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 through G proteins but they also initiate signals via other entities (Davenport et al., 2013). Many ligands, including hormones, 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 H1 histamine receptor antagonist, Cozaar an AT1 angiotensin receptor antagonist, Neurontin a GABA B receptor antagonist, Plavix a P2Y12 ADP receptor antagonist, Singulair a CysLT1 leukotriene receptor antagonist, and Zyprexa a mixed D2/D1/5-HT2 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).

Key Words: Sphingosine 1-phosphate, G protein-coupled receptor, Fingolimod, FTY720, Drug discovery, S1P agonist
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 discovery 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<sub>1</sub> (formerly known as Edg-1) in 1998 along with four other S1P<sub>a</sub> 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 Pfeflschifer, 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<sub>1</sub> 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<sub>1</sub>.

DEVELOPMENT OF S1P<sub>1</sub> RECEPTOR MODULATORS

Fingolimod (FTY720, Gilenya<sup>®</sup>, 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<sub>1</sub>, was found to directly alter the trafficking of naïve and antigen-activated CD4<sup>+</sup> 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<sub>1</sub>, S1P<sub>3</sub>, S1P<sub>4</sub>, and S1P<sub>5</sub>) (Billich et al., 2003). Second, effects of fingolimod on lymphocyte egress involve not agonistic but rather functional antagonistic activity against S1P<sub>1</sub> (Fig. 1) (Mattouibian et al., 2004). Both S1P and fingolimod-phosphate have been reported to induce lymphopenia via the agonistic activation of S1P<sub>1</sub> and subsequent internalization of S1P<sub>1</sub> in the lymphocytes (Brinkmann et al., 2004; Thangada et al., 2010). In fact, the absence of S1P<sub>1</sub> 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<sub>1</sub> (Brinkmann et al., 2004; Chiba, 2009). In the case of S1P<sub>1</sub> agonist like S1P, internalized S1P<sub>1</sub> recycled back to the cell surface within several hours. However, in the case of S1P<sub>1</sub> modulators like fingolimod-phosphate, internalized S1P<sub>1</sub> undergoes proteosomal degradation, resulting in long-time absence of S1P<sub>1</sub> 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 autoimmune 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<sub>1</sub> 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<sub>1</sub> (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 (Bie et al., 2008; Marsolais et al., 2011; Trifliett and Fozard, 2012). Furthermore, in low-density lipoprotein
(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 (S1P1, 3-5) (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).

**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.

**SEW2871**

SEW2871 is a highly selective S1P 1 agonist, which does not act on S1P 2-6. 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
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 S1P1 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 (Aβ1-42)-induced spatial memory impairment and hippocampal neuronal loss, indicating the S1P1 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-

### 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     | S1P1/S1P3/S1P5 | Relapsing remitting multiple sclerosis (RRMS) | Approved       | (2010)             | NCT02575365          |
|                |             | Neurodegeneration                              | Phase IV       | Active             | NCT02159023          |
|                |             | 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        | S1P1        | Subacute cutaneous lupus erythematosus         | Phase II       | Active             | NCT01294774          |
|                |             | Hematological malignancies                     |                |                    |                      |
| Siponimod      | S1P1/S1P5   | Secondary progressive multiple sclerosis       | Phase III      | Active             | NCT01665144          |
|                |             | RRMS                                           | Phase II       | Completed (2012)   | NCT00879658          |
|                |             | - (Extension) -                                |                | - Active            | NCT011851821         |
|                |             | 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        | S1P1        | Multiple sclerosis                             | Phase I        | Completed (2010)   | NCT00616733          |
| Ponesimod      | S1P1        | 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)   | NCT1208090           |
| Ozanimod       | S1P1/S1P5   | Multiple sclerosis                             | Phase III      | Active             | NCT02047734          |
|                |             | Ulcerative colitis                             | Phase III      | Active             | NCT02435992          |
|                |             | Crohn's disease                                | Phase II       | Active             | NCT02531113          |
| Ceralifimod    | S1P1/S1P5   | RRMS                                           | Phase II       | Completed (2011)   | NCT01081782          |
|                |             | - (Extension) -                                | Phase II       | Terminated (2015)  | NCT01226745          |
| GSK2018682     | S1P1/S1P5   | RRMS                                           | Phase I        | Completed (2011)   | NCT01466322          |
| MT-1303        | S1P1        | 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.
lagent α 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 (Sammann et al., 2010). Therefore, multiple applications have been suggested for S1P₁ agonists.

**KRP-203**

KRP-203 is an immunosuppressive S1P₁ 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 S1P₁. Furthermore, KRP-203-phosphate has an agonistic effect on S1P₁ like S1P₂, but not S1P₃, (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-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 S1P₁ 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 (Bie 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 S1P₁ modulators on respiratory disorders require careful interpretation.

**Siponimod (BAF312)**

Siponimod (also known as BAF312) is a novel alkoxyimino derivative and an agonist of S1P₁ and S1P₅ (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 TNF-α/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 (NCT00879658, NCT01185821) (Selmaï 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 S1P₁ 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 S1P₁ agonist like fingolimod (Nishi et al., 2011). CS-0777 has also been investigated in multiple sclerosis, like almost all other S1P₁ 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 S1P₁ agonist that induces sequestration of lymphocytes into lymphoid organs (Bollig 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 S1P₁ 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 S1P₁/S₅ dual modulator, and inhibits 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 S1P₁ 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 S1P₁/S₅ 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 S1P₁, and S1P₅ 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 S1P₁ 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 S1P₂ 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 S1P₂ 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. S1P2 signaling is involved in microvascular permeability, and it has been reported JTE-013 inhibition of S1P2 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 haemorrhage, 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, S1P2 signaling plays an important role in microvascular permeability and muscle growth.

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. S1P2 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 (Trifilli 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 S1P2 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 S1P2 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 obse diabetic mouse model under high-fat diet conditions (Japik et al., 2015). Levels of plasmogen activator inhibitor-1, which is produced from adipocytes, are increased in obese individuals. Interestingly, JTE-013 suppressed plasmogen activator inhibitor-1 increases in mouse 3T3-L1 adipocytes (Ito et al., 2013), and S1P2 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 S1P2 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 S1P2 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 S1P1 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 S1P1 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 S1P1, S1P2, and as a result S1P3, monoselective agonists, such as AUY924, CS-0777, KRP-203, SEW2871, ponesimod, and MT-1303, have been developed. Dual agonists on S1P1 and S1P2 have also been produced, such as, cerafimod, siponimod, ozanimod, and GS0018682. The first clinical approval issued for fingolimod was for the treatment of relapsing multiple sclerosis. Currently, fingolimod and other S1P1 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 S1P1 than fingolimod, it is hoped that they will cause fewer adverse effects and be more effective. We are confident that in near future, more S1P1 receptor modulators will be approved for the treatment of disorders associated with autoimmune and inflammation.

**ACKNOWLEDGMENTS**

This research was supported by the Basic Science Research Program of the Korean National Research Foundation funded by the Korean Ministry of Education, Science and Technology (NRF-2016R1D1A1A009917086) and by the Korean National Research Foundation funded by the Korean government (MSIP) (Grant no. 2009-0083538).

**REFERENCES**

Adachi, K. and Chiba, K. (2008) FTY720 story. Its discovery and the following accelerated development of sphingosine 1-phosphate receptor agonists as immunomodulators based on reverse pharmacology. *Perspect. Medizin. Chem.* 1, 11-23.

An, S., Bleu, T., Huang, W., Hallmark, O. G., Coughlin, S. R. and Goetzl, E. J. (1997) Identification of cDNAs encoding two G protein-coupled receptors for lysosphingolipids. *FEBS Lett.* 417, 279-282.

Asle-Rousta, M., Oryan, S., Ahmadiani, A. and Rahmema, M. (2013) Activation of sphingosine 1-phosphate receptor-1 by SEW2871 improves cognitive function in Alzheimer’s disease model rats. *EXCLI J.* 12, 449-461.

Awad, A. S., Ye, H., Huang, L., Li, L., Foss, F. W., Jr., Macdonald, T. L., Lynch, K. R. and Okusa, M. D. (2006) Selective sphingosine 1-phosphate receptor 1 receptor activation reduces ischemia-reperfusion injury in mouse kidney. *Am. J. Physiol. Renal Physiol.* 290, F1516-F1524.

Billich, A., Bornancin, F., Devay, P., Metchtchevikova, D., Urtz, N. and Billich A1, Bornancin, F., Dévay, P., Metchtchevikova, D., Urtz, N.
and Baumrucker, T. (2003) Phosphorylation of the immunomodulatory drug FTY720 by sphingosine kinases. J. Biol. Chem. 278, 47408-47415.

Ble, F. X., Cannel, C., Zurburreg, S., Gerard, C., Frossard, N., Beckmann, N. and Triffleif, A. (2009) Activation of the lung S1P receptor reduces allergen-induced plasma leakage in mice. Br. J. Pharmacol. 158, 1295-1301.

Boll, M. H., Abele, S., Binkert, C., Bravo, R., Buehnmann, S., Bur, D., Gatfield, J., Hess, P., Kohl, C., Mangold, C., Mathys, B., Menny, K., Muller, C., Nayler, O., Scherz, M., Schmidt, G., Sippel, V., Steiner, B., Strasser, D., Treiber, A. and Weiler, T. (2010) 2-iminothiazolidin-4-one derivatives as potent, orally active S1P receptor agonists. J. Med. Chem. 53, 4198-4211.

Brinkmann, V., Cyster, J. G. and Hla, T. (2004) FTY720: sphingosine 1-phosphate receptor-1 in the control of lymphocyte egress and endothelial barrier function. Am. J. Transplant. 4, 1019-1025.

Brinkmann, V., Davis, M. D., Heise, C. E., Albert, R., Cottens, S., Hof, R., Bruns, C., Prieschel, E., Baumrucker, T., Hiestand, P., Foster, C. A., Zollinger, M. and Brinkmann, V., Davis, M. D., Heise, C. E., Albert, R., Cottens, S., Hof, R., Bruns, C., Prieschel, E., Baumrucker, T., Hiestand, P., Foster, C. A., Zollinger, M. and Lynch, K. R. (2002) The immune modulator FTY720 targets sphingosine 1-phosphate receptors. J. Biol. Chem. 277, 21453-21457.

Bunemann, M., Brandts, B., zu Heringdorf, D. M., van Koppen, C. J., Brinkmann, V., Cyster, J. G. and Hla, T. (2004) FTY720: sphingosine 1-phosphate receptor modulator. J. Pharmacol. Ther. 108, 308-319.

Chiba, K. (2005) New therapeutic approach for autoimmune diseases in the central nervous system. Biochim. Biophys. Acta 1831, 20-32.

Chiba, K. (2009) New therapeutic approach for autoimmune diseases by the sphingosine 1-phosphate receptor modulator, fingolimod (FTY720). Yakugaku zasshi 129, 655-665.

Choi, J. W. and Chun, J. (2013) Lysophospholipids and their receptors in guinea-pig airway myocytes by sphingosine-1-phosphate. J. Physiol. 489, 701-707.

Calabresi, P. A., Radue, E. W., Goodin, D., Jeffery, D., Rammohan, K. W., Reder, A. T., Vollmer, T., Agius, M. A., Kappos, L., Stites, T., Li, B., Cappiello, L., von Rosenstiel, P. and Lublin, F. D. (2014) Safety and efficacy of fingolimod in patients with relapsing-remitting multiple sclerosis (FREEDOMS II): a double-blind, randomised, placebo-controlled, phase 3 trial. Lancet Neurology 13, 545-556.

Camm, J., Hla, T., Bakshi, R. and Brinkmann, V. (2014) Cardiac and vascular effects of fingolimod: mechanistic basis and clinical implications. Am. Heart J. 168, 632-644.

Chiba, K. (2005) FTY720, a new class of immunomodulator, inhibits lymphocyte egress from secondary lymphoid tissues and thymus by agonistic activity at sphingosine 1-phosphate receptors. Pharmacol. Ther. 108, 308-319.

Chiba, K. (2009) New therapeutic approach for autoimmune diseases by the sphingosine 1-phosphate receptor modulator, fingolimod (FTY720). Yakugaku zasshi 129, 655-665.

Choi, J. W. and Chun, J. (2013) Lysophospholipids and their receptors in the central nervous system. Biochim. Biophys. Acta 1831, 20-32.

Choi, J. W., Gardell, S. E., Herr, D. R., Rivera, R., Lee, C. W., Zollinger, M. and Mobley, K. W. (2011) FTY720 (fingolimod) efficacy in an animal model of multiple sclerosis (S1PR1(-/-)): a double-blind, randomised, placebo-controlled, phase 3 trial. Lancet Neurology 10, 1002-1101.

Fujishiro, J., Kudou, S., Iwai, S., Takahashi, M., Hakamata, Y., Kinosita, M., Iwamami, S., Izawa, S., Yasue, T., Hashizume, K., Murakami, T. and Kobayashi, E. (2006) Use of sphingosine-1-phosphate 1 receptor agonist, KRP-203, in combination with a subtherapeutic dose of cyclosporine A for rat renal transplantation. Transplantation 82, 804-812.

Gardell, S. E., Dubin, A. E. and Chun, J. (2006) Emerging medicinal roles for lysophospholipid signaling. Trends Mol. Med. 12, 65-75.

Gergely, P., Nuesselein-Hideshus, B., Guerini, D., Brinkmann, V., Traebert, M., Bruns, C., Pan, S., Gray, N. S., Hinterdinger, K., Cooke, N. G., Groeneewegen, A., Vitaliti, A., Sing, T., Luttriger, O., Yang, J., Gardin, A., Wang, N., Crumb, W. J., Jr., Saltzman, M., Rosenberg, M. and Wallstrom, E. (2012) The selective sphingosine 1-phosphate receptor modulator BAF312 redirects lymphocyte distribution and has species-specific effects on heart rate. Br. J. Pharmacol. 167, 1035-1047.

Germinario, E., Peron, S., Toniolo, L., Betto, R., Cencetti, F., Donati, C., Bruni, P. and Danieli-Betto, D. (2012) S1P2 receptor promotes mouse skeletal muscle regeneration. J. Appl. Physiol. 113, 707-713.

Gollmann, G., Neuwirt, H., Tripp, C. H., Mueller, H., Konwalinka, G., Heuffer, C., Romani, N. and Tiefenthaler, M. (2008) Sphingosine-1-phosphate receptor type-1 agonism impairs blood dendritic cell chemotaxis and skin dermal cell migration to lymph nodes under inflammatory conditions. Int. Immunol. 20, 911-923.

Goodemote, K. A., Mattie, M. E., Berger, A. and Spiegel, S. (1995) Involvement of a pertussis toxin-sensitive G protein in the mitogenic signaling pathways of sphingosine 1-phosphate. J. Biol. Chem. 270, 10272-10277.

Hashimoto, M., Wang, X., Mao, L., Kobayashi, T., Kawasaki, S., Mori, N., Toews, M. L., Kim, J. H., Cerutis, D. R., Liu, X. and Rennard, S. I. (2008) Sphingosine 1-phosphate potentiates human lung fibroblast chemotaxis through the S1P1 receptor. Am. J. Respir. Cell Mol. Biol. 39, 356-363.

Hofmann, U., Burkard, N., Vogt, C., Thoma, A., Frantz, S., Ertl, G., Ritter, O. and Bonz, A. (2009) Protective effects of sphingosine-1-phosphate receptor agonist treatment after myocardial ischaemia-reperfusion. Cardiovasc. Res. 83, 285-293.

Hou, J., Chen, Q., Zhang, K., Cheng, B., Xie, G., Wu, X., Luo, C., Chen, L., Liu, H., Zhao, B., Dai, K. and Fang, X. (2015) Sphingosine-1-phosphate receptor 2 signaling suppresses macrophage phagocytosis and impairs host defense against sepsis. Anesthesiology 123, 409-422.

Howard, A. D., McAllister, G., Feighner, S. D., Liu, Q., Nargund, R. P., Van der Ploeg, L. H. and Patchett, A. A. (2001) Orphan G-protein-coupled receptors and natural ligand discovery. Trends Pharmacol.
K. E. and Vollmer, T. L. (2012a) Pharmacological effects of CS-0777, a selective sphingosine-1-phosphate receptor-1 modulator: results from a 12-week, open-label pilot study in multiple sclerosis patients. J. Neuroimmunol. 246, 100-107.

Moberly, J. B., Rohatagi, S., Zahir, H., Noveck, R. J. and Trut, K. E. (2012b) Pharmacological modulation of peripheral T and B lymphocytes by a selective sphingosine-1-phosphate receptor-1 modulator. J. Clin. Pharmacol. 52, 996-1006.

Moonenar, W. H. and Hla, T. (2012) SnapShot: Bioactive lysosphospholipids. Cell 148, 378-378.e2.

Mutoh, T., Rivera, R. and Chun, J. (2012) Insights into the pharmacological relevance of lysosphospholipid receptors. Br. J. Pharmacol. 165, 829-844.

Nishi, T., Miyazaki, S., Takemoto, T., Suzuki, K., Iio, Y., Nakajima, K., Ohnuki, T., Kawase, Y., Nara, F., Inaba, S., Izumi, T., Yuita, H., Os-hima, K., Doi, H., Inoue, R., Tomisato, W., Kagiari, T. and Shimo-zato, T. (2011) Discovery of CS-0777: a potent, selective, and orally active S1P agonist. ACS Med. Chem. Lett. 2, 386-387.

Noda, H., Takeuchi, H., Mizuno, T. and Suzumura, A. (2013) Fingolimod phosphate promotes the neuroprotective effects of microglia. J. Neuroimmunol. 256, 13-18.

Nofer, J. R., Bot, M., Brodde, M., Taylor, P. J., Salm, P., Brinkmann, V., van Berkel, T., Assmann, G. and Biessen, E. A. (2007) FTY720, a synthetic sphingosine 1-phosphate receptor agonist, inhibits development of atherosclerosis in low-density lipoprotein receptor-deficient mice. Circulation 115, 501-508.

O’Sullivan, C., Schubart, A., Mir, A. K. and Dev, K. K. (2016) The dual S1PR1/S1PR2 drug BAF312 (Siponimod) attenuates demyelination in organotypic slice cultures. J. Neuroinflammation 13, 31.

Ogawa, R., Takahashi, M., Hirose, S., Morimoto, H., Ise, H., Murakami, J. B., Rohatagi, S., Zahir, H., Hsu, C., Noveck, R. J. and Trudósada, M., Yatomi, Y., Ohmori, T., Ikeda, H. and Ozaki, Y. (2002) Oo, M. L., Thangada, S., Wu, M. T., Liu, C. H., Macdonald, T. L., Lynch, Olsson, T., Boster, A., Fernandez, O., Freedman, M. S., Pozzilli, C., Olivera, A. and Spiegel, S. (1993) Sphingosine-1-phosphate as a second messenger in the murine mast cell: function and receptor activation. Lab. Invest. 90, 1209-1224.

Park, S. W., Kim, M., Chen, S. W., Brown, K. M., D’Agati, V. D. and Lee, H. T. (2010) Sphingosine-1-phosphate protects kidney and liver after hepatic ischemia and reperfusion in mice through S1P receptor activation. J. Pharmacol. Exp. Ther. 337, 547-556.

Paugh, S. W., Payne, S. G., Barbour, S. E., Milstien, S. and Spiegel, S. (2003) The immunosuppressant FTY720 is phosphorylated by sphingosine kinase type 2. FEBS Lett. 554, 189-193.

Plati, L., Froidevaux, S., Hess, P., Nayler, O., Bolli, M. H., Schlosser, E., Kohl, C., Steiner, B. and Clozel, M. (2011) The selective sphingo-sine-1-phosphate receptor 1 agonist posesmide protects against lymphocyte-mediated tissue inflammation. J. Pharmacol. Exp. Ther. 337, 138-145.

Pott, F., Costa, S., Bergonzini, V., Galletti, M., Pignatti, E., Weber, C., Simoni, M. and Nofer, J. R. (2012) Effect of sphingosine-1-phosphate (S1P) receptor agonists FTY720 and CYM5442 on atherosclerosis development in LDL receptor deficient (LDL-R−/−) mice. Vasc. Pharmacol. 57, 56-64.

Pott, F., Guittieri, F., Sacchi, S., Weissenn-Plenz, G., Varga, G., Brodde, M., Weber, C., Simoni, M. and Nofer, J. R. (2013) KRP-203, sphingosine-1-phosphate receptor type 1 agonist, ameliorates atherosclerosis in LDL-R−/− mice. Arterioscler. Thromb. Vasc. Biol. 33, 1505-1512.

Proia, R. L. and Hla, T. (2015) Emerging biology of sphingosine-1-phosphate: its role in pathogenesis and therapy. J. Clin. Invest. 125, 1379-1387.

Pyne, N. J., Tonelli, F., Lim, K. G., Long, J. S., Edwards, J. and Pyne, S. (2012) Sphingosine-1-phosphate signalling in cancer. Biochem. Soc. Trans. 40, 94-100.

Rivera-Nieves, J. (2015) Strategies that target leukocyte traffic in inflammatory bowel diseases: recent developments. Curr. Opin. Gastroenterol. 31, 441-448.

Sammani, S., Moreno-Vinasco, L., Mirzapoiavzoz, T., Singleton, M. A., Schumpelick, V., David, J. B., Logsdon, M. B., Ohmori, T., Ikeda, H. and Ozaki, Y. (2002) Enhancement of sphingosine-1-phosphate-induced migration of vascular endothelial cells and smooth muscle cells by an EDG-5 antagonist. Biochem. Biophys. Res. Commun. 299, 483-487.

Oskeritzian, C. A., Price, M. M., Hilt, N. C., Kapitonov, D., Falanga, Y. T., Morales, J. K., Ryan, J. J., Milstien, S. and Spiegel, S. (2010) Essential roles of sphingosine-1-phosphate receptor 2 in human mast cell activation, anaphylaxis, and pulmonary edema. J. Exp. Med. 207, 465-474.

Overington, J. P., Al-Lazikani, B. and Hopkins, A. L. (2006) How many drug targets are there? Nat. Rev. Drug Discov. 5, 993-996.

Pan, S., Mi, Y., Pally, C., Beerli, C., Chen, A., Guerin, D., Hintterd, K., Nusslein-Hilshesse, B., Tumtland, T., Lefebvre, S., Liu, Y., Gao, W., Chu, A., Brinkmann, V., Bruns, C., Streiff, M., Cnnen, C., Cooke, N. and Gray, N. (2006) A monoselective sphingosine-1-phosphate receptor-1 agonist prevents allograft rejection in a stringent rat heart transplantation model. Chem. Biol. 13, 1227-1234.

Park and Im. S1P Receptor Modulators and Drug Discovery
man, M. S., Stuve, O., Rieckmann, P., Montalban, X., Ziemssen, T., Auberson, L. Z., Pohlmann, H., Mercier, F., Dahlke, F. and Wallstrom, E. (2013) Sipimod for patients with relapsing-remitting multiple sclerosis (BOLD): an adaptive, dose-ranging, randomised, phase 2 study. *Lancet Neurol.* **12**, 756-767.

Shea, B. S., Brooks, S. F., Fontaine, B. A., Chun, J., Luster, A. D. and Tager, A. M. (2010) Prolonged exposure to sphingosine 1-phosphate receptor-1 agonists exacerbates vascular leak, fibrosis, and mortality after lung injury. *Am. J. Respir. Cell Mol. Biol.* **43**, 662-673.

Shimizu, H., Takahashi, M., Kaneko, T., Murakami, T., Hakamata, Y., Kudou, S., Kishi, T., Fukuchi, K., Iwamani, S., Kurniawan, K., Yasue, T., Enosawa, S., Matsumoto, K., Takeyoshi, I., Morishita, Y. and Kobayashi, E. (2005) KRP-203, a novel synthetic immunosuppressant, prolongs graft survival and attenuates chronic rejection in rat skin and heart allografts. *Circulation* **111**, 222-229.

Skoura, A., Michaud, J., Im, D. S., Thangada, S., Xiong, Y., Smith, J. D. and Hla, T. (2011) Sphingosine-1-phosphate receptor-2 function in myeloid cells regulates vascular inflammation and atherosclerosis. *Artersioles. Thromb. Vasc. Biol.* **31**, 81-85.

Song, J., Matsuda, C., Kai, Y., Nishida, T., Nakajima, K., Mizushima, T., Kinoshita, M., Yasue, T., Sawa, Y. and Itö, T. (2008) A novel sphingosine 1-phosphate receptor agonist, 2-amino-2-propanediol hydrochloride (KRP-203), regulates chronic colitis in interleukin-10 gene-deficient mice. *J. Pharmacol. Exp. Ther.* **324**, 276-283.

Stone, M. L., Sharma, A. K., Zhao, Y., Charles, E. J., Hueter, M. E., Johnston, W. F., Kron, I. L., Lynch, K. R. and Laubach, V. E. (2015) Sphingosine-1-phosphate receptor 1 agonism attenuates lung ischemia-reperfusion injury. *Am. J. Physiol. Lung Cell Mol. Physiol.* **308**, L1245-L1252.

Subei, A. M. and Cohen, J. A. (2015) Sphingosine 1-phosphate receptor modulators in multiple sclerosis. *CNS Drugs* **29**, 565-573.

Takahashi, M., Shimizu, H., Murakami, T., Enosawa, S., Suzuki, C., Takeno, Y., Hakamata, Y., Kudou, S., Izawa, S., Yasue, T. and Kobayashi, E. (2005) A novel immunomodulator KRP-203 combined with cyclosporine prolongs graft survival and abrogated transplant vasculopathy in rat heart allografts. *Transplant. Proc.* **37**, 143-145.

Thangada, S., Khanna, K. M., Blaho, V. A., Oo, M. L., Im, D. S., Guo, C., Lefrancos, L. and Hla, T. (2010) Cell-surface residence of sphingosine 1-phosphate receptor 1 on lymphocytes determines lymphocyte egress kinetics. *J. Exp. Med.* **207**, 1475-1483.

Trifilieff, A., Baur, F. and Fozard, J. R. (2009) Role of sphingosine-1-phosphate (S1P) and the S1P1 receptor in allergen-induced, mast cell-dependent contraction of rat lung parenchymal strips. *Naunyn Schmiedebergs Arch. Pharmacol.* **380**, 303-309.

Trifilieff, A. and Fozard, J. R. (2012) Sphingosine-1-phosphate-induced airway hyper-reactivity in rodents is mediated by the sphingosine-1-phosphate type 3 receptor. *J. Pharmacol. Exp. Ther.* **342**, 399-406.

Vaclavkova, A., Chimenti, S., Arenberger, P., Hollo, P., Sator, P. G., Burcklen, M., Stefani, M. and D’Ambrosio, D. (2014) Oral ponesimod in patients with chronic plaque psoriasis: a randomised, double-blind, placebo-controlled phase 2 trial. *Lancet* **384**, 2036-2045.

Van Brocklyn, J. R., Grale, M. H., Bernhardt, G., Hobson, J. P., Lipp, M. and Spiegel, S. (2000) Sphingosine-1-phosphate is a ligand for the G protein-coupled receptor EDG-6. *Blood* **95**, 2624-2629.

van Koppen, C., Meyer zu Heringdorf, M., Laser, K. T., Zhang, C., Jacobs, K. H., Bunemann, M. and Pott, L. (1996) Activation of a high affinity G1 protein-coupled plasma membrane receptor by sphingo-1-phosphate. *J. Biol. Chem.* **271**, 2082-2087.

Wang, M., Lu, L., Liu, Y., Gu, G. and Tao, R. (2014) FTY720 attenuates hypoxia-reoxygenation-induced apoptosis in cardiomyocytes. *Exp. Mol. Pathol.* **97**, 218-224.

Wei, S. H., Rosen, H., Matheu, M. P., Sanna, M. G., Wang, S. K., Jos, E., Wong, C. H., Parker, I. and Cahalan, M. D. (2005) Sphingosine 1-phosphate type 1 receptor agonism inhibits transendothelial migration of medullary T cells to lymphatic sinuses. *Nat. Immunol.* **6**, 1228-1235.

Whetzel, A. M., Bolick, D. T., Shinivasan, S., Macdonald, T. L., Morris, M. A., Ley, K. and Hedrick, C. C. (2006) Sphingosine-1-phosphate prevents monocyte/endothelial interactions in type 1 diabetic NOD mice through activation of the S1P1 receptor. *Circ. Res.* **99**, 731-739.

Xie, J. H., Nomura, N., Koprak, S. L., Quackenbush, E. J., Forrest, M. J. and Rosen, H. (2003) Sphingosine-1-phosphate receptor agonism impairs the efficiency of the local immune response by altering trafficking of naive and antigen-activated CD4+ T cells. *J. Immunol.* **170**, 3662-3670.

Xu, J., Gray, F., Henderson, A., Hicks, K., Yang, J., Thompson, P. and Oliver, J. (2014) Safety, pharmacokinetics, pharmacodynamics, and bioavailability of GSK2018682, a sphingosine-1-phosphate receptor modulator, in healthy volunteers. *Clin. Pharmacol. Drug Dev.* **3**, 170-178.

Yamazaki, Y., Kon, J., Sato, K., Tomura, H., Sato, M., Yoneya, T., Okazaki, H., Okajima, F. and Ohta, H. (2000) Edg-6 as a putative sphingosine 1-phosphate receptor coupling to Ca2+ signaling pathway. *Biochem. Biophys. Res. Commun.* **268**, 583-589.

Yokoo, E., Yatomi, Y., Takahata, T., Osada, M., Okamoto, Y. and Ozaki, Y. (2004) Sphingosine 1-phosphate inhibits migration of RBL-2H3 cells via S1P1: cross-talk between platelets and mast cells. *J. Biochem.* **135**, 673-681.

You, S., Piali, L., Kuhn, C., Steinier, B., Sauvaget, V., Valette, F., Clozel, M., Bach, J. F. and Chatenoud, L. (2013) Therapeutic use of a selective S1P receptor modulator ponesimod in autoimmune diabetes. *Diabetes* **62**, 443-455.

Zhang, G., Yang, L., Kim, G. S., Ryan, K., Lu, S., O’Donnell, R. K., Spokes, K., Shapiro, N., Aird, W. C., Kluks, M. J., Yano, K. and Sanchez, T. (2013) Critical role of sphingosine-1-phosphate receptor 1 (S1PR1) in acute vascular inflammation. *Blood* **122**, 443-455.

Zhang, Z. Y., Zhang, Z. and Schleszensky, H. J. (2009a) FTY720 attenuates lesional interleukin-17+ cell accumulation in rat experimental autoimmune neuritis. *Neuropathol. Appl. Neurobiol.* **35**, 487-495.

Zhang, Z. Y., Zhang, Z., Zuc, C., Neuses-Heilmes, B., Leppert, D. and Schleszensky, H. J. (2009b) AUY954, a selective S1P modulator, prevents experimental autoimmune neuritis. *J. Neuroimmunol.* **216**, 59-65.

Zhu, Z., Fu, Y., Tian, D., Sun, N., Han, W., Chang, G., Dong, Y., Xu, L., Liu, Q., Huang, D. and Shi, F. D. (2015) Combination of the immune modulator fingolimod with atelapase in acute ischemic stroke: a pilot trial. *Circulation* **132**, 1104-1112.