The sphingosine 1-phosphate receptor modulator fingolimod as a therapeutic agent: recent findings and new perspectives

Andrea Huwiler, Uwe Zangemeister-Wittke

PII: S0163-7258(17)30285-1
DOI: doi:10.1016/j.pharmthera.2017.11.001
Reference: JPT 7151

To appear in: *Pharmacology and Therapeutics*

Please cite this article as: Huwiler, A. & Zangemeister-Wittke, U., The sphingosine 1-phosphate receptor modulator fingolimod as a therapeutic agent: recent findings and new perspectives, *Pharmacology and Therapeutics* (2017), doi:10.1016/j.pharmthera.2017.11.001

P&T 23191

The sphingosine 1-phosphate receptor modulator fingolimod as a therapeutic agent: recent findings and new perspectives.

Andrea Huwiler* and Uwe Zangemeister-Wittke

Institute of Pharmacology, University of Bern, Inselspital INO-F, CH-3010 Bern, Switzerland

[#] address for correspondence:

Prof. Dr. Andrea Huwiler, email: Huwiler@pki.unibe.ch

Contents

Abstract

The immunomodulatory drug fingolimod (FTY720, Gilenya^{R}) was approved for oral treatment of relapsing-remitting multiple sclerosis, due to its impressive efficacy and good tolerability. Pharmacologically, it acts as an unselective agonist of sphingosine 1-phosphate receptors (S1PR) and as a selective functional antagonist of the $S1P_1$ subtype by induction of receptor downregulation. Since $S1P_1$ is crucial for the regulation of lymphocyte trafficking, its downregulation causes redistribution of the immune cells to secondary lymphoid tissues, resulting in the depletion from the circulation and hence immunosuppression. Numerous preclinical studies have since been performed with the aim to increase the spectrum of potential indications for fingolimod with emphasis on other autoimmune disorders and diseases associated with inflammation and uncontrolled cell proliferation, including cancer. As an alternative to fingolimod, novel S1PR modulators with a more selective receptor activation profile and improved pharmacokinetic performance and tolerability have also been developed. Preclinical and clinical studies are ongoing to investigate their therapeutic potential for various indications. This review discusses the most relevant preclinical and clinical findings from S1PR-targeting and from less-well defined off-target effects reported in the literature, and reveals perspectives for using fingolimod and functionally-related derivatives and new formulations in the management of an increasing number of diseases.

Keywords: Fingolimod, S1P receptor modulators, preclinical developments, pharmacology, clinical indications, adverse events

List of abbreviations:

ALS, amyotrophic lateral sclerosis; BDNF, brain-derived neurotropic factor; CerS, ceramide synthase; CNS, central nervous system; CPL, cecal ligation and puncture; $cPLA_2$, cytosolic phospholipase A_2; CSA, cyclosporine; CTL, cytotoxic T lymphocytes; CYP450, cytochrome P450; DAMPs, damage-associated molecular patterns; EAE, experimental autoimmune encephalomyelitis; ERK, extracellular signal-regulated protein kinase; FTY720, fingolimod; HDAC, histone deacetylase; HDL, high density lipoprotein; ISP-1, myriocin; KO, knockout; LPA, lysophosphatidic acid; LPP3, lysophospholipid phosphatase 3; MECP2, methyl-CpG-binding protein 2; MMF, mycofenolate mofetil; MS, multiple sclerosis; NK, natural killer cells; PECAM, platelet endothelial cell adhesion molecule; PP2A, protein phosphatase 2A; PPMS, primary progressive multiple sclerosis; RRMS, relapsing-remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis; SphK, sphingosine kinase; S1P, sphingosine 1-phosphate; Sgpl1, S1P lyase; SIRS, systemic inflammatory response syndrome; SLE, systemic lupus erythematosus; SPP1, S1P phosphatase 1; T1D, type 1 diabetes mellitus; T2D, type 2 diabetes mellitus; Th, T helper cell; TLR, Toll-like receptor; TNBS, trinitrobenzene sulfonic acid; TRPM7, transient receptor potential cation channel M member 7.

1. Historic development of fingolimod

Fingolimod (FTY720, Gilenya^{R}, 2-amino-2[2-(4-octylphenyl)ethyl]-1,3-propanediol) was originally synthesized by the Japanese chemist Tetsuro Fujita from Yoshitomi Pharmaceutical Industries Ltd. (present Mitsubishi Tanabe Pharma Corporation, Japan) (Adachi *et al.*, 1995) using the natural compound myriocin (ISP-1) as a lead. Myriocin was previously isolated by the same group from the culture broth of *Isaria sinclairii*, the imperfect or asexual stage of the genus *Cordyceps sinclarii* (cordycipitaceae), a subfamily of parasitic fungi. It was described as an immunosuppressant 10-100 times more potent than cyclosporin A (Chiba *et al.*, 1998; Fujita *et al.*, 1996). Remarkably, extract and powder from the near relative *Cordyceps sinensis* has been widely used in Traditional Chinese Medicine due to its energy boosting effect and to grant eternal youth. Based on the chemical structure of myriocin, a series of derivatives were synthesized from which FTY720 turned out to be an even more potent immunosuppressant when tested *in vitro* in a mouse allogenic mixed lymphocyte reaction assay, and *in vivo* in rat and dog transplantation models where allograft survival was prolonged for several weeks (Chiba *et al.*, 1998; Fujita *et al.*, 1996; Suzuki *et al.*, 1996b).

2. Mechanism of action of fingolimod

The main immunomodulatory mechanism of action of fingolimod is based on its effect on lymphocyte homing. It reversibly redistributes T and B cells from the circulation to secondary lymphoid organs like peripheral and mesenteric lymph nodes and Peyer's patches, thereby causing a state of peripheral lymphopenia (Chiba *et al.*, 1998). Since fingolimod resembles in chemical structure the sphingolipid molecule sphingosine, it can serve as a substrate for sphingosine kinase (SphK) to become phosphorylated to the active metabolite fingolimod-P (Brinkmann *et al.*, 2002). Thus, fingolimod is a pro-drug which requires SphK to become active. Two subtypes of SphK, SphK1 and SphK2, exist and in principle both enzymes can phosphorylate fingolimod *in vitro* (Billich *et al.*, 2003). However, SphK2 is 30-fold more efficient due to a lower Km value of fingolimod for SphK2 compared to SphK1 (Billich *et al.*, 2003). Furthermore, SphK2 is the only enzyme which activates fingolimod *in vivo*, since only *Sphk2* knockout mice are resistant to fingolimod-induced lymphopenia (Zemann *et al.*, 2006) and lack fingolimod-mediated protection from disease symptoms in experimental autoimmune encephalomyelitis (EAE), a widely used animal model for multiple sclerosis (Imeri *et al.*, 2016).

Fingolimod-P mimics sphingosine 1-phosphate (S1P) in structure and thus, not surprisingly, also binds to S1P receptors (Brinkmann *et al.*, 2002) (Mandala *et al.*, 2002). S1P receptors

were originally described as endothelial differentiation genes (edg) and build a subclass of G protein-coupled lipid receptors, which are most homologous to the lysophosphatidic acid (LPA) receptors. So far, 5 subtypes of S1P receptors have been identified, denoted $S1P_{1-5}$. They have all been determined to bind S1P with high affinity (Kihara *et al.*, 2014) with binding constants ranging from 1 to 10 nM, except for $S1P_4$, which has a ten-fold lower affinity (Mandala *et al.*, 2002). Fingolimod-P binds with similar affinity as S1P to $S1P_1$, $S1P_3$, and $S1P_5$, but shows much better binding to $S1P_4$ than S1P (6 nM versus 90 nM) (Mandala *et al.*, 2002). Fingolimod is not a ligand for $S1P_2$.

Due to the diversity of S1P receptor subtypes with their distinct function and ubiquitous expression in the body (Kihara *et al.*, 2014; O'Sullivan *et al.*, 2017), in theory multiple effects can be expected from using fingolimod, which may either have therapeutic benefit or cause adverse events.

3. Specific Fingolimod targets

3.1. The S1P receptor family

The $S1P_1$ receptor

As outlined above and because of its binding to and activation of the various S1P receptor subtypes, except for $S1P_2$, „active“ fingolimod is pharmacologically considered an unselective S1P receptor agonist (Brinkmann, 2007; Brinkmann *et al.*, 2002; Mandala *et al.*, 2002). Moreover, it causes sustained desensitization of the $S1P_1$-mediated signaling pathway by inducing receptor internalization and degradation, which on the cellular level results in functional antagonism. This effect of fingolimod on $S1P_1$ is unique and not seen with the endogenous ligand S1P, which also internalizes $S1P_1$ upon binding but then dissociates in endosomes and the receptor recycles back to the plasma membrane. Similarly, $S1P_{3,4,\text{ and }5}$ are also internalized upon fingolimod-P binding and then redistribute back to the cell surface. Particularly, downregulation of $S1P_1$ on T cells is supposed to account for the immunosuppressive effect of fingolimod, whereas downregulation of $S1P_1$ in other cell types, notably in endothelial cells, is likely responsible for the adverse events observed under long-term fingolimod treatment (Brinkmann, 2007). The agonistic effect of fingolimod on $S1P_{3,4\text{ and }5}$ may account for additional biological effects with unknown consequences as outlined below.

These early pioneering studies have fueled research on $S1P_1$ receptor biology to better understand its role in human diseases. Since $S1P_1$ is ubiquitously expressed in almost every cell type, its downregulation by prolonged fingolimod treatment is expected to have multiple consequences on cellular responses and tissue homeostasis. The generation of systemic

S1pr1 knockout mice corroborated the vital biological function of $S1P_1$, since embryos die in the developmental stage of day E12.5-E14.5, due to impaired vessel maturation and hemorrhagic bleedings.

Besides its role in immune cell trafficking and vascular development, $S1P_1$ is implicated in the regulation of vascular integrity. Increased vascular permeability is a typical feature of inflammation and allergy, and therefore a possible barrier protective effect of S1P signaling is of therapeutic interest. Various *in vitro* and *in vivo* studies have investigated the effect of S1P and fingolimod on the endothelial barrier function. It was shown that endotoxin-induced microvascular permeability and inflammation leading to acute lung and kidney injury in mice can be reduced by direct application of either S1P or fingolimod (Peng *et al.*, 2004). On the contrary, application of a $S1P_1$ receptor antagonist (W146), induced loss of capillary integrity in mouse skin and lung (Sanna *et al.*, 2006), and the most recently developed potent $S1P_1$ selective antagonist NIBR-0213 induced transient lung and heart permeability defects in rats, which promoted chronic inflammatory remodeling (Bigaud *et al.*, 2016). Furthermore, a conditional gene deletion approach was used to demonstrate that plasma S1P is crucial for vascular integrity. Since *Sphk1/Sphk2* double knockout mice are embryonically lethal, a conditional double knockout (KO) mouse was generated, which contains one conditional *Sphk1* allele and one null *Sphk1* allele in a *Sphk2* null background ($Sphk^{f/-}$ $Sphk2^{-/-}$) and carries a Cre transgene. By inducing Cre, plasma S1P levels became undetectable. These „pS1Pless“ mice showed a several-fold increased vascular leakage and reduced survival compared to wildtype mice upon administration of platelet activating factor (PAF), and the symptoms could be relieved by injection of a selective $S1P_1$ agonist (AUY954) (Camerer *et al.*, 2009).

Improved endothelial barrier function resulting from $S1P_1$ activation by S1P and fingolimod was also demonstrated in cultures of human endothelial cells *in vitro* (Dudek *et al.*, 2007). The molecular mechanisms underlying the barrier enhancing effect are not yet fully understood, but the role of adherens junction molecules, such as VE-cadherin and platelet-endothelial cell adhesion molecule (PECAM-1), appears to be crucial. In this regard, it was shown that S1P and fingolimod-P can stimulate the translocation of VE-cadherin to cell-cell contact sites of endothelial cells in cultures (Sanchez *et al.*, 2003), and *in vivo* in *S1pr1* knockout mice the retinal vasculature lost VE-cadherin staining (Gaengel *et al.*, 2012). For PECAM-1, upregulation was detected in endothelial cells overexpressing SK-1, which is supposed to increase S1P levels (Limaye *et al.*, 2005). Furthermore, $S1P_1$ silencing was shown to reduce expression of PECAM-1 and VE-cadherin (Krump-Konvalinkova *et al.*, 2005), and deregulation of these adherens junction molecules by fingolimod was also seen in a mouse model of multiple sclerosis, i.e. EAE (Imeri *et al.*, 2016). On the other hand, the barrier enhancing effect of fingolimod seems to be dose-dependent, since higher

concentrations in the range of 10 to 100 μM in cultures of human umbilical vein endothelial cells rather compromised the barrier function, and *in vivo* in mechanically ventilated mice even aggravated lung injury (Muller *et al.*, 2011). Similarly, in a bleomycin-induced lung injury model in mice, prolonged exposure to fingolimod resulted in vascular leak, fibrosis and increased mortality (Shea *et al.*, 2010).

Altogether, these studies suggest that $S1P_1$ activation improves the endothelial barrier function, whereas $S1P_1$ antagonism, notably also by prolonged fingolimod treatment, disrupts it, thereby increasing permeability and vascular leakage.

The $S1P_3$ receptor

A more detailed characterization of $S1P_3$ activation by fingolimod-P showed that it has an EC_{50} value of 7 to 10 nM for $S1P_3$, which is comparable to the endogeneous ligand S1P. However, its efficacy reached only 50% of S1P, suggesting a partial agonistic effect (Riddy *et al.*, 2012). By definition, a partial agonist in the presence of a full agonist produces an antagonistic output. This could mean that *in vivo*, fingolimod can also antagonize $S1P_3$ signaling rather than stimulate it, depending on the local S1P concentration. This idea is appealing and warrants further investigation, particularly in view of the potential anti-tumor activity of fingolimod discussed in chapter 5.3. In this context it is noteworthy that $S1P_3$ was recently shown to have prometastatic properties in breast and lung carcinoma cells (Filipenko *et al.*, 2016; Zhang *et al.*, 2013), and high expression is frequently found in tumors from breast cancer patients where it correlates with poor prognosis (Watson *et al.*, 2010). Since plasma S1P levels are often elevated in cancer patients (Alberg *et al.*, 2013; Sutphen *et al.*, 2004; Zhang *et al.*, 2015), the requirements for fingolimod to act as a partial $S1P_3$ agonist and thus impede metastatic growth might be fulfilled.

The $S1P_4$ receptor

The role of $S1P_4$ in physiological processes is still poorly understood and therefore effects of fingolimod mediated by $S1P_4$ are unclear. S1P and fingolimod-P associate with $S1P_4$ with binding constants of 95 nM and 6 nM, respectively, which means that fingolimod is a much better ligand for this receptor than the endogenous ligand (Mandala *et al.*, 2002). $S1P_4$ expression is restricted in the body and mainly found in lymphocytes and tissues of the immune and hematopoietic system (Graler *et al.*, 1998).

Knockout of *S1pr4* in mice and zebrafish consistently revealed a reduction of the number of circulating neutrophils, suggesting a role for $S1P_4$ in immunity and infection (Allende *et al.*, 2011; Pankratz, 2016). *S1pr4* deficient mice also show differential reactions to inflammation

with exacerbated T helper (Th)2 cell responses and decreased responses of Th1 cells (Allende *et al.*, 2011). Moreover, megakaryocytes generated from *S1pr4* deficient mice showed atypical and reduced formation of proplatelets in vitro, and the recovery of platelet numbers after experimental thrombocytopenia was significantly delayed, suggesting a role for $S1P_4$ in thrombopoiesis (Golfier *et al.*, 2010). Of note, in humans, a rare missense variant of $S1P_4$ (Arg365Leu) was reported, which generates a loss-of-function receptor and is associated with reduced white blood cells and neutrophil counts (Pankratz, 2016), thus corroborating the preclinical data from mice (Allende *et al.*, 2011).

The $S1P_5$ receptor

The $S1P_5$ receptor was originally cloned as rat nerve growth factor-regulated G protein-coupled receptor Nrg-1(Glickman *et al.*, 1999) and later found to be identical to $S1P_5$/edg8. It is predominantly expressed in the brain and spleen (Im *et al.*, 2000; Malek *et al.*, 2001), and in these tissues, it is further concentrated in oligodendrocytes and natural killer (NK) cells (O'Sullivan *et al.*, 2017).

Fingolimod-P and S1P bind to $S1P_5$ with equally high affinity. In oligodendrocytes this triggers two distinct functional responses depending on the developmental stage of the cells. It leads to retraction in pre-oligodendrocytes, whereas it increases the survival of mature cells (Jaillard *et al.*, 2005). In addition, migration of oligodendrocyte precursor cells (OPCs), which normally migrate over considerable distances during brain development, is inhibited by $S1P_5$ activation (Novgorodov *et al.*, 2007). Fingolimod was also shown to protect human oligodendrocytes from apoptosis induced by serum and glucose deprivation, suggesting a neuroprotective effect by activating $S1P_5$ (Miron *et al.*, 2008). Moreover, $S1P_5$ is also expressed on brain microcapillary endothelial cells where it contributes to the blood-brain barrier function and maintains the immunoquiescent state of brain endothelial cells (van Doorn *et al.*, 2012).

In *S1pr5* knockout mice the number of circulating NK cells is decreased (Walzer *et al.*, 2007) proposing a function of $S1P_5$ is to promoting NK cell egress from bone marrow and lymph nodes into the blood, and recruiting them to sites of inflammation (Jenne *et al.*, 2009; Walzer *et al.*, 2007). *S1pr5* knockout mice also lack circulating Ly6C-negative peripheral monocytes, but maintain normal levels in the bone marrow (Debien *et al.*, 2013).

3.2. Other targets of Fingolimod

Besides S1P receptors, which are activated by fingolimod at low nM concentrations, other intracellular targets of either fingolimod or fingolimod-P have been described. These include

SphK1, S1P lyase (Sgpl1), ceramide synthase (CerS) 2, histone deacetyases (HDACs), cytosolic phospholipase A_2, protein phosphatase A2 (PP2A), and the cation channel TRPM7. However, these off-targets respond only at much higher concentrations in the μM range. It was recently reported that fingolimod can in principle accumulate to high μM concentrations in cells *in vitro* to achieve concentrations required for such "off-target" effects (Schroder *et al.*, 2015). However, it is unlikely and evidence has never been provided that passive tissue targeting of fingolimod occurs *in vivo.* Moreover, the low target selectivity of fingolimod at such high concentrations is not appropriate for therapeutic use.

SphK1

As determined *in vitro*, fingolimod is a substrate of SphK-2 and is also phosphorylated by SphK-1, but with much lower efficiency (Billich *et al.*, 2003). *In vivo*, however, SphK2 is the only kinase which activates fingolimod as demonstrated in Sphk1-and Sphk2-deficient mice. fingolimod, but not fingolimod-P, acts also as a direct inhibitor of SphK1, but very high concentrations of 50 μM are needed to achieve 50% inhibition (Tonelli *et al.*, 2010; Vessey *et al.*, 2007). As a pharmacological target with oncogenic potential, SphK1 is of interest for cancer therapy (see chapter 5.3.). Considering the toxicity profile of fingolimod at 0.5 mg per day in patients (**Table 2**), however, it is unrealistic to believe that such high doses can be tolerated. Nevertheless, numerous preclinical studies have shown pro-apoptotic and anti-tumor effects of fingolimod with tumor cell lines *in vitro* and in tumor models in mice, which could be partly attributed to inhibition of SphK1 (Pchejetski *et al.*, 2010).

S1P lyase

S1P lyase (Sgpl1) irreversibly degrades S1P to hexadecenal and phosphoethanolamine as the final step for eliminating sphingolipids in the cell in addition to secretion. In contrast to S1P, fingolimod is not a substrate for Sgpl1 and was even shown to inhibit it (Bandhuvula *et al.*, 2005). Moreover, treatment of mice with fingolimod inhibits tissue Sgpl1 within 12 h, resulting in stable or even increased S1P levels. It was therefore proposed that disruption of S1P metabolism by inhibiting Sgpl1 accounts for some of the effects of fingolimod on immune cells (Bandhuvula *et al.*, 2005). In support of this, inhibition of Sgpl1 using novel selective inhibitors can mimic the effect of fingolimod on lymphocyte homing (Bagdanoff *et al.*, 2010; Weiler *et al.*, 2014).

Ceramide synthase 2

Ceramide synthases (CerS) comprise a family of enzymes that acylate dihydrosphingosine to dihydroceramides and therefore are crucial for de-novo synthesis of ceramides (Park *et al.*, 2014; Wegner *et al.*, 2016) and consequently, also the downstream metabolite S1P. Six subtypes of CerS have been identified which all have certain substrate preferences and utilize a restricted subset of fatty acid-CoAs for dihydroceramide synthesis, thereby generating ceramides of different chain lengths (Wegner *et al.*, 2016). In vitro activity studies revealed non-competitive inhibition of CerS2 by S1P with an IC_{50} value of approx. 20 μM (Laviad *et al.*, 2008). This inhibition may represent a negative feedback regulation ensuring that the overproduction of cellular S1P turns off de-novo synthesis of the precursor ceramide and thus ensures a balanced sphingolipid homeostasis. Fingolimod, but not the phosphorylated form, was shown to inhibit CerS only at high concentrations in a cell-free assay with an IC_{50} value of 30 to 50 μM (Lahiri *et al.*, 2009), and also in experiments with human pulmonary artery endothelial cells (Berdyshev *et al.*, 2009).

Protein phosphatase 2A

The protein phosphatase 2A (PP2A) is an important cellular Ser/Thr phosphatase that regulates multiple signal transduction pathways by dephosphorylating protein kinases and other enzymes (Millward *et al.*, 1999). Its role as a tumor suppressor became evident for the first time when it was found to be strongly inhibited by the tumor promoter ocadaic acid (Bialojan *et al.*, 1988). PP2A is a heterotrimeric complex built by three subunits, including a scaffolding subunit, a regulatory subunit and a catalytic subunit. Each subunit again exists in several isoforms and additional splice variants which all together allow the existence of 80 different subforms of the PP2A holoenzyme. This finally directs its subcellular localization and allows for such a huge diversification of substrates.

The first observation that PP2A is affected by sphingolipids was reported in 1993 showing that short-chain C2-ceramide could increase PP2A activity in vitro, whereas other sphingolipids such as dihydro-C2-ceramide or sphingosine had no activating effect (Dobrowsky *et al.*, 1993). Later on, activity studies using the purified PP2A holoenzyme revealed a weak effect of fingolimod on PP2A activation in the range of a 60% increase at 10 μM, which was sufficient to inhibit Akt and induce apoptosis in leukemia cells (Matsuoka *et al.*, 2003). The relevance of PP2A as a target of fingolimod in cancer therapy is discussed in chapter 5.3.

Histone deacetylases (HDAC)

Histone acetylation is implicated in the epigenetic regulation of gene expression. The acetylation reaction is catalyzed by histone acetyltransferases (HAT) and associates with active gene transcription because acetylation of histones loosens the contact between core nucleosome proteins and DNA and facilitates the binding of transcription factors to their respective binding sites (Eckschlager *et al.*, 2017; Kelly *et al.*, 2013). On the contrary, histone deacetylases (HDAC) remove acetyl groups from histones, thereby tightening the chromatin structure, which hinders binding of transcription factors and hence suppress gene transcription. Numerous studies demonstrated that HDAC inhibitors have therapeutic potential to treat diseases such as cancer, inflammation, fibrosis and even cognitive disorders (Eckschlager *et al.*, 2017; Fischer *et al.*, 2010; Hull *et al.*, 2016; Liu *et al.*, 2015). Notably, certain HDAC inhibitors are already FDA approved and used for the treatment of relapsed and refractory cutaneous T-cell lymphoma (vorinostat), peripheral T cell lymphoma (belinostat) and multiple myeloma (panobinostat) (Eckschlager *et al.*, 2017). Recently, it was reported that S1P generated by Sphk2 in the nucleus binds directly to HDAC and inhibits its activity. This results in hyperacetylation of histones and regulates expression of certain genes such as the cyclin-dependent kinase inhibitor p21 and c-fos (Hait *et al.*, 2009). A similar effect was also exerted by fingolimod-P (Hait *et al.*, 2014) suggesting that SphK2 is a driver of epigenetic gene regulation.

The phospholipase A_2

The phospholipase A_2 (PLA_2) hydrolyses phospholipids at the sn2 position and thereby liberates fatty acids and lysophospholipids. Particularly, the liberation of arachidonic acid, which serves as a precursor for all eicosanoids including prostaglandins, leukotrienes and thromboxane, is a crucial and rate limiting step in inflammatory reactions (Dennis *et al.*, 2011). PLA_2 exists as a family of several subtypes subclassified as secreted low molecular weight enzymes ($sPLA_2$s), cytosolic Ca^{2+}-dependent enzymes ($cPLA_2$s) and Ca^{2+}-independent enzymes ($iPLA_2$s). Inhibition of $sPLA_2$s and $cPLA_2$ was shown to reduce inflammatory reactions *in vitro* and *in vivo* (Dennis *et al.*, 2011; Kokotou *et al.*, 2017) and the development of subtype specific PLA_2 inhibitors as anti-inflammatory drugs and as alternatives to cyclooxygenase inhibitors is of great interest. In mast cells, a central cell type involved in inflammatory allergic disorders, fingolimod, but not fingolimod-P, inhibited antigen-stimulated arachidonic acid release (by 30% at 1 μM) and even more potently prostaglandin D_2 synthesis (EC_{50} approx. 100 nM) (Payne *et al.*, 2007). Furthermore, in renal mesangial cells, which are implicated in chronic inflammatory kidney diseases like glomerulonephritis, fingolimod downregulated cytokine-stimulated $sPLA_2$ production and secretion (Xin *et al.*, 2007), which, however, was not due to direct inhibition but rather to

inhibition of a transcriptional mechanism. Thus, in addition to its immunomodulatory role, fingolimod has direct anti-inflammatory effects and thus potential for treating inflammatory and allergic diseases.

The cation channel TRPM7

Transient receptor potential cation channel subfamily M member 7 (TRPM7) is a plasma membrane ion channel that contains a cytosolic protein kinase domain. It has a variety of physiological functions, including the regulation of Ca^{2+} signalling, Mg^{2+} homeostasis, cell migration, proliferation and differentiation and immune responses . Inhibition or depletion of TRPM7 causes anoxic neuronal death, cardiac fibrosis, tumour progression and macrothrombocytopenia (Chubanov *et al.*, 2017). Recently, it was shown that in HEK293 cells overexpressing TRPM7 or in cardiac fibroblasts, TRPM7 is dose-dependently inhibited by fingolimod and the endogenous analog sphingosine with IC_{50} values of 720 nM and 300 nM, respectively (Qin *et al.*, 2013). No inhibition was seen by the phosphorylated species S1P and fingolimod-P. It was suggested that TRPM7 inhibition by fingolimod contributes to the drug's anti-proliferative and anti-migratory effects (Qin *et al.*, 2013).

4. Pharmacokinetic characteristics of Fingolimod

The pharmacokinetic characteristics of fingolimod were summarized from the report NDA 22-527 submitted to the FDA for drug approval. A major advantage of fingolimod as a therapeutic agent is the possibility of its oral application. Absorption is food-independent and slow (maximal plasma concentration after 12-16 h), but extensive, and its bioavailability is high (93%). It reaches steady-state concentrations after 1-2 months during daily intake (Clinical Pharmacology and Biopharmaceutics Review- Application number : NDA 22-527; https://www.accessdata.fda.gov/drugsatfda_docs/nda/2009/022115s0).

Fingolimod shows high plasma protein binding (>99.7%), mainly to albumin, and in contrast to S1P, there is no evidence for fingolimod binding to ApoM/HDL. It has a large volume of distribution of approx. 20 L/kg and shows slow blood clearance (6.3±2.3 L/h), resulting in a half-life of 6-9 days.

In the blood, there is a stable equilibrium between fingolimod and fingolimod-P and both plasma levels decrease in parallel following similar elimination kinetics. The enzymes involved in regulating the equilibrium between the inactive and active compound are Sphk2 and ecto-phosphatase lysophospholipid phosphatase 3 (LPP3). It is noteworthy, however, that fingolimod-P can also be dephosphorylated intracellulary by the S1P phosphatase 1 (SPP1) (Mechtcheriakova *et al.*, 2007), and then reenters the fingolimod blood pool. In

addition to phosphorylation, two further pathways of fingolimod biotransformation exist which are depicted in **Fig. 2**.

(1) Cytochrome P450 (CYP450)-mediated ω-hydroxylation/oxidation followed by fatty acid-like-β-oxidation steps in the liver. fingolimod blood clearance is slow with 6.3 ± 2.3 L/h. The main CYP450 isoform identified is CYP4F2 with only minor contributions of other CYP450 isoforms. Inactive metabolites are then eliminated by the renal system (81%).

(2) Fingolimod acylation by a still unknown acyltransferase to form atypical ceramides. Ceramides have attracted attention due to their pro-apoptotic effect on various cell types, and it is possible that such atypical ceramides are involved.

The main metabolites detectable in the blood are M3 and M4 from the CYP4F2 route and M29 and M30 from the acylation route (**Fig. 2**). Quantification of the metabolites in blood analysis after a single oral dose of radiolabeled ^{14}C-Fingolimod revealed that 23.3% of the radioactivity was bound to fingolimod, 10.3% to fingolimod-P, 8.3% to M3, 8.9% to M29 and 7.3% to M30.

The finding that CYP4F2 is a key enzyme in the biotransformation of fingolimod has prompted studies investigating the role of CYP4F2 inhibitors and possible drug interactions. Indeed, co-administration of the CYP4F2 inhibitor and antimycotic drug Ketoconazole was shown to increase the concentrations of fingolimod and fingolimod-P in the blood. Other drug interactions which may affect bioavailability have not been reported so far. Since fingolimod is metabolized mainly in the liver, dosing must be adjusted in case of liver insufficiency.

5. Fingolimod as a therapeutic agent

From its main effect to deplete peripheral lymphocytes, it is obvious that fingolimod has potential for the treatment of diseases associated with inappropriate immune responses, such as in allograft rejection and autoimmunity. First preclinical studies in rats, mice and dogs revealed a delay in allograft rejection with fingolimod alone and an even synergistic effect when used together with cyclosporine A (CSA) (Chiba *et al.*, 1996; Suzuki *et al.*, 1996a). It was then tested in several phase 1 to phase 3 clinical trials (Tedesco-Silva *et al.*, 2004) (Mulgaonkar *et al.*, 2006) (NCT00239811) (Tedesco-Silva *et al.*, 2006) (Salvadori *et al.*, 2006) for kidney transplantation. In the phase 3 trials, *de-novo* renal transplant recipients were randomized to receive either 5 mg fingolimod plus a reduced dose CSA, 2.5 mg fingolimod plus a full dose CSA or mycofenolate mofetil (MMF) plus a full dose CSA over 1 year. Primary endpoints were incidence of acute rejection, graft loss, death or discontinuation of treatment. Unfortunately, these trials could not prove a superior effect of fingolimod/CSA treatment compared to the standard therapy with MMF/CSA, nor did fingolimod allow

reduction of the CSA dose. In addition, more complications were reported in the fingolimod/CSA groups, including decreased renal function and macula edema, leading to discontinuation of the studies.

On the other hand, fingolimod could prove successful for treating multiple sclerosis, and under the name Gilenya^{R} has been approved for oral treatment of RRMS. RRMS currently remains as the only approved indication for fingolimod, all other indications of interest are still under preclinical or phase 1-2 clinical investigation.

5.1. Multiple sclerosis

Multiple sclerosis (MS) is an autoimmune and chronic neurodegenerative disease that disrupts the normal functioning of the brain, optic nerves and spinal cord through inflammation-mediated white matter demyelination and axonal loss (Dutta *et al.*, 2014; Milo *et al.*, 2010). This ultimately leads to a progressive decay of both physical and cognitive functions. There are three main types of MS: the relapsing-remitting MS (RRMS), the primary progressive MS (PPMS) and the secondary progressive MS (SPMS). The transition from RRMS to SPMS is thought to occur when axonal loss outweighs the compensatory capacity of the central nervous system (CNS) such as neuroprotection and remyelination (Dutta *et al.*, 2014; Milo *et al.*, 2010).

Early on, it was recognized that fingolimod can reduce disease symptoms in various animal models of multiple sclerosis. These promising results prompted testing in clinical trials of RRMS. It is of note that for this indication fingolimod can be applied as monotherapy and at a ten-fold lower dose than used in the renal transplantation studies, thereby minimizing adverse events. Several clinical trials were performed from phase 1 to 3, where fingolimod proved to be effective in reducing disease symptoms and the number of relapses. In 2010, the FDA and EMA granted approval for this indication.

In addition, fingolimod was tested in clinical studies for PPMS. However, the primary endpoint delay of MS disability progression compared to placebo, was not met. Altogether, fingolimod represents a clear improvement in the therapy of RRMS, mainly due to its oral applicability and good tolerability at clinically effective doses (**Table 2**). New S1PR modulators with higher selectivity for $S1P_1$ or dual activity against $S1P_{1+5}$ are currently under investigation for various other indications for which S1PR modulation represents a reasonable therapeutic concept (**Table 3**).

5.2. Inflammatory bowel diseases

Chronic inflammatory bowel disease (IBD), which mainly includes Crohn's disease and ulcerative colitis, is an idiopathic disease caused by multiple factors of genetic, inflammatory or environmental origin (Peyrin-Biroulet *et al.*, 2017). The detailed mechanisms of IBD pathogenesis are not completely understood. It is characterized by intestinal inflammation and a dysregulated immune response to host intestinal microflora (Baumgart *et al.*, 2007). Since T cell infiltration into the mucosa is a common feature, it is expected that fingolimod by redistributing immune cells to secondary lymphoid organs, can relieve disease symptoms and progression (Peyrin-Biroulet *et al.*, 2017).

First promising data with fingolimod came from a mouse model of chronic colitis. In this model, the disease was caused by depletion of interleukin 10 using gene knockout. Treatment of mice with fingolimod for 4 weeks efficiently reduced the number of CD4+ T cells in the colonic lamina propria and decreased the production of IFNγ production in colonic lymphocytes thereby mitigating the severity of the disease (Mizushima *et al.*, 2004). In other experimental models of colitis, such as colitis induced by dextran sulfate sodium (DSS), trinitrobenzene sulfonic acid (TNBS) or by colitogenic T cell transfer, similar protective effects of fingolimod were reported (Daniel *et al.*, 2007; Deguchi *et al.*, 2006; Mizushima *et al.*, 2004). However, in other preclinical models of colitis the therapeutic effect of fingolimod could not be confirmed, suggesting this indication to be more critically investigated to clarify this inconsistency (Montrose *et al.*, 2013; Radi *et al.*, 2011).

Recent data from using the new $S1P_{1+5}$-selective immunomodulator ozanimod support the idea of targeting $S1P_{1/5}$ in the treatment of IBD. In this regard, ozanimod was shown to decrease inflammation and disease parameters in TNBS-induced colitis and colitis induced by adoptive T cell transfer in mice (Scott *et al.*, 2016). It was then further tested in a phase 2 clinical study (NCT02435992) in patients with moderately or severe active ulcerative colitis. Daily oral treatment with 1 mg ozanimod achieved higher clinical response rates and remissions, and improved mucosal healing resulting in lower Mayo Clinic scores compared to placebo. This effect was already seen 8 weeks after the start of treatment and was maintained up to week 32 (Sandborn *et al.*, 2016). Similarly, the new $S1P_1$-selective agonist KRP-203 (2-amino-2-{2-[4-(3-benzyloxyphenylthio)-2-chlorophenyl]ethyl}-1,3-propanediol hydrochloride) (Fujishiro *et al.*, 2006; Shimizu *et al.*, 2005) also proved to be effective in mitigating the symptoms of colitis (Song *et al.*, 2008). A clinical phase 2 study (NCT01375179) to assess its potential in the treatment of ulcerative colitis was conducted but terminated early due to non-convincing results at a first interim analyses after 18 months (according to the company's clinical trial results database https://www.novctrd.com/).

More recently, the next generation $S1P_{1+4+5}$-selective modulator etrasimod (APD334) (Buzard *et al.*, 2014) also entered a phase 2 study (NCT03139032) in IBD patients and it remains to be seen whether this compound holds more promise to reduce disease symptoms.

5.3. Psoriasis

Psoriasis is a chronic inflammatory skin disease characterized by infiltration of T cells into skin lesions and by focal formation of inflamed raised plaques which constantly shed scales derived from hyperproliferating skin epithelial cells. First-line and still the most effective therapy is the use of glucocorticoids, which classically act by immune suppression and by reducing keratinocyte proliferation (Das *et al.*, 2009).

In principle, fingolimod could be useful in the treatment of psoriasis by redistribution of the T cells away from the skin and by blocking migration of skin dendritic cells to draining lymph nodes where antigen presentation to naïve lymphocytes occurs (Han *et al.*, 2015). Unfortunately, since animal models of psoriasis do not exist, efficacy of fingolimod has not been demonstrated. Nevertheless, second-generation S1PR modulators with higher selectivity have been developed. One is ponesimod which was tested in a phase 2 study (NCT01208090) in patients with moderate to severe chronic plaque psoriasis. The primary endpoint was defined as 75% reduction in psoriatic area after 16 weeks. The conclusion from the study was that treatment with ponesimod resulted in clinical responses after 16 weeks and symptoms improved further when it was continued (Vaclavkova *et al.*, 2014).

5.4. Rheumatoid arthritis

Rheumatoid arthritis is another autoimmune disease that mainly affects the joints and leads to a progressive disability (McInnes *et al.*, 2011). It is characterized by synovial inflammation and hyperplasia, autoantibody production, cartilage and bone destruction, but is also associated with systemic complications such as cardiovascular, pulmonary and skeletal disorders (McInnes *et al.*, 2011). Reactive T cells are implicated in the pathogenesis of the disease and it is plausible to assume that fingolimod has therapeutic benefit. Indeed, in various rat and mouse models of arthritis fingolimod was shown to reduce the incidence of arthritis, and to reduce hindpaw swelling and bone destruction (Matsuura *et al.*, 2000; Tsunemi *et al.*, 2010; Wang *et al.*, 2007; Yoshida *et al.*, 2013). Clinical studies with fingolimod or novel S1PR modulators in patients with arthritis have not been performed yet.

5.5. Cancer

5.5.1. *S1PR-dependent effects*

The immune suppressive effect of fingolimod results from impeding egress of naïve and central memory T cells from lymph nodes upon $S1P_1$ binding of its phosphorylated analog fingolimod-P generated by SphK2 (Brinkmann *et al.*, 2010; Kharel *et al.*, 2005; Mehling *et al.*, 2008; Zemann *et al.*, 2006). Whereas a decrease of circulating lymphocytes and reduced cell trafficking to sites of inflammation like in the CNS accounts for efficacy in MS, immune suppression is counterproductive in cancer patients. Even if it may affect also regulatory T cells implicated in downregulating anti-tumor immune responses (Wolf *et al.*, 2009), the decline of circulating cytotoxic T cells (CTLs) and certain NK cells (Johnson *et al.*, 2011) potentially hampers tumor rejection and immune surveillance. To overcome the immunosuppressive effect of fingolimod, derivatives were generated which cannot be phosphorylated and bind to $S1P_1$, but still maintain biological activity in cells (Omar *et al.*, 2011; Segura-Ulate *et al.*, 2017). In addition to inducing a decline in circulating immune cells by $S1P_1$ binding, data from preclinical mouse models suggest that fingolimod also stimulates the accumulation of myeloid-derived suppressor cells in tumors via $S1P_3$-dependent stimulation of GM-CSF release, which would favor immune escape and tumor growth (Li *et al.*, 2017). In this context, it is noteworthy that $S1P_3$ signaling was recently shown to have prometastatic properties in breast and lung carcinoma cells (Filipenko *et al.*, 2016; Zhang *et al.*, 2013). Since fingolimod-P was found to act only as a partial agonist of $S1P_3$ with about 50% of the activity of S1P (Riddy *et al.*, 2012), it would be interesting to see if it has anti-metastatic activity by antagonizing $S1P_3$ in the presence of high levels of S1P.

5.5.2. S1PR-independent effects (off-target)

Fingolimod was shown to inhibit cancer-relevant signal transduction pathways independent of S1PR binding, in part due to its structural similarity to sphingosine. The heterogeneity of responses to fingolimod includes increased cell death, decreased cell proliferation, angiogenesis, migration, invasion, metastasis and inflammation (Patmanathan *et al.*, 2015; White *et al.*, 2016). Thus, by targeting multiple oncogenic signaling pathways and compensatory pathways implicated in drug resistance, fingolimod in principle holds promise for drug repurposing as an anticancer agent for various oncology indications characterized by different molecular abnormalities.

The mechanisms underlying the S1PR-independent anti-tumor activity of fingolimod are less well defined and all were measured at relatively high µM concentrations. One of the better characterized effects is inhibition and proteasomal degradation of the oncoprotein SphK1 (Tonelli *et al.*, 2010) and hence downmodulation of various well-characterized pro-

tumorigenic processes associated with this pathway (Lim *et al.*, 2011; Pchejetski *et al.*, 2010; Vessey *et al.*, 2007).

Another target of fingolimod in cancer is PP2A, a tumor suppressor with decreased activity in various human tumors (Cristobal *et al.*, 2016; Grech *et al.*, 2016; Perrotti *et al.*, 2013). Inhibition of PP2A occurs by mutations, hyperphosphorylation of the catalytic subunit or deregulation of the endogenous inhibitors SET and CIP2A (Chen *et al.*, 1992; Cristobal *et al.*, 2016; Saddoughi *et al.*, 2013), leading to constitutive activation of survival signaling pathways (Pitman *et al.*, 2012). Surface plasmon resonance (SPR) analysis indeed demonstrated that fingolimod binds to I2PP2A/SET with a K_d of 11 nM (Saddoughi *et al.*, 2013) but biological activity was again only measured at µM concentrations. Further effects of fingolimod on PP2A function seem to include CIP2A downregulation and PP2A hypophosphorylation (Rincon *et al.*, 2015), but these mechanisms need to be explored in more detail. In BCL/ABL-transformed leukemia cells, 2.5 µM fingolimod was shown to increase cellular PP2A activity by 400 % and consequently dephosphorylate and degrade BCR/ABL with an EC_{50} value of 80 nM (Neviani *et al.*, 2007). It was suggested that this mechanism accounts for the anti-tumor effect in Philadelphia chromosome (Ph1)-positive leukemia. Fingolimod was also shown to inhibit activation of the chemokine receptor CXCR4 in multiple myeloma. CXCR4 and its ligand CXCL12 are crucial for multiple myeloma cell proliferation and regulate the migration and homing to the bone marrow (Azab *et al.*, 2009). The anti-leukemic effect of fingolimod occurred in cooperation with bortezomib and the molecular data suggest a cross-talk between the S1P and the CXCR4 pathways (Beider *et al.*, 2017). However, inhibition of CXCR4 determined by analysis of intracellular survival signaling via extracellular signal-regulated protein kinase (ERK)1/2 and S6 proteins was measured at a concentration of 20 µM fingolimod without showing data from lower doses at which cell viability was already decreased. Anti-leukemic activity of fingolimod was also measured in mice, but only after daily injections of very high doses of 10 mg/kg, which makes it difficult to predict the clinical potential of the treatment.

Current preclinical data suggest that a downside of fingolimod for cancer therapy is its low efficacy and potency. *In vitro*, biological responses in cancer cells were reported at µM concentrations, and in tumor xenograft models in mice, anti-tumor activity was always measured after daily injections of aberrantly high doses, ranging from 2.5-10 mg/kg (Beider *et al.*, 2017; Cristobal *et al.*, 2014; Gstalder *et al.*, 2016; Pchejetski *et al.*, 2010; Rincon *et al.*, 2015). This is up to 1400-fold more than used in patients to treat MS, and it is clear that at such high doses numerous targets are affected which are crucial for normal tissue function. The safety data for fingolimod shown for the 0.5 mg daily schedule (Table 1) make it

unrealistic to believe that such high doses can be applied to patients without escalation of toxicity.

To improve the efficacy and tolerability of fingolimod, second-generation derivatives and complex pharmaceutical formulations were engineered and tested in various preclinical tumor models. These include the analog OSU-2S, which shows reduced S1PR modulation and increased inhibition of tumor cell proliferation (Omar *et al.*, 2011), lipid-encapsulated ingolimod for oral delivery (Estella-Hermoso de Mendoza *et al.*, 2015), nanoparticles combining fingolimod with docetaxel (Alshaker *et al.*, 2017; Wang *et al.*, 2017), and fingolimod-loaded nanocarriers targeting CD19, CD20 or CD37 (Mao *et al.*, 2014). However, again, these new developments were tested at clinically unrealistic mg/kg doses and therefore conclusions for anti-tumor activity in patients cannot be drawn.

Altogether, these preclinical studies reveal that very high concentration of fingolimod are needed to reduce cancer cell viability *in vitro* and affect tumor growth in xenograft models *in vivo*. At such high doses, effects of low specificity are common and indeed multiple low-affinity targets of fingolimod have been described, increasing the risk of unpredictable complications. We therefore conclude that fingolimod will likely fail to achieve clinical responses in cancer patients at tolerable doses.

5.6. Stroke

Ischemic stroke occurs as a result of occlusion of a cerebral artery by a blood clot, which causes oxygen and glucose deprivation and results in neuronal death, either by apoptosis or necrosis, and in metabolic and functional deficits. Dying cells then release factors such as damage-associated molecular patterns (DAMPs) that stimulate the pathogen recognition receptors toll-like receptor (TLR)-2 and -4, activate NF-κB and the synthesis of various pro-inflammatory mediators, including IL-1β, TNF-α, and chemokines. These in turn recruit immune cells to the affected brain area to clear cell debris and promote healing. Immune cells infiltrating during the first phase consist of macrophages and neutrophils, and in a second delayed phase of T- and B cells (Santos Samary *et al.*, 2016). However, ischemia/reperfusion injury is not restricted to the brain but occurs also in other organs, and it was first shown that fingolimod is capable of reducing tissue injury in kidney and liver upon ischemia/reperfusion induction (Anselmo *et al.*, 2002; Troncoso *et al.*, 2001). In view of this finding, animal models of stroke were performed where it was demonstrated that fingolimod reduces lesion size and improves neurological outcome after experimental stroke (Czech *et al.*, 2009). In the meantime, several more preclinical studies using fingolimod in animal models of stroke were reported, and a systematic review and a meta-analysis of the data was conducted to prove the drug's efficiency in such animal models. Results indicated an

overall beneficial efficacy of fingolimod in stroke models with one negative study that used a permanent middle cerebral artery occlusion model (Liu *et al.*, 2013). However, it should be kept in mind that animal experiments are routinely not performed in aged mice which would resemble the situation in humans with comorbidities like diabetes and hypertension (Liu *et al.*, 2013).

A pilot trial and a clinical phase 2 study (NCT02002390, NCT02956200) were performed to provide proof-of-concept for the efficacy of fingolimod, also in combination with alteplase and thrombectomy, to reduce brain inflammation and improve clinical outcomes of acute ischemic stroke in patients. The data revealed that patients treated with alteplase plus fingolimod 0.5 mg daily for 3 consecutive days within 4.5 hours after the onset of ischemic stroke showed a significant reduction of neurologic impairment compared to patients in the standard therapy receiving group. Neurologic functions improved in these patients during the first week in coincidence with a reduction of circulating lymphocyte counts. After 3 months, more patients in the fingolimod group achieved full recovery of their neurologic capability (Fu *et al.*, 2014; Zhu *et al.*, 2015)

5.7. Systemic inflammatory response syndrome /sepsis

Systemic inflammatory response syndrome (SIRS), sepsis and septic shock are typically characterized by a strong inflammatory reaction and dysregulated vascular permeability, leading to hypovolaemia and severe tissue edema finally culminating in multiorgan failure. Numerous options to block the inflammatory response, i.e. by neutralizing or antagonizing TNFα or IL-1β, have been explored over years, but all failed due to the multifactorial nature of the disease. Moreover, hypovolaemia is normally treated by volume substitution, but due to the increased vascular permeability the added fluid can even aggravate edema formation. Therefore, likely more promising is to directly target the vascular endothelial cells and increase their barrier function. Since $S1P_1$ activation is well described as a barrier enhancing mechanism, the activation of this receptor signaling system is desirable. Interestingly, in sepsis, serum levels of S1P (Coldewey *et al.*, 2016; Frej *et al.*, 2016; Winkler *et al.*, 2015) and its carrier ApoM/HDL (Christoffersen *et al.*, 2012; Wu *et al.*, 2004) are downregulated, which further suggests the usefulness of increasing S1P/$S1P_1$ signaling to reduce symptoms of sepsis. In this context it was previously reported that application of reconstituted HDL attenuates organ injury in a rat model of endotoxic shock (McDonald *et al.*, 2003). Although not explicitly investigated, it is likely that S1P, which is coupled to HDL in the blood, accounts for the protective effect of HDL. Furthermore, fingolimod could prove protective also in preclinical models of sepsis. For instance, in an acute LPS-induced lung injury model in mice, symptoms were significantly relieved by applied S1P (at 1 μM) or fingolimod (at 0.1

mg/kg) (Peng *et al.*, 2004), and, in a cecal ligation and puncture (CLP)-induced sepsis model in rats, fingolimod, at 0.2 mg/kg decreased volume loss by 30% (Lundblad *et al.*, 2013). Moreover, it improved LPS- and (CLP)-induced cardiac impairment in mice (Coldewey *et al.*, 2016), and reduced vascular and immune defects (Hemdan *et al.*, 2016). On the other hand, the $S1P_1$ agonist SEW2871, which was claimed to be selective for $S1P_1$, but manifold less potent than S1P and lacking a functional antagonistic effect (Jo *et al.*, 2005), could not substitute for fingolimod in a rat model of sepsis, but instead caused severe cardiac complications and no improvement of the vascular barrier function (Flemming *et al.*, 2017). This is probably due to low potency and off-target effects caused by the use of higher doses. Collectively, more data are needed to rate the therapeutic potential of fingolimod in the treatment of SIRS and septic shock in patients.

5.8. Chronic inflammatory kidney diseases

Systemic lupus erythematosus:

Systemic lupus erythematosus (SLE) is a systemic and poly-etiological autoimmune disease that is associated with a broad spectrum of clinical and immunologic manifestations. Early symptoms most frequently affect the skin and joints, whereas morbidity and mortality is mainly due to kidney damage (Bai *et al.*, 2017; Yu *et al.*, 2017). The development of nephritis in patients with SLE involves multiple pathways including aberrant apoptosis, autoantibody production, immune complex deposition, complement activation and inflammatory cytokines and interferon-γ production (Bai *et al.*, 2017; Yu *et al.*, 2017). Additional typical renal features are glomerular crescents, podocyte injury, tubulointerstitial lesions and vascular injury. Although outcomes for patients with lupus nephritis have improved over the past 30 years, curative treatment of this disease still remains a challenge (Bai *et al.*, 2017; Yu *et al.*, 2017).

Several animal models exist where the autoimmune disease spontaneously develops. Most commonly used are NZB/W F1, MRL-lpr/lpr and BXBS mice (Perry *et al.*, 2011). In all three models, fingolimod was tested and found effective in reducing disease pathology and improving renal function (Alperovich *et al.*, 2007; Ando *et al.*, 2010; Okazaki *et al.*, 2002).

Notably, the novel $S1P_1$-selective agonist KRP-203 was also tested in the MRL-lpr/lpr mice. When these mice received KRP-203 for 8-12 weeks, a significant reduction of kidney injury was observed, which was explained by its ability to block T-cell infiltration into the kidney (Wenderfer *et al.*, 2008). The novel $S1P_1$-selective cenerimod (ACT-334441) is currently under investigation in a phase 1 study (NCT02914223), and it will be interesting to see whether this compound holds the promise to mitigate disease symptoms and the progression of SLE.

Other forms of inflammatory kidney diseases:

Fingolimod was also found effective in other inflammatory kidney diseases such as in mesangioproliferative glomerulonephritis or tubulointerstitial fibrosis, which have no autoimmune character. In rats in which chronic progressive glomerulosclerosis was induced, the treatment with oral fingolimod, at 0.3mg/kg daily, resulted in an effective reduction of blood lymphocyte counts and renal lymphocyte infiltration and proteinuria and matrix expansion were reduced and renal function improved (Martini *et al.*, 2007; Peters *et al.*, 2004). In another study in mice, fingolimod administered orally at 1mg/kg for 5 days relieved tubulointerstitial fibrosis induced by unilateral ureter obstruction (Thangada *et al.*, 2014).

5.9. Atherosclerosis

Atherosclerosis is a multifactorial disease of inflammatory nature and associated with increased vascular plaque formation that at some point causes rupture and thrombus formation. Patients therefore have a strongly increased risk for cardiovascular complications including coronary artery disease, myocardial infarcts and stroke (Ross, 1999). Risk factors for the pathogenesis of atherosclerosis include high serum levels of cholesterol/LDL, smoking, hypertension and diabetes mellitus (Ross, 1999). On the cellular level, atherosclerosis is mainly characterized by endothelial dysfunction, but other cell types are also involved such as macrophages, T cells and smooth muscle cells. The activated endothelial cells show increased permeability and adhesion of immune cells, and change their normally anticoagulant state into a pro-coagulant state (Dahal *et al.*, 2017; Ross, 1999). In addition, the inflammatory environment stimulates proliferation and migration of vascular smooth-muscle cells leading to thickening and remodeling of the vascular wall (Ross, 1999).
To date, the standard of care for this disease still aims at reducing plasma cholesterol levels with statins and fibrates. However, new strategies are underway to more specifically reduce vascular inflammation, for instance by inhibiting or neutralizing IL-1β (Ridker *et al.*, 2017). Since S1P modulators like fingolimod have demonstrated anti-inflammatory potential in the clinical setting, their use in the treatment of atherosclerosis is suggested.

Mice are resistant to atherosclerosis by nature and hence require certain genetic manipulations to establish suitable preclinical models. Particularly, ApoE knockout (KO) mice, LDL receptor KO mice, hepatic lipase KO mice or human cholesterol-ester transfer protein transgenic mice were shown to develop atherosclerosis with high incidence (Kapourchali *et al.*, 2014). Unfortunately, however, when fingolimod was tested in ApoE KO and LDL receptor KO mice under various diet conditions, outcomes were controversial and

difficult to explain. On the one hand, in mice kept on a cholesterol-rich diet, fingolimod attenuated the development of atherosclerosis, which was attributed to its effect on the distribution and activation of T cells and macrophages in plaques, and to reduce plasma cytokine levels (Keul *et al.*, 2007; Nofer *et al.*, 2007). On the other hand, in ApoE KO mice kept under standard diet (Klingenberg *et al.*, 2007), and in LDL receptor KO mice fed with a low cholesterol Western-like diet (0.25%) (Poti *et al.*, 2012), two procedures causing milder hypercholesterolaemia, smaller lesions and no increase of plasma cytokines, fingolimod could not prevent atherosclerotic lesions, although lymphocyte counts were reduced. The conflicting data of the studies were discussed (Poti *et al.*, 2012), and it was proposed that fingolimod may be protective only under conditions of massively increased cholesterol burden and exacerbated inflammation. In another study, the $S1P_1$ agonist KRP-203 also improved atherosclerosis lesions in LDL-R-deficient mice fed a cholesterol-rich diet (Poti *et al.*, 2013), but because of these controversial findings it remains unclear whether clinical studies with S1PR modulators in atherosclerosis are warranted.

5.10. Diabetes mellitus

Two types of diabetes mellitus exist of which type 1 (T1D) has a juvenile onset and accounts for 10% of all cases of diabetes. It is characterized as an autoimmune destruction of pancreatic beta-cells in the islets of Langerhans. Consequently, there is a loss of insulin secretion and the main therapeutic option is to substitute insulin by administration. In contrast, type 2 diabetes (T2D) has a adult onset and covers 90% of diabetics. It is mainly characterized by the development of insulin resistance in peripheral tissues, and obesity and associated inflammation are the prevalent risk factors (Ng *et al.*, 2017). In T2D, there is no evidence for autoimmune-mediated destruction of beta-cells, although amyloid deposition in the islets frequently occurs which may interfere with beta-cell function and insulin secretion.

Beta-cell apoptosis: Since apoptosis of beta-cells is crucial in the development of T1D, understanding the regulation of apoptosis in this cell type is mandatory. The trigger for beta-cell death comes from auto-reactive T cells that are directed against multiple islet antigens, and therefore, therapeutic interference with the activated immune cells seems promising. Several immunomodulatory drugs, including anti-CD3, anti-CD20, and CTLA4-Ig, were tested in clinical trials of new-onset T1D patients (Orban *et al.*, 2011; Pescovitz *et al.*, 2009; Sherry *et al.*, 2011). Although these treatments resulted in short-term improvement of beta-cell function for several months, durable improvement was not observed. This was explained by the low regenerative capacity of human beta-cells (Maganti *et al.*, 2014). Fingolimod was so far only tested in animal models of autoimmune- and drug-induced diabetes, and in many of these models, it was found effective to normalize blood glucose levels and to protect islet

graft destruction (Fu *et al.*, 2002; Maki *et al.*, 2002; Suzuki *et al.*, 1998; Tsuji *et al.*, 2012; Yang *et al.*, 2003). Interestingly, fingolimod also increased beta-cell survival and improved their regeneration in vivo in hyperglycemic db/db mice (Moon *et al.*, 2013; Zhao *et al.*, 2012). Whether fingolimod deserves further investigation in clinical trials for T1D, particularly also in view of disappointing long-term results with other immunomodulators, is disputable.

Alternatively, beta-cell destruction could be impeded by targeting the molecular pathways involved in the death response, possibly in combination with immunomodulators like fingolimod. In this context, the cellular sphingolipid rheostat has attracted attention as the balance between pro-apoptotic ceramide and pro-survivial S1P was shown to affect the cell's life/death decision. In numerous studies with beta-cells, inhibition of ceramide formation or stimulation of SphK1/S1P signaling could protect cells from apoptosis (Jessup *et al.*, 2011) (Ng *et al.*, 2017) (Meikle *et al.*, 2017).

Insulin resistance: Insulin restistance is the main risk factor for T2D. There is ample evidence from cell culture and animal experiments that the deregulation of ceramides, glucosylceramide and gangliosides is involved in insulin resistance (Chaurasia *et al.*, 2015; Chavez *et al.*, 2012). Procedures to block or deplete enzymes in the de-novo synthesis of ceramide, gucosylceramide synthase, or GM3 synthase, all improve glucose tolerance and prevent insulin resistance and diabetes (Chavez *et al.*, 2012). Furthermore, it is was recently reported that adiponectin, a peptide hormone secreted by adipocytes and acting via adiponectin receptors (AdipoR)/AMPK to stimulate glucose uptake and fatty acid oxidation, has insulin-sensitizing, anti-inflammatory and anti-atherogenic activity by modulating ceramides. Interestingly, AdipoR has an intrinsic ceramidase activity (Holland *et al.*, 2011), and thereby, reduces cellular ceramide levels by converting it to sphingosine and further to the prosurvival S1P. The prosurvival effect of S1P on beta-cells was further supported by data from SphK1 knockout mice. When fed with high-fat diet, SphK1-deficient islets were more susceptible to lipotoxic stress factors (Qi *et al.*, 2013). Fingolimod also reversed insulin resistance in animal models (Bruce *et al.*, 2013; Kendall *et al.*, 2008) and it was proposed that this effect was due to reduced ceramide formation in muscles (Bruce *et al.*, 2013).

Altogether, based on available data, fingolimod seems to have some short-term benefit in T1D by downregulating the immune response against beta-cell, but long-term responses are missing as it fails to halt disease progression. Furthermore, it must be kept in mind that in diabetic patients the risk for developing macula edema under fingolimod is increased (Moss, 2017). This risk must be weighed against its rather limited benefit as an insulin sensitizer in diabetes.

5.11. Rett's syndrome

The Rett's syndrome is a progressive neurodevelopmental disorder that predominantly affects young femals and manifests with autistic-like behavior and severe mental retardation. Typical symptoms include motor impairment, such as ataxia, apraxia and tremor, stereotypic hand movements, seizures, hyperventilation, apnea and even cardiac dysrhythmias (Cronk *et al.*, 2016; Liyanage *et al.*, 2014; Pecorelli *et al.*, 2016). The disorder is mainly characterized as a genetic disease which in most cases is caused by a mutation in the X-linked gene coding for the methyl-CpG-binding protein 2 (*MECP2*). In rare cases, other mutations, such as in the genes coding for the cyclin-dependent kinase-like 5 protein (*CDKL5*) and forkhead box G1 (*FOXG1*), were also described. The MECP2 protein is known to regulate the transcriptional activity of various genes and modulate the chromatin structure (Liyanage *et al.*, 2014). A more global function as genome-wide epigenetic modulator has also been proposed (Liyanage *et al.*, 2014). A well described target gene of MECP2 is the brain-derived neurotropic factor (BDNF) (Liyanage *et al.*, 2014). Notably, MECP2 knockout mice, which represent a model for Rett syndrome and consequently develop neuronal and motor deficits comparable to Rett patients, showed reduced BNDF expression in various brain regions including hippocampus, striatum and cerebellum (Deogracias *et al.*, 2012). In these mice, application of fingolimod not only increased BDNF protein expression in the brain, but also improved neuronal and motor deficits, and even prolonged the life-expectancy of the animals (Deogracias *et al.*, 2012). Furthermore, in a rat model of valproic acid-induced autism, fingolimod mitigated learning and memory impairments and social deficits (Wu *et al.*, 2017). A phase 1 study (NCT02061137) currently investigates the safety and efficacy of fingolimod in children older than 6 years with Rett syndrome, and it will be interesting to see if fingolimod can mitigate the regression of motor and language skills.

5.12. Fear extinction memory

Since fingolimod-P was previously shown to directly inhibit HDAC activity in cell nuclei *in vitro* as well as *in vivo* in mouse brain (Hait *et al.*, 2014), and in view of the finding that HDAC inhibitors can improve cognitive disorders (Fischer *et al.*, 2010), it may be hypothesized that fingolimod can have beneficial cognitive and memory enhancing effects. In a mouse model of fear extinction memory testing, fingolimod application over 3 days followed by electric foot shock leads to a continuous reduction of the freezing time (a measure of fear towards the electric shock). When an extinction session was introduced after day 2 in which mice were reexposed to the conditioned stimulus but without footshock, they returned to a higher freezing time upon footshock on day 3. In contrast, fingolimod treated mice remained at the

low freezing time, suggesting that the extinction of aversive memories was improved (Hait *et al.*, 2014). Since these experiments were carried out in immunocompromised SCID mice, which are deficient in both B and T cell responses, the involvement of immune-mediated mechanisms in the fingolimod effect was excluded, and particularly involvement of HADC inhibition in the hippocampus was suggested as a possible mechanism. More cognitive tests are needed to better rate the potential of fingolimod as enhancer of cognitive ability and memory.

5.13. Other potential indications for fingolimod

The above mentioned indications for fingolimod have been extensively investigated in preclinical studies or have already moved forward to clinical trials. In addition, fingolimod is under consideration for various other disorders as well, most notable are disorders associated with neuroinflammatory processes, such as Amyotrophic Lateral Sclerosis (ALS) (Potenza *et al.*, 2016), Alzheimer (Takasugi *et al.*, 2013), Parkinson (Zhao *et al.*, 2017) and cerebral malaria (Finney *et al.*, 2011). Currently, a clinical phase 2 study (NCT01786174) is ongoing to determine safety and tolerability of fingolimod in patients with ALS. It will be interesting to see whether it can mitigate the clinical symptoms of this still incurable neurodegenerative disease.

6. Adverse effects of fingolimod

Table 2 summarizes some of the adverse events reported from patients with relapsing-remitting MS treated with low doses of 0.5 mg fingolimod daily for 24 months (FREEDOMS II). Those seen more frequently with fingolimod than with placebo included lymphopenia, increased liver transaminases, herpes viral infections, hypertension, and initial bradycardia and first degree AV block both as first-dose effects. Of note, analysis of pooled safety data from various phase 2 and phase 3 studies revealed that the risk for macular edema, which in FREEDOMS II occurred in 1% of the patients in both the fingolimod and the placebo group, was seven times more common in individuals with coexisting diabetes or history of uveitis when treated with 1.25 mg fingolimod (Moss, 2017; Zarbin *et al.*, 2013).

Also, data derived from the phase II trials on renal transplant recipients, which received fingolimod at 10 times higher doses and also in combination with other immunosuppressive drugs like CSA and MMF, additional adverse effects to the ones summarized in table 2, were reported including reduced lung function and renal function (Budde *et al.*, 2006; Salvadori *et al.*, 2006).

Here, we want to point out that based on published preclinical data much higher doses of fingolimod would be required to achieve anti-tumor effects in patients. To illustrate this, in tumor-xenografted mice doses ranging from 2.5. to 10 mg/kg daily were used, which would correspond to doses of 175 to 700 mg per day in cancer patients. Even though this simplified calculation does not fully respect differences in pharmacokinetics and drug metabolism between mouse and man, and between i.p. and oral delivery, considering the safety data shown in **Table 2** for 0.5 mg fingolimod, it goes without saying that such aberrantly high doses will result in many more complications.

Rebound syndrome upon drug discontinuation

Fingolimod discontinuation may be necessary due to different reasons, the most common are severe adverse effects, breakthrough disease activity or pregnancy. In a recent study involving 50 patients, fingolimod treatment was discontinued in 5 patients who developed a severe disease recurrence known as rebound phenomenon (Hatcher *et al.*, 2016). Such a frequency is indeed clinically relevant and deserves more detailed investigation. Symptoms of recurrence usually start 4-12 weeks after drug withdrawal when lymphocytes reenter the circulation and the CNS. The mechanism underlying the rebound phenomenon is still unclear, but it has been speculated that different T cell subsets repopulate the circulating pool of immune cells with different kinetics. This is supported by the finding that in two patients with severe rebound after fingolimod withdrawal $CD8^+$ T cells recovered more rapidly than the $CD4^+$ and the $CD19^+$ subsets (Hatcher *et al.*, 2016).

In an approach to investigate possible changes that occur in the immune system upon fingolimod withdrawal, the experimental autoimmune encephanomyelitis (EAE) model was performed in mice. Interestingly, T cells of mice under fingolimod withdrawal showed (1) a several-fold increase of $S1P_1$ mRNA and (2) increased phospho-Akt signaling. Furthermore, compared to mice under continuous drug exposure, (3) an increased TH_{17}/TH_1 cell ratio, as analyzed in the spinal cord, and (4) reduced Treg cell function in lymph nodes was reported (Cavone *et al.*, 2015). Functional rebound is often associated with treatment discontinuation and has also been described for various other immunosuppressive agents after abrupt dose reduction or withdrawal (Sueki *et al.*, 2017). Clearly, further studies are warranted to pinpoint the type of immune cells responsible for the clinical symptoms after fingolimod withdrawal.

7. Perspectives

Fingolimod was the first S1PR modulator reported to have immunosuppressive activity and it is so far the only one that was approved for clinical use. However, fingolimod is a nonspecific

S1PR agonist and therefore new functionally related compounds have been developed, which better discriminate between the various S1PR subtypes and hence are expected to have improved efficacy and tolerability. These include the $S1P_1$ selective compounds KRP-203, ponesimod and cenerimod, the $S1P_{1+5}$ selective siponimod and ozanimod, and the $S1P_{1+4+5}$ selective amiselimod and etrosimod. Due to their higher selectivity, these new drug developments hold great promise for clinical benefit in a broad range of inflammatory and autoimmune diseases.

On the other hand, for cancer therapy, the $S1P_1$-dependent immunosuppressive effect of fingolimod is supposed to be counterproductive and therefore numerous preclinical investigations have focused on S1PR-independent (off-target) effects. In addition to fingolimod, several new formulations with improved tumor-targeting properties have been tested, all of them achieved measurable cytotoxic effects only at high concentrations in the µM range. Remarkably, to achieve anti-tumor effects *in vivo* doses of 10 mg/kg fingolimod per day were used, which is far beyond the dose of 0.5-1.25 mg per day at which adverse events already occur in patients treated for multiple sclerosis. We therefore conclude that fingolimod and the currently available new formulations will fail to achieve clinical responses in cancer patients at tolerable doses.

Acknowledgements

This work was supported by the Swiss National Science Foundation (3100A0-111806).

Conflict of interest

none

References

Adachi K, Kohara T, Nakao N, Arita M, Chiba K, Mishina T*, et al.* (1995). Design, synthesis, and structure-activity-relationships of 2-substituted-2-amino-1,3-propanediols - discovery of a novel immunosuppressant, FTY720. *Bioorg Med Chem Lett* **5**(8)**:** 853-856.

Alberg AJ, Armeson K, Pierce JS, Bielawski J, Bielawska A, Visvanathan K*, et al.* (2013). Plasma sphingolipids and lung cancer: a population-based, nested case-control study. *Cancer Epidemiol Biomarkers Prev* **22**(8)**:** 1374-1382.

Allende ML, Bektas M, Lee BG, Bonifacino E, Kang J, Tuymetova G*, et al.* (2011). Sphingosine-1-phosphate lyase deficiency produces a pro-inflammatory response while impairing neutrophil trafficking. *J Biol Chem* **286**(9)**:** 7348-7358.

Alperovich G, Rama I, Lloberas N, Franquesa M, Poveda R, Goma M*, et al.* (2007). New immunosuppresor strategies in the treatment of murine lupus nephritis. *Lupus* **16**(1)**:** 18-24.

Alshaker H, Wang Q, Srivats S, Chao Y, Cooper C, Pchejetski D (2017). New FTY720-docetaxel nanoparticle therapy overcomes FTY720-induced lymphopenia and inhibits metastatic breast tumour growth. *Breast Cancer Res Treat* **165**(3)**:** 531-543.

Ando S, Amano H, Amano E, Minowa K, Watanabe T, Nakano S*, et al.* (2010). FTY720 exerts a survival advantage through the prevention of end-stage glomerular inflammation in lupus-prone BXSB mice. *Biochem Biophys Res Commun* **394**(3)**:** 804-810.

Anselmo D, Amersi FF, Shen XD, Gao F, Katori M, Ke B*, et al.* (2002). FTY720: a novel approach to the treatment of hepatic ischemia-reperfusion injury. *Transplant Proc* **34**(5)**:** 1467-1468.

Azab AK, Runnels JM, Pitsillides C, Moreau AS, Azab F, Leleu X*, et al.* (2009). CXCR4 inhibitor AMD3100 disrupts the interaction of multiple myeloma cells with the bone marrow microenvironment and enhances their sensitivity to therapy. *Blood* **113**(18)**:** 4341-4351.

Bagdanoff JT, Donoviel MS, Nouraldeen A, Carlsen M, Jessop TC, Tarver J*, et al.* (2010). Inhibition of sphingosine 1-phosphate lyase for the treatment of rheumatoid arthritis: discovery of (E)-1-(4-((1R,2S,3R)-1,2,3,4-tetrahydroxybutyl)-1H-imidazol-2-yl)ethanone oxime (LX2931) and (1R,2S,3R)-1-(2-(isoxazol-3-yl)-1H-imidazol-4-yl)butane-1,2,3,4-tetraol (LX2932). *J Med Chem* **53**(24)**:** 8650-8662.

Bai Y, Tong Y, Liu Y, Hu H (2017). Self-dsDNA in the pathogenesis of systemic lupus erythematosus. *Clin Exp Immunol***, in press. doi: 10.111/cei.13041.**

Bandhuvula P, Tam YY, Oskouian B, Saba JD (2005). The immune modulator FTY720 inhibits sphingosine-1-phosphate lyase activity. *J Biol Chem* **280**(40)**:** 33697-33700.

Baumgart DC, Carding SR (2007). Inflammatory bowel disease: cause and immunobiology. *Lancet* **369**(9573)**:** 1627-1640.

Beider K, Rosenberg E, Bitner H, Shimoni A, Leiba M, Koren-Michowitz M*, et al.* (2017). The Sphingosine-1-Phosphate Modulator FTY720 Targets Multiple Myeloma via the CXCR4/CXCL12 Pathway. *Clin Cancer Res* **23**(7)**:** 1733-1747.

Berdyshev EV, Gorshkova I, Skobeleva A, Bittman R, Lu X, Dudek SM*, et al.* (2009). FTY720 inhibits ceramide synthases and up-regulates dihydrosphingosine 1-phosphate formation in human lung endothelial cells. *J Biol Chem* **284**(9)**:** 5467-5477.

Bialojan C, Takai A (1988). Inhibitory effect of a marine-sponge toxin, okadaic acid, on protein phosphatases. Specificity and kinetics. *Biochem J* **256**(1)**:** 283-290.

Bigaud M, Dincer Z, Bollbuck B, Dawson J, Beckmann N, Beerli C*, et al.* (2016). Pathophysiological Consequences of a Break in S1P1-Dependent Homeostasis of Vascular Permeability Revealed by S1P1 Competitive Antagonism. *PLoS One* **11**(12)**:** e0168252.

Billich A, Bornancin F, Devay P, Mechtcheriakova D, Urtz N, Baumruker T (2003). Phosphorylation of the immunomodulatory drug FTY720 by sphingosine kinases. *J Biol Chem* **278**(48)**:** 47408-47415.

Brinkmann V (2007). Sphingosine 1-phosphate receptors in health and disease: mechanistic insights from gene deletion studies and reverse pharmacology. *Pharmacol Ther* **115**(1)**:** 84-105.

Brinkmann V, Billich A, Baumruker T, Heining P, Schmouder R, Francis G*, et al.* (2010). Fingolimod (FTY720): discovery and development of an oral drug to treat multiple sclerosis. *Nat Rev Drug Discov* **9**(11)**:** 883-897.

Brinkmann V, Davis MD, Heise CE, Albert R, Cottens S, Hof R*, et al.* (2002). The immune modulator FTY720 targets sphingosine 1-phosphate receptors. *J Biol Chem* **277**(24)**:** 21453-21457.

Bruce CR, Risis S, Babb JR, Yang C, Lee-Young RS, Henstridge DC*, et al.* (2013). The sphingosine-1-phosphate analog FTY720 reduces muscle ceramide content and improves glucose tolerance in high fat-fed male mice. *Endocrinology* **154**(1)**:** 65-76.

Budde K, Schutz M, Glander P, Peters H, Waiser J, Liefeldt L*, et al.* (2006). FTY720 (fingolimod) in renal transplantation. *Clin Transplant* **20 Suppl 17:** 17-24.

Buzard DJ, Kim SH, Lopez L, Kawasaki A, Zhu X, Moody J*, et al.* (2014). Discovery of APD334: Design of a clinical stage functional antagonist of the sphingosine-1-phosphate-1 receptor. *ACS Med Chem Lett* **5**(12)**:** 1313-1317.

Calabresi PA, Radue EW, Goodin D, Jeffery D, Rammohan KW, Reder AT*, et al.* (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 Neurol* **13**(6)**:** 545-556.

Camerer E, Regard JB, Cornelissen I, Srinivasan Y, Duong DN, Palmer D*, et al.* (2009). Sphingosine-1-phosphate in the plasma compartment regulates basal and inflammation-induced vascular leak in mice. *J Clin Invest* **119**(7)**:** 1871-1879.

Camm J, Hla T, Bakshi R, Brinkmann V (2014). Cardiac and vascular effects of fingolimod: mechanistic basis and clinical implications. *Am Heart J* **168**(5)**:** 632-644.

Cavone L, Felici R, Lapucci A, Buonvicino D, Pratesi S, Muzzi M*, et al.* (2015). Dysregulation of sphingosine 1 phosphate receptor-1 (S1P1) signaling and regulatory lymphocyte-dependent immunosuppression in a model of post-fingolimod MS rebound. *Brain Behav Immun* **50:** 78-86.

Chaurasia B, Summers SA (2015). Ceramides - lipotoxic inducers of metabolic disorders. *Trends Endocrinol Metab* **26**(10)**:** 538-550.

Chavez JA, Summers SA (2012). A ceramide-centric view of insulin resistance. *Cell Metab* **15**(5)**:** 585-594.

Chen J, Martin BL, Brautigan DL (1992). Regulation of protein serine-threonine phosphatase type-2A by tyrosine phosphorylation. *Science* **257**(5074)**:** 1261-1264.

Chiba K, Hoshino Y, Suzuki C, Masubuchi Y, Yanagawa Y, Ohtsuki M*, et al.* (1996). FTY720, a novel immunosuppressant possessing unique mechanisms. I. Prolongation of skin allograft survival and synergistic effect in combination with cyclosporine in rats. *Transplant Proc* **28**(2)**:** 1056-1059.

Chiba K, Yanagawa Y, Masubuchi Y, Kataoka H, Kawaguchi T, Ohtsuki M*, et al.* (1998). FTY720, a novel immunosuppressant, induces sequestration of circulating mature lymphocytes by acceleration of lymphocyte homing in rats. I. FTY720 selectively decreases the number of circulating mature lymphocytes by acceleration of lymphocyte homing. *J Immunol* **160**(10)**:** 5037-5044.

Christoffersen C, Nielsen LB (2012). Apolipoprotein M--a new biomarker in sepsis. *Crit Care* **16**(3)**:** 126.

Chubanov V, Ferioli S, Gudermann T (2017). Assessment of TRPM7 functions by drug-like small molecules. *Cell Calcium* **67:** 166-173.

Cohen JA, Barkhof F, Comi G, Hartung HP, Khatri BO, Montalban X*, et al.* (2010). Oral fingolimod or intramuscular interferon for relapsing multiple sclerosis. *N Engl J Med* **362**(5)**:** 402-415.

Coldewey SM, Benetti E, Collino M, Pfeilschifter J, Sponholz C, Bauer M*, et al.* (2016). Elevation of serum sphingosine-1-phosphate attenuates impaired cardiac function in experimental sepsis. *Sci Rep* **6:** 27594.

Cristobal I, Madoz-Gurpide J, Manso R, Gonzalez-Alonso P, Rojo F, Garcia-Foncillas J (2016). Potential anti-tumor effects of FTY720 associated with PP2A activation: a brief review. *Curr Med Res Opin* **32**(6)**:** 1137-1141.

Cristobal I, Manso R, Rincon R, Carames C, Senin C, Borrero A*, et al.* (2014). PP2A inhibition is a common event in colorectal cancer and its restoration using FTY720 shows promising therapeutic potential. *Mol Cancer Ther* **13**(4)**:** 938-947.

Cronk JC, Derecki NC, Litvak V, Kipnis J (2016). Unexpected cellular players in Rett syndrome pathology. *Neurobiol Dis* **92**(Pt A)**:** 64-71.

Czech B, Pfeilschifter W, Mazaheri-Omrani N, Strobel MA, Kahles T, Neumann-Haefelin T*, et al.* (2009). The immunomodulatory sphingosine 1-phosphate analog FTY720 reduces lesion size and improves neurological outcome in a mouse model of cerebral ischemia. *Biochem Biophys Res Commun* **389**(2)**:** 251-256.

Dahal U, Sharma D, Dahal K (2017). An Unsettled Debate About the Potential Role of Infection in Pathogenesis of Atherosclerosis. *J Clin Med Res* **9**(7)**:** 547-554.

Daniel C, Sartory N, Zahn N, Geisslinger G, Radeke HH, Stein JM (2007). FTY720 ameliorates Th1-mediated colitis in mice by directly affecting the functional activity of CD4+CD25+ regulatory T cells. *J Immunol* **178**(4)**:** 2458-2468.

Das RP, Jain AK, Ramesh V (2009). Current concepts in the pathogenesis of psoriasis. *Indian J Dermatol* **54**(1)**:** 7-12.

Debien E, Mayol K, Biajoux V, Daussy C, De Aguero MG, Taillardet M*, et al.* (2013). S1PR5 is pivotal for the homeostasis of patrolling monocytes. *Eur J Immunol* **43**(6)**:** 1667-1675.

Deguchi Y, Andoh A, Yagi Y, Bamba S, Inatomi O, Tsujikawa T*, et al.* (2006). The S1P receptor modulator FTY720 prevents the development of experimental colitis in mice. *Oncol Rep* **16**(4)**:** 699-703.

Dennis EA, Cao J, Hsu YH, Magrioti V, Kokotos G (2011). Phospholipase A2 enzymes: physical structure, biological function, disease implication, chemical inhibition, and therapeutic intervention. *Chem Rev* **111**(10)**:** 6130-6185.

Deogracias R, Yazdani M, Dekkers MP, Guy J, Ionescu MC, Vogt KE*, et al.* (2012). Fingolimod, a sphingosine-1 phosphate receptor modulator, increases BDNF levels and improves symptoms of a mouse model of Rett syndrome. *Proc Natl Acad Sci U S A* **109**(35)**:** 14230-14235.

Dobrowsky RT, Kamibayashi C, Mumby MC, Hannun YA (1993). Ceramide activates heterotrimeric protein phosphatase 2A. *J Biol Chem* **268**(21)**:** 15523-15530.

Dudek SM, Camp SM, Chiang ET, Singleton PA, Usatyuk PV, Zhao Y*, et al.* (2007). Pulmonary endothelial cell barrier enhancement by FTY720 does not require the S1P1 receptor. *Cell Signal* **19**(8)**:** 1754-1764.

Dutta R, Trapp BD (2014). Relapsing and progressive forms of multiple sclerosis: insights from pathology. *Curr Opin Neurol* **27**(3)**:** 271-278.

Eckschlager T, Plch J, Stiborova M, Hrabeta J (2017). Histone deacetylase inhibitors as anticancer drugs. *Int J Mol Sci* **18**(7).

Estella-Hermoso de Mendoza A, Castello-Cros R, Imbuluzqueta E, Cirauqui C, Pippa R, Odero MD*, et al.* (2015). Lipid nanosystems enhance the bioavailability and the therapeutic efficacy of FTY720 in acute myeloid leukemia. *J Biomed Nanotechnol* **11**(4)**:** 691-701.

Filipenko I, Schwalm S, Reali L, Pfeilschifter J, Fabbro D, Huwiler A*, et al.* (2016). Upregulation of the S1P3 receptor in metastatic breast cancer cells increases migration and invasion by induction of PGE2 and EP2/EP4 activation. *Biochim Biophys Acta* **1861**(11)**:** 1840-1851.

Finney CA, Hawkes CA, Kain DC, Dhabangi A, Musoke C, Cserti-Gazdewich C*, et al.* (2011). S1P is associated with protection in human and experimental cerebral malaria. *Mol Med* **17**(7-8)**:** 717-725.

Fischer A, Sananbenesi F, Mungenast A, Tsai LH (2010). Targeting the correct HDAC(s) to treat cognitive disorders. *Trends Pharmacol Sci* **31**(12)**:** 605-617.

Flemming S, Burkard N, Meir M, Schick MA, Germer CT, Schlegel N (2017). Sphingosine-1-phosphate receptor-1 agonist SEW2871 causes severe cardiac side effects and does not improve microvascular barrier breakdown in sepsis. *Shock***, in press. doi: 10.1097/SHK.0000000000000908.**

Frej C, Linder A, Happonen KE, Taylor FB, Lupu F, Dahlback B (2016). Sphingosine 1-phosphate and its carrier apolipoprotein M in human sepsis and in Escherichia coli sepsis in baboons. *J Cell Mol Med* **20**(6)**:** 1170-1181.

Fu F, Hu S, Deleo J, Li S, Hopf C, Hoover J*, et al.* (2002). Long-term islet graft survival in streptozotocin- and autoimmune-induced diabetes models by immunosuppressive and potential insulinotropic agent FTY720. *Transplantation* **73**(9)**:** 1425-1430.

Fu Y, Zhang N, Ren L, Yan Y, Sun N, Li YJ*, et al.* (2014). Impact of an immune modulator fingolimod on acute ischemic stroke. *Proc Natl Acad Sci U S A* **111**(51)**:** 18315-18320.

Fujishiro J, Kudou S, Iwai S, Takahashi M, Hakamata Y, Kinoshita M*, et al.* (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**(6)**:** 804-812.

Fujita T, Hirose R, Yoneta M, Sasaki S, Inoue K, Kiuchi M*, et al.* (1996). Potent immunosuppressants, 2-alkyl-2-aminopropane-1,3-diols. *J Med Chem* **39**(22)**:** 4451-4459.

Gaengel K, Niaudet C, Hagikura K, Lavina B, Muhl L, Hofmann JJ*, et al.* (2012). The sphingosine-1-phosphate receptor S1PR1 restricts sprouting angiogenesis by regulating the interplay between VE-cadherin and VEGFR2. *Dev Cell* **23**(3)**:** 587-599.

Glickman M, Malek RL, Kwitek-Black AE, Jacob HJ, Lee NH (1999). Molecular cloning, tissue-specific expression, and chromosomal localization of a novel nerve growth factor-regulated G-protein-coupled receptor, nrg-1. *Mol Cell Neurosci* **14**(2)**:** 141-152.

Golfier S, Kondo S, Schulze T, Takeuchi T, Vassileva G, Achtman AH*, et al.* (2010). Shaping of terminal megakaryocyte differentiation and proplatelet development by sphingosine-1-phosphate receptor S1P4. *Faseb J* **24**(12)**:** 4701-4710.

Graler MH, Bernhardt G, Lipp M (1998). EDG6, a novel G-protein-coupled receptor related to receptors for bioactive lysophospholipids, is specifically expressed in lymphoid tissue. *Genomics* **53**(2)**:** 164-169.

Grech G, Baldacchino S, Saliba C, Grixti MP, Gauci R, Petroni V*, et al.* (2016). Deregulation of the protein phosphatase 2A, PP2A in cancer: complexity and therapeutic options. *Tumour Biol* **37**(9)**:** 11691-11700.

Gstalder C, Ader I, Cuvillier O (2016). FTY720 (Fingolimod) inhibits HIF1 and HIF2 signaling, promotes vascular remodeling, and chemosensitizes in renal cell carcinoma animal model. *Mol Cancer Ther* **15**(10)**:** 2465-2474.

Hait NC, Allegood J, Maceyka M, Strub GM, Harikumar KB, Singh SK*, et al.* (2009). Regulation of histone acetylation in the nucleus by sphingosine-1-phosphate. *Science* **325**(5945)**:** 1254-1257.

Hait NC, Wise LE, Allegood JC, O'Brien M, Avni D, Reeves TM*, et al.* (2014). Active, phosphorylated fingolimod inhibits histone deacetylases and facilitates fear extinction memory. *Nat Neurosci* **17**(7)**:** 971-980.

Han Y, Li X, Zhou Q, Jie H, Lao X, Han J*, et al.* (2015). FTY720 abrogates collagen-induced arthritis by hindering dendritic cell migration to local lymph nodes. *J Immunol* **195**(9)**:** 4126-4135.

Hatcher SE, Waubant E, Nourbakhsh B, Crabtree-Hartman E, Graves JS (2016). Rebound Syndrome in Patients With Multiple Sclerosis After Cessation of Fingolimod Treatment. *JAMA Neurol* **73**(7)**:** 790-794.

Hemdan NY, Weigel C, Reimann CM, Graler MH (2016). Modulating sphingosine 1-phosphate signaling with DOP or FTY720 alleviates vascular and immune defects in mouse sepsis. *Eur J Immunol* **46**(12)**:** 2767-2777.

Holland WL, Miller RA, Wang ZV, Sun K, Barth BM, Bui HH*, et al.* (2011). Receptor-mediated activation of ceramidase activity initiates the pleiotropic actions of adiponectin. *Nat Med* **17**(1)**:** 55-63.

Hull EE, Montgomery MR, Leyva KJ (2016). HDAC inhibitors as epigenetic regulators of the immune system: Impacts on cancer therapy and inflammatory diseases. *Biomed Res Int* **2016:** 8797206.

Im DS, Ungar AR, Lynch KR (2000). Characterization of a zebrafish (Danio rerio) sphingosine 1-phosphate receptor expressed in the embryonic brain. *Biochem Biophys Res Commun* **279**(1)**:** 139-143.

Imeri F, Schwalm S, Lyck R, Zivkovic A, Stark H, Engelhardt B*, et al.* (2016). Sphingosine kinase 2 deficient mice exhibit reduced experimental autoimmune encephalomyelitis: Resistance to FTY720 but not ST-968 treatments. *Neuropharmacology* **105:** 341-350.

Jaillard C, Harrison S, Stankoff B, Aigrot MS, Calver AR, Duddy G*, et al.* (2005). Edg8/S1P5: an oligodendroglial receptor with dual function on process retraction and cell survival. *J Neurosci* **25**(6)**:** 1459-1469.

Jenne CN, Enders A, Rivera R, Watson SR, Bankovich AJ, Pereira JP*, et al.* (2009). T-bet-dependent S1P5 expression in NK cells promotes egress from lymph nodes and bone marrow. *J Exp Med* **206**(11)**:** 2469-2481.

Jessup CF, Bonder CS, Pitson SM, Coates PT (2011). The sphingolipid rheostat: a potential target for improving pancreatic islet survival and function. *Endocr Metab Immune Disord Drug Targets* **11**(4)**:** 262-272.

Jo E, Sanna MG, Gonzalez-Cabrera PJ, Thangada S, Tigyi G, Osborne DA*, et al.* (2005). S1P1-selective in vivo-active agonists from high-throughput screening: off-the-shelf chemical probes of receptor interactions, signaling, and fate. *Chem Biol* **12**(6)**:** 703-715.

Johnson TA, Evans BL, Durafourt BA, Blain M, Lapierre Y, Bar-Or A*, et al.* (2011). Reduction of the peripheral blood CD56(bright) NK lymphocyte subset in FTY720-treated multiple sclerosis patients. *J Immunol* **187**(1)**:** 570-579.

Kapourchali FR, Surendiran G, Chen L, Uitz E, Bahadori B, Moghadasian MH (2014). Animal models of atherosclerosis. *World J Clin Cases* **2**(5)**:** 126-132.

Kelly RD, Cowley SM (2013). The physiological roles of histone deacetylase (HDAC) 1 and 2: complex co-stars with multiple leading parts. *Biochem Soc Trans* **41**(3)**:** 741-749.

Kendall MR, Hupfeld CJ (2008). FTY720, a sphingosine-1-phosphate receptor modulator, reverses high-fat diet-induced weight gain, insulin resistance and adipose tissue inflammation in C57BL/6 mice. *Diabetes Obes Metab* **10**(9)**:** 802-805.

Keul P, Tolle M, Lucke S, von Wnuck Lipinski K, Heusch G, Schuchardt M*, et al.* (2007). The sphingosine-1-phosphate analogue FTY720 reduces atherosclerosis in apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol* **27**(3)**:** 607-613.

Kharel Y, Lee S, Snyder AH, Sheasley-O'neill S L, Morris MA, Setiady Y*, et al.* (2005). Sphingosine kinase 2 is required for modulation of lymphocyte traffic by FTY720. *J Biol Chem* **280**(44)**:** 36865-36872.

Kihara Y, Maceyka M, Spiegel S, Chun J (2014). Lysophospholipid receptor nomenclature review: IUPHAR Review 8. *Br J Pharmacol* **171**(15)**:** 3575-3594.

Klingenberg R, Nofer JR, Rudling M, Bea F, Blessing E, Preusch M*, et al.* (2007). Sphingosine-1-phosphate analogue FTY720 causes lymphocyte redistribution and hypercholesterolemia in ApoE-deficient mice. *Arterioscler Thromb Vasc Biol* **27**(11)**:** 2392-2399.

Kokotou MG, Limnios D, Nikolaou A, Psarra A, Kokotos G (2017). Inhibitors of phospholipase A2 and their therapeutic potential: an update on patents (2012-2016). *Expert Opin Ther Pat* **27**(2)**:** 217-225.

Krump-Konvalinkova V, Yasuda S, Rubic T, Makarova N, Mages J, Erl W*, et al.* (2005). Stable knock-down of the sphingosine 1-phosphate receptor S1P1 influences multiple functions of human endothelial cells. *Arterioscler Thromb Vasc Biol* **25**(3)**:** 546-552.

Lahiri S, Park H, Laviad EL, Lu X, Bittman R, Futerman AH (2009). Ceramide synthesis is modulated by the sphingosine analog FTY720 via a mixture of uncompetitive and noncompetitive inhibition in an Acyl-CoA chain length-dependent manner. *J Biol Chem* **284**(24)**:** 16090-16098.

Laviad EL, Albee L, Pankova-Kholmyansky I, Epstein S, Park H, Merrill AH, Jr.*, et al.* (2008). Characterization of ceramide synthase 2: tissue distribution, substrate specificity, and inhibition by sphingosine 1-phosphate. *J Biol Chem* **283**(9)**:** 5677-5684.

Li Y, Zhou T, Wang Y, Ning C, Lv Z, Han G*, et al.* (2017). The protumorigenic potential of FTY720 by promoting extramedullary hematopoiesis and MDSC accumulation. *Oncogene* **36**(26)**:** 3760-3771.

Lim KG, Tonelli F, Li Z, Lu X, Bittman R, Pyne S*, et al.* (2011). FTY720 analogues as sphingosine kinase 1 inhibitors: enzyme inhibition kinetics, allosterism, proteasomal degradation, and actin rearrangement in MCF-7 breast cancer cells. *J Biol Chem* **286**(21)**:** 18633-18640.

Limaye V, Li X, Hahn C, Xia P, Berndt MC, Vadas MA*, et al.* (2005). Sphingosine kinase-1 enhances endothelial cell survival through a PECAM-1-dependent activation of PI-3K/Akt and regulation of Bcl-2 family members. *Blood* **105**(8)**:** 3169-3177.

Liu J, Zhang C, Tao W, Liu M (2013). Systematic review and meta-analysis of the efficacy of sphingosine-1-phosphate (S1P) receptor agonist FTY720 (fingolimod) in animal models of stroke. *Int J Neurosci* **123**(3)**:** 163-169.

Liu N, Zhuang S (2015). Treatment of chronic kidney diseases with histone deacetylase inhibitors. *Front Physiol* **6:** 121.

Liyanage VR, Rastegar M (2014). Rett syndrome and MeCP2. *Neuromolecular Med* **16**(2)**:** 231-264.

Lundblad C, Axelberg H, Grande PO (2013). Treatment with the sphingosine-1-phosphate analogue FTY 720 reduces loss of plasma volume during experimental sepsis in the rat. *Acta Anaesthesiol Scand* **57**(6)**:** 713-718.

Maganti A, Evans-Molina C, Mirmira R (2014). From immunobiology to beta-cell biology: the changing perspective on type 1 diabetes. *Islets* **6**(2)**:** e28778.

Maki T, Gottschalk R, Monaco AP (2002). Prevention of autoimmune diabetes by FTY720 in nonobese diabetic mice. *Transplantation* **74**(12)**:** 1684-1686.

Malek RL, Toman RE, Edsall LC, Wong S, Chiu J, Letterle CA*, et al.* (2001). Nrg-1 belongs to the endothelial differentiation gene family of G protein-coupled sphingosine-1-phosphate receptors. *J Biol Chem* **276**(8)**:** 5692-5699.

Mandala S, Hajdu R, Bergstrom J, Quackenbush E, Xie J, Milligan J*, et al.* (2002). Alteration of lymphocyte trafficking by sphingosine-1-phosphate receptor agonists. *Science* **296**(5566)**:** 346-349.

Mao Y, Wang J, Zhao Y, Wu Y, Kwak KJ, Chen CS*, et al.* (2014). A novel liposomal formulation of FTY720 (fingolimod) for promising enhanced targeted delivery. *Nanomedicine* **10**(2)**:** 393-400.

Martini S, Kramer S, Loof T, Wang-Rosenke Y, Daig U, Budde K*, et al.* (2007). S1P modulator FTY720 limits matrix expansion in acute anti-thy1 mesangioproliferative glomerulonephritis. *Am J Physiol Renal Physiol* **292**(6)**:** F1761-1770.

Matsuoka Y, Nagahara Y, Ikekita M, Shinomiya T (2003). A novel immunosuppressive agent FTY720 induced Akt dephosphorylation in leukemia cells. *Br J Pharmacol* **138**(7)**:** 1303-1312.

Matsuura M, Imayoshi T, Okumoto T (2000). Effect of FTY720, a novel immunosuppressant, on adjuvant- and collagen-induced arthritis in rats. *Int J Immunopharmacol* **22**(4)**:** 323-331.

McDonald MC, Dhadly P, Cockerill GW, Cuzzocrea S, Mota-Filipe H, Hinds CJ*, et al.* (2003). Reconstituted high-density lipoprotein attenuates organ injury and adhesion molecule expression in a rodent model of endotoxic shock. *Shock* **20**(6)**:** 551-557.

McInnes IB, Schett G (2011). The pathogenesis of rheumatoid arthritis. *N Engl J Med* **365**(23)**:** 2205-2219.

Mechtcheriakova D, Wlachos A, Sobanov J, Bornancin F, Zlabinger G, Baumruker T*, et al.* (2007). FTY720-phosphate is dephosphorylated by lipid phosphate phosphatase 3. *FEBS Lett* **581**(16)**:** 3063-3068.

Mehling M, Brinkmann V, Antel J, Bar-Or A, Goebels N, Vedrine C*, et al.* (2008). FTY720 therapy exerts differential effects on T cell subsets in multiple sclerosis. *Neurology* **71**(16)**:** 1261-1267.

Meikle PJ, Summers SA (2017). Sphingolipids and phospholipids in insulin resistance and related metabolic disorders. *Nat Rev Endocrinol* **13**(2)**:** 79-91.

Millward TA, Zolnierowicz S, Hemmings BA (1999). Regulation of protein kinase cascades by protein phosphatase 2A. *Trends Biochem Sci* **24**(5)**:** 186-191.

Milo R, Kahana E (2010). Multiple sclerosis: geoepidemiology, genetics and the environment. *Autoimmun Rev* **9**(5)**:** A387-394.

Miron VE, Jung CG, Kim HJ, Kennedy TE, Soliven B, Antel JP (2008). FTY720 modulates human oligodendrocyte progenitor process extension and survival. *Ann Neurol* **63**(1)**:** 61-71.

Mizushima T, Ito T, Kishi D, Kai Y, Tamagawa H, Nezu R*, et al.* (2004). Therapeutic effects of a new lymphocyte homing reagent FTY720 in interleukin-10 gene-deficient mice with colitis. *Inflamm Bowel Dis* **10**(3)**:** 182-192.

Montrose DC, Scherl EJ, Bosworth BP, Zhou XK, Jung B, Dannenberg AJ*, et al.* (2013). S1P(1) localizes to the colonic vasculature in ulcerative colitis and maintains blood vessel integrity. *J Lipid Res* **54**(3)**:** 843-851.

Moon H, Chon J, Joo J, Kim D, In J, Lee H*, et al.* (2013). FTY720 preserved islet beta-cell mass by inhibiting apoptosis and increasing survival of beta-cells in db/db mice. *Diabetes Metab Res Rev* **29**(1)**:** 19-24.

Moss HE (2017). Visual consequences of medications for multiple sclerosis: the good, the bad, the ugly, and the unknown. *Eye Brain* **9:** 13-21.

Mulgaonkar S, Tedesco H, Oppenheimer F, Walker R, Kunzendorf U, Russ G*, et al.* (2006). FTY720/cyclosporine regimens in de novo renal transplantation: a 1-year dose-finding study. *Am J Transplant* **6**(8)**:** 1848-1857.

Muller HC, Hocke AC, Hellwig K, Gutbier B, Peters H, Schonrock SM*, et al.* (2011). The Sphingosine-1 Phosphate receptor agonist FTY720 dose dependently affected endothelial integrity in vitro and aggravated ventilator-induced lung injury in mice. *Pulm Pharmacol Ther* **24**(4)**:** 377-385.

Neviani P, Santhanam R, Oaks JJ, Eiring AM, Notari M, Blaser BW*, et al.* (2007). FTY720, a new alternative for treating blast crisis chronic myelogenous leukemia and Philadelphia chromosome-positive acute lymphocytic leukemia. *J Clin Invest* **117**(9)**:** 2408-2421.

Ng ML, Wadham C, Sukocheva OA (2017). The role of sphingolipid signalling in diabetes associated pathologies (Review). *Int J Mol Med* **39**(2)**:** 243-252.

Nofer JR, Bot M, Brodde M, Taylor PJ, Salm P, Brinkmann V*, et al.* (2007). FTY720, a synthetic sphingosine 1 phosphate analogue, inhibits development of atherosclerosis in low-density lipoprotein receptor-deficient mice. *Circulation* **115**(4)**:** 501-508.

Novgorodov AS, El-Alwani M, Bielawski J, Obeid LM, Gudz TI (2007). Activation of sphingosine-1-phosphate receptor S1P5 inhibits oligodendrocyte progenitor migration. *Faseb J* **21**(7)**:** 1503-1514.

O'Sullivan S, Dev KK (2017). Sphingosine-1-phosphate receptor therapies: Advances in clinical trials for CNS-related diseases. *Neuropharmacology* **113**(Pt B)**:** 597-607.

Okazaki H, Hirata D, Kamimura T, Sato H, Iwamoto M, Yoshio T*, et al.* (2002). Effects of FTY720 in MRL-lpr/lpr mice: therapeutic potential in systemic lupus erythematosus. *J Rheumatol* **29**(4)**:** 707-716.

Omar HA, Chou CC, Berman-Booty LD, Ma Y, Hung JH, Wang D*, et al.* (2011). Antitumor effects of OSU-2S, a nonimmunosuppressive analogue of FTY720, in hepatocellular carcinoma. *Hepatology* **53**(6)**:** 1943-1958.

Orban T, Bundy B, Becker DJ, DiMeglio LA, Gitelman SE, Goland R*, et al.* (2011). Co-stimulation modulation with abatacept in patients with recent-onset type 1 diabetes: a randomised, double-blind, placebo-controlled trial. *Lancet* **378**(9789)**:** 412-419.

Pankratz N (2016). Meta-analysis of rare and common exome chip variants identifies S1PR4 and other loci influencing blood cell traits. *Nat Genet* **48**(8)**:** 867-876.

Park JW, Park WJ, Futerman AH (2014). Ceramide synthases as potential targets for therapeutic intervention in human diseases. *Biochim Biophys Acta* **1841**(5)**:** 671-681.

Patmanathan SN, Yap LF, Murray PG, Paterson IC (2015). The antineoplastic properties of FTY720: evidence for the repurposing of fingolimod. *J Cell Mol Med* **19**(10)**:** 2329-2340.

Payne SG, Oskeritzian CA, Griffiths R, Subramanian P, Barbour SE, Chalfant CE*, et al.* (2007). The immunosuppressant drug FTY720 inhibits cytosolic phospholipase A2 independently of sphingosine-1-phosphate receptors. *Blood* **109**(3)**:** 1077-1085.

Pchejetski D, Bohler T, Brizuela L, Sauer L, Doumerc N, Golzio M*, et al.* (2010). FTY720 (fingolimod) sensitizes prostate cancer cells to radiotherapy by inhibition of sphingosine kinase-1. *Cancer Res* **70**(21)**:** 8651-8661.

Pecorelli A, Cervellati C, Hayek J, Valacchi G (2016). OxInflammation in Rett syndrome. *Int J Biochem Cell Biol* **81**(Pt B)**:** 246-253.

Peng X, Hassoun PM, Sammani S, McVerry BJ, Burne MJ, Rabb H*, et al.* (2004). Protective effects of sphingosine 1-phosphate in murine endotoxin-induced inflammatory lung injury. *Am J Respir Crit Care Med* **169**(11)**:** 1245-1251.

Perrotti D, Neviani P (2013). Protein phosphatase 2A: a target for anticancer therapy. *Lancet Oncol* **14**(6)**:** e229-238.

Perry D, Sang A, Yin Y, Zheng YY, Morel L (2011). Murine models of systemic lupus erythematosus. *J Biomed Biotechnol* **2011:** 271694.

Pescovitz MD, Greenbaum CJ, Krause-Steinrauf H, Becker DJ, Gitelman SE, Goland R*, et al.* (2009). Rituximab, B-lymphocyte depletion, and preservation of beta-cell function. *N Engl J Med* **361**(22)**:** 2143-2152.

Peters H, Martini S, Wang Y, Shimizu F, Kawachi H, Kramer S*, et al.* (2004). Selective lymphocyte inhibition by FTY720 slows the progressive course of chronic anti-thy 1 glomerulosclerosis. *Kidney Int* **66**(4)**:** 1434-1443.

Peyrin-Biroulet L, Christopher R, Behan D, Lassen C (2017). Modulation of sphingosine-1-phosphate in inflammatory bowel disease. *Autoimmun Rev* **16**(5)**:** 495-503.

Pitman MR, Woodcock JM, Lopez AF, Pitson SM (2012). Molecular targets of FTY720 (fingolimod). *Curr Mol Med* **12**(10)**:** 1207-1219.

Potenza RL, De Simone R, Armida M, Mazziotti V, Pezzola A, Popoli P*, et al.* (2016). Fingolimod: A disease-modifier drug in a mouse model of amyotrophic lateral sclerosis. *Neurotherapeutics* **13**(4)**:** 918-927.

Poti F, Costa S, Bergonzini V, Galletti M, Pignatti E, Weber C*, et al.* (2012). Effect of sphingosine 1-phosphate (S1P) receptor agonists FTY720 and CYM5442 on atherosclerosis development in LDL receptor deficient (LDL-R(-)/(-)) mice. *Vascul Pharmacol* **57**(1)**:** 56-64.

Poti F, Gualtieri F, Sacchi S, Weissen-Plenz G, Varga G, Brodde M*, et al.* (2013). KRP-203, sphingosine 1-phosphate receptor type 1 agonist, ameliorates atherosclerosis in LDL-R-/- mice. *Arterioscler Thromb Vasc Biol* **33**(7)**:** 1505-1512.

Qi Y, Chen J, Lay A, Don A, Vadas M, Xia P (2013). Loss of sphingosine kinase 1 predisposes to the onset of diabetes via promoting pancreatic beta-cell death in diet-induced obese mice. *Faseb J* **27**(10)**:** 4294-4304.

Qin X, Yue Z, Sun B, Yang W, Xie J, Ni E*, et al.* (2013). Sphingosine and FTY720 are potent inhibitors of the transient receptor potential melastatin 7 (TRPM7) channels. *Br J Pharmacol* **168**(6)**:** 1294-1312.

Radi ZA, Heuvelman DM, Masferrer JL, Benson EL (2011). Pharmacologic evaluation of sulfasalazine, FTY720, and anti-IL-12/23p40 in a TNBS-induced Crohn's disease model. *Dig Dis Sci* **56**(8)**:** 2283-2291.

Riddy DM, Stamp C, Sykes DA, Charlton SJ, Dowling MR (2012). Reassessment of the pharmacology of Sphingosine-1-phosphate S1P3 receptor ligands using the DiscoveRx PathHunter and Ca2+ release functional assays. *Br J Pharmacol* **167**(4)**:** 868-880.

Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C*, et al.* (2017). Antiinflammatory Therapy with Canakinumab for Atherosclerotic Disease. *N Engl J Med* **377**(12)**:** 1119-1131.

Rincon R, Cristobal I, Zazo S, Arpi O, Menendez S, Manso R*, et al.* (2015). PP2A inhibition determines poor outcome and doxorubicin resistance in early breast cancer and its activation shows promising therapeutic effects. *Oncotarget* **6**(6)**:** 4299-4314.

Ross R (1999). Atherosclerosis--an inflammatory disease. *N Engl J Med* **340**(2)**:** 115-126.

Saddoughi SA, Gencer S, Peterson YK, Ward KE, Mukhopadhyay A, Oaks J*, et al.* (2013). Sphingosine analogue drug FTY720 targets I2PP2A/SET and mediates lung tumour suppression via activation of PP2A-RIPK1-dependent necroptosis. *EMBO Mol Med* **5**(1)**:** 105-121.

Salvadori M, Budde K, Charpentier B, Klempnauer J, Nashan B, Pallardo LM*, et al.* (2006). FTY720 versus MMF with cyclosporine in de novo renal transplantation: a 1-year, randomized controlled trial in Europe and Australasia. *Am J Transplant* **6**(12)**:** 2912-2921.

Sanchez T, Estrada-Hernandez T, Paik JH, Wu MT, Venkataraman K, Brinkmann V*, et al.* (2003). Phosphorylation and action of the immunomodulator FTY720 inhibits vascular endothelial cell growth factor-induced vascular permeability. *J Biol Chem* **278**(47)**:** 47281-47290.

Sandborn WJ, Feagan BG (2016). Ozanimod treatment for ulcerative colitis. *N Engl J Med* **375**(8)**:** e17.

Sanna MG, Wang SK, Gonzalez-Cabrera PJ, Don A, Marsolais D, Matheu MP*, et al.* (2006). Enhancement of capillary leakage and restoration of lymphocyte egress by a chiral S1P1 antagonist in vivo. *Nat Chem Biol* **2**(8)**:** 434-441.

Santos Samary C, Pelosi P, Leme Silva P, Rieken Macedo Rocco P (2016). Immunomodulation after ischemic stroke: potential mechanisms and implications for therapy. *Crit Care* **20**(1)**:** 391.

Schroder M, Arlt O, Schmidt H, Huwiler A, Angioni C, Pfeilschifter JM*, et al.* (2015). Subcellular distribution of FTY720 and FTY720-phosphate in immune cells - another aspect of Fingolimod action relevant for therapeutic application. *Biol Chem* **396**(6-7)**:** 795-802.

Scott FL, Clemons B, Brooks J, Brahmachary E, Powell R, Dedman H*, et al.* (2016). Ozanimod (RPC1063) is a potent sphingosine-1-phosphate receptor-1 (S1P1) and receptor-5 (S1P5) agonist with autoimmune disease-modifying activity. *Br J Pharmacol* **173**(11)**:** 1778-1792.

Segura-Ulate I, Belcher TK, Vidal-Martinez G, Vargas-Medrano J, Perez RG (2017). FTY720-derivatives do not induce FTY720-like lymphopenia. *J Pharmacol Sci* **133**(3)**:** 187-189.

Shea BS, Brooks SF, Fontaine BA, Chun J, Luster AD, Tager AM (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**(6)**:** 662-673.

Sherry N, Hagopian W, Ludvigsson J, Jain SM, Wahlen J, Ferry RJ, Jr.*, et al.* (2011). Teplizumab for treatment of type 1 diabetes (Protege study): 1-year results from a randomised, placebo-controlled trial. *Lancet* **378**(9790)**:** 487-497.

Shimizu H, Takahashi M, Kaneko T, Murakami T, Hakamata Y, Kudou S*, et al.* (2005). KRP-203, a novel synthetic immunosuppressant, prolongs graft survival and attenuates chronic rejection in rat skin and heart allografts. *Circulation* **111**(2)**:** 222-229.

Song J, Matsuda C, Kai Y, Nishida T, Nakajima K, Mizushima T*, et al.* (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**(1)**:** 276-283.

Sueki H, Mizukawa Y, Aoyama Y (2017). Immune reconstitution inflammatory syndrome in non-HIV immunosuppressed patients. *J Dermatol* **in press. doi: 10.1111/1346-8138.14074.**

Sutphen R, Xu Y, Wilbanks GD, Fiorica J, Grendys EC, Jr., LaPolla JP*, et al.* (2004). Lysophospholipids are potential biomarkers of ovarian cancer. *Cancer Epidemiol Biomarkers Prev* **13**(7)**:** 1185-1191.

Suzuki K, Yan H, Li XK, Amemiya H, Suzuki S, Hiromitsu K (1998). Prevention of experimentally induced autoimmune type I diabetes in rats by the new immunosuppressive reagent FTY720. *Transplant Proc* **30**(4)**:** 1044-1045.

Suzuki S, Enosawa S, Kakefuda T, Li XK, Mitsusada M, Takahara S*, et al.* (1996a). Immunosuppressive effect of a new drug, FTY720, on lymphocyte responses in vitro and cardiac allograft survival in rats. *Transpl Immunol* **4**(3)**:** 252-255.

Suzuki S, Enosawa S, Kakefuda T, Shinomiya T, Amari M, Naoe S*, et al.* (1996b). A novel immunosuppressant, FTY720, with a unique mechanism of action, induces long-term graft acceptance in rat and dog allotransplantation. *Transplantation* **61**(2)**:** 200-205.

Takasugi N, Sasaki T, Ebinuma I, Osawa S, Isshiki H, Takeo K*, et al.* (2013). FTY720/fingolimod, a sphingosine analogue, reduces amyloid-beta production in neurons. *PLoS One* **8**(5)**:** e64050.

Tedesco-Silva H, Mourad G, Kahan BD, Boira JG, Weimar W, Mulgaonkar S*, et al.* (2004). FTY720, a novel immunomodulator: efficacy and safety results from the first phase 2A study in de novo renal transplantation. *Transplantation* **77**(12)**:** 1826-1833.

Tedesco-Silva H, Pescovitz MD, Cibrik D, Rees MA, Mulgaonkar S, Kahan BD*, et al.* (2006). Randomized controlled trial of FTY720 versus MMF in de novo renal transplantation. *Transplantation* **82**(12)**:** 1689-1697.

Thangada S, Shapiro LH, Silva C, Yamase H, Hla T, Ferrer FA (2014). Treatment with the immunomodulator FTY720 (fingolimod) significantly reduces renal inflammation in murine unilateral ureteral obstruction. *J Urol* **191**(5 Suppl)**:** 1508-1516.

Tonelli F, Lim KG, Loveridge C, Long J, Pitson SM, Tigyi G*, et al.* (2010). FTY720 and (S)-FTY720 vinylphosphonate inhibit sphingosine kinase 1 and promote its proteasomal degradation in human pulmonary artery smooth muscle, breast cancer and androgen-independent prostate cancer cells. *Cell Signal* **22**(10)**:** 1536-1542.

Troncoso P, Ortiz M, Martinez L, Kahan BD (2001). FTY 720 prevents ischemic reperfusion damage in rat kidneys. *Transplant Proc* **33**(1-2)**:** 857-859.

Tsuji T, Inoue M, Yoshida Y, Fujita T, Kaino Y, Kohno T (2012). Therapeutic approach for type 1 diabetes mellitus using the novel immunomodulator FTY720 (fingolimod) in combination with once-daily injection of insulin glargine in non-obese diabetic mice. *J Diabetes Investig* **3**(2)**:** 132-137.

Tsunemi S, Iwasaki T, Kitano S, Imado T, Miyazawa K, Sano H (2010). Effects of the novel immunosuppressant FTY720 in a murine rheumatoid arthritis model. *Clin Immunol* **136**(2)**:** 197-204.

Vaclavkova A, Chimenti S, Arenberger P, Hollo P, Sator PG, Burcklen M*, et al.* (2014). Oral ponesimod in patients with chronic plaque psoriasis: a randomised, double-blind, placebo-controlled phase 2 trial. *Lancet* **384**(9959)**:** 2036-2045.

van Doorn R, Lopes Pinheiro MA, Kooij G, Lakeman K, van het Hof B, van der Pol SM*, et al.* (2012). Sphingosine 1-phosphate receptor 5 mediates the immune quiescence of the human brain endothelial barrier. *J Neuroinflammation* **9:** 133.

Vessey DA, Kelley M, Zhang J, Li L, Tao R, Karliner JS (2007). Dimethylsphingosine and FTY720 inhibit the SK1 form but activate the SK2 form of sphingosine kinase from rat heart. *J Biochem Mol Toxicol* **21**(5)**:** 273-279.

Walzer T, Chiossone L, Chaix J, Calver A, Carozzo C, Garrigue-Antar L*, et al.* (2007). Natural killer cell trafficking in vivo requires a dedicated sphingosine 1-phosphate receptor. *Nat Immunol* **8**(12)**:** 1337-1344.

Wang F, Tan W, Guo D, He S (2007). Reduction of CD4 positive T cells and improvement of pathological changes of collagen-induced arthritis by FTY720. *Eur J Pharmacol* **573**(1-3)**:** 230-240.

Wang Q, Alshaker H, Bohler T, Srivats S, Chao Y, Cooper C*, et al.* (2017). Core shell lipid-polymer hybrid nanoparticles with combined docetaxel and molecular targeted therapy for the treatment of metastatic prostate cancer. *Sci Rep* **7**(1)**:** 5901.

Watson C, Long JS, Orange C, Tannahill CL, Mallon E, McGlynn LM*, et al.* (2010). High expression of sphingosine 1-phosphate receptors, S1P1 and S1P3, sphingosine kinase 1, and extracellular signal-regulated kinase-1/2 is associated with development of tamoxifen resistance in estrogen receptor-positive breast cancer patients. *Am J Pathol* **177**(5)**:** 2205-2215.

Wegner MS, Schiffmann S, Parnham MJ, Geisslinger G, Grosch S (2016). The enigma of ceramide synthase regulation in mammalian cells. *Prog Lipid Res* **63:** 93-119.

Weiler S, Braendlin N, Beerli C, Bergsdorf C, Schubart A, Srinivas H*, et al.* (2014). Orally active 7-substituted (4-benzylphthalazin-1-yl)-2-methylpiperazin-1-yl]nicotinonitriles as active-site inhibitors of sphingosine 1-phosphate lyase for the treatment of multiple sclerosis. *J Med Chem* **57**(12)**:** 5074-5084.

Wenderfer SE, Stepkowski SM, Braun MC (2008). Increased survival and reduced renal injury in MRL/lpr mice treated with a novel sphingosine-1-phosphate receptor agonist. *Kidney Int* **74**(10)**:** 1319-1326.

White C, Alshaker H, Cooper C, Winkler M, Pchejetski D (2016). The emerging role of FTY720 (Fingolimod) in cancer treatment. *Oncotarget* **7**(17)**:** 23106-23127.

Winkler MS, Nierhaus A, Holzmann M, Mudersbach E, Bauer A, Robbe L*, et al.* (2015). Decreased serum concentrations of sphingosine-1-phosphate in sepsis. *Crit Care* **19:** 372.

Wolf AM, Eller K, Zeiser R, Durr C, Gerlach UV, Sixt M*, et al.* (2009). The sphingosine 1-phosphate receptor agonist FTY720 potently inhibits regulatory T cell proliferation in vitro and in vivo. *J Immunol* **183**(6)**:** 3751-3760.

Wu A, Hinds CJ, Thiemermann C (2004). High-density lipoproteins in sepsis and septic shock: metabolism, actions, and therapeutic applications. *Shock* **21**(3)**:** 210-221.

Wu H, Wang X, Gao J, Liang S, Hao Y, Sun C*, et al.* (2017). Fingolimod (FTY720) attenuates social deficits, learning and memory impairments, neuronal loss and neuroinflammation in the rat model of autism. *Life Sci* **173:** 43-54.

Xin C, Ren S, Eberhardt W, Pfeilschifter J, Huwiler A (2007). FTY720 suppresses interleukin-1beta-induced secretory phospholipase A2 expression in renal mesangial cells by a transcriptional mechanism. *Br J Pharmacol* **150**(7)**:** 943-950.

Yang Z, Chen M, Fialkow LB, Ellett JD, Wu R, Brinkmann V*, et al.* (2003). The immune modulator FYT720 prevents autoimmune diabetes in nonobese diabetic mice. *Clin Immunol* **107**(1)**:** 30-35.

Yoshida Y, Tsuji T, Watanabe S, Matsushima A, Matsushima Y, Banno R*, et al.* (2013). Efficacy of combination treatment with fingolimod (FTY720) plus pathogenic autoantigen in a glucose-6-phosphate isomerase peptide (GPI325-339)-induced arthritis mouse model. *Biol Pharm Bull* **36**(11)**:** 1739-1746.

Yu F, Haas M, Glassock R, Zhao MH (2017). Redefining lupus nephritis: clinical implications of pathophysiologic subtypes. *Nat Rev Nephrol* **13**(8)**:** 483-495.

Zarbin MA, Jampol LM, Jager RD, Reder AT, Francis G, Collins W*, et al.* (2013). Ophthalmic evaluations in clinical studies of fingolimod (FTY720) in multiple sclerosis. *Ophthalmology* **120**(7)**:** 1432-1439.

Zemann B, Kinzel B, Muller M, Reuschel R, Mechtcheriakova D, Urtz N*, et al.* (2006). Sphingosine kinase type 2 is essential for lymphopenia induced by the immunomodulatory drug FTY720. *Blood* **107**(4)**:** 1454-1458.

Zhang L, Wang X, Bullock AJ, Callea M, Shah H, Song J*, et al.* (2015). Anti-S1P Antibody as a Novel Therapeutic Strategy for VEGFR TKI-Resistant Renal Cancer. *Clin Cancer Res* **21**(8)**:** 1925-1934.

Zhang W, Zhao J, Lee JF, Gartung A, Jawadi H, Lambiv WL*, et al.* (2013). ETS-1-mediated transcriptional up-regulation of CD44 is required for sphingosine-1-phosphate receptor subtype 3-stimulated chemotaxis. *J Biol Chem* **288**(45)**:** 32126-32137.

Zhao P, Yang X, Yang L, Li M, Wood K, Liu Q*, et al.* (2017). Neuroprotective effects of fingolimod in mouse models of Parkinson's disease. *Faseb J* **31**(1)**:** 172-179.

Zhao Z, Choi J, Zhao C, Ma ZA (2012). FTY720 normalizes hyperglycemia by stimulating beta-cell in vivo regeneration in db/db mice through regulation of cyclin D3 and p57(KIP2). *J Biol Chem* **287**(8)**:** 5562-5573.

Zhu Z, Fu Y, Tian D, Sun N, Han W, Chang G*, et al.* (2015). Combination of the immune modulator fingolimod with alteplase in acute ischemic stroke: A pilot trial. *Circulation* **132**(12)**:** 1104-1112.

Table 1: Pharmacokinetic parameters of fingolimod

PK parameters	Values
Dosing	0.5 mg daily p.o.
Bioavailablity	93%
Absorption	Slow, high, food independent
Metabolism	Liver: by CYP4F2
Elimination	Renal, 81%
Bound in plasma	99.7% (mainly to albumin)
Blood clearance	Low, (6.3 L/h)
Distribution volume	20 L/kg
Half-live	6 - 9 days
Max. plasma conc.	12-16 hours
Steady state after	1-2 months
Drug interactions	Ketoconazole

Table 2: Selected adverse events reported from patients with relapsing-remitting MS treated with 0.5 mg fingolimod daily for 24 months

Event*	**No. of patients fingolimod (N = 358) vs. placebo (N=355)**	**Remarks**
Lymphopenia	27 (8%) vs. 0	After one month, peripheral blood lymphocyte counts dropped by an average of 75 %. This is an indication for a positive biological response and was not reported as adverse event. Lymphopenia as a complication was defined as less than 0.2 x 10^9 lymphocytes per liter.
Increased alanine amino transferase	29 (8%) vs. 6 (2%)	
Increased gamma glutamyltransferase	23 (6%) vs. 2 (14%)	
Herpes viral infections	30 (8%) vs. 19 (5%)	Two fatal cases were reported from a previous study after treatment with 1.25 mg fingolimod daily (Cohen *et al.*, 2010)
Hypertension	32 (9%) vs. 11 (3%)	Small increases in systolic/diastolic blood pressure (~3/~1 mm Hg) after 2-6 months (Camm *et al.*, 2014)
Bradycardia	5 (1%) vs. 1 (<0.5%)	Mild to moderate with a mean decrease of 8 beats per minute in the first 6 h after treatment initiation.

		Initial $S1P_1$ agonism activates GIRK channels in atrial myocytes in the sinoatrial node, leading to hyperpolarization and reduction in excitability (Camm *et al.*, 2014)
First-degree AV block	17 (5%) vs. 7 (2%)	In the first 6 h after treatment initiation, transient and asymptomatic. Same molecular effect on atrial myocytes in the AV node as described for bradycardia in the sinoatrial node (Camm *et al.*, 2014).

*Data were taken from Calabresi et al (Calabresi *et al.*, 2014) based on the FREEDOMS II study (ClinicalTrials.gov number NCT00355134). Only complications are listed which occurred more frequently than in the placebo group and comprised >1% of the treated patients.

Table 3: Fingolimod and novel S1PR modulators in clinical studies

Compound name	**S1PR profile**	**approved**	**Phase 3**	**Phase 2**	**Phase 1**
Fingolimod (FTY720)	$S1P_{1,3,4,5}$	RRMS	PPMS	Stroke Uveitis Schizophrenia ALS	Rett's syndrome
KRP-203	$S1P_1$			UC (terminated) SLE (terminated)	
Ponesimod (ACT-128800)	$S1P_1$			RRMS Psoriasis Graft rejection	
Cenerimod (ACT-334441)	$S1P_1$			SLE	
Siponimod (BAF312)	$S1P_{1+5}$		SPMS	RRMS Dermatomyositis Polymyositis	
Ozanimod (RPC-1063)	$S1P_{1+5}$		RRMS UC	Crohn's disease	
Amiselimod (MT-1303)	$S1P_{1,4,5}$			RRMS Crohn's disease Psoriasis	SLE
Etrasimod (APD334)	$S1P_{1,4,5}$			UC	

ALS, amyotrophic lateral sclerosis; PPMS, primary progressive multiple sclerosis ; RRMS, relapsing-remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis ; SLE, systemic lupus erythematosus ; UC, ulcerative colitis.

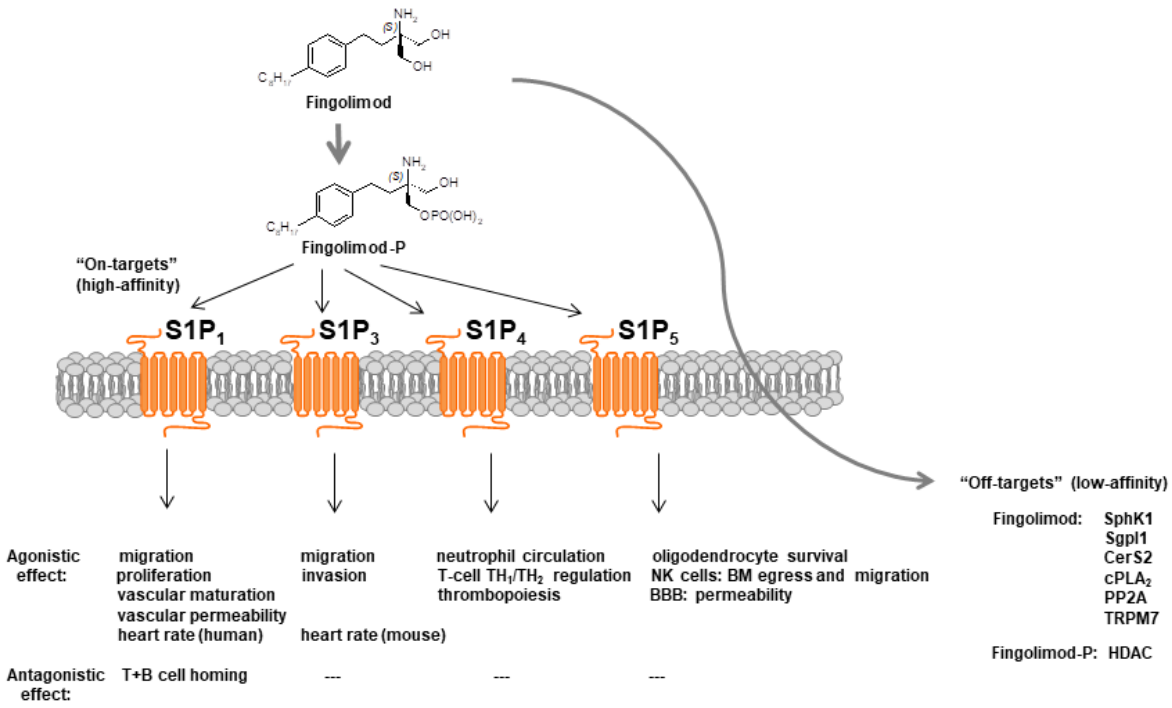

Fig.1

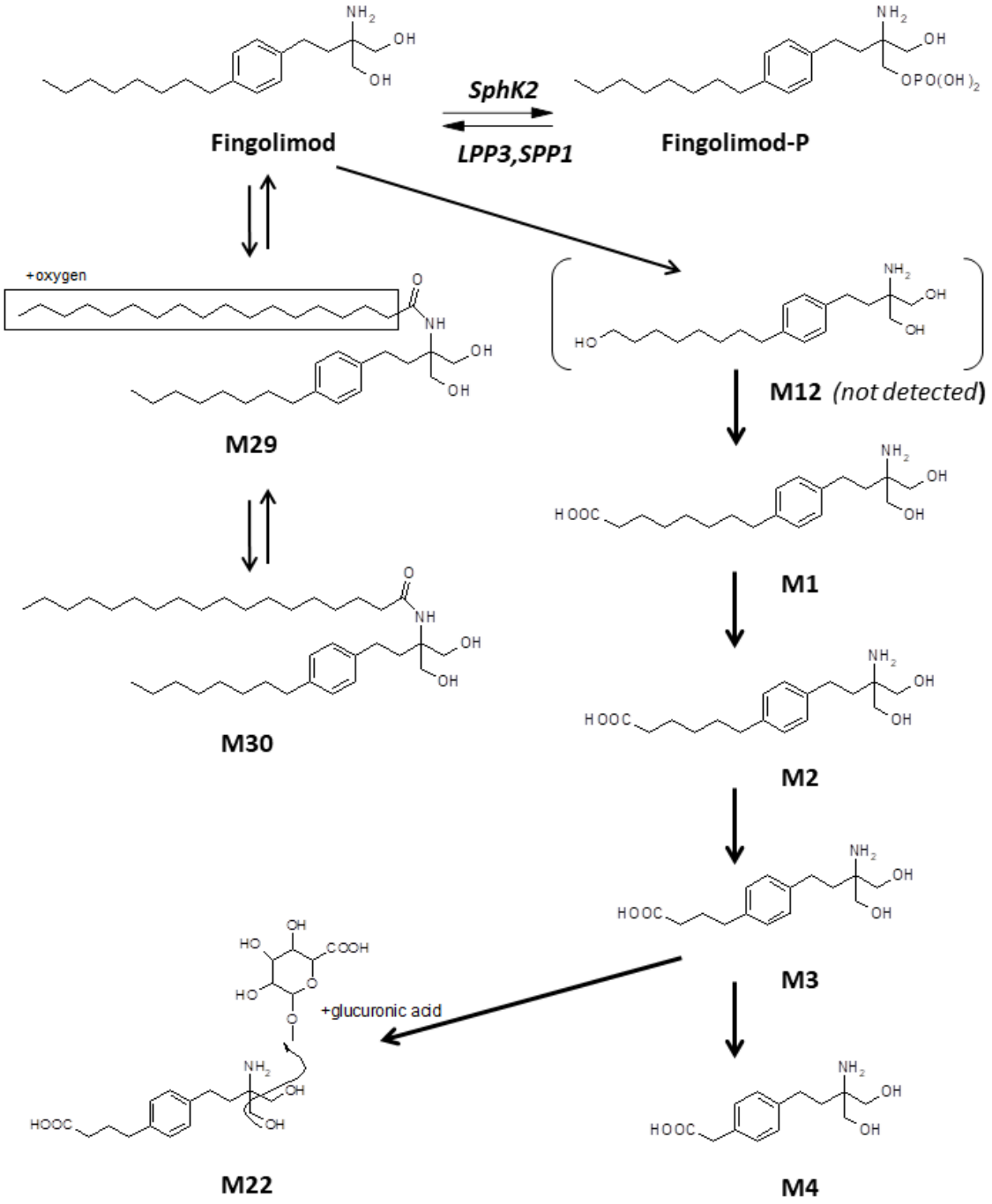

Fig. 2

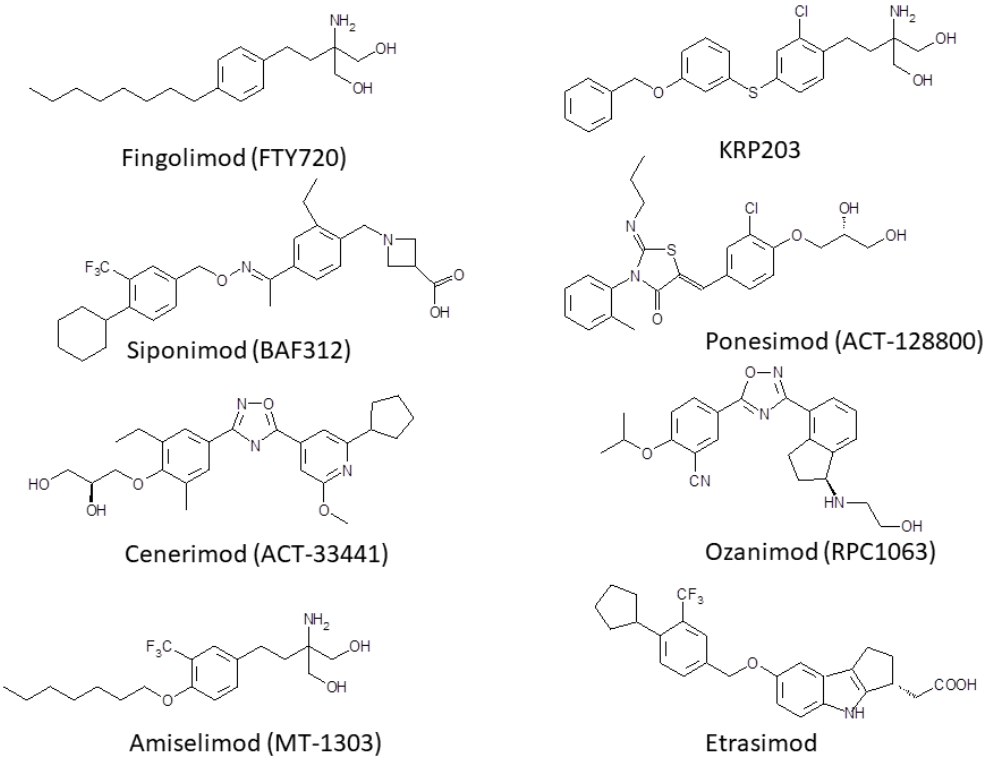

Fig. 3