuticals Ltd. developed a diol–based, potent, orally administrable, highly selective S1P₁ agonist, ACT-128800, or ponesimod.^{112,113} Preliminary studies showed that ponesimod, which does not require phosphorylation for its bioactivity, caused maximal reduction of lymphocyte egress at an oral dose of 3 mg/kg in rats and displayed 69% bioavailability in beagle dogs.¹¹³ An advantage of ponesimod towards fingolimod is the rapid lymphocyte recovery upon discontinuation of the agent; lymphocyte concentrations are reported to return to basic levels 36 hrs after halting ponesimod treatment even at doses as

high as 100 mg/kg.¹¹³ Phase I studies determined the dose-dependence of ponesimod as the decrease in lymphocyte counts was proportional to the dose administered.¹¹⁴ In a clinical phase II trial ponesimod proved to induce an average lymphocyte depletion by 62% after 8 days when administered in daily doses of 20 and 40 mg orally to RRMS patients, resulting in a significant decrease of new T1 lesions from 6.2 (placebo) to 1.1 (ponesimod).¹¹⁵ Since June 2015, ponesimod has been evaluated in a clinical phase III trial for treatment of RRMS and secondary progressive MS (SPMS), as it delivers promising results in terms of reduction of MS relapses, low variability, and good tolerance.^{114–116}

Most recent synthetic S1P₁ agonists:

Another series of S1P₁-specific substrates based on oxadiazole moieties embedded in the very core of the molecule was synthesized to challenge autoimmune disorders. One compound created by Astellas Pharma Inc. is ASP4058 (Table 3).¹¹⁷ This relatively nonpolar compound missing a distinct head group displayed preferential agonism on S1P₁ and S1P₅ in GTPγS binding assays *in vitro*. ASP4058 is reported to reduce PBL counts with ED₅₀ values of 0.10 mg/kg 24 hrs after initial oral dosing and 0.023 mg/kg after 21 days of continuous treatment, making it initially less potent than fingolimod (EC₅₀(FTY720) = 0.041 mg/kg after initial oral dose) but equipotent over a longer period of time (EC₅₀(FTY720) = 0.020 mg/kg after 21 days).¹¹⁷ ASP4058 readily enters the CNS and is capable of reducing EAE onsets in rats and prevents relapses of RRMS in mice while displaying fewer safety concerns regarding bradycardia and bronchoconstriction in the rodent model.¹¹⁷

The mechanism of action of ASP4058 involves the internalization of S1P₁ as found in human carotid artery endothelial cells (HCtAECs) by Yamamoto *et al.*; 1 hour after *in vitro* treatment, HCtAEC S1P₁ expression is reported to drop to 86 ± 14% upon a 100 nM dose. A 1,000 nM treatment causes a S1P₁ expression decrease to 23 ± 4.3%.¹¹⁸ ASP4058 also promotes endothelial cell barrier integrity by inducing tightening of cell-cell junctions between HCtAECs and proved to suppress the formation of intracranial aneurysm (IA) in a non-human primate model (*Macaca fascicularis*).¹¹⁸ Despite these promising data, after a phase I study on healthy human subjects that started in 2010, Astellas Pharma Inc. decided to discontinue the development of ASP4058 in 2012 due to “strategic reasons” as well as results from the phase I trial.^{119,120}

Almost exclusive S1P₁ activity has been achieved with CYM5442, an allosteric S1P receptor agonist with 10,000-fold greater selectivity for S1P₁ over S1P₅.¹²¹ CYM5442 is reported to be approximately 10- to 50-fold more potent than SEW2871 and its S1P₁ agonism is noncompetitively inhibited by W146.¹²¹ *In vivo* studies in the murine model provided extensive lymphopenia data: CYM5442 elicits internalization, polyubiquitination, and degradation of S1P₁, resulting in dose-dependent depletion of B and T cells in blood. B220⁺ B lymphocyte counts decrease by 63% with an ED₅₀ value of 1.0 mg/kg, CD4⁺ T cell counts are diminished by 83% with an ED₅₀ value of 0.5 mg/kg, and CD8⁺ T lymphocytes are depleted by 84% with the ED₅₀ value being 2.0 mg/kg. CNS entry of CYM5442 has also been reported.¹²¹ The S1P₁ modulator proved to diminish the production of chemokines and the cytokines IFNα, CCL2, IL-6, TNFα, and IFNγ, as well as reducing the mortality of

mice with H1N1 infection by suppression of the innate immune response.¹²² CYM5442 was also found to retard but not prevent acute graft-*versus*-host disease (aGVHD) through reduction of macrophage infiltration in mice.¹²³ A close derivative of CYM5442 is compound RP-001, featuring a carboxylic acid instead of the terminal alcohol of CYM5442 as well as isopropoxy and nitrile substituents at the aromatics instead of two former ethoxy moieties. S1P₁ agonism of RP-001 proved to be highly potent with an EC₅₀ value of 9 pM; S1P₂₋₄ activity is reported to be low, S1P₅ agonism is considered moderate.¹²⁴ Unlike CYM5442, RP-001 acts as a competitive S1P₁ substrate with W146.¹²⁴ The mechanism of action on the receptor is similar, as RP-001 agonism on S1P₁ leads to internalization and degradation by polyubiquitination.¹²⁴ RP-001 causes dose-dependent lymphopenia of CD4⁺ T cells in particular (EC₅₀ = 0.03 mg/kg) with maximal lymphocyte sequestration from blood after 2 hrs post treatment; rapid lymphocyte level recovery has been observed as lymphocyte counts return to basic levels 8h after a final dose of 0.3 mg/kg.¹²⁴ The short duration of lymphopenia as well as its half-life render RP-001 and its precursor impractical for clinical use.¹²⁴

Exchange of the carboxylic acid in RP-001 for a terminal hydroxyl group as well as shortening of its free tail chain by a single methylene group leads to the presumably most prominent potential drug candidate for autoimmune disorders: Developed by Celgene, RPC1063, trivially named ozanimod, represents one of the most potent, receptor-specific S1P₁ modulators.¹²⁵⁻¹²⁷ Displaying a similar mechanism of action based on S1P receptor internalization, followed by its degradation, ozanimod has proven to reduce inflammation in preliminary EAE animal experiments through reduction of cytokine production as well as significant depletion of circulating B lymphocytes, CD4⁺ CCR7⁺ T lymphocytes, and CD8⁺ CCR7⁺ lymphocytes.¹²⁶ When compared to fingolimod, siponimod, and ponesimod, ozanimod is superior in receptor specificity and reduction of adverse effects; its 10,000-fold greater agonism on S1P₁ than on S1P₃ contributes to ozanimod's small margin of cardiac side effects.¹²⁶ The compound does not require bioactivation through phosphorylation and readily permeates into the CNS where it engages in S1P₁ and S1P₅ agonism. EC₅₀ values range from 0.16 ± 0.06 nM on S1P₁ cAMP generation receptor and 0.41 ± 0.16 nM in S1P₁ GTPγS binding assays.¹²⁶ Ozanimod has successfully completed clinical phase II SUNBEAM trial and combined RADIANCE phase II/III studies where it proved to significantly reduce the average cumulative number of magnetic resonance imaging (MRI) lesions in MS patients to 1.5 with treatment, compared to 11.1 with placebo.^{127,128} Ozanimod has a half-life of 19 hrs in the human body and depleted lymphocyte counts recover 3 days after the final dose, making the drug candidate more attractive for variable treatment than fingolimod, which has a half-life of 168 hrs and a 4–8 week duration of lymphocyte recovery.¹²⁶ However, when Celgene filed a New Drug Approval (NDA) for ozanimod in February 2018, the Food and Drug Administration (FDA) rejected ozanimod as most of its efficacy in MS treatment is due to a metabolite *in vivo*, as disclosed by Celgene at the annual American Academy of Neurology meeting.^{129,130} The rejection occurred due to insufficient data regarding the metabolite, known as RP-101075, which accounts for 90% of ozanimod's efficacy due to similar agonism on S1P₁.^{125,129,130} After this setback, Celgene publicly announced another NDA for ozanimod in the first quarter of 2019.¹³¹

Modulation of the S1P receptor 1 through synthetic S1P-derived or structurally very distinct small molecule agents offers an alluring method of treatment for autoimmune diseases like MS. Since the discovery of fingolimod in 1992, a broad array of agonists and antagonists have been developed to target S1P receptors with an emphasis on S1P₁, as this receptor is most strongly linked to the presence of symptoms and the progression of autoimmune disorders. Ever since, receptor specificity and a proper time frame of physiological action of the potential drug candidates have been pursued. Interestingly, the development of S1P₁ modulators like SEW2871 showed that exclusive receptor specificity can also introduce side effects just as receptor promiscuity can since the S1P signaling pathway consists of a complex interaction of presumably multiple, if not all of the S1P receptor subtypes. Likewise, excellent pharmacological characteristics may not necessarily be accredited to the drug that was administered; the actual agonist responsible for the desired effect can be a metabolite formed *in vivo*, *c.f.* fingolimod or ozanimod. Taking this broader view is indispensable in the search for further optimized small molecule autoimmune modulators.

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Scheme 1.

Sphingolipid metabolism. Sphingolipid *de novo* biosynthesis begins with L-serine and palmitoyl-CoA being condensed to 3-ketosphinganine by 3-ketosphinganine synthase. 3-Ketosphinganine is then reduced by 3-ketosphinganine reductase and NADPH to sphinganine. *N*-Acylation of sphinganine through CoA-sphinganine acyl transferase with palmitic acid produces dihydroceramide which is then oxidized in its C4 position to ceramide by *N*-acyl sphinganine dehydrogenase in the presence of FAD. Ceramide serves as the basis for either sphingomyelin production by CMP-phosphorylcholine transferase or ganglioside biosynthesis through glycosylation with various sugars at the C1 hydroxyl position. Deacylation by ceramidase forms sphingosine, which can be converted back to ceramide when *N*-acylated with palmitic acid. Phosphorylation of sphingosine by sphingosine kinases 1 and 2 gives S1P, which can be also dephosphorylated by respective S1P phosphatases 1 and 2. Irreversible degradation of sphingolipids occurs through the breakdown of S1P by S1P lyase into phosphoethanolamine and hexadecenal. Hexadecenal is converted by fatty aldehyde dehydrogenase ALDH3A2 into hexadecenoate which in turn is a substrate for palmitoyl-CoA.^{1,6,8–17}

Figure 1.
D-threo-PDMP (a) and *L*-threo-PDMP (b).⁶²

Figure 2.
Metabolite RP-101075 of ozanimod.¹²⁵

Table 1.Fingolimod and FTY720-based S1P₁ agonists and antagonists.^{40–54,63–80}

Name	Structure
FTY720 (Fingolimod) ^{40–53}
(S)-FTY720-(<i>E</i>)-Vinylphosphonate ^{54–56}
(S)-FTY720-Phosphonate ^{54–56}
AAL-R ^{63–69}
VPC22277 ⁷⁰
VPC22173 ⁷⁰
VPC23019 ^{70,71}
VPC44116 ⁷³
VPC03090 ⁷⁴
W146 ^{75,76}
KRP-203 ^{77–80}

Table 2.

Structurally divergent S1P₁ modulators.^{81–116}

Name	Structure
NIBR-0213 ^{81–85}	
AUY954 ^{86–88}	
Carbamoyl-nicotinamide ^{89–90}	
SEW2871 ^{82,92–94}	
AMG 369 ⁹⁵	
BAF312 (Siponimod) ^{96–104}	
ONO-4641 (Ceralifimod) ^{105–111}	
ACT-128800 (Ponesimod) ^{112–116}	

Oxadiazole-based S1P₁ agonists.^{117–128}

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