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Targeting the sphingosine-1-phosphate axis in cancer, inflammation and beyond

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Abstract

The bioactive lipid sphingosine-1-phosphate (S1P) is involved in multiple cellular signalling systems and has a pivotal role in the control of immune cell trafficking. As such, S1P has been implicated in disorders such as cancer and inflammatory diseases. This Review discusses the ways in which S1P might be therapeutically targeted — for example, via the development of chemical inhibitors that target the generation, transport and degradation of S1P and via the development of specific S1P receptor agonists. We also highlight recent conflicting results observed in preclinical studies targeting S1P and discuss ongoing clinical trials in this field.

Since the discovery of the sphingolipid metabolite sphingosine-1-phosphate (S1P) as a bioactive signalling molecule more than 20 years ago[1], a plethora of its functions that are important for health and disease have been identified. The numerous biological functions of S1P include regulation of cellular proliferation, survival, migration, invasion, differentiation and cellular architecture, as well as the control of immune cell trafficking, angiogenesis and vascular integrity[2–6]. Therefore, it is not surprising that S1P affects the immune system, central nervous system and cardiovascular system and has been implicated in a broad range of diseases, including atherosclerosis, respiratory distress, diabetes and, most importantly, cancer[7] and inflammatory disorders[8]. The control of immune cell trafficking is one of the hallmarks of the involvement of S1P in these diseases[9].

S1P is formed intracellularly by the phosphorylation of sphingosine (which is derived from the deacylation of ceramide), a process that is catalysed by two sphingosine kinases: SPHK1 and SPHK2. S1P is then exported out of cells where it can act on five specific G protein-coupled receptors (S1P receptor 1 (S1PR1) to S1PR5) and can also act on some direct intracellular targets before being broken down by S1P lyase. Each of these steps that makes up the so-called S1P axis could be therapeutically targeted (BOX 1). Given the large number of roles of S1P, it is crucial that S1PRs or tissue-specific S1P production or degradation are specifically targeted to ensure specificity and reduce side effects.

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Box 1

The sphingosine-1-phosphate axis

The sphingosine-1-phosphate (S1P) axis refers to the signalling molecule S1P, its receptors and intracellular targets, as well as the proteins that synthesize, transport and degrade S1P. Many stimuli have been shown to activate S1P synthesis inside cells, which can then either act on intracellular targets or be secreted to act on cell surface receptors. The latter process is termed 'inside-out' signalling and occurs when S1P acts in an autocrine and/or paracrine fashion. An S1P gradient also exists, with high S1P levels in the circulation and low S1P levels in tissues. This gradient is maintained by a balance between the synthesis of S1P — which probably occurs in red blood cells, platelets and endothelial cells — and the degradation of S1P in tissues. The S1P gradient promotes the trafficking of haematopoietic cells from lymphoid tissues into the blood and is dependent on the expression of S1P receptors.

Although it has been suggested since the mid-1990s that compounds targeting the S1P axis would be of therapeutic benefit[10], several S1P modulators have only recently reached the clinic and demonstrated the utility of targeting the S1P axis. Indeed, fingolimod (also known as FTY720), which acts as a functional antagonist of S1PR1, is an oral therapeutic that is approved for the treatment of multiple sclerosis[11]. In this Review we discuss the development of chemical inhibitors targeting S1P generation, transport and degradation, and highlight the development of specific agonists and antagonists that target cell surface S1PRs. We focus on inflammatory disorders and cancer in which there is the strongest evidence for the importance of the S1P axis, and also describe ongoing clinical trials.

Cell surface receptors and intracellular targets

Most of the functions of S1P have been attributed to its activation of the cell surface receptors S1PR1 to S1PR5. These receptors are coupled to several — often overlapping — heterotrimeric G proteins, which accounts for both the diversity and, at times, the opposing effects of S1P on cells[6]. Although much of the research to date has focused on S1P signalling through S1PRs, for many years there have been observations suggesting the existence of direct intracellular targets.

More recently, several proteins have been shown to directly bind to S1P, demonstrating important roles for S1P as a localized second messenger within cells; these proteins include TNF receptor-associated factor 2 (TRAF2; an E3 ubiquitin ligase that is a key component of the nuclear factor-κB (NF-κB) pathway)[12] as well as the histone deacetylases HDAC1 and HDAC2 (REF. 13), which regulate gene expression. The activity of other proteins, including the β-amyloid precursor protein cleaving enzyme 1 (BACE1), which has been implicated in Alzheimer's disease[14], has been shown to be modulated *in vitro* by S1P. Therefore, the potential clinical implications of targeting the S1P axis and its receptors have attracted much attention.

S1P synthesis, degradation and export

Cellular levels of S1P are controlled by its synthesis and degradation. S1P is irreversibly degraded by S1P lyase, an enzyme that is localized in the endoplasmic reticulum and cleaves the sphingoid base into ethanolamine phosphate and hexadecenal. S1P can also be dephosphorylated by two phosphatases localized in the endoplasmic reticulum: S1P phosphatase 1 (SGPP1) and SGPP2, which are members of the lipid phosphate phosphohydrolase (LPP) family. It is possible that other phosphatases are also able to

dephosphorylate S1P. The resultant sphingosine can be reused for the synthesis of ceramide and complex sphingolipids (FIG. 1).

Signalling of S1P through its cell surface S1PRs is further controlled through localization: S1P formed inside the cell must be secreted or flopped out of the cytoplasm to bind to and activate these receptors in paracrine or autocrine manners. Although it has been shown that several ABC transporters, including ABCA1, ABCC1 and ABCG2 (REF. 15), transport S1P, the identity of the transporter in red blood cells or platelets remains unclear. Interestingly, recent studies have demonstrated that the SPNS2 protein, which belongs to the large major facilitator superfamily of transporters, regulates S1P release from endothelial and lymphendothelial cells, and controls S1P levels in plasma and lymph[16–20]. Because lymphocyte egress is suppressed in *Spns2*-deficient mice[16–20], targeting SPNS2 could be a new therapeutic avenue for autoimmune diseases.

Experimental evidence suggests that the S1P axis is controlled by synthesis, secretion and degradation. The activation of SPHKs and subsequent S1P-dependent activation of S1PRs is required for the full effects of many signalling molecules such as growth factors and cytokines[7,8]; this is referred to as ‘inside-out’ signalling (BOX 1). In addition, the S1P gradient between the blood and lymphoid organs is required for S1PR1-mediated egress of lymphocytes, and either disruption of this gradient or S1PR1 inhibition (using fingolimod) induces lymphopenia and immunosuppression in mice[9]. Moreover, recent studies have demonstrated that the secretion of S1P by SPNS2 expressed on endothelial cells regulates T and B lymphocyte egress from their respective primary lymphoid organs[17–20]. It has also been suggested that LPP3 promotes efficient export of mature T cells from the thymus into the circulation by destroying thymic S1P[21].

Together, these studies — which were carried out in mice — demonstrate that the generation, destruction and secretion of S1P is tightly regulated and that the S1P gradient is crucial for lymphocyte trafficking. Therefore, as we discuss below, the S1P axis could be targeted at the level of synthesis, secretion and degradation.

Targeting S1P synthesis and degradation

Studies from knockout mice have provided valuable information about the effects of disrupting the activity of enzymes involved in the synthesis of S1P. Mice in which either SPHK1 or SPHK2 is knocked out (*Sphk1*- or *Sphk2*-knockout mice) are viable and fertile with no obvious phenotypic changes, but circulating levels of S1P are decreased in *Sphk1*-knockout mice and elevated in *Sphk2*-knockout mice, probably owing to compensatory upregulation of SPHK1 (REF. 22). Double knockout mice (in which both SPHK1 and SPHK2 are knocked out) are embryonic lethal, which indicates that the ability to synthesize S1P is essential for development[23]. However, studies using isoform-specific silencing RNAs and knockout mice have indicated that SPHK1 and SPHK2 do have some distinct and non-redundant functions involved in pathophysiology. This has spurred the search for more effective isoform-specific inhibitors of SPHK1 and SPHK2.

SPHK1

Several studies, involving overexpression, small interfering RNA (siRNA) knockdown and inhibitors (TABLE 1) in cell culture and animal tumour models, have indicated that increased SPHK1 activity promotes cell growth and inhibits apoptosis. The studies have also shown that increased SPHK1 activity regulates several pathological processes such as inflammation and cancer, and that upregulation of SPHK1 correlates with poor cancer prognosis[3,4,8]. These results have substantiated the sphingolipid rheostat concept, in which SPHK1 produces S1P that promotes growth and inhibits apoptosis while decreasing levels

of the precursors sphingosine and ceramide that inhibit growth and promote apoptosis[10]. Consequently, several studies have investigated the possibility of tipping the balance from S1P production towards sphingosine and ceramide production in order to promote apoptosis and inhibit growth (reviewed in REF. 24).

SPHK1 and S1P in cancer and inflammation

More recent studies have indicated that the actions of SPHK1 and S1P are complex, especially with regard to the involvement of SPHK1 and S1P in inflammation and cancer. Upregulation of SPHK1 increases the production of S1P, and this molecule links chronic intestinal inflammation to colitis-associated cancer through the stimulation of S1PR1, which leads to the activation of the master transcription factors NF-κB and STAT3 (signal transducer and activator of transcription 3) in a malicious feed-forward amplification loop[22]. This S1PR1–STAT3 signalling axis has been previously found in breast cancer[25] and lymphomas[26] and is also crucial for myeloid cell colonization at future metastatic sites in prostate cancer and melanoma[27]. Of particular relevance, RNA-based downregulation of SPHK1 or S1PR1 has been shown to block the persistent activation of STAT3 and reduce cancer progression and levels of inflammatory mediators in animal models[22,27].

These studies raise several questions. For example, what cell types produce S1P — is it the tumour cells, cells of the tumour microenvironment or tumour-associated immune cells? What is the role of systemic S1P in cancer and inflammation? Earlier studies using mouse models of cancer[28,29] and patient samples[30–32] suggested that the tumours themselves, in which SPHK1 is upregulated, may be a key source of S1P. However, a recent study has suggested that local tumour growth is regulated by both S1P from the tumour and systemic S1P, whereas lung colonization and metastasis is selectively controlled via systemic S1P and downregulation of breast cancer metastasis suppressor 1 (BRMS1; a master suppressor of metastasis) through S1PR2 signalling[29].

These findings have implications for targeting the S1P axis. Indeed, neutralization of systemic S1P with a specific monoclonal antibody (known as sphingomab) suppressed lung metastasis, which suggests a new therapeutic strategy to prevent cancer metastasis[137]. Sonepcizumab, the humanized version of sphingomab, has recently completed Phase I clinical trials in cancer (TABLE 2) and advanced into Phase II safety and efficacy trials. Thus, targeting S1P production in the tumour and the host would help reduce both growth and metastasis, respectively.

Encouraging results such as these have driven the pursuit of effective SPHK1 inhibitors for cancer chemotherapy. Although several SPHK1 inhibitors have shown promise in preclinical studies[7,33], two new selective SPHK1 inhibitors had no effect on cancer cell growth, which seems to contradict the current dogma. An amidine-based inhibitor that had 15-fold higher selectivity for SPHK1 over SPHK2 and a K_i (inhibition constant) value of 100 nM[34] rapidly reduced S1P levels in cells but did not potently inhibit cell growth. Its administration to mice resulted in a rapid decrease in S1P levels in the blood, which indicates that there is a rapid turnover of circulating S1P levels[34]; however, the effects of this amidine-based inhibitor on tumour growth *in vivo* have not yet been reported. An even more potent SPHK1 inhibitor, PF-543, which has a nanomolar K_i value and 100-fold selectivity for SPHK1 over SPHK2, has been identified[35]. PF-543 also rapidly reduced S1P levels in cells, but it too had no effect on cell growth at the same or higher concentrations.

The lack of effect of PF-543 on cell growth might be due to its inability to increase levels of pro-apoptotic ceramide, as was typically observed when SPHK1 was inhibited or downregulated[7,33]. It is also possible that the potency of SPHK1 inhibitors may depend in part on their ability to induce the proteasomal degradation of SPHK1, as has been

demonstrated for some of these inhibitors such as Ski[36] (2-(*p*-hydroxyanilino)-4-(*p*-chlorophenyl) thiazole; also known as SKI-II). Alternatively, S1P that is produced and secreted as a result of SPHK1 upregulation may promote cancer progression by tumour-induced angiogenesis and lymphangiogenesis[28], and it could be crucial for myeloid cell colonization at future metastatic sites[27] *in vivo* without affecting tumour growth. Although PF-543 appears to be a useful tool for inhibiting SPHK1 *in vitro*, its effects were not investigated *in vivo*.

The recently solved X-ray crystal structure of SPHK1 revealed that the active site is located in a cleft between two domains with a hydrophobic lipid-binding pocket buried in the carboxy-terminal domain[37]. It remains to be determined how the substrate sphingosine — which has a hydrocarbon tail that may be associated with membranes — can tunnel into this site in a tail-to-head manner. Elucidation of the structural basis of SPHK1 substrate recognition and catalysis will lead to a better understanding of how this important enzyme can be regulated. In addition, it might clarify the seemingly contradictory findings observed with different SPHK1 inhibitors and could accelerate the development of high-potency inhibitors for therapeutic uses. Owing to the many important roles of S1P in physiological processes, further studies are also needed to determine whether there are any adverse effects associated with the long-term inhibition of SPHK1 and decrease in S1P levels.

SPHK2

Knowledge of the pathophysiological roles of SPHK2 is not as advanced as that of SPHK1, perhaps owing to the fewer numbers of studies carried out on SPHK2, its localization in several subcellular compartments and its ambiguous nature in promoting pathology in some disorders and preventing it in others[5]. Although SPHK1 is generally localized in the cytosol and is translocated to the plasma membrane upon activation, SPHK2 is expressed in several organelles, including the nucleus in many cell types. S1P produced by the actions of SPHK2 in the nucleus binds to and inhibits HDAC1 and HDAC2, which suggests that S1P is an endogenous HDAC inhibitor that contributes to epigenetic regulation of gene expression[13].

Although it has been reported that knockdown of SPHK2 induces apoptosis[7], somewhat surprisingly it was recently suggested that mitochondrial SPHK2 is pro-apoptotic; it produces S1P that is degraded by S1P lyase to hexadecenal, which then binds to the apoptosis regulator BAX, promoting its oligomerization and the release of cytochrome *c*[38]. However, contrary to the view that SPHK2 is pro-apoptotic, studies of *Sphk2*-null mice have revealed that it is required for ischaemic pre- and post-conditioning as well as cardioprotection[39,40]. Moreover, S1P produced by mitochondrial SPHK2 binds to the scaffold protein prohibitin 2 (REF. 41) — a protein that is important for respiration and the assembly of complex IV. Studies involving conditional deletions of SPHK2 might clarify the functions of this protein and help determine whether specific SPHK2 inhibitors might be clinically useful.

Targeting SPHK2

An SPHK2 inhibitor, ABC294640, has been described that inhibits the growth of cancer cells in culture and reduces S1P levels and the growth of mammary tumours in nude mice[42]. However, care should be taken in interpreting results obtained from this compound, as more recent research has shown that ABC294640 also binds to the oestrogen receptor and has anti-oestrogenic effects[43]. A newer SPHK2 inhibitor, SLR080811, decreased S1P levels in fibroblasts[44], but administration of the compound to mice caused an unexpected rapid increase in blood S1P levels. Although this elevation in S1P levels could be due to the off-target effects of SLR080811 on S1P transporters, this observation resembles the increase in

circulating basal levels of S1P seen in *Sphk2*-null mice[22], which indicates that deletion as well as inhibition of SPHK2 leads to compensatory increases in the activity of SPHK1.

This increase in SPHK1 levels caused by the downregulation or deletion of SPHK2 might explain why the data on the roles of SPHK2 in inflammation are also conflicting. There was greater disease severity in *Sphk2*-knockout mice with colitis[22] (induced by dextran sulphate sodium); disease severity was also higher in severe combined immunodeficient (SCID) mice adoptively transferred with *Sphk2*-null T cells[45] than with wild-type T cells. Conversely, inhibition of SPHK2 with ABC294640 reduced the severity of colitis[46] and colitis-associated cancer[47]. Several studies have also examined the role of SPHK2 in models of inflammatory arthritis. Although genetic ablation of SPHK2 had no effect on arthritis progression, administration of ABC294640 increased the severity of arthritis in one study[48] and protected against the development of arthritis in another study[49]. Such observations remain puzzling. Together, these results suggest that ABC29460 may not provide a valid approach for investigating the role of SPHK2 *in vivo* and so the development of new specific inhibitors is eagerly awaited.

HDAC inhibitors are used in psychiatry and various brain disorders, and are being investigated as potential treatments for several other diseases, particularly cancer[50–52]. Because SPHK2 produces nuclear S1P that inhibits HDACs[13], SPHK2 inhibitors could have opposite effects to HDAC inhibitors, which might not be beneficial. The development of sphingosine analogues that are phosphorylated *in vivo* by SPHK2 to produce S1P mimetics is an approach that might inhibit HDACs with greater specificity than current clinically used pan-HDAC inhibitors. Overall, however, targeting SPHK2 should be approached with caution.

S1P lyase

S1P lyase catalyses the irreversible cleavage of S1P into phosphoethanolamine and hexadecenal, which are precursors for phospholipid synthesis (FIG. 1). A recent report demonstrated that aldehyde dehydrogenase family 3 member A2 (*ALDH3A2*), the causative mammalian gene for Sjögren–Larsson syndrome, is responsible for the conversion of hexadecenal to hexadecenoic acid, which suggests that the accumulation of hexadecenal may contribute to neurological and cognitive defects, as well as ichthyosis, in the pathogenesis of Sjögren–Larsson syndrome[53]. It remains to be determined whether S1P lyase inhibition could reduce symptoms in affected patients.

S1P lyase regulates the cellular pool of S1P that is available for signalling in S1P-dependent physiological and pathological processes[54]. As the terminal step in the degradation of all sphingolipids, it not only controls levels of bioactive sphingolipid metabolites but is also the link between sphingolipid and phospholipid metabolism. S1P lyase deficiency (by gene ablation) or RNA-based inhibition is associated with elevated nuclear S1P levels and reduced HDAC activity[55]. In addition to enhanced histone acetylation, it is possible that downregulation of HDAC isoenzymes may contribute to the dysregulation of calcium homoeostasis that is observed in S1P lyase-null cells[55].

S1P lyase in lymphocyte function and immunosuppression

Seminal studies by Schwab and Cyster[56] showed that lymphocyte egress from lymphoid organs is mediated by S1P gradients — that are maintained at least in part by S1P lyase — between the circulation and tissues. They found that S1P levels in lymphoid tissues are low and are dramatically increased following the administration of THI (2-acetyl-4-tetrahydroxybutylimidazole; a food colourant), which is a compound that inhibits the degradation of S1P by S1P lyase. Moreover, increased cellular levels of S1P and disruption

of the S1P gradient induced lymphopenia probably through the downregulation of S1PR1 expression on lymphocytes[56]. This study indicates that S1P lyase may represent a novel immunosuppressant drug target.

Indeed, derivatives of THI have been developed that prevent the development and reduce the severity of rheumatoid arthritis in mice[57]. A Phase II clinical trial was recently completed for one of these compounds, LX3305 (TABLE 2), examining its efficacy in the treatment of rheumatoid arthritis. However, the mechanism by which THI or any of its derivatives inhibit S1P lyase *in vivo* merits additional studies as none of these compounds has been shown to directly inhibit lyase activity *in vitro*.

The crystal structure of the yeast S1P lyase highlighted residues that are involved in activity and substrate binding[58], and so this knowledge might aid the development of inhibitors that specifically target the S1P lyase active site rather than its pyridoxal cofactor (which could induce side effects by inhibiting other pyridoxal-dependent enzymes).

S1P lyase and muscle function

Suppression of S1P lyase may also be an effective way to promote muscle regeneration. S1P is a trophic factor for muscle regeneration and can activate quiescent muscle stem cells known as satellite cells[59], which maintain muscle homeostasis and are needed for muscle repair. Intriguingly, S1P lyase is upregulated in injured skeletal muscle and in muscles of *mdx* mice[60]. THI treatment elevated muscle S1P levels, resulting in enhanced recruitment and proliferation of satellite cells as a result of S1PR2 and STAT3 activation, which led to suppression of cell cycle inhibitors and skeletal muscle regeneration[60].

Studies in *Drosophila melanogaster* have shown that genetic elevation of S1P (caused by the deletion of S1P lyase) suppresses dystrophic muscle phenotypes[61]. Because there are no known S1PR homologues in *D. melanogaster*, it was suggested that localized intracellular S1P elevation directly promotes the suppression of muscle wasting in fruitflies[61]. Thus, it is possible that inhibitors of S1P lyase may provide a new therapeutic strategy for myopathies. However, further work needs to be carried out to understand the role of S1P in mammalian muscle development and regeneration.

Targeting S1PRs

First- and second-generation agonists and antagonists that are specific for one or a subset of S1PRs have been developed (TABLE 1) and are discussed below. Fingolimod has been clinically approved for the treatment of relapsing and remitting multiple sclerosis in the United States and Europe[11], and several other compounds are in clinical trials (TABLE 2).

Efforts to develop highly specific and efficacious drugs will be greatly enhanced by the recent report of the crystal structure of S1PR1 complexed with an antagonist[62]. Intriguingly, this structure indicates that, at least for this member of the S1PR family, the ligand binding pocket is covered by an amino-terminal helix. This suggests that to access the binding pocket S1P must slide laterally within the plane of the bilayer between a pair of transmembrane helixes. Ultimately, this structure will both assist with the development of S1PR1 targeted compounds that have greater specificity and provide a basis for determining the structure of other S1PRs.

S1PR1 agonism and antagonism

Fingolimod is a sphingosine analogue that is phosphorylated primarily by SPHK2 to form phosphorylated fingolimod, which is an agonist at all of the S1PRs except for S1PR2 (REFS 63,64). However, persistent activation of S1PR1 by phosphorylated fingolimod causes

S1PR1 internalization and degradation, and so fingolimod acts as a functional antagonist at this receptor[11,65]. Drug-induced downregulation of the expression of cell surface S1PRs on lymphocytes prevents their egress from lymphoid organs and induces lymphopenia and immunosuppression[11,66]; these effects are advantageous for the treatment of autoimmune diseases such as multiple sclerosis.

Although phosphorylated fingolimod targets multiple S1PRs, several next-generation agonists and antagonists have been developed and are in clinical trials that more specifically target S1PR1 (TABLE 2). The use of fingolimod as a lead compound and its optimization for potency at S1PR1 (REF. 67) led to the development of siponimod (also known as BAF312)[68], which is now in Phase III trials for multiple sclerosis. Other examples of S1PR1-directed drugs include ONO-4641 (REF. 69), which is a novel selective agonist for both S1PR1 and S1PR5, and ponesimod (ACT-128800)[70], which is a potent selective S1PR1 modulator; both of these drugs have been effective in rodent models and are now in Phase II clinical trials for multiple sclerosis and moderate-to-severe chronic plaque psoriasis, respectively (TABLE 2). Other modulators of S1PRs are being investigated in several preclinical disease models (TABLE 1), including viral responses, cancer treatments and modulation of angiogenesis.

The issue of receptor specificity must be borne in mind, as transient bradycardia is the major adverse effect of fingolimod in humans and is also observed with siponimod and other S1PR1 modulators. This suggests that S1PR1 modulators contribute to this effect (FIG. 2). Therefore, S1PR1 modulators could potentially have the same adverse effects in patients as have been reported for fingolimod, including first-dose bradycardia, macular oedema and infection[71,72]. However, S1PR1 modulators might still be an effective treatment option for patients with serious disease, provided they are selected and monitored appropriately[71]. A major remaining challenge is to gain a deeper knowledge of any beneficial as well as adverse side effects of targeting S1PRs and to understand how potential therapeutics modulate the functions and mechanisms of action of S1PRs.

The understanding of the mechanism of action of S1PR1 modulators focuses on preventing S1PR1 function on lymphocytes, either by functional antagonism (for example, phosphorylated fingolimod)[11,66] or with a competitive antagonist (for example, NIBR-0213), in order to prevent lymphocytes from recognizing S1P egress signals[73]. However, it has been suggested that some of the advantageous effects of the compounds that modulate S1PR1-mediated functions are independent of their effects on lymphocytes[74]. Using the experimental autoimmune encephalomyelitis (EAE) mouse model of multiple sclerosis, it was shown that loss of S1PR1 in astrocytes alone reduced disease severity, demyelination, axonal loss and astrogliosis, and made the mice non-responsive to fingolimod treatment[74]. So, in addition to inhibiting the migration of specific lymphocyte subsets into the central nervous system, the therapeutic activity of fingolimod could be due to its direct effects on neural cells, particularly astrocytes[11].

It is also assumed that some of the reported adverse effects of S1PR1 modulators, such as macular oedema, might be due to vascular leakage as S1PR1 is important for the maintenance of vascular integrity. This effect may limit the therapeutic window of some antagonists more than that of agonists[75]. In this regard, the selective S1PR1 agonist CYM-5442 modulates S1PR1 on the pulmonary endothelium, inhibiting the cytokine storm and enhancing survival — following the infection of mice with human pathogenic influenza virus — independently of lymphocyte S1PR1 activation[76].

There is no doubt that an increased understanding of S1PR1 signalling and of the cell types that mediate the effects of S1PR1 signalling will advance the development of additional

S1P-based therapeutics for the treatment of multiple sclerosis and potentially other diseases. However, the potential for S1P analogues to have multiple targets remains a challenge for pharmacological intervention.

S1PR-independent functions of fingolimod

It has been known for several years that the prodrug fingolimod itself can induce apoptosis of several types of cancer cells independently of its phosphorylation or its S1PR-dependent effects[67,77–79]. More recently, fingolimod was shown to activate protein phosphatase 2A (PP2A), a tumour suppressor that dephosphorylates many oncogenic signalling proteins including AKT, leading to mitochondria-dependent apoptosis. This led to the suggestion that fingolimod might be an alternative treatment for blast crisis in chronic myelogenous leukaemia and Philadelphia chromosome-positive acute lymphocytic leukaemia — two *BCR–ABL*-driven leukaemias against which ABL kinase inhibitors fail to induce a long-term response[67,78,79].

Recently, it was found that fingolimod, but not phosphorylated fingolimod, directly binds to the PP2A inhibitor (I2PP2A; also known as SET) — a protein that inhibits PP2A function and thus results in PP2A reactivation — and, surprisingly, causes caspase-independent death of lung cancer cells through RIPK1 (receptor-interacting serine/threonine protein kinase 1)-dependent necroptosis. It was known that RIPK1 activates RIPK3 and, subsequently, leads to increased phosphorylation and activation of mixed lineage kinase domain-like protein (MLKL). MLKL, in turn, activates mitochondrial phosphoglycerate mutase family member 5 (PGAM5), which leads to dynamin 1-like protein (DNM1L; also known as DRP1)-dependent mitochondrial fission and subsequent cell death[80,81]. However, PP2A-dependent necroptosis occurs independently of PGAM5, which indicates that fingolimod might induce a novel type of necrotic cell death programme. Further studies to elucidate this pathway may be of great importance as fingolimod administration has been shown to suppress tumour progression *in vivo*[36].

S1PR1 and STAT3

Several studies have suggested that S1PR1 has a crucial role in the persistent activation of STAT3 (REFS 22,25–27), a transcription factor that is constitutively active in — and associated with — multiple types of cancers. Historically, it has been difficult to therapeutically target STAT3 (REFS 82–84). STAT3 induces the transcription of S1PR1, which then reciprocally activates STAT3, resulting in its persistent activation and interleukin-6 (IL-6) production[25] (FIG. 3). This unique S1PR1-dependent axis may be an attractive target for intervention, as several reports have indicated that disruption of S1PR1 signalling abrogates this cycle of STAT3 amplification[22,82–84].

Targeting of S1PR1 using short hairpin RNA (shRNA) or via the administration of fingolimod reduces S1PR1 expression and downregulates STAT3 activity in the activated B cell-like subtype of diffuse large B cell lymphoma, which reduces the growth of lymphoma tumour cells *in vitro* and *in vivo*[26]. Moreover, SPHK1 and S1PR1 were upregulated in chronic intestinal inflammation and associated cancers[26]. Indeed, S1P was essential for the production of the multifunctional NF-κB-regulated cytokine IL-6, the persistent activation of STAT3 and the consequent upregulation of S1PR1. In this case, treatment with fingolimod decreased not only S1PR1 expression but also SPHK1 levels and, by doing so, it eliminated the NF-κB–IL-6–STAT3 amplification cascade and the development of colitis-associated cancer in mice (FIG. 3). Therefore, it was suggested that the SPHK1–S1P–S1PR1 axis forms the nexus between NF-κB and STAT3, which connects chronic inflammation with colitis-associated cancer, and that fingolimod may be useful in treating colon cancer in patients with colitis[22].

A more recent study introduced the notion that the S1PR1–STAT3 signalling axis is involved in tumour metastasis; namely, it suggested that levels of signalling molecules involved in this axis are elevated in distant organs before the arrival of tumour cells, which empowers myeloid cells to invade, proliferate and resist apoptosis at pre-metastatic sites[27]. These myeloid cells then influence other cells, such as fibroblasts, to produce factors that facilitate the formation of pre-metastatic niches for tumour cell metastasis. An important aspect of this notion is the therapeutic potential of targeting the S1PR1–STAT3 signalling axis to eliminate and/or reduce preformed pre-metastatic niches, thereby preventing tumour metastasis[27].

Constitutive activation of STAT3 through S1PR1 therefore allows for both a survival advantage and an increased metastatic potential. Targeting S1PR1 with an antagonist can thus potentially decrease two clinically relevant functions of cancer development at the same time; that is, it can inhibit tumour cell proliferation and survival while simultaneously decreasing the ability of the cancer to spread. Further studies using other specific S1PR1 modulators are needed to confirm these possibilities.

S1PR1 and angiogenesis

Angiogenesis has multiple physiological roles in development and disease. The importance of S1PR1 in angiogenesis and vascular maturation was first realized with the observation that S1PR1-knockout animals die *in utero* owing to severe haemorrhaging and incomplete vasculogenesis[85]. It has recently been shown that S1PR1 is involved in the termination of sprouting angiogenesis[86] (FIG. 4) and that endothelial S1PR1 stabilizes the primary vascular network during development and homeostasis[87,88]. Severe aberrations in vessel size and excessive sprouting were observed in the limbs of mice in which S1PR1 was deleted on endothelial cells, which indicates that the effect of S1PR1 on sprouting is endothelial cell-autonomous. Similar effects were observed with S1PR1 knockdown in zebrafish, which suggests that this is an evolutionarily conserved mechanism[86].

S1PR1 activation counteracts vascular endothelial growth factor (VEGF) function and positively regulates endothelial cell–cell adhesion. These results establish a functional antagonism between S1PR1 and VEGF receptor 2 (VEGFR2) at the levels of sprouting angiogenesis and junctional stability[87]. Moreover, it was shown that vascular endothelial cadherin is a crucial downstream mediator of S1PR1 function. Together, these studies suggest that S1P carried by blood flow closes a negative feedback loop that inhibits sprouting angiogenesis once the vascular bed is established and functional.

In addition to responding to S1P, S1PR1 responds to laminar shear stress to transduce blood flow-mediated signalling involving the extracellular signal-regulated kinase (ERK)–mitogen-activated protein kinase (MAPK) pathway and the AKT–endothelial nitric oxide (eNOS) pathway in endothelial cells *in vitro* and *in vivo*. Moreover, activation of these pathways was suppressed by fingolimod[88]. Interestingly, the same study showed that S1PR1 can be activated in a ligand-independent manner by laminar shear stress, which suggests that S1PR1 can respond not only to blood-borne S1P but also to biomechanical signals independently of its ligand. This raises several intriguing questions. If S1PR1 is always mechanosensitive, what is the function of S1P binding? And what is the relationship between mechanotransduction and sprouting?

The regulation of vascular hypersprouting by S1PR1 may have implications for the clinical use of S1PR1 modulators. For example, it is possible that exaggerated VEGF signalling in pathological conditions — such as tumour angiogenesis, age-related macular degeneration and rheumatoid arthritis — could be reduced by agonists that activate S1PR1 signalling. However, it is still not clear whether S1PR1 functions similarly in pathology-driven

angiogenesis as it does during development. This is an important area that merits further study.

Targeting other S1PRs

In contrast to the large amount of information that is available on the functions of S1PR1, less is known about the biological and pathological roles of the other S1PRs, and so substantially less progress has been made in developing specific agonists and antagonists of these receptors. A few clues have surfaced that suggest that targeting other S1PRs might be beneficial in certain diseases. Below, we address the emerging pathophysiological roles of specific, individual S1PRs that still need receptor-specific pharmacological validation.

S1PR2 in osteoporosis

Bone is continuously remodelled throughout life by the balanced actions of osteoblasts that form bone and osteoclasts that resorb it. In conditions such as osteoporosis, osteolysis and rheumatoid arthritis, this balance is tipped in favour of osteoclasts. Osteoclasts are derived from precursor monocytes that dynamically migrate from the blood into bone and back into the blood. This migration is dependent on S1P-mediated ligation of specific S1PRs. S1PR1 promotes the migration of osteoclasts from the bone to the blood along an upward S1P gradient[89], whereas S1PR2 inhibits their migration, leading to increased numbers of osteoclasts on bone and subsequent bone resorption[90]. Accordingly, it was recently shown that the steroid hormone calcitriol — the active form of vitamin D, which is an established treatment for osteoporosis — acts in part by reducing the expression of S1PR2 on osteoclast precursor cells[91].

Effective antagonism of S1PR2 might be useful for the treatment of osteoporosis as inhibition of S1PR2 with the S1PR2 antagonist JTE-013 relieved osteoporosis in a mouse model by limiting the location of osteoclast precursor cells and reducing the number of mature osteoclasts that were attached to the bone surface[90]. However, although many studies have shown JTE-013 to be useful for targeting S1PR2, this compound also antagonizes S1PR4 (REF. 92), and other studies have suggested that it might also have off-target effects[93]. Therefore, the results obtained with JTE-013 should be validated by genetic deletion or downregulation of S1PR2.

S1PR3 in cancer and sepsis

Several reports have implicated S1PR3 in cancer. Early studies showed that estradiol stimulates SPHK1 in human breast cancer cells and that ligation of S1PR3 by released S1P transactivated the epidermal growth factor receptor in a matrix metalloproteinase-dependent manner[94]. Thus, these findings reveal that SPHK1 has a key role in the coupling of signals among three membrane-spanning events induced by estradiol, S1P and epidermal growth factor[94]. Moreover, increased expression of S1PR3 correlated with shorter disease-free survival times in patients with oestrogen receptor-positive breast cancer[95]. The notion that S1PR3 might be involved in cancer progression is supported by the observation that an S1PR3-specific inhibitory monoclonal antibody (7H9) decreased tumour growth in a xenograft model of breast cancer[96].

In addition to cancer, S1PR3 has been implicated in promoting sepsis. S1PR3 activation promotes lipopolysaccharide (LPS)-induced vascular leakage and lung oedema[97], as well as regulating the amplification of inflammation in sepsis syndrome[98]. Moreover, whereas S1PR1 was shown to be crucial for endothelial barrier enhancement, S1PR3 expression was involved in barrier disruption[97]. Recent studies have shown that increased S1PR3 expression is associated with the mortality of severely ill patients with sepsis or acute lung injury; these studies suggest that S1PR3 is a candidate biomarker and a target for future therapeutic

strategies against acute lung injury[99]. The potential of S1PR3 inhibition in sepsis treatment was substantially advanced when it was shown that the S1PR3-specific inhibitory monoclonal antibody 7H9 increased the survival of LPS-treated mice[96]. Further studies with a humanized version of this antibody are required to demonstrate possible efficacy in patients with sepsis or cancer.

S1PR4 and dendritic cell function

Although it is predominantly expressed on lymphocytic and haematopoietic cells, the role of S1PR4 in immune homeostasis is still poorly understood. S1PR4 is involved in the regulation of dendritic cell function and T_H17 cell differentiation[100], and has been shown to modify the course of several immune diseases in murine models[100]. Moreover, this receptor could be involved in neutropenia and inflammation[101]. Therefore, S1PR4 may be an interesting target for influencing the course of several autoimmune pathologies using newly developed selective S1PR4 antagonists[102].

S1PR5 and natural killer cells

S1PR5 is required for the trafficking of natural killer cells — which are involved in the clearance of infectious agents and antitumour surveillance — from the bone marrow and lymph nodes into tissue[103]. This indicates that S1PR5 agonists may be useful for promoting natural killer cell-dependent clearance of tumours, whereas S1PR5 antagonists may reduce transplant rejection. However, there is currently no evidence to support this notion. Although some selective and orally active S1PR5 agonists have been synthesized[104], their *in vivo* effects have not yet been reported.

Concluding remarks and future perspectives

Given the success of targeting S1PR1, we expect that a new generation of selective S1PR-targeted drugs will be developed as the roles of these receptors in health and disease become more apparent. However, some of the pathogenic roles of the S1P axis have been linked to multiple S1PRs, possibly owing to the coupling of different S1PRs to the same Gα proteins in particular cell types[105]. Thus, for further progress in this area it is necessary to bear in mind the need for molecular and pharmacological validation of the specific roles of individual S1PRs.

Based on the recently solved X-ray crystal structure of S1PR1, we expect that the structures of the other S1PRs will soon be solved. A better understanding of structure–function relationships within this subfamily will enable the rational design of more selective and potent modulators with useful pharmacokinetics. The development of these tools will provide a fundamental understanding of S1P signalling as well as new therapeutics for treating many human disorders.

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Glossary

Angiogenesis	The development of new blood vessels. Angiogenesis is required in development and tissue repair, as well as pathologically for tumour progression

Fingolimod	A sphingosine-1-phosphate receptor (S1PR) agonist. However, sustained activation of S1PR1 by fingolimod leads to degradation of S1PR1. Thus, fingolimod is often referred to as a 'functional antagonist' of S1PR1, particularly in its role in lymphocyte trafficking
Histone deacetylases	(HDACs). Proteins that remove acetyl groups from specific histone lysine residues, thus altering gene transcription
ABC transporters	(ATP-binding cassette transporters). A family of proteins that transport small molecules across the membrane, including drugs and lipids. Several of these proteins have been shown to transport sphingosine-1-phosphate
'Inside-out' signalling	A model whereby agonists such as growth factors promote the production of sphingosine-1-phosphate (S1P) within the cell. This S1P is then exported outside the cell to signal through cell surface S1P receptors in an autocrine and/or paracrine manner
Lymphopenia	An abnormally low level of lymphocytes in the blood
Sphingolipid rheostat concept	A concept that describes how the metabolic balance between sphingosine-1-phosphate (S1P) and ceramide regulates cell fate. S1P-mediated signals mostly regulate cell survival and proliferation, whereas ceramide-mediated signals regulate growth inhibition and apoptosis
Myeloid cell	A blood cell type that includes macrophages, monocytes, neutrophils, basophils, eosinophils, erythrocytes, megakaryocytes and dendritic cells but not lymphocytes
Lymphangiogenesis	The development of new lymph vessels. Lymphangiogenesis is required in development and for tissue repair, as well as for the metastasis of some tumours, such as breast cancer tumours
Sjögren–Larsson syndrome	An autosomal recessive form of ichthyosis (scaly, dry, thickened skin) that is characterized by spastic paraplegia and mild to moderate intellectual disability
Ichthyosis	A family of mostly genetic skin disorders characterized by dry, thickened, scaly skin, often with cracks
***mdx* mice**	A mouse model of Duchenne muscular dystrophy, which is a muscle-wasting disease that is caused by a mutation in the X-linked dystrophin gene, leading to loss of expression of the dystrophin protein
Plaque psoriasis	An autoimmune disorder of the skin in which the patient typically presents with scaly patches of skin with a red and/or white hue
Bradycardia	A decreased resting heart rate that results in in dizziness, weakness and fatigue
Macular oedema	The accumulation of fluid and protein in the macula (visual field), leading to swelling and loss of vision

Experimental autoimmune encephalomyelitis	(EAE). An animal model of inflammation-induced demyelinating disease, often used as a proxy for human multiple sclerosis
Cytokine storm	A potentially fatal immune reaction consisting of a positive feedback loop between highly elevated levels of many cytokines with immune cells
Sprouting angiogenesis	The process of developing new blood vessels in which angiogenic factors bind to receptors on endothelial cells of a blood vessel. These cells grow out and form sprouts connecting to other blood vessels
Laminar shear stress	The stress on tissues derived from the flow of a fluid through the vessel
Osteoporosis	A bone-thinning disease that is characterized by overactive bone resorption by osteoclasts, reduced bone formation by osteoblasts, or both
Sepsis syndrome	A life-threatening systemic response to severe infection that is characterized by vascular leakage and oedema, hypo- or hyperthermia, low blood pressure and reduced lung function
Dendritic cell	A cell type that has a central role in the adaptive immune response by presenting antigens to lymphocytes and causing their activation
T_H17 cell	T helper 17 cell; a subset of T helper cells that secrete pro-inflammatory cytokines.

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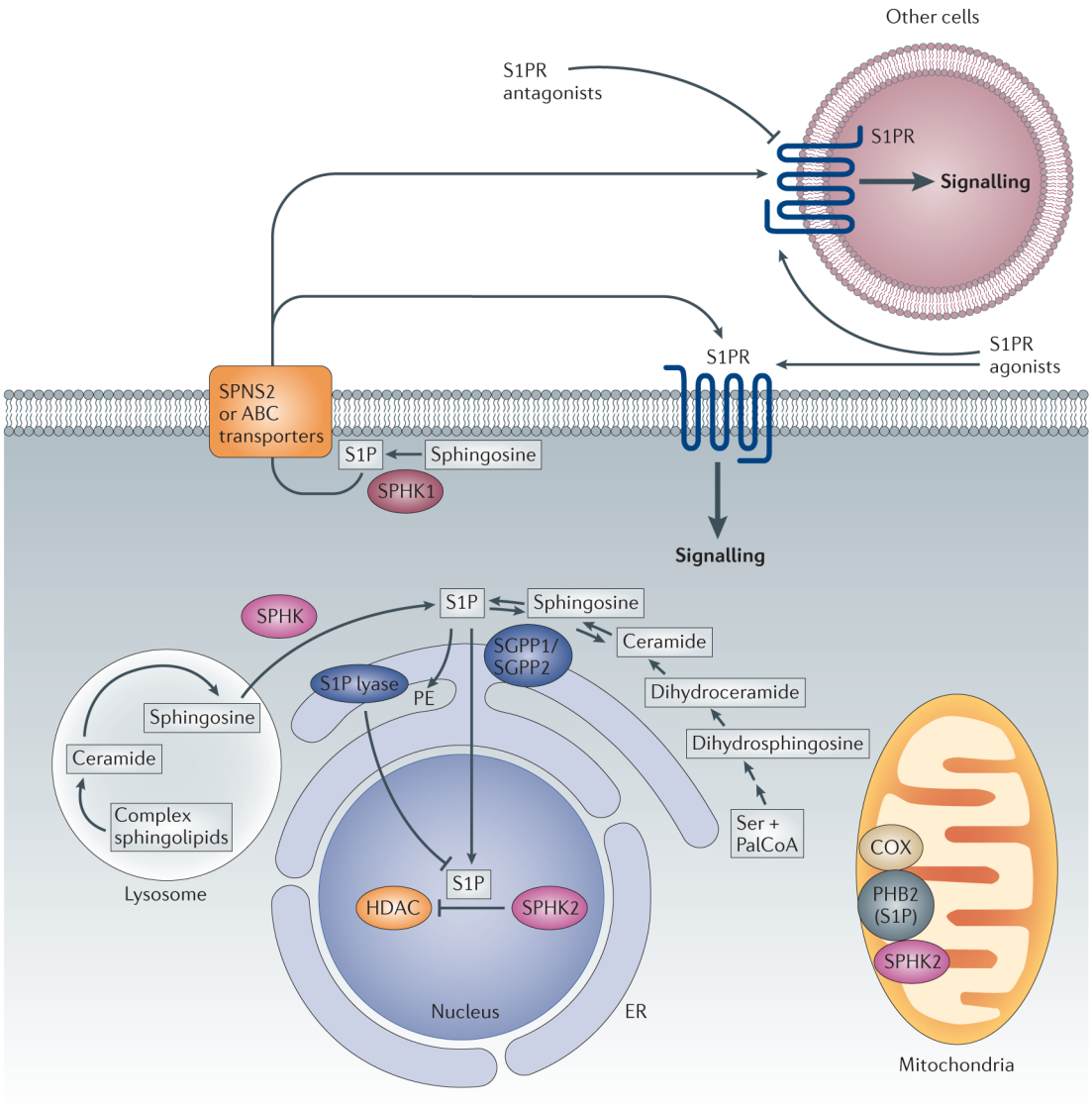

Figure 1. S1P biosynthesis, degradation, export and signalling

Sphingosine, the substrate of sphingosine kinases (SPHKs), is not generated *de novo* but through the degradation of complex sphingolipids and ceramide, which can occur in the lysosome as well as on the endoplasmic reticulum (ER) and other membranes. SPHK1 is mainly located in the cytosol and is translocated to the plasma membrane upon activation. This leads to the formation of sphingosine-1-phosphate (S1P), which can be exported out of the cell by specific transporters. Binding to S1P receptors (S1PRs) initiates downstream signalling pathways. SPHK2 is localized to the ER, mitochondria and nucleus. At the ER, S1P is irreversibly degraded by S1P lyase or dephosphorylated by S1P phosphatases to sphingosine, which is reused for the synthesis of ceramide. S1P produced in the mitochondria and nucleus by SPHK2 also has direct intracellular targets. These include prohibitin 2 (PHB2), which stabilizes cytochrome *c* oxidase (COX), and histone deacetylases (HDACs), which remove acetyl groups from histones. ABC, ATP-binding cassette; PalCoA, palmitoyl-CoA; PE, phosphoethanolamine; SGPP1, S1P phosphatase 1; SGPP2, S1P phosphatase 2.

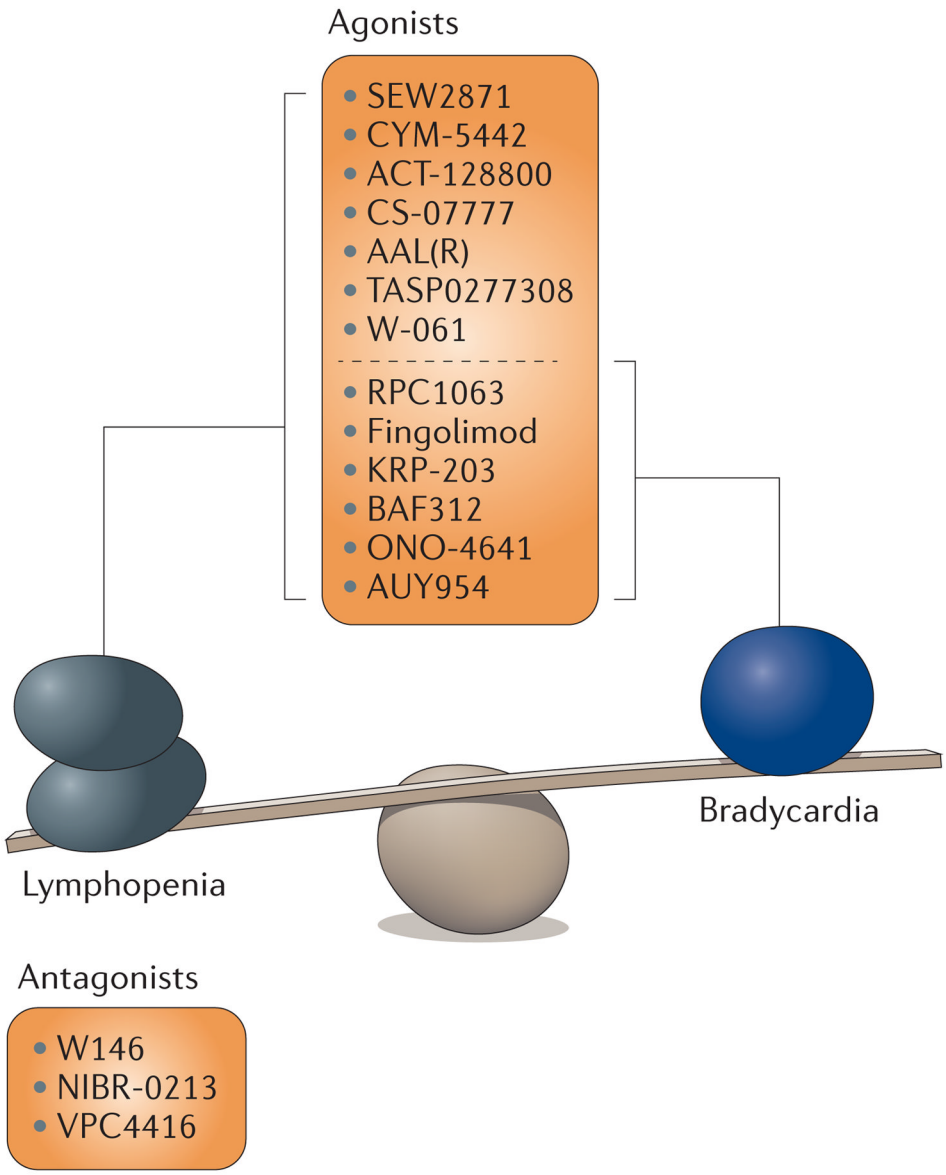

Figure 2. The balance between beneficial and detrimental effects of S1PR1 agonists and antagonists

Agonists and antagonists of sphingosine-1-phosphate receptor 1 (S1PR1) can induce both lymphopenia and the bradycardic side effects. Fingolimod acts as both an agonist and functional antagonist of S1PR1.

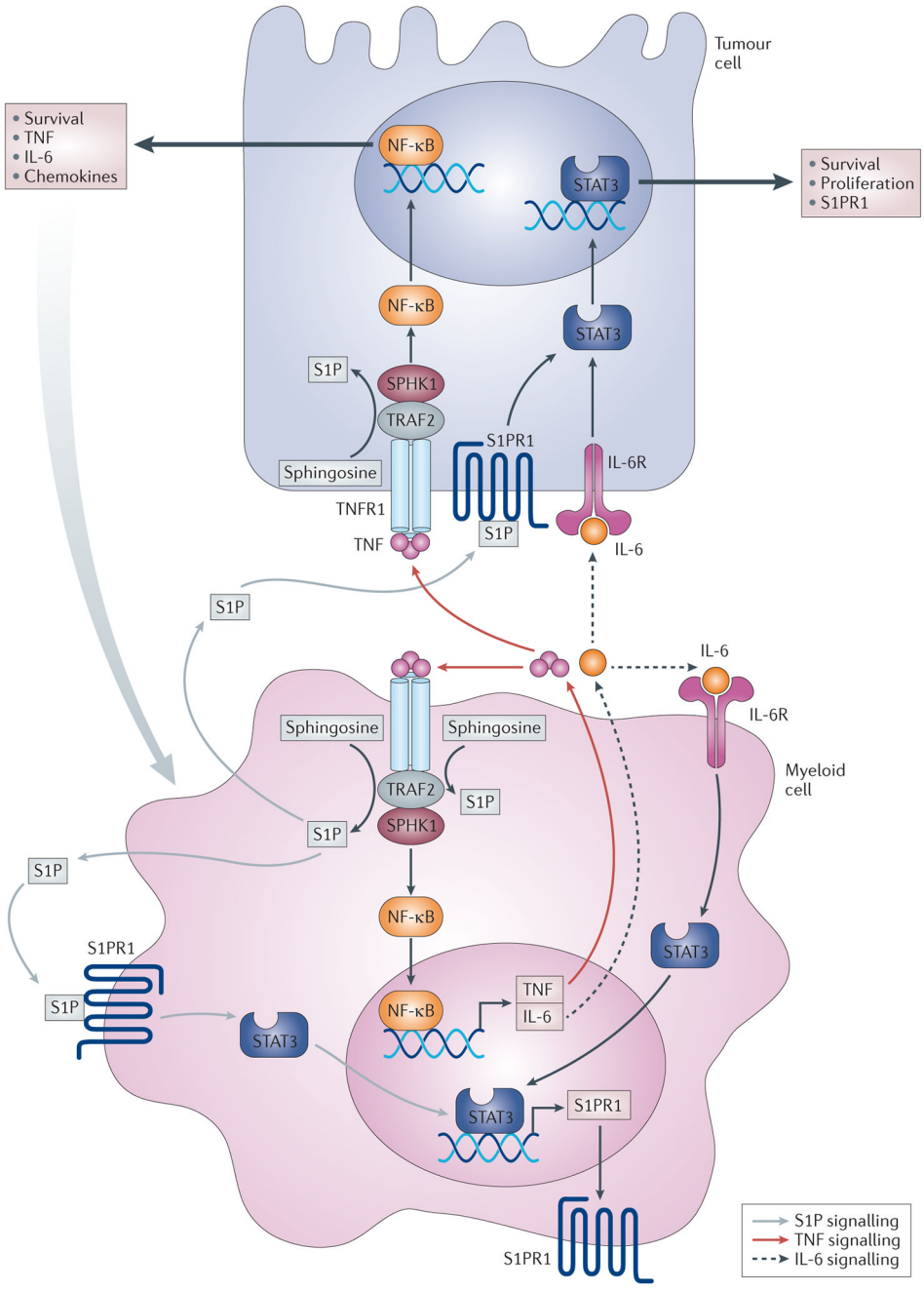

Figure 3. The S1PR1–STAT3 axis linking inflammation and cancer

Sphingosine kinase 1 (SPHK1) is upregulated in tumour cells to produce sphingosine-1-phosphate (S1P); this activates S1P receptor 1 (S1PR1), which leads to the activation of signal transducer and activator of transcription 3 (STAT3). Reciprocally, STAT3 enhances the transcription of its target genes, including *S1PR1*. S1P is also involved in the activation of nuclear factor-κB (NF-κB), which regulates the transcription of the pro-inflammatory cytokines tumour necrosis factor (TNF) and interleukin-6 (IL-6). TNF stimulates SPHK1 to further maintain NF-κB activation, and IL-6 induces STAT3 activation. In addition to upregulating SPHK1 in tumour cells, inflammation upregulates SPHK1 in inflammatory and/or myeloid cells in a manner similar to that in tumour cells. Communication among

tumour cells, the host microenvironment and inflammatory cells via systemic S1P regulates metastasis. Targeting SPHK1 and S1PR1 — for example, with fingolimod — interferes with these amplification cascades and cancer progression. IL-6R, IL-6 receptor; TNFR, TNF receptor; TRAF2, TNF receptor-associated factor 2.

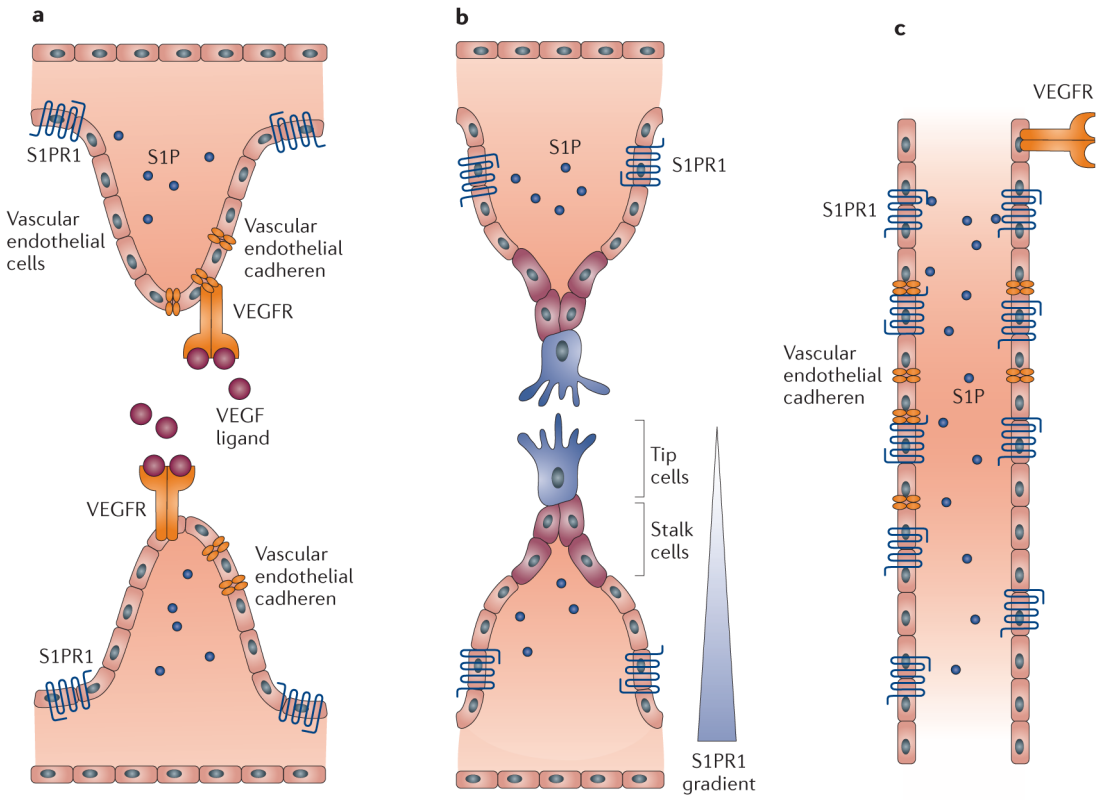

Figure 4. S1PR1-mediated suppression of sprouting angiogenesis and stabilization of blood vessels

a | Sphingosine-1-phosphate receptor 1 (S1PR1) is expressed in vascular endothelial cells and colocalizes with vascular endothelial cadherin in regions of normal blood flow, but is internalized in regions with turbulent blood flow (not shown). **b** | Engagement of vascular endothelial growth factor (VEGF) signalling in the growing vascular front induces the formation of sprouts consisting of tip cells that extend out from the blood vessel and stalk cells. The sprouting vascular front, the tip and stalk cell region express very low levels of S1PR1 (REF. 88); alternatively, S1PR1 is expressed on tip cells but they are not exposed to S1P present in the bloodstream[86]. **c** | After its fusion into a primary vascular loop, which is a key step in the initiation of blood flow, S1P activates S1PR1. Activation of S1PR1 enhances the formation of adherens junctions, inhibits VEGF signalling, suppresses sprouting and stabilizes new vascular connections. VEGFR, VEGF receptor.

Table 1

Compounds that target the S1P axis

Compounds (alternative names)	Targets	Mechanism of action	Preclinical effects in animal models of disease	Refs
SKI-I	SPHK1	SPHK1-specific inhibitor (K_i = 10 μM)	Decreases cancer progression, angiogenesis, lymphangiogenesis and airway hyperresponsiveness	28,33,106,107
Safingol	SPHK1, SPHK2	SPHK1 (K_i = 5 μM) and PKC inhibitor	Decreases cancer progression	108
SKi (2-(*p*-hydroxyanilino)-4-(*p*-chlorophenyl)thiazole or SKI-II)	SPHK1, SPHK2	SPHK inhibitor (IC_{50} = 16 μM for SPHK1; IC_{50} = 8 μM for SPHK2)	Decreases cancer progression	109
PF-543	SPHK1	SPHK1-specific inhibitor (K_i = 3.6 nM)	No effect observed on cell growth	35
ABC294640	SPHK2	SPHK2-specific inhibitor (K_i = 9.8 μM), partial oestrogen receptor antagonist	Decreases cancer progression, liver transplant graft injury and rheumatoid arthritis	42,110,111
LX3305 and LX2931	S1P lyase	Both compounds inhibit S1P lyase activity	Reduces rheumatoid arthritis and cerebral malaria	57,112
THI (2-acetyl-4-tetrahydroxybutylimidazole)	S1P lyase	Inhibits S1P lyase activity	Reduces muscular dystrophy	60
Fingolimod and phosphorylated fingolimod	S1PR1, S1PR3, S1PR4, S1PR5	S1PR1 agonist and functional antagonist (IC_{50} = 0.2–6 nM for S1PR1, S1PR3, S1PR4 and S1PR5)	Suppresses EAE, inhibits lymphocyte trafficking, prevents transplant rejection and decreases colitis and cancer progression	11,22,25,63, 113,114
KRP-203 and phosphorylated KRP-203	S1PR1, S1PR4	S1PR1 agonist and functional antagonist (ED_{50} = 0.84 nM)	Decreases rejection of heart allografts, colitis, atherosclerosis and renal injury	115–119
AUY954	S1PR1	Agonist (EC_{50} = 1.2 nM)	Decreases experimental autoimmune neuritis, heart transplant rejection and EAE	120,121
SEW2871	S1PR1	Agonist (EC_{50} = 14–140 nM)	Decreases ischaemic renal failure and blocks diabetic nephropathy	122–124
CS-0777 and phosphorylated CS-0777	S1PR1	S1PR1 agonist and functional antagonist (EC_{50} = 1.1 nM)	Decreases EAE	125
AAL(R) and phosphorylated AAL(R)	S1PR1, S1PR3, S1PR4, S1PR5	Agonist (EC_{50} = 1 nM)	Inhibits cytokine storm	64,126
TASP0277308	S1PR1	Antagonist (IC_{50} 2 nM)	Ameliorates collagen-induced arthritis	127
CYM-5442	S1PR1	Agonist (EC_{50} = 1.35 nM)	Inhibits cytokine storm resulting from viral infection and decreases EAE	66,76,128
VPC23019	S1PR1, S1PR3	Antagonist (pK_i = 7.9 for S1PR1; pK_i = 5.9 for S1PR3)	Used for receptor function testing in cells and *ex vivo* tissue preparations	129
W146	S1PR1	Antagonist (K_i = 10–20 nM)	Induces lymphopenia and inhibits hyperalgesia	75,130,131

Compounds (alternative names)	Targets	Mechanism of action	Preclinical effects in animal models of disease	Refs
VPC44116	S1PR1	Antagonist (K_i = 30 nM)	Decreases Hodgkin's lymphoma	132,133
JTE-013	S1PR2	Antagonist (K_i = 17 nM)	Decreases osteoporosis and atherosclerosis	90,134,135
Ponesimod (ACT-128800)	S1PR1	S1PR1-specific agonist (EC_{50} = 5–9.1 nM)	Decreases delayed-type hypersensitivity and arthritis	70,136
ASONEP and iSONEP	S1P	S1P-blocking antibody (K_d = 100 pM)	Decreases cancer progression, angiogenesis and choroidal neovascularization	137,138
Siponimod (BAF312)	S1PR1, S1PR5	Agonist (EC_{50} = 0.4 nM for S1PR1)	Decreases EAE	68
ONO-4641	S1PR1, S1PR5	Agonist (EC_{50} = 0.03 nM S1PR1)	Decreases EAE and colitis	69,139
VPC23153	S1PR4	Agonist (K_d = 38 nM)	Induces vasoconstriction	140,141
W-061	S1PR1, S1PR4, S1PR5	Agonist (K_i = 4 μM for S1PR1; 65 μM for S1PR4; 10 μM for S1PR5)	Decreases colitis and graft-versus-host disease	139,142
NIBR-0213	S1PR1	Antagonist (IC_{50} = 2 nM)	Decreases EAE	73

EAE, experimental autoimmune encephalomyelitis; EC_{50}, half-maximal effective concentration; ED_{50}, half-maximal effective dose; IC_{50}, half-maximal inhibitory concentration; K_d, dissociation constant; K_i, inhibition constant; PKC, protein kinase C; pK_i, negative log of the K_i value; S1P, sphingosine-1-phosphate; S1PR, S1P receptor; SPHK, sphingosine kinase.

Table 2

Drugs in clinical trials targeting the S1P axis

Drug	Mechanism of action	Indications	ClinicalTrials.gov identifier	Phase
Fingolimod (Gilenya; Novartis)	S1PR modulator, S1PR1 functional antagonist	Relapsing–remitting multiple sclerosis	-	Approved
		Acute, non-infectious intermediate, posterior and pan-uveitis	NCT01791192	II
		Amyotrophic lateral sclerosis	NCT01786174	II
		Schizophrenia	NCT01779700	I
		Acute demyelinating optic neuritis	NCT01757691	II
		Relapsing–remitting multiple sclerosis with depression, in combination with antidepressants	NCT01436643	IV
		Chronic inflammatory demyelinating polyradiculoneuropathy	NCT01625182	III
		Kidney transplant	NCT00099801	III
Safingol	Sphingosine derivative, PKC inhibitor	Solid tumours, combined with fenretinide	NCT01553071	I
		Solid tumours, combined with cisplatin	NCT00084812	I (completed)
Sonepcizumab	S1P-specific monoclonal antibody	Exudative age-related macular degeneration	NCT01414153	II
		Pigment epithelial detachment	NCT01334255	I (terminated)
		Neovascular age-related macular degeneration	NCT00767949	I
		Solid tumours	NCT00661414	I (completed)
		Unresectable and refractory renal cell carcinoma	NCT01762033	II
ABC294640	SPHK2 inhibitor	Pancreatic cancer	NCT01488513	I
KRP203	S1PR1 agonist	Sub-acute cutaneous lupus erythematosus	NCT01294774	II (terminated)
		Ulcerative colitis	NCT01375179	II (terminated)
		Haematological malignancies	NCT01830010	I
Siponimod (BAF312)	S1PR1 and S1PR5 modulator	Hepatic impairments	NCT01565902	I
		Relapsing–remitting multiple sclerosis	NCT00879658	II
		Relapsing–remitting multiple sclerosis	NCT01185821	II
		Secondary progressive multiple sclerosis	NCT01665144	III
		Polymyositis, dermatomyositis	NCT01148810	II (terminated)
RPC1063	S1PR1 modulator	Relapsing–remitting multiple sclerosis	NCT01628393	II
		Ulcerative colitis	NCT01647516	II
ONO-4641	S1PR1 and S1PR5 agonist	Multiple sclerosis	NCT01226745	II
LX3305	S1P lyase inhibitor	Rheumatoid arthritis	NCT00847886	I (completed)
		Rheumatoid arthritis	NCT00903383	II (completed)
GSK2018682	S1PR1 agonist	Relapsing–remitting multiple sclerosis	NCT01466322	I (completed)
		Relapsing–remitting multiple sclerosis	NCT01431937	I (completed)

Drug	Mechanism of action	Indications	ClinicalTrials.gov identifier	Phase
Ponesimod ACT-128800	S1PR1 agonist	Plaque psoriasis	NCT00852670	II (completed)
		Relapsing–remitting multiple sclerosis	NCT01093326	II
		Psoriasis	NCT01208090	II (completed)
		Relapsing–remitting multiple sclerosis	NCT01006265	II (completed)

PKC, protein kinase C; S1P, sphingosine-1-phosphate; S1PR, S1P receptor; SPHK, sphingosine kinase.