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Sphingosine 1-Phosphate Receptor Modulators and Drug Discovery

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Abstract

Initial discovery on sphingosine 1-phosphate (S1P) as an intracellular second messenger was faced unexpectedly with roles of S1P as a first messenger, which subsequently resulted in cloning of its G protein-coupled receptors, $S1P_{1-5}$. The molecular identification of S1P receptors opened up a new avenue for pathophysiological research on this lipid mediator. Cellular and molecular *in vitro* studies and *in vivo* studies on gene deficient mice have elucidated cellular signaling pathways and the pathophysiological meanings of S1P receptors. Another unexpected finding that fingolimod (FTY720) modulates S1P receptors accelerated drug discovery in this field. Fingolimod was approved as a first-in-class, orally active drug for relapsing multiple sclerosis in 2010, and its applications in other disease conditions are currently under clinical trials. In addition, more selective S1P receptor modulators with better pharmacokinetic profiles and fewer side effects are under development. Some of them are being clinically tested in the contexts of multiple sclerosis and other autoimmune and inflammatory disorders, such as, psoriasis, Crohn's disease, ulcerative colitis, polymyositis, dermatomyositis, liver failure, renal failure, acute stroke, and transplant rejection. In this review, the authors discuss the state of the art regarding the status of drug discovery efforts targeting S1P receptors and place emphasis on potential clinical applications.

Key Words: Sphingosine 1-phosphate, G protein-coupled receptor, Fingolimod, FTY720, Drug discovery, S1P agonist

GPCR IN DRUG DISCOVERY

G protein-coupled receptors (GPCRs) constitute the largest superfamily of receptors for signaling molecules, ligands, and currently comprise some 865 receptors (Im, 2002, 2013; Fredriksson *et al*., 2003; Kihara *et al*., 2015). GPCRs are also known as 7TM receptors, because they have seven transmembrane domains. These receptors may signal though G proteins but they also initiate signals via other entities (Davenport *et al*., 2013). Many ligands, including hormones, autacoids, neurotransmitters, and very small molecules to large proteins can bind and activate GPCRs, and their activations lead to a multitude of physiological processes (Howard *et al*., 2001; Overington *et al*., 2006; Im, 2013).

GPCRs represent a major drug target in all clinical areas. Currently, about 40% of drugs on the market target GPCRs and regulate their activities positively or negatively, because a variety of GPCRs offer selectivity and specificity for many human diseases (Im, 2013). Examples of their applications include Claritin a H_1 histamine receptor antagonist, Cozaar an AT_1 angiotensin receptor antagonist, Neurontin a $GABA_B$ γ-aminobutyric acid receptor agonist, Plavix a $P2Y_{12}$ ADP receptor antagonist, Singulair a $CysLT_1$ leukotriene D_4 receptor antagonist, Zantac a H_2 histamine receptor antagonist, and Zyprexa a mixed D_2/D_1/5-HT_2 dopamine/serotonin receptors antagonist. About 55 GPCRs have been cloned and identified as receptors for intercellular lipid mediators, and recent studies have unearthed their functional roles under both physiological and pathological conditions (Im, 2004, 2009, 2013). Furthermore, the number of drug discovery studies being conducted on GPCRs have increased in many fields including cancer, cardiac dysfunction, central nervous system disorders, inflammatory diseases, metabolic disorders, and obesity, (Im, 2004, 2009; Mutoh *et al*., 2012; Pyne *et al*., 2012; Choi and Chun, 2013; Makide *et al*., 2014; Proia and Hla, 2015). Here, we summarize current knowledge on one specific aspect of drug discovery involving interactions between GPCRs and the intercellular lipid mediator, sphingosine 1 phosphate (S1P).

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SPHINGOSINE 1-PHOSPHATE AND ITS GPCRs

S1P is a bioactive lysophospholipid metabolite that can act as an intercellular lipid mediator (Moolenaar and Hla, 2012). Initially, S1P was reported to be a second messenger that mediates increases in intracellular calcium levels as a result of PDGF and IgE signaling (Olivera and Spiegel, 1993; Choi *et al*., 1996). However, S1P was also unexpectedly found to function as an intercellular first messenger like autacoids. The presence of S1P GPCRs in the plasma membrane was suggested by the pertussis toxin sensitivity in S1P-induced actions (Bunemann *et al*., 1995; Goodemote *et al*., 1995; Im *et al*., 1997; Okajima *et al*., 1997; van Koppen *et al*., 1996). The discovery of $S1P_1$ (formerly known as Edg-1) in 1998 along with four other $S1P_{2\text{-}5}$ receptors represents a milestone in sphingolipid biology (An *et al*., 1997; Lee *et al*., 1998; Lynch and Im, 1999; Okamoto *et al*., 1999; Im *et al*., 2000; Van Brocklyn *et al*., 2000; Yamazaki *et al*., 2000), and leads to the identification of a variety of biological functions mediated by interactions between S1P and S1P receptors (Sanchez and Hla, 2004). In particular, S1P regulates the response and function of various cellular and organ systems, such as, cell differentiation, cell migration, cell proliferation, immune response, trafficking of T and B cells, and vascular stability (Gardell *et al*., 2006; Huwiler and Pfeilschifter, 2008). S1P receptors exhibit overlapping or distinct expression patterns in various cells and tissues, and as a result, the various cellular functions of S1P have been assigned to S1P receptor subtypes. Furthermore, because S1P plays critical roles in autoimmune diseases, cancer, and diseases related to the cardiovascular, immune, nervous, and reproductive systems, S1P receptors become important treatment targets (Kihara *et al*., 2015). In addition, the discovery of S1P receptors made the screening and development of S1P agonists and antagonists accessible. Furthermore, discoveries of S1P receptor subtype-selective agonists or antagonists could provide novel therapeutic candidates (Im, 2010).

The discovery that fingolimod (also known as FTY720) is an agonist of four S1P receptor subtypes by researchers in immune therapeutics field accelerated the drug discovery in this area. Fingolimod has been approved as a first-in-class drug targeting $S1P_1$ and several selective S1P receptor modulators are being subjected to clinical trials. In this review, we focus on drug discovery involving S1P receptors, especially $S1P_1$.

DEVELOPMENT OF $S1P_1$ RECEPTOR MODULATORS

Fingolimod (FTY720, Gilenya®, Novartis)

Fingolimod is a well-known success of drug discovery in the S1P research field. In 1995, fingolimod was produced from the immunosuppressive natural product myriocin, which was isolated from the fungus *Isaria sinclairii* (Billich *et al*., 2003; Im, 2003; Paugh *et al*., 2003). In 2002, the mechanism responsible for the immunosuppressive activity of fingolimod was determined to be due to the regulation of lymphocyte trafficking in a rodent model of multiple sclerosis (Brinkmann *et al*., 2002). Fingolimod, through $S1P_1$, was found to directly alter the trafficking of naïve and antigen-activated $CD4^+$ T cells and to control egress of lymphocytes from secondary lymphoid tissues and endothelial barrier function (Xie *et al*., 2003; Brinkmann *et al*., 2004; Chiba, 2005). In 2010, the Food and Drug Administration (FDA) approved fingolimod as the first oral disease-modifying drug to treat relapses of multiple sclerosis (Kihara *et al*., 2015). Multiple sclerosis is a demyelinating disease that damages axonal myelin sheaths in the brain and spinal cord. The primary action mechanism of fingolimod is to reduce lymphocyte egress from secondary lymphoid organ, thymus, and bone marrow, resulting in lymphopenia (Adachi and Chiba, 2008). Thereby, lymphopenia contributes to inhibit axon myelin sheath damage.

Fingolimod is a unique drug in two respects. First, fingolimod is a prodrug (Fig. 1). *In vivo* fingolimod is phosphorylated by sphingosine kinases (SK1 and SK2) and then phosphorylated fingolimod acts as an agonist on four S1P receptor subtypes ($S1P_1$, $S1P_3$, $S1P_4$ and $S1P_5$) (Billich *et al*., 2003). Second, effects of fingolimod on lymphocyte egress involve not agonistic but rather functional antagonistic activity against $S1P_1$ (Fig. 1) (Matloubian *et al*., 2004). Both S1P and fingolimod-phosphate have been reported to induce lymphopenia via the agonistic activation of $S1P_1$ and subsequent internalization of $S1P_1$ in the lymphocytes (Brinkmann *et al*., 2004; Thangada *et al*., 2010). In fact, the absence of $S1P_1$ on the cell surface blocks re-circulation of lymphocytes from secondary lymphoid organs to blood, because lymphocytes egress by chemotactic response to S1P concentration gradient (high in blood and low in lymph node) through $S1P_1$ (Brinkmann *et al*., 2004; Chiba, 2009). In the case of $S1P_1$ agonist like S1P, internalized $S1P_1$ recycled back to the cell surface within several hours. However, in the case of $S1P_1$ modulators like fingolimod-phosphate, internalized $S1P_1$ undergoes proteosomal degradation, resulting in long-time absence of $S1P_1$ until de novo synthesized (Oo *et al*., 2007). This kind of functional antagonism of fingolimod means it has a long action time, which can sometimes be disadvantageous (Subei and Cohen, 2015). The immune modulatory action of fingolimod has also been reported in autoimmune diseases other than multiple sclerosis, such as spontaneous autoimmnune polyneuropathy and experimental autoimmune neuritis (Kim *et al*., 2009; Zhang *et al*., 2009a).

Other applications of fingolimod have also been suggested, such as, for the treatment of ischemia/reperfusion injury, which is the cellular damage that results from ischemia and re-supply of blood to infarcted tissues. In fact, it has been estimated that systemic inflammatory response after ischemia/reperfusion may account for 30-40% of intensive care unit mortalities (Eltzschig and Collard, 2004). In one animal study, fingolimod significantly inhibited leukocyte infiltration, peripheral blood lymphocyte counts, and vascular permeability in renal ischemia/reperfusion injury (Awad *et al*., 2006). In other studies, fingolimod attenuated arterial pressure and improved organ function when it was applied to heart or lung ischemia/reperfusion (Hofmann *et al*., 2009; Stone *et al*., 2015). Recently, fingolimod was reported to inhibit hypoxia/reperfusion-induced cardiomyocyte apoptosis by inhibiting caspase 3 activation, and by activating Akt and Erk signaling through $S1P_{1/3}$ activation (Wang *et al*., 2014). These preclinical studies indicate that fingolimod has potential to be used for the treatment of ischemia/reperfusion injury and autoimmune disorders.

Fingolimod has been reported to attenuate neuroinflammation by regulating the activation and neuroprotective effects of microglia mainly via $S1P_1$ (Jackson *et al*., 2011; Noda *et al*., 2013; Kolahdooz *et al*., 2015). In addition, fingolimod is known to inhibit allergen-induced airway inflammation and hyper-reactivity in mice (Ble *et al*., 2009; Marsolais *et al*., 2011; Trifilieff and Fozard, 2012). Furthermore, in low-density lipoprotein

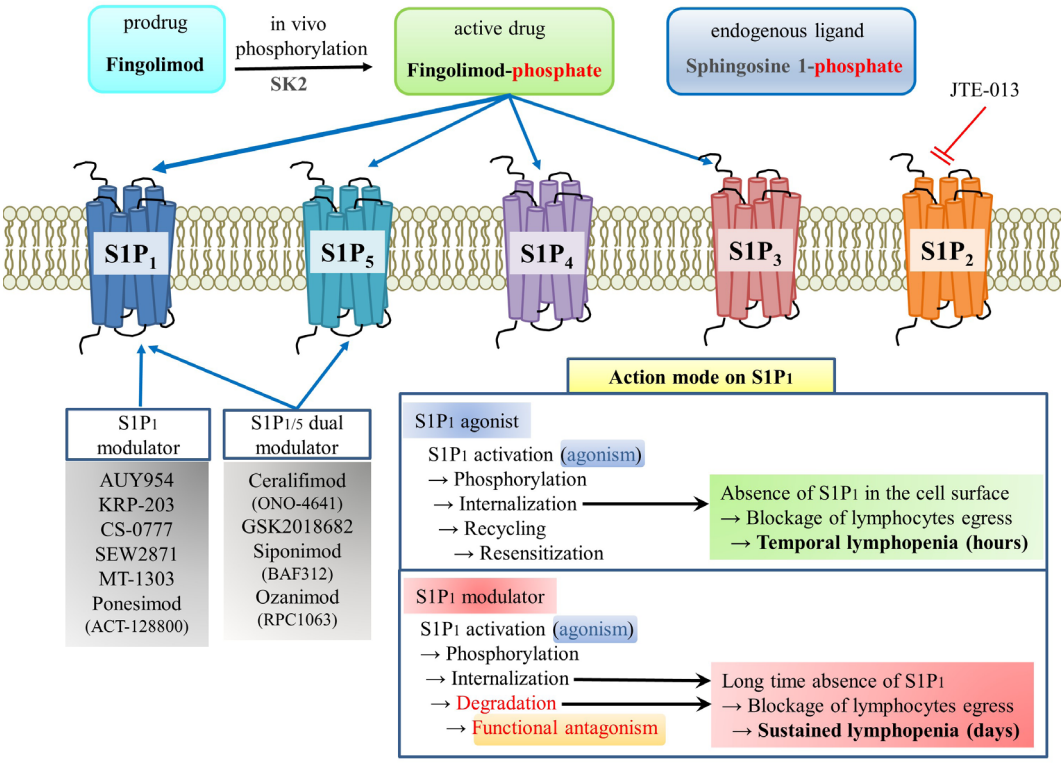

Fig. 1. Action mechanism of fingolimod and other S1P receptor modulators. Fingolimod is transformed to fingolimod-phosphate *in vivo* by sphingosine kinases. Fingolimod-phosphate can activate $S1P_1$, $S1P_3$, $S1P_4$, and $S1P_5$, and the fingolimod activation of $S1P_1$ in lymphocytes leads to GRK2-mediated phosphorylation of C-terminal tail of $S1P_1$, which recruits β-arrestin and induces $S1P_1$ internalization. This internalization exposes $S1P_1$ to proteosomal degradation, which prevents the recycling of $S1P_1$ and results in the loss of $S1P_1$ from the plasma membrane. This absence of $S1P_1$ blocks lymphocyte egression from secondary lymphoid organs and reduces T and B cell counts in the blood. Lymphopenia is presumed to be the main mechanism whereby fingolimod causes immune suppression in autoimmune diseases like relapsing multiple sclerosis.

(LDL) receptor-deficient mice, fingolimod significantly attenuated atherosclerotic lesion formation, necrotic core formation, and lymphocyte function (Nofer *et al*., 2007). And peritoneal macrophages isolated from fingolimod-treated mice showed more of the M2 macrophage phenotype and less of the M1 macrophage phenotype (Nofer *et al*., 2007). However, Poti *et al*. (2012) reported in LDL receptor-deficient mice fed a Western diet, fingolimod reduced macrophage function, weight gain, and white adipose tissue amount, but failed to affect atherosclerosis.

A phase 3 clinical trial on fingolimod for primary progressive multiple sclerosis (INFORMS; NCT00731692) showed that it has anti-inflammatory effects but fails to reduce disease progression (Lublin *et al*., 2016). In addition, a phase 2 clinical trial for its effects on pulmonary function in moderate asthma patients was completed in 2009 (NCT00785083).

In a pilot study conducted in patients with acute ischemic stroke, fingolimod/alteplase combination therapy was well tolerated, attenuated reperfusion injury, and improved clinical outcomes (Fu *et al*., 2014; Li *et al*., 2015; Zhu *et al*., 2015). Fingolimod is now under a phase 2 clinical trial for acute stroke (NCT02002390). In addition, fingolimod is currently under phase 2 clinically testing in Rett's syndrome (NCT02061137), phase 2 schizophrenia (NCT01779700), phase 4 neurodegeneration (NCT02575365), and multiple sclerosis (Table 1).

Several side effects of fingolimod have been reported in three phase 3 trials (FREEDOMS, FREEDOM II, and TRANSFORMS) (Kappos *et al*., 2010; Calabresi *et al*., 2014; Cohen *et al*., 2016b). The common adverse effects were bradycardia at the first dose or atrioventricular block, macular edema, hypertension, headache, cough, dyspnea, back pain, influenza, and diarrhea (Subei and Cohen, 2015). First dose bradycardia is believed to be mediated via transient $S1P_1$ activation in atrial myocytes, which would disappear by down-regulation of $S1P_1$ (Camm *et al*., 2014). Unlike in man, $S1P_3$ in atrial myocytes causes bradycardia in mice (Forrest *et al*., 2004; Sanna *et al*., 2004). Other adverse effects of fingolimod may be due to off-target effects via other S1P receptors, as it is a non-selective S1P agonist ($S1P_{1, 3\text{-}5}$) (Brinkmann *et al*., 2002). Therefore, currently several $S1P_1$ selective agonists/modulators are being developed as drug candidates in the wake of fingolimod (Fig. 1).

SEW2871

SEW2871 is a highly selective $S1P_1$ agonist, which does not act on $S1P_{2\text{-}5}$. As was expected, SEW2871 has been reported to reduce lymphocyte numbers in blood (Wei *et al*., 2005; Kim *et al*., 2009). As like fingolimod, SEW2871 also ameliorated ischemic acute renal failure after ischemia/reperfusion injury in mice (Lien *et al*., 2006). SEW2871 was also found to protect heart and liver tissues after myocardial or hepatic ischemia/reperfusion injury and these protective effects were ascribed

Table 1. Summary of S1P receptor modulators currently undergoing or completed in clinical trials (based on data at http://www.clinicaltrials.gov/ in July 2016)

Chemical	Target	Condition	Stage	Status	NLM ID
Fingolimod (FTY-720)	$S1P_{1/3/4/5}$	Relapsing remitting multiple sclerosis (RRMS)		Approved (2010)	
		Neurodegeneration	Phase IV	Active	NCT02575365
		Schizophrenia	Phase II	Active	NCT01779700
		Rett Syndrome	Phase II	Active	NCT02061137
		Acute Stroke	Phase II	Active	NCT02002390
		Amyotrophic lateral sclerosis	Phase II	Completed (2015)	NCT01786174
		Primary progressive multiple sclerosis	Phase III	Completed (2014)	NCT00731692
		Renal insufficiency	Phase I	Completed (2011)	NCT00731523
		Moderate asthma	Phase II	Completed (2009)	NCT00785083
		Renal/Kidney transplantation	Phase III	Completed (2006)	NCT00099801
KRP-203	$S1P_1$	Subacute cutaneous lupus erythematosus	Phase II	Active	NCT01294774
		Hematological malignancies	Phase I	Active	NCT01830010
Siponimod (BAF312)	$S1P_{1/5}$	Secondary progressive multiple sclerosis	Phase III	Active	NCT01665144
		RRMS	Phase II	Completed (2012)	NCT00879658
		- (Extension) -		- Active -	NCT01185821
		Polymyositis	Phase II	Active	NCT01801917
		Active dermatomyositis	Phase II	Completed (2016)	NCT02029274
		Hepatic impairment	Phase I	Completed (2014)	NCT01565902
		Renal impairment	Phase I	Completed (2014)	NCT01904214
CS-0777	$S1P_1$	Multiple sclerosis	Phase I	Completed (2010)	NCT00616733
Ponesimod (ACT-128800)	$S1P_1$	RRMS	Phase II	Active	NCT01093326
		Ponesimod *vs* teriflunomide in RRMS	Phase III	Active	NCT02425644
		Chronic GVHD	Phase II	Active	NCT02461134
		Psoriasis	Phase II	Completed (2012)	NCT01208090
Ozanimod (RPC1063)	$S1P_{1/5}$	Multiple sclerosis	Phase III	Active	NCT02047734
		Ulcerative colitis	Phase III	Active	NCT02435992
		Crohn's disease	Phase II	Active	NCT02531113
Ceralifimod (ONO-4641)	$S1P_{1/5}$	RRMS	Phase II	Completed (2011)	NCT01081782
		- (Extension) -	Phase II	Terminated(2015)	NCT01226745
GSK2018682	$S1P_{1/5}$	RRMS	Phase I	Completed (2011)	NCT01466322
MT-1303	$S1P_1$	Crohn's disease	Phase II	Active	NCT02378688
		Systemic lupus erythematosus	Phase I	Active	NCT02307643
		RRMS	Phase II	Completed (2014)	NCT01742052
		Plaque psoriasis	Phase II	Completed (2014)	NCT01987843
		Inflammatory bowel disease	Phase I	Completed (2014)	NCT01666327

RRMS, relapsing remitting multiple sclerosis.

to $S1P_1$ activation (Hofmann *et al*., 2009; Park *et al*., 2010).

Several preclinical studies have shown SEW2871 has therapeutic implications in contexts of diabetes, Alzheimer's disease, liver fibrosis, and inflammatory responses. In a non-obese mouse model of type 1 diabetes, SEW2871 inhibited monocyte adhesion to diabetic aortas and prevented monocyte/endothelial interactions, which suggests SEW2871-induced $S1P_1$ signaling has potential for treatment of the vascular complications of type 1 diabetes (Whetzel *et al*., 2006). In a rat model of Alzheimer's disease, chronic SEW2871 administration inhibited β amyloid ($A\beta_{1-42}$)-induced spatial memory impairment and hippocampal neuronal loss, indicating the $S1P_1$ signaling pathway offers a novel therapeutic target for the prevention of neurodegenerative disorders (Asle-Rousta *et al*., 2013).

SEW2871 was also found to modulate liver fibrosis by directly regulating the migration of human hepatic myofibroblasts into the damaged areas (Li *et al*., 2011). In LX-2 cells (a human hepatic stellate cell line), SEW2871 exerted a powerful migratory effect by increasing smooth muscle α-actin, procol-

lagen αI and αIII, and total hydroxyproline contents (Liu *et al*., 2011). It has also been reported SEW2817 has the following anti-inflammatory effects; it inhibits dendritic cell chemotaxis and migration to lymph nodes, causes switching to the M2 macrophage phenotype, and decreases proinflammatory cytokine levels under inflammatory conditions (Gollmann *et al*., 2008; Hughes *et al*., 2008). The intravenous administration of SEW2871 was found to attenuate LPS-induced acute inflammatory lung injury (Sammani *et al*., 2010). Therefore, multiple applications have been suggested for $S1P_1$ agonists.

KRP-203

KRP-203 is an immunosuppressive $S1P_1$ agonist with a molecular structure similar to that of fingolimod (Shimizu *et al*., 2005). Like fingolimod and SEW2871, KRP-203 can regulate lymphocyte homing and has an immunosuppressive activity (Shimizu *et al*., 2005). The action mechanism of KRP-203 is identical to that of fingolimod, that is, it involves the *in vivo* phosphorylation and functional antagonism of $S1P_1$. Furthermore, KRP-203-phosphate has an agonistic effect on $S1P_5$ like $S1P_1$, but not $S1P_{2\text{-}4}$ (Lukas *et al*., 2014). KRP-203 has been developed for use in organ transplantation. In 2005, KRP-203 was reported to prolong graft survival significantly and to reduce chronic rejection and graft vasculopathy in rat skin and heart allografts (Shimizu *et al*., 2005; Takahashi *et al*., 2005), and in 2006, it was suggested to be a potential immune modulator after rat renal transplantation (Fujishiro *et al*., 2006).

KRP-203 has also been applied in several experimental models for autoimmune disorders and inflammatory bowel diseases. In a rat experimental autoimmune myocarditis model, KRP-203 significantly inhibited the infiltrations of macrophages and $CD4^+$ T cells into myocardium, and reduced areas of inflammation (Ogawa *et al*., 2007). In a murine model of concanavalin A-induced autoimmune hepatitis, KRP-203 increased lymphocyte sequestration in secondary lymph nodes and decreased numbers of $CD4^+$ lymphocytes in liver (Kaneko *et al*., 2006).

The effects of KRP-203 have also been examined in inflammatory disorders, such as, Crohn's disease and atherosclerosis. In an interleukin (IL)-10 gene-deficient ($IL\text{-}10^{-/-}$) mouse model of chronic colitis, KRP-203 inhibited body weight loss and proinflammatory cytokine production, suppressed lymphocyte infiltration at inflammatory sites, and prevented chronic colitis (Song *et al*., 2008). In LDL receptor-deficient mice on cholesterol-rich diet, KRP-203 dramatically suppressed atherosclerotic lesion formation and induced lymphopenia, and *in vitro*, inhibited tumor necrosis factor-α, IL-6, and interferon-γ-induced protein-10 (Poti *et al*., 2013).

Currently, KRP-203 is undergoing on a phase 2 clinical trial for subacute lupus erythematosus (NCT01294774), and a phase 1 clinical trial to evaluate its safety, tolerability, pharmacokinetics, and efficacy in patients undergoing stem cell transplantation for hematological malignancies (NCT01830010) (Table 1).

AUY954

AUY954 is an aminocarboxylate analogue of fingolimod and a potent and selective $S1P_1$ agonist (Pan *et al*., 2006). AUY954 has been demonstrated to have beneficial effects after rat heart transplantation, in experimental autoimmune neuritis, and on lung inflammation. In a stringent rat transplantation model, AUY954 decreased circulating lymphocytes and prolonged cardiac allograft survival (Pan *et al*., 2006). In addition, in an animal model of experimental autoimmune neuritis (a T cell-mediated autoimmune inflammatory demyelinating disease of nervous system), AUY954 sequestered lymphocytes into secondary lymphoid tissues and significantly inhibited inflammatory demyelination, immune cell infiltration, and expressions of IL-17 and metalloproteinase-9 in rat sciatic nerves (Zhang *et al*., 2009b).

The actions of AUY954 in respiratory disorders are complicated. In an allergen-induced airway inflammation model, intranasal administration of AUY954 inhibited lymphocyte accumulation in bronchoalveolar lavage fluid, but no effect on eosinophils (Ble *et al*., 2009). On the other hand, AUY954 inhibited airway chemokine release and accumulations of activated T cells and eosinophils in ovalbumin-induced eosinophilic airway inflammation (Marsolais *et al*., 2011). However, prolonged exposure of AUY954 dramatically worsened lung injury, vascular leak, and mortality in a mouse model of bleomycin-induced lung injury, and repeated AUY954 administration increased pulmonary fibrosis by inducing vascular leak (Shea *et al*., 2010). This cautions the effects of $S1P_1$ modulators on respiratory disorders require careful interpretation.

Siponimod (BAF312)

Siponimod (also known as BAF312) is a novel alkoxyimino derivative and an agonist of $S1P_1$ and $S1P_5$ (Gergely *et al*., 2012). Siponimod is being investigated in the context of multiple sclerosis (Subei and Cohen, 2015). Like fingolimod, siponimod induces lymphopenia by preventing lymphocyte egress from lymph nodes (Fryer *et al*., 2012). In addition, siponimod was found to completely suppress experimental autoimmune encephalomyelitis in a rat model (Gergely *et al*., 2012), and in another study, to inhibit LPC-induced demyelination in organotypic slice cultures and attenuate LPS or TNFα/IL-17-induced IL-6 production in astrocytes and microglia (O'Sullivan *et al*., 2016). These findings suggest that siponimod and fingolimod may act directly through brain cells as well as through lymphopenia (Choi *et al*., 2011; O'Sullivan *et al*., 2016).

In healthy individuals, siponimod reduced T and B cell numbers in blood within 4-6 h, and numbers recovered to basal levels within a week after stopping treatment (Gergely *et al*., 2012). Siponimod may be an effective treatment for immune-mediated diseases. Initial and extended phase 2 clinical trials in patients with relapsing-remitting multiple sclerosis (BOLD) have been successfully completed (NCT 00879658, NCT01185821) (Selmaj *et al*., 2013; Kappos *et al*., 2016).

Currently, siponimod is undergoing a phase 3 efficacy and safety clinical trial in patients with secondary progressive multiple sclerosis (NCT01665144) along with mechanistic studies of phase 3 trial (NCT02330965). Also a phase 2 efficacy and tolerability clinical trial for polymyositis is undergoing (NCT01801917). A phase 2 clinical trial for dermatomyositis (NCT02029274), and two phase 1 pharmacokinetic trials for renal and hepatic impairments (NCT01904214 and NCT01565902) have been completed (Table 1).

CS-0777

CS-0777 is a selective $S1P_1$ modulator that is currently being developed for the treatment of autoimmune diseases such as multiple sclerosis (Moberly *et al*., 2012a). CS-0777 is phosphorylated *in vivo*, and the phosphorylated CS-0777 acts as

a selective $S1P_1$ agonist like fingolimod (Nishi *et al*., 2011). CS-0777 has also been investigated in multiple sclerosis, like almost all other $S1P_1$ agonists, it induces lymphopenia and suppresses experimental autoimmune encephalomyelitis (Nishi *et al*., 2011). In an open-label, pilot phase 1, clinical trial on healthy individuals and multiple sclerosis patients, oral CS-0777 decreased numbers of lymphocytes and $CD4^+$ T cells in blood, and levels returned to normal condition within 4 weeks of discontinuation (NCT00616733) (Moberly *et al*., 2012a, 2012b) (Table 1).

Ponesimod (ACT-128800)

Ponesimod is an orally active, selective $S1P_1$ agonist that induces sequestration of lymphocytes into lymphoid organs (Bolli *et al*., 2010). In contrast to the long half-life and slow elimination of fingolimod, ponesimod is eliminated within 1 week after discontinuation and its pharmacological effects are rapidly reversible (D'Ambrosio *et al*., 2016).

The clinical pharmacology of ponesimod has been described in several studies. In lymphocyte-mediated inflammatory diseases, ponesimod reduced several types of inflammation response, including edema formation, inflammatory cell infiltration, and proinflammatory cytokine levels (Piali *et al*., 2011). In addition, in a non-obese diabetic mouse model of autoimmune diabetes, ponesimod protected against disease development by reducing numbers of B and T cells in blood and spleen (You *et al*., 2013). Ponesimod is viewed as a potential new therapeutic strategy for autoimmune disorders. Once-daily treatment (10, 20 or 40 mg) significantly reduced the number of new T1 Gd+ lesions and had beneficial effects on clinical endpoints in relapsing-remitting multiple sclerosis (NCT01006265) (Olsson *et al*., 2014). Currently, a long-term safety phase 2 clinical trial in relapsing-remitting multiple sclerosis (NCT01093326) and a phase 3 oral ponesimod vs teriflunomide trial in relapsing multiple sclerosis (NCT02425644) are being conducted.

Psoriasis is a long-lasting autoimmune disease characterized by patches of abnormal skin, which are typically itchy, red, and scaly. Because psoriasis is a T cell-mediated inflammatory skin disease, studies on ponesimod have been conducted in this context. In particular, a randomized, double-blind, placebo-controlled phase 2 clinical trial showed the efficacy, safety, and tolerability of oral ponesimod in chronic plaque psoriasis (NCT01208090) (Vaclavkova *et al*., 2014).

Ponesimod is also under phase 2 clinical trial for chronic graft-*versus*-host disease (GVHD) (NCT02461134), which is a complication encountered after stem cell or bone marrow transplantation. GVHD has autoimmune-like features and chronic GVHD involves both autoreactive and alloreactive T and B cells. In GVHD, newly transplanted donor cells attack the transplant recipient's body. As $S1P_1$ modulators suppress vascular damage and immune cell accumulation in skin, and thus, reduce immune response (Huu *et al*., 2013), they offer potential means to targeting GVHD.

Ozanimod (RPC1063)

Ozanimod is an oral selective $S1P_{1/5}$ dual modulator, and induces lymphopenia and regulates immune response (Scott *et al*., 2016). In three models of autoimmune diseases that is, experimental autoimmune encephalitis, TNBS-induced colitis, and $CD4^+$ CD45RBhi T cell adaptive transfer colitis, oral ozanimod diminished inflammation parameters. This finding supports the clinical development of ozanimod for multiple sclerosis (Cohen *et al*., 2016a; Scott *et al*., 2016).

Inflammatory bowel disease is a disease of the small intestine and colon and is classified as Crohn's disease or ulcerative colitis. Crohn's disease affects the entire gastrointestinal tract, whereas ulcerative colitis usually affects colon and rectum. Because inflammatory bowel disease is a type of autoimmune disease, it is considered immunosuppression may allow control of its symptoms. Ozanimod induces peripheral lymphocyte sequestration and reduces circulating lymphocyte counts in the gastrointestinal tract (Rivera-Nieves, 2015). In a double-blind, placebo-controlled phase 2 trial in ulcerative colitis, ozanimod induced a significantly higher rate of clinical remission than a placebo (NCT01647516) (Sandborn *et al*., 2016), which suggests $S1P_1$ modulation offers a means of treating inflammatory bowel disease. Currently, ozanimod is under phase 3 trials in relapsing multiple sclerosis (NCT02047734) and moderate to severe ulcerative colitis (NCT02435992), and a phase 2 trial in moderate to severe Crohn's disease (NCT02531113) (Table 1).

Ceralifimod (ONO-4641)

Ceralifimod is a selective $S1P_{1/5}$ dual agonist (Ohno *et al*., 2010). Like fingolimod, it suppresses peripheral blood lymphocyte counts in rats by inhibiting lymphocyte egress from secondary lymphoid tissues (Ohno *et al*., 2010). Ceralifimod was also found to prevent relapsing-remitting experimental autoimmune encephalomyelitis in a non-obese diabetic mouse model (Ohno *et al*., 2010; Komiya *et al*., 2013). A phase 2 clinical trial of ceralifimod was completed for relapsing-remitting multiple sclerosis (NCT01081782) in 2011. However, a phase 2 safety and efficacy extension study of ceralifimod was terminated by developers (NCT01226745) (Subei and Cohen, 2015).

GSK2018682

GSK2018682 is a potent $S1P_1$ and $S1P_5$ agonist (Xu *et al*., 2014). In an experimental autoimmune encephalomyelitis mouse model, GSK2018682 and fingolimod exhibited similar efficacy. A phase 1 clinical trial on GSK2018682 for relapsing-remitting multiple sclerosis was completed in 2011 (NCT01466322).

MT-1303

MT-1303 is a selective $S1P_1$ modulator (a functional antagonist) that is undergoing development. MT-1303 has been subjected to a phase 2 study in moderate to severe chronic plaque psoriasis (NCT01987843), a phase 2 study of MT-1303 in relapsing-remitting multiple sclerosis (NCT01742052), and a phase 1 for inflammatory bowel disease (NCT01666327). Currently, phase 2 clinical trials are being conducted for Crohn's disease (NCT02378688) and systemic lupus erythematosus (NCT02307643) (Table 1 and www.clinicaltrials.gov).

DEVELOPMENT OF S1P RECEPTOR ANTAGONIST

JTE-013

JTE-013 is a potent, selective $S1P_2$ antagonist that has no effect on the other four S1P receptors (Osada *et al*., 2002), and has been mainly used to study the roles and functions of $S1P_2$ in different cell types and diseases.

It has been established that S1P affects various cellular

responses in endothelial cells, such as, cytoskeletal re-structuring and cell-extracellular/intracellular matrix interactions. $S1P_2$ signaling is involved in microvascular permeability, and it has been reported JTE-013 inhibition of $S1P_2$ significantly inhibited microvascular permeability in an *in vivo* animal model and regulated endothelial tight junctions and barrier function *in vitro* (Lee *et al*., 2009). In a recent preclinical study, JTE-013 modulated the responses of brain endothelium by inhibiting cerebrovascular permeability, the development of intracerebral heamorrhage, and neurovascular injury in an experimental model of stroke (Kim *et al*., 2015). After injecting the myotoxic drug notexin to induce muscle degeneration, JTE-013 treatment delayed regeneration of muscle and reduced levels of myogenin (a muscle differentiation marker), and the phosphorylation of Akt (a key marker of muscle growth) (Germinario *et al*., 2012). Therefore, $S1P_2$ signaling plays an important role in microvascular permeability and muscle regeneration.

During allergic response in lung, mast cells contain many granules rich in histamine and heparin, and thus, play central roles in various allergic diseases and anaphylaxis. $S1P_2$ expressed in mast cells was found to be involved in S1P-induced RBL-2H3 mast cell migration (Yokoo *et al*., 2004). JTE-013 also inhibited responses to ovalbumin and allergen-induced mast cell activation in rats (Trifilieff *et al*., 2009). Treatment with JTE-013 attenuated IgE-stimulated anaphylactic responses and pulmonary edema in mice (Oskeritzian *et al*., 2010), and in *in vitro* and *in vivo* studies, JTE-013 reduced mast cell activation, airway infiltration, and the serum levels of histamine and several cytokines (Oskeritzian *et al*., 2015). In addition, JTE-013 has been reported to inhibit S1P-induced fibroblast chemotaxis, Rho activation, and focal adhesion kinase phosphorylation, which all affect tissue repair after injury (Hashimoto *et al*., 2008). JTE-013 also blocked S1P-induced inhibition of migration and Rac1-dependent signaling pathway in human bronchial smooth muscle cells (Kawata *et al*., 2005). These findings suggest that $S1P_2$ antagonism offers a novel means of treating airway allergic diseases.

Pancreatic β cell dysfunction contributes to the development of insulin resistance and of type 2 diabetes, and several authors have reported relations between $S1P_2$ and type 2 diabetes. In a murine model, JTE-013 suppressed streptozotocin-induced blood glucose increases, pancreatic β cell apoptosis, and the incidence of diabetes (Imasawa *et al*., 2010). Furthermore, JTE-013 protected pancreatic β cells in a New Zealand obese diabetic mouse model under high-fat diet conditions (Japtok *et al*., 2015). Levels of plasminogen activator inhibitor-1, which is produced from adipocytes, are increased in obese individuals. Interestingly, JTE-013 suppressed plasminogen activator inhibitor-1 increases in mouse 3T3-L1 adipocytes (Ito *et al*., 2013), and $S1P_2$ deficient mice fed a high-fat diet had better glucose/insulin tolerance test results and smaller epididymal adipocytes (Kitada *et al*., 2016). These results suggest JTE-013 might be useful for treating obesity and type 2 diabetes.

Treatment with JTE-013 also reduced plasma levels of IL-1β and IL-18 (endotoxin-induced inflammatory cytokines) in $ApoE^{-/-}$ mice and $S1P_2$ gene deficiency reduced atherosclerosis (Skoura *et al*., 2011). Furthermore, JTE-013 modulated the permeability and inflammatory responses of the vascular endothelium during endotoxemia (Zhang *et al*., 2013), and $S1P_2$ was suggested to be a critical receptor in macrophages due to its impairment of phagocytosis and antimicrobial defense during the pathogenesis of sepsis (Hou *et al*., 2015). These findings indicate JTE-013 offers a novel means of treating inflammatory disorders, such as, atherosclerosis and sepsis.

CLOSING REMARK

The discovery of S1P receptors and subsequent finding of fingolimod as a modulator of $S1P_1$ receptor successfully linked to the FDA approval of fingolimod as a first orally active drug treating relapsing multiple sclerosis. The commercial release of fingolimod stimulated pharmaceutical researchers to develop better drugs targeting $S1P_1$ in terms of efficacy and safety, and these efforts have resulted in multiple drug candidates for clinical trials (Table 1). Much effort has been made to overcome the non-selectivity of fingolimod, which acts on $S1P_1$ and $S1P_{3\text{-}5}$, and as a result $S1P_1$ monoselective agonists, such as AUY954, CS-0777, KRP-203, SEW2871, ponesimod, and MT-1303, have been developed. Dual agonists on $S1P_1$ and $S1P_5$ have also been produced, such as, ceralifimod, siponimod, ozanimod, and GSK2018682. The first clinical approval issued for fingolimod was for the treatment of relapsing multiple sclerosis. Currently, fingolimod and other $S1P_1$ modulators are being developed for autoimmune disease and for inflammatory disorders, such as, plaque psoriasis, dermatomyositis, Crohn's disease, ulcerative colitis, polymyositis, liver failure, renal failure, acute stroke, GVHD, and transplant rejection. Because these exhibit greater selectivity for $S1P_1$ than fingolimod, it is hoped that they will cause fewer adverse effects and be more effective. We are confident that in near future, more S1P receptor modulators will be approved for the treatment of disorders associated with autoimmune and inflammation.

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