



Review

Modulation of sphingosine-1-phosphate in inflammatory bowel disease

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ABSTRACT

Inflammatory bowel diseases (IBD), including ulcerative colitis and Crohn's disease, involve an inappropriate immune reaction in the digestive tract, causing a variety of disabling symptoms. The advent of monoclonal antibodies (anti-tumor necrosis factor, anti-integrin, anti-interleukin –23) has revolutionized IBD management. Nevertheless, these agents, with potential for immunogenicity, are associated with high rates of response loss and disease relapse over time. They are also associated with high production costs.

Sphingosine-1-phosphate (S1P), a membrane-derived lysophospholipid signaling molecule, is implicated in a vast array of physiological and pathophysiological processes, primarily via extracellular activation of S1P1–S1P5 receptors. S1P1, S1P4 and S1P5 are involved in regulation of the immune system, while S1P2 and S1P3 may be associated with cardiovascular, pulmonary, and theoretical cancer-related risks. Targeting S1P receptors for inflammatory conditions has been successful in clinical trials leading to approval of the non-selective S1P modulator, fingolimod, for relapsing forms of multiple sclerosis. However, the association of this non-selective S1P modulator with serious adverse events provides the rationale for developing more selective S1P receptor modulators. Until recently, three S1P modulators with differing selectivity for S1P receptors were in clinical development for IBD: ozanimod (RPC1063), etrasimod (APD334) and amiselimod (MT-1303). The development of amiselimod has been stopped as Biogen are currently focusing on other drugs in its portfolio. Following encouraging results from the Phase 2 TOUCHSTONE trial, a Phase 3 trial of the S1P modulator ozanimod in patients with moderate-to-severe ulcerative colitis is ongoing. Etrasimod is also being tested in a phase 2 trial in ulcerative colitis. These pipeline medications can be administered orally and may avoid the formation of anti-drug antibodies that can lead to treatment failure with injectable biologic therapies for IBD. Data from ongoing clinical trials will establish the relationship between the selectivity of S1P modulators and their safety and efficacy in IBD, as well as their potential place in the clinical armamentarium for IBD.

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Abbreviations: IBD, inflammatory bowel disease; MS, multiple sclerosis; PML, progressive multifocal leukoencephalopathy; RBS, rectal bleeding subscore; S1P, sphingosine-1-phosphate; TNF, tumor necrosis factor; ULN, upper limit of normal.

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1. Introduction

Inflammatory bowel diseases (IBD), including ulcerative colitis and Crohn's disease, are chronic, disabling conditions [1] that can cause progressive damage to the lining of the digestive tract [2]. Medical therapy for IBD aims to suppress the inappropriate inflammatory response, heal the lining of the digestive tract, maintain corticosteroid-free remission, and improve quality of life [2,3]. Pharmacotherapies for IBD include glucocorticoids, aminosalicylates, immunomodulators (thiopurines and methotrexate), as well as relatively newer biologic therapies, e.g. tumor necrosis factor (TNF) inhibitors, gut-selective integrin antagonists, and the recently Food and Drug Administration (FDA)-approved interleukin-12 and -23 inhibitor, ustekinumab [4–7]. However, these treatment options for IBD have limitations in terms of patient response, efficacy, side effects, and routes of administration, as well as being costly to use.

Poor efficacy with conventional therapies was highlighted in a recent, multicenter, European cohort study assessing disease burden and unmet clinical needs in adults with moderate-to-severe ulcerative colitis (Mayo score ≥ 6 , treatment excluding biologic therapies and surgery). Among the study population, 75% were receiving aminosalicylates and 63% were receiving thiopurines. A high proportion of the patients (87%) had uncontrolled ulcerative colitis and one quarter reported unmet clinical needs [3]. Almost half (48%) were dissatisfied with their current treatment, and moderate-to-severe symptoms were a predictor of this dissatisfaction [8].

Intravenously or subcutaneously administered biologic therapies, introduced nearly two decades ago [4], show good initial clinical response rates but are associated with high rates of response loss and disease relapse over time [9]. All monoclonal antibodies have the potential for immunogenicity and anti-drug antibodies are associated with an increased risk of losing response to therapy [9]. Costs associated with the biologics are also limiting the use of these agents [10]. A recent web-based survey of 1315 patients with Crohn's disease or ulcerative colitis, across 14 hospitals in the Netherlands, identified anti-TNF use as being the main driver behind healthcare costs in IBD, accounting for 64% and 31% of total costs among these respective patient groups [11].

Small molecule drugs, with molecular weights of <1 kDa (often below 500 Da), are able to diffuse easily through cell membranes, therefore providing potential advantages over the larger biologics in terms of route of administration, pharmacokinetic features, and antigenicity [12]. Furthermore, these small molecules are also simpler to produce, compared with the more complex production of biologics, and overall drug costs are expected to be lower [12]. Sphingosine-1-phosphate (S1P) receptor modulators are among these small molecule therapies currently in clinical development for IBD [4]. These novel, oral pipeline medications not only have the advantage of a more convenient route of administration, but also have the potential to avoid the formation of anti-drug antibodies, which requires the need for frequent testing and often leads to treatment failure.

This article reviews the physiological and pathophysiological roles of S1P and S1P1–5 receptor subtypes, and discusses how these may relate to the efficacy and safety of S1P modulators. It also offers perspectives on the development of the clinical armamentarium for IBD.

2. Molecular aspects of S1P action

S1P is a membrane-derived lysophospholipid signaling molecule [13]. Although intracellular roles for S1P have been described, it acts

primarily as an extracellular signaling molecule, activating five different subtypes of G protein-coupled receptors, S1P1–5 [13,14]. The expression, downstream signaling molecules, and functions of these five receptors are summarized in Fig. 1 [13,15–24]. S1P1–3 are widely expressed, whereas the expression of S1P4 and S1P5 is restricted to distinct cell types [13]. These receptors are involved in many physiological processes, and are particularly important for the regulation of the immune, cardiovascular, and nervous systems [13]. They have also been implicated in pathological conditions, and preclinical work has implicated theoretical risks such as cancer pathogenesis [13].

2.1. S1P1

In the immune system, S1P1 regulates the trafficking of lymphocytes out of the secondary lymphoid organs into the blood and lymph (Fig. 2; panel A) [13,25]. Naïve T-cells enter lymph nodes and egress in an S1P/S1P1-dependent mechanism through the sinus-lining endothelium via the efferent lymph into the blood [25]. However, when a productive antigen encounter occurs, the T-cells become activated and transiently down-modulate S1P1. This renders the cells unresponsive to the egress signal provided by S1P and the proliferating cells remain in the lymph node. Therefore, S1P1 activation leads to the sequestration of lymphocyte subpopulations in the peripheral lymphoid organs, preventing them from being trafficked to inflamed tissues, thereby modulating immunity [26].

Dendritic and endothelial cells also express S1P1, which may mediate effects on dendritic cell migration and vascular barrier function [15].

S1P1 may also play a role in nociception, acute bradycardia and proliferation [19,20–22,27]. In estrogen receptor-positive breast cancer cells, high expression of S1P1 has been linked with poor prognosis and decreased expression of pro-apoptotic markers [22,28].

2.2. S1P2 and S1P3

S1P2 often exerts cellular functions that are opposed to the functions of the S1P1 receptor, and the pro-inflammatory roles of the S1P2 receptor are well documented in the literature [13,29]. Nevertheless, the manner by which S1P2 regulates the underlying migratory events of the different cell types is complicated, and evidence can appear to be contradictory [29].

In addition to a pro-inflammatory role, the S1P2 receptor is involved in smooth muscle contraction and fibrosis. S1P2 induces contraction of diverse types of smooth muscle (including vascular, bronchial, intestinal, and bladder smooth muscle) by increasing intracellular Ca^{2+} concentrations and by activation of the Rho/Rho kinase [29–32]. Both S1P2 and S1P3 receptors mediate vasoconstriction in the vascular system, with differential responses in different vascular beds [29]. Notably, S1P2 may play an injurious role in renal ischemia-reperfusion injury [33] and S1P3 receptor may be responsible for the hypertension associated with the non-selective S1P receptor agonist, fingolimod (FTY720) [19].

Both S1P2 and S1P3 receptors are involved in pro-fibrotic pathways induced by S1P and fingolimod-phosphate (the active metabolite of fingolimod) in normal human lung fibroblasts [23]. Activation of the S1P2/Rho/ROCK pathway by fingolimod-phosphate leads to contraction of human lung myofibroblasts [24]. Fibroblast contraction is observed in many fibrotic disorders, and contributes to tissue stiffness and organ

S1P receptors	S1PR1	S1PR2	S1PR3	S1PR4	S1PR5
Expression	Broad; including B, T, and dendritic cells, endothelium, cardiac tissue, neurons	Broad; including vascular smooth muscle, endothelium, cardiac tissue, lung fibroblasts, tumor cells	Broad; including vascular smooth muscle, endothelium, cardiac tissue, lung fibroblasts	Restricted; predominantly immune e.g., T cells, dendritic cells; breast cancer cells	Restricted; natural killer cells, endothelial cells, oligodendrocytes
Signaling	ERK Akt Rac	Rho/ROCK Akt NFkB	NFkB	ERK	Rho/ROCK Akt
Biological outcome	Lymphocyte migration, dendritic cell migration, vascular barrier function, bradycardia, nociception, proliferation	Vasoconstriction, inflammation, fibrosis, inhibition of B-cell survival, proliferation	Vasoconstriction, fibrosis, proliferation	Inhibition of effector cytokines, secretion of IL-10 (suppressive cytokine)	Natural killer cell migration, blood-brain barrier integrity, oligodendrocyte function (process retraction, myelination and survival)
Clinical relevance*	Autoimmune modulation, bradycardia, tumor maintenance, theoretical breast cancer prognosis	Renal injury, fibroblast contraction, tumor maintenance	Hypertension, tumor maintenance, theoretical breast cancer prognosis	Autoimmune modulation, theoretical breast cancer prognosis	Autoimmune modulation, myelination

Fig. 1. Expression, downstream signaling molecules and function of S1P receptor subtypes [13,15–24]. ERK, extra cellular signal-regulated kinase; IL-10, interleukin-10; S1P, sphingosine-1-phosphate. *Based on a combination of animal and human data.

dysfunction [24]. Furthermore, S1P2 and S1P3 have both been linked to NFkB signaling, which is one of the transcription factors/coactivators implicated in pathophysiological processes including fibrosis, inflammation, angiogenesis, and cancer cell growth [34].

The role of S1P2 in cancer pathology is complex, and evidence indicates that it controls mechanisms that both promote and prevent

tumor growth [29]. S1P2 inhibits B-cell survival, and aged mice lacking S1P2 expression develop diffuse large B cell lymphoma [35]. However, S1P2 activates pro-survival pathways in prostate adenocarcinoma and increases proliferation in chronic myeloid leukemia cells [13,36]. S1P also leads to increased proliferation and invasion in lung adenocarcinoma cell lines through S1P3-mediated expression of epidermal growth

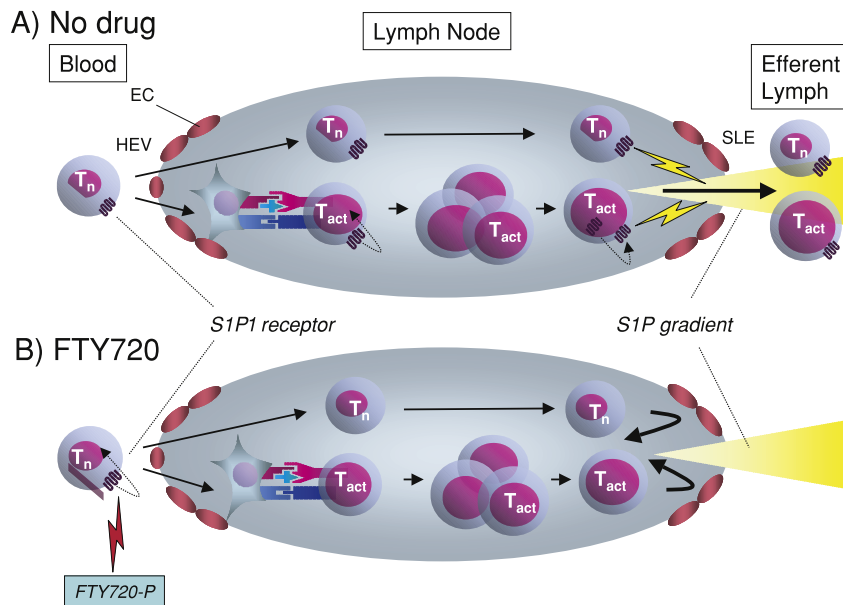


Fig. 2. The S1P/S1P1-dependent egress of T-cells from lymph nodes. From Brinkmann V, Cyster JG, Hla T. FTY720: sphingosine-1-phosphate receptor-1 in the control of lymphocyte egress and endothelial barrier function. *Am J Transplant* 2004;4(7):1019–25. Reprinted with permission from AAAS [25]. Panel A: T_n regularly circulate between blood and lymphatic tissue. T_n enter LN via HEV and egress in an S1P/S1P1-dependent step through the SLE via the efferent lymph into blood. When productive antigen encounter occurs in the LN, T_n become activated (T_{act}) and transiently down-modulate S1P1 - this renders cells unresponsive to the egress signal provided by S1P and the proliferating cells remain in the LN. At the end of the proliferation phase, T_{act} up-regulate S1P1 and egress from LN in an S1P/S1P1-dependent step. Panel B: after phosphorylation, fingolimod (FTY720), which is the only S1P receptor modulator currently approved, acts as a 'super agonist' at S1P1 on T_n and T_{act} and induces aberrant internalization of the receptor. This renders these T-cells unresponsive to S1P, and they become 'trapped' in the LN and unable to recirculate to peripheral tissues. HEV, high endothelial venules; LN, lymph nodes; S1P, sphingosine-1-phosphate; SLE, sinus-lining endothelium; Tact, activated Tn; T_n , naive T-cells.

factor receptor [13]. In estrogen receptor-positive breast cancer cells, high expression of S1P3 has been linked with poor prognosis [22,28].

2.3. S1P4

S1P4 inhibits proliferation and secretion of effector cytokines and enhances secretion of the suppressive cytokine interleukin-10, thereby mediating immunosuppressive effects of S1P [17]. In addition, the S1P4 receptor is expressed on dendritic cells and dendritic cell migration, and cytokine release was profoundly affected in mice that are deficient in S1P4. These S1P4^{-/-} deficient mice also had improved pathology in a dextran-sulfate induced animal model of IBD [37].

In estrogen receptor-negative breast cancer cells, high expression of S1P4 correlated with poor prognosis [38]. These data suggest that an S1P4 functional antagonism would be desirable for the treatment of IBD.

2.4. S1P5

S1P5 is expressed on endothelial cells within the blood-brain barrier, where it maintains barrier integrity in *in vitro* models [18]. Oligodendrocytes also express S1P5, which plays a role in oligodendrocyte progenitor migration [39], as well as oligodendrocyte myelination survival and process retraction [13,40]. Mice lacking S1P5 expression have decreased numbers of natural killer cells in the periphery and increased numbers in the lymph nodes and bone marrow [13,41,42], highlighting a role in immune regulation. However, the role of S1P5 on oligodendrocyte function may depend on the state of cell differentiation and maturation [43,44]. Therefore, the role of S1P5 requires further research and characterization [13].

The inflammatory roles played by the S1P receptor subtypes in different organs and tissues lend themselves to a variety of therapeutic applications. However, use of S1P modulators will undoubtedly lead to unintended side effects [29]. Furthermore, the outcome of drugs that interact with S1P receptors will ultimately depend on the degree of functional antagonism achieved via receptor internalization balanced against their ability to stimulate signaling that persists even after the receptors have been internalized inside the cell [45]. All of these considerations must be taken into account when balancing potential receptor signaling that could contribute to efficacy against theoretical safety risks.

3. Modulation of S1P in non-IBD disease

Targeting S1P receptors for the treatment of inflammatory conditions was first established in clinical studies of the non-selective S1P modulator, fingolimod, in multiple sclerosis (MS). Based on three Phase III trials (FREEDOMS, FREEDOMS II and TRANSFORMS) [46–48], fingolimod was approved in 2010 in the USA for the treatment of patients with relapsing forms of MS [49], and in 2011 in the EU for adults with highly active relapsing-remitting MS under certain conditions [50].

The mechanism by which fingolimod exerts its therapeutic effects in MS is yet to be fully elucidated, but may involve a reduction of lymphocyte migration into the central nervous system [43,49]. After phosphorylation, fingolimod is thought to act as a 'super agonist' at S1P1 on naïve and activated T cells, which induces aberrant internalization of the receptor (Fig. 2; panel B) [25]. In this way, the T cells become unresponsive to the S1P gradient, thus becoming 'trapped' in the lymph nodes and unable to recirculate to peripheral tissues [25].

However, fingolimod-phosphate also binds with high affinity to S1P1, S1P3, S1P4 and S1P5 [49] and activation of S1P2 by fingolimod-phosphate has been reported (Table 1) [14,23,24]. This non-selectivity of fingolimod may explain some of the adverse events that have been reported with this agent; it carries label warnings for several potential adverse effects, including infections, bradyarrhythmias and atrioventricular blocks, increased blood pressure, respiratory adverse effects, liver injury, and basal cell carcinoma [49]. Furthermore, fingolimod

Table 1

Affinity of S1P and S1P modulators for human S1P receptor subtypes [14]. pEC₅₀, the negative logarithm to base 10 of the half maximum effective concentration of an agonist.

pEC ₅₀	S1P1	S1P2	S1P3	S1P4	S1P5
S1P	7.1–9.4	8.1–8.5	8.4–9.8	7.2–8.1	7.4–8.9
Fingolimod-phosphate	8.1–9.5	7.5	7.8–9.4	6.6–9.2	8.2–9.5
Ozanimod (RPC1063)	9.8	No response ^a	No response ^a	No response ^a	7.3
Etrasimod (APD334) ^b	6.10	No response	No response	147	24.4

S1P, sphingosine-1-phosphate; NR, not reported by Spiegel 2016; no published data available.

^a NR, not reported by Spiegel 2016; no response was based on *in vitro* potency and selectivity preclinical studies of human S1P receptor subtypes in various assays [51].

^b Data reported in preclinical studies on recombinant human receptor subtypes in a β-arrestin assay [52].

has a long elimination half-life: 6–9 days, with about 81% of the dose excreted as inactive metabolites in urine [49,50]. Therefore, it takes 6 weeks to completely clear this agent [50] from the system, which makes managing adverse events and switching treatment more challenging.

To preserve the efficacy of fingolimod while minimizing adverse events related to S1P receptor subtypes, selective S1P receptor modulators are in various stages of clinical trials for MS (Table 2) [53–62]. These selective S1P receptor agents appear to have similar efficacy to fingolimod but with the advantage of a shorter half-life and more rapid lymphocyte recovery post-discontinuation [53,63–65]. Although there are still concerns related to first-dose bradycardia and conduction block, Phase 3 trials are ongoing for siponimod (BAF312) in secondary progressive MS and for ozanimod (RPC1063) and ponesimod (ACT-128800) in relapsing MS, based on promising Phase 2 trial data [53,55–57,59,66].

S1P modulators also have therapeutic potential for the management of psoriasis (Table 2) [56,67]. In a Phase 2 trial involving 326 patients with moderate-to-severe chronic plaque psoriasis, ponesimod significantly increased the proportion of patients achieving ≥75% improvement in Psoriasis Area and Severity Index after 16 weeks, compared with placebo (ponesimod 20 mg once daily, 46.0%; ponesimod 40 mg once daily, 48.1%; placebo, 13.4%; *P* < 0.0001 for both ponesimod doses vs. placebo) [56]. No further clinical trials have been registered for ponesimod in this disease area; however, the finding from these Phase 2 studies support further research of selective S1P1 receptor modulators in the treatment of other autoimmune conditions [56].

4. Modulation of S1P in preclinical models of IBD

S1P levels may be modulated as part of a contributory or compensatory mechanism during IBD. In preclinical studies, critical enzymes that phosphorylate sphingosine (kinases: Sphk1, Sphk2), dephosphorylate S1P (phosphatases: Sgpp1, Sgpp2), degrade S1P (lyase: Sgp1), as well as the transporter that transfers S1P from intracellular to extracellular compartments (Spns2), were found to be dysregulated in inflamed tissue in mice and humans, with a greater tendency toward synthesis than degradation [15]. Data from preclinical models also point to a role for S1P4 in experimental colitis. S1P4 deficiency decreased TH17 differentiation of T cells, increased mucosal immunoglobulin A levels under inflammatory conditions, and alleviated dextran sulfate sodium-induced colitis in a murine model [37].

The efficacy of S1P modulators has been demonstrated in models of colitis. Treatment with fingolimod reduced the clinical and histopathologic severity of oxazolone-induced colitis in mice, with improvements observed in body weight loss, diarrhea, and intestinal inflammation [68]. Similarly, the S1P1/S1P5 agonist ozanimod decreased inflammation and disease parameters in two animal models of colitis (2,4,6-trinitrobenzenesulfonic acid colitis and CD4(+) CD45RB(hi) T cell adoptive transfer colitis) [26]. In the latter study, ozanimod also induced S1P1 receptor internalization *in vitro* and reduced circulating B and

Table 2

A summary of S1P receptor modulators under investigation for non-IBD inflammatory disorders [53–62].

Agent	Company	S1P receptor target	Disease/population	Study phase	Key clinical trials	Key efficacy findings	Key safety findings
Ponesimod (ACT-128800)	Actelion Pharmaceuticals	S1P1	RRMS	III	OPTIMUM (NCT01006265)	Significant reduction in new GdE lesions from week 12–24 with ponesimod vs. placebo; 83%, 20 mg; 77%, 40 mg [55]	Bradycardia (~15 beats/min decrease) in 2% of patients with ponesimod; 1.2% and 0.9% had first and second-degree heart block, respectively, on day 1 (within 2–3 h of 10 mg dosing). Other AEs included: anxiety, dizziness, dyspnea, increased ALT (>3 × ULN), influenza, insomnia, and peripheral edema. Dyspnea and peripheral edema were dose-dependent; 7 patients discontinued study due to dyspnea [55]
			Chronic plaque psoriasis (moderate-to-severe)	II	Phase IIa (NCT01208090)	Ponesimod QD significantly increased % of patients achieving ≥75% improvement in PASI after 16 weeks, vs. placebo: 20 mg, 46.0%; 40 mg, 48.1%; placebo, 13.4% ($P < 0.0001$ for both doses vs. placebo) [56]	AEs included raised liver enzyme concentrations and dizziness. Mean maximum heart rate decreased by ~13 beats/min on day 1 (within 2 h of 10 mg dosing) vs. pre-administration (~70 beats per min); recovery started after 4 h, close to baseline by week 2. Changes in other ECG variables were not clinically relevant, except transient prolongation of PR interval on day 1 (10 mg). Second-degree AV block and bradycardia or sinus bradycardia events on day 1; recurred in 1 patient. Four patients discontinued due to transient second-degree AV block on day 1 (10 mg dose). No cardiac AEs required treatment; all events resolved spontaneously. Little change in mean blood pressure [56]
Siponimod (BAF312)	Novartis Pharma AG	S1P1 S1P5	Secondary progressive MS	II	BOLD MS (NCT00879658)	Reduction in new GdE and new/enlarged T2 lesions (without double counting) by 72% with 2 mg and 82% with 10 mg over first 3 months, vs. placebo [57]	Second degree AV block in 5% of patients with siponimod (10 and 2 mg; within 2 h of first dose; all made a full recovery). Bradycardia in 17% (10 and 2 mg). One death, 27 days post discontinuation (–1.25 mg dose; patient with a history of coronary artery disease), and 1 nonfatal myocardial infarction, 45 days post discontinuation (10 mg dose). Elevations in ALT (>3 × ULN) more frequent in the higher doses [57]
				III	EXPAND (NCT01665144) [58]	N/A	N/A
Ozanimod (RPC1063)	Receptos Inc. acquired by Celgene Corporation	S1P1 S1P5	Relapsing MS	II/III	RADIANCE (NCT01628393/NCT02047734)	Met primary endpoint: 84% and 89% reductions in cumulative number of GdE lesions from weeks 12 to 24 with ozanimod 0.5 and 1 mg, respectively, vs. placebo [59]	Three serious AEs unrelated to therapy (0.5 mg dose): optic neuritis, somatoform autonomic dysfunction, and cervical squamous metaplasia (HPV). No serious infectious or cardiac AEs. No macular edema [59]
				III	SUNBEAM (NCT02294058) [60]	N/A	N/A
Amiselimod (MT-1303)	Mitsubishi Tanabe Pharma/Biogen	S1P1	RRMS	II	MOMENTUM; extension planned (NCT01742052/NCT01890655)	Median total number of GdE T1-weighted lesions from weeks 8 to 24 significantly lower with 0.2 and 0.4 mg amiselimod QD vs. placebo; 0.0 lesions and –1.0 median difference vs. placebo in both groups; $P = 0.0021$ and $P = 0.0003$, respectively [61]	Most common treatment-emergent AEs: headache (10% with amiselimod vs. 4% with placebo); nasopharyngitis (7–10% versus 8%). No serious treatment-emergent AEs for >1 patient in any group; no clinically significant reductions in heart rate [61]
GSK2018682	GlaxoSmithKline	S1P1	Healthy volunteers	I	Three trials (NCT01466322/NCT01387217/NCT01431937)	Safety, pharmacokinetics and pharmacodynamics of single (up to 24 mg) and repeat dosing (up to 6 mg/day) for 28 days [62]	Higher incident of gastrointestinal and cardiovascular AEs vs. placebo: abdominal pain, 7.1% vs. 0%; AV block and bradycardia, 3.5% vs. 0% [62]

AE, adverse event; ALT, alanine aminotransaminase; AV, atrioventricular; HPV, human papilloma virus; ECG, electrocardiogram; GdE, gadolinium-enhanced; MS, multiple sclerosis; PASI, Psoriasis Area and Severity Index; QD, once daily; RRMS, relapsing-remitting multiple sclerosis; S1P, sphingosine-1-phosphate; ULN, upper limit of normal.

CCR7(+) T lymphocyte levels in rodents, supporting a role for S1P1 and lymphopenia in the effects of ozanimod on experimental colitis.

Etrasimod, designed to selectively target S1P1, S1P4 and S1P5 receptors, produced dose-dependent blood lymphopenia in adult mice who were administered single injections of this S1P modulator at doses between 0.03 and 1 mg/kg [52]. Importantly, plasma concentrations of etrasimod and resulting lymphopenia were shown to be inversely related. Etrasimod also provided systemic immune cell modulation and attenuated disease in an *in vivo* model of ulcerative colitis: using the CD4(+) CD45RB(hi) T cell adoptive transfer colitis model, etrasimod 3 mg/kg decreased mucosal thickness and infiltration of immune cells in colon of SCID mice, compared with those receiving the vehicle control [52]. At this dose, etrasimod also prevented the increase in colon weight:length ratio found in the vehicle control group.

The anti-inflammatory properties of these selective S1P agonists might be due to potential effects on dendritic cell migration and vascular barrier function, as well as their lymphopenic effects [15]. Based on investigations in preclinical IBD models and intestinal biopsies from patients with this disease, Karupuchamy and colleagues hypothesized that this family of drugs have a tripartite mechanism of action, i.e. one that combines the retention of naïve T cells at secondary lymphoid organs with the mobilization of effector T cells from the intestine and subsets of activated dendritic cells to inductive sites, and modification of the endothelial barrier function [15]. Overall, this has a net effect of attenuating intestinal inflammation [15].

Regardless of the exact mode of action, these preclinical studies provide preliminary evidence that S1P modulators have potential to treat inflammation associated with colitis, and establish the rationale for further clinical development in IBD.

5. S1P modulators in IBD clinical trials

No S1P modulators are currently approved for the management of IBD, but two with differing S1P receptor selectivity profiles have been studied in clinical trials for these conditions.

5.1. Amiselimod

Amiselimod (MT-1303) is an oral, selective modulator of S1P1 [60]. It was under investigation for moderate-to-severe, active Crohn's disease in a 14-week, randomized, placebo-controlled, Phase 2 trial [69] with an open-label extension of ≥ 22 weeks [70]. Amiselimod was also under evaluation for ulcerative colitis, MS, and other autoimmune conditions [71]; however, Biogen recently announced that it is discontinuing its development [72].

5.2. Ozanimod

Ozanimod (RPC1063) is an oral agonist of S1P1 and S1P5 [26,73], which may be taken with or without food [74]. The Phase 2 TOUCHSTONE clinical trial investigated ozanimod in 197 adults with moderate-to-severe ulcerative colitis [73]. Patients were randomized to receive oral ozanimod 0.5 mg, ozanimod 1 mg, or placebo, once daily; patients underwent dose escalation during the first week after randomization. Ozanimod 1 mg once daily was associated with slightly higher rate of clinical remission of ulcerative colitis than placebo after 8 weeks (16% vs. 6%; $P = 0.048$). Recently published findings from the open-label extension of this study suggest that the efficacy of this S1P agonist occurs rapidly, with improvements in partial Mayo scores shown within 4–8 weeks, and is maintained or durable over the long term [75]. In this phase of the study, patients ($n = 170$; 86%) were switched to or continued to receive the ozanimod 1 mg dose [75]. Partial Mayo scores improved in all patient groups, particularly in those which had originally received placebo or the lower dose of ozanimod, with changes in scores at Week 44 of -2.6 , -2.7 and -1.3 in the placebo, 0.5 mg and 1 mg groups, respectively [75]. At Week 44, 90.9% ($n =$

119/131) of patients had little or no active disease based on the physicians global assessment (0 or 1); 84.7% ($n = 111/131$) had no blood in their stool (rectal bleeding subscore [RBS], 0), with 98.4% of patients ($n = 129/131$) having little or no blood in their stool (RBS, 0 or 1); and 80.2% ($n = 105/131$) had little or no increase in the number of stools (stool frequency subscore, 0 or 1) [75].

A Phase 3, randomized, placebo-controlled trial to assess ozanimod 1 mg once daily as therapy for moderate-to-severe ulcerative colitis over 52 weeks is currently in recruitment, with an estimated final enrollment of 900 individuals [76]. An open-label extension of this trial is planned [77] that will provide data on the efficacy and safety of ozanimod over up to 5 years.

5.3. Etrasimod

Etrasimod (APD334) is an oral, next-generation S1P modulator in clinical development for the chronic management of autoimmune diseases, including ulcerative colitis [78]. Etrasimod selectively targets S1P1, S1P4, and S1P5 *in vitro* signaling assays providing the potential for effective systemic and local immune cell modulation [52]. The safety and efficacy of etrasimod are being evaluated over 48 weeks in adults with moderate-to-severe, active ulcerative colitis in the Phase 2 OASIS clinical study [79], a randomized, placebo-controlled trial with a 34-week extension [78,80]. The OASIS study results are expected during 2017.

6. Safety of S1P modulators in non-IBD disease and IBD

Selective S1P receptor modulators were first developed to avoid the major side effects associated with the non-selective modulator, fingolimod, in patients with MS: in particular, bradycardia and atrioventricular block [65]. Despite these reported side effects, no increased cardiovascular risk with fingolimod use in MS patients has been found in the most recent safety data analysis of the Phase 3 study data [81]. Progressive multifocal leukoencephalopathy (PML), a rare but potentially fatal condition, is also a concern with fingolimod use in patients with MS [82]. To date, 17 suspected cases of PML have been reported [82,83]. Nevertheless, in a recent study of 119,000 patients with MS, no evidence of increased PML risk was found with this agent [66,84].

Bradycardia and atrioventricular block were originally attributed to the action of fingolimod on S1P3 [85]; however, in humans, bradycardia may be due to a mechanistic effect through initial S1P1 agonism prior to receptor down-regulation [86]. All three selective S1P receptor modulator agents currently in Phase 3 trials for MS (i.e. ponesimod, siponimod and ozanimod) were well tolerated, although as previously mentioned, there have been reports of bradycardia and first- and second-degree heart block with each of these agents (Table 2) [55–57].

The two selective S1P receptor modulators in development for IBD have the potential to avoid those S1P receptors that are associated with adverse effects, while targeting the receptors related to these diseases [52,73]. However, full safety data for the agents are not yet available. Phase 2 safety trials for etrasimod in IBD are ongoing and trial completion is not expected until later this year.

Findings from the Phase 2 TOUCHSTONE trial of ozanimod (agonist of S1P1 and S1P5) for ulcerative colitis have been published (Table 3) [73]. The most common adverse events were ulcerative colitis flare (3–4% with ozanimod vs. 8% with placebo), anemia (0–5% vs. 6%) and headache (0–3% vs. 5%). First-degree atrioventricular block and sinus bradycardia developed on Day 8 in 1 patient who was treated with ozanimod. Elevations in hepatic aminotransferase levels of >3 times the upper limit of normal (ULN) range were observed in 4 patients (3%) during ozanimod treatment. Squamous-cell carcinoma of the skin developed in 1 patient in the ozanimod 1 mg arm. Adverse pulmonary effects were not reported. Adverse events reported in the extension study were generally similar to those reported in the original study, although anemia and ulcerative flare were reported as serious adverse

Table 3
A summary of S1P receptor modulators under investigation for IBD inflammatory disorders [73,79,80,87].

Agent	Company	S1P receptor target	Disease	Study phase	Key clinical trials	Key efficacy findings	Key safety findings
Ozanimod (RPC1063)	Receptos Inc., acquired by Celgene Corporation	S1P1 S1P5	Ulcerative colitis (moderate-to-severe)	III	TOUCHSTONE (NCT01647516)	Slightly higher rate of clinical remission of ulcerative colitis after 8 weeks ozanimod (1 mg QD) vs. placebo (16% vs. 6%; $P = 0.048$); effect maintained after 32 weeks [73]	Most common AEs: ulcerative colitis flare (3–4% with ozanimod vs. 8% with placebo); anemia (0–5% vs. 6%) and headache (0–3% vs. 5%). First-degree AV block and sinus bradycardia on day 8 in 1 patient (0.5 mg dose; had preexisting bradycardia) Elevations in hepatic aminotransferase levels ($>3 \times$ ULN) in 4 patients (3%) with ozanimod. Squamous-cell carcinoma in one patient [73]
			Crohn's disease (moderate-to-severe)	II	Phase II (NCT02531113) [87]	N/A	N/A
Etrasimod (APD334)	Arena	S1P1 S1P4 S1P5	Ulcerative colitis	II	OASIS (NCT02447302; NCT02536404) [79,80]	N/A	N/A

AE, adverse event; AV, atrioventricular; IBD, irritable bowel disease; NA, not applicable; QD, once daily; S1P, sphingosine-1-phosphate; ULN, upper limit of normal.

events in ≥ 2 patients [75]. Elevations in hepatic aminotransferase levels of > 3 times ULN, observed in 2.7% (4/170) patients, were asymptomatic, transient, and resolved while treatment was continued [75]. Larger-scale and longer-term trials are required to fully characterize the safety profile of ozanimod [73].

The time it takes for lymphocytes to return to normal levels after drug withdrawal is important for physicians and patients, especially if therapy needs to be halted or if a switch in therapy is required. In this regard, withdrawal of both etrasimod and ozanimod have been shown to result in a rapid recovery of lymphocytes to baseline within approximately 1 week [21,88] whereas lymphocyte recovery for fingolimod takes significantly longer due to its long half-life [64].

Ongoing clinical studies will help establish how the selectivity of S1P modulators translates into safety in the population of patients with IBD.

7. Clinical perspectives on S1P modulators in IBD

The new generation of small molecules, such as S1P modulators, may represent a second therapeutic revolution in IBD following that of monoclonal antibodies, which occurred in the late 1990s. Small molecules, including S1P modulators, benefit from convenient oral administration and have therapeutic potential in IBD. Activation of S1P1, S1P4, and S1P5 may decrease intestinal inflammation, while modulating S1P2 and S1P3 may be associated with cardiovascular, pulmonary, and theoretical cancer-related risks. Novel, orally-available S1P modulators have therapeutic potential in IBD, and may avoid some of the limitations associated with current therapies. Available Phase 2 data for selective S1P modulators in patients with ulcerative colitis are encouraging. Results from further Phase 2 and 3 studies evaluating the use of S1P

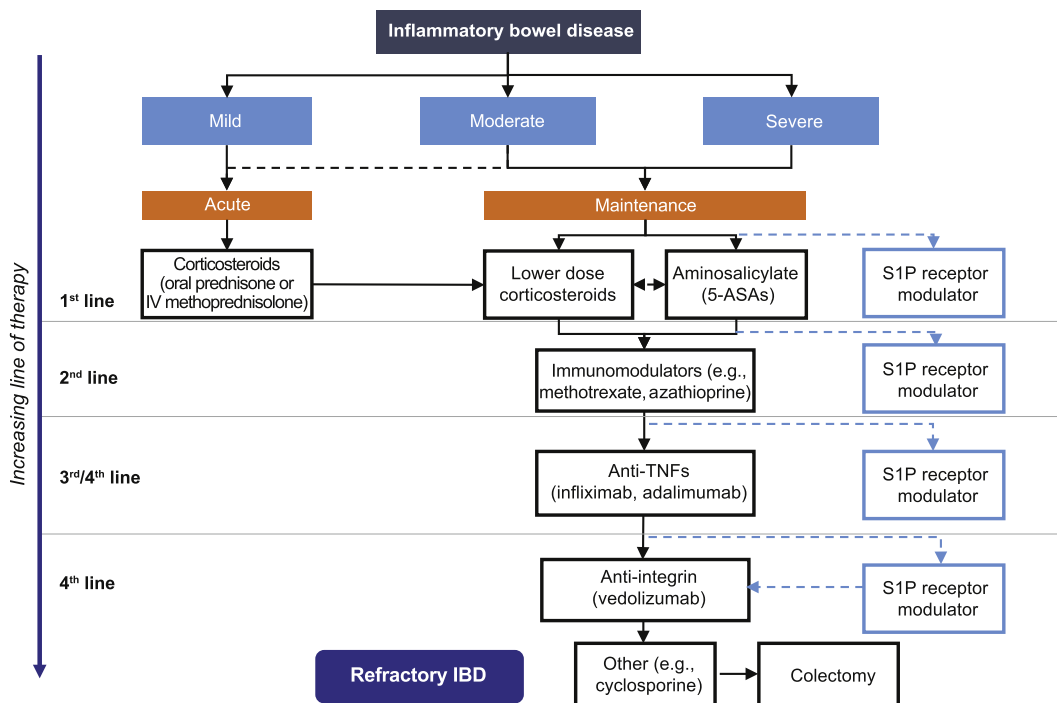


Fig. 3. Illustrative diagram showing how S1P receptor modulators could potentially fit into current treatment practice for ulcerative colitis. IBD, irritable bowel disease; IV, intravenous; S1P, sphingosine-1-phosphate; TNF, tumor necrosis factor.

modulators in IBD are expected over the next few years; these data will add to the emerging body of evidence and establish the relationship between the selectivity of S1P modulators and their safety and efficacy. Subject to supporting clinical evidence and regulatory approvals, it is possible that these pipeline medications will be added to the existing clinical armamentarium (Fig. 3) over the coming years, either as alternatives to established medications or as add-on therapies. The availability of novel, oral IBD therapies would increase the options for patients who prefer this route of administration, as well as for patients in whom other IBD medications are contraindicated, not well tolerated, or ineffective (e.g. secondary to the development of anti-drug antibodies). A relatively large pharmacological armamentarium for IBD would provide more scope for individualizing the treatment of these conditions.

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Conflicts of interest

Laurent Peyrin-Biroulet: honoraria from Merck, AbbVie, Janssen, Genentech, Mitsubishi, Ferring, Norgine, Tillots, Vifor, Hospira/Pfizer, Celltrion, Takeda, Biogaran, Boehringer-Ingelheim, Lilly, HAC-Pharma, Index Pharmaceuticals, Amgen, Sandoz, Forward Pharma GmbH, Celgene, Biogen, Lycera, Samsung Bioepis; Dominic Behan is Chair of the Arena Scientific Board; Ronald Christopher and Cheryl Lassen are employees of Arena Pharmaceuticals Inc.

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