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Emerging medicinal roles for lysophospholipid signaling

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The two lysophospholipids (LPs) lysophosphatidic acid and sphingosine 1-phosphate (S1P) regulate diverse biological processes. Over the past decade, it has become clear that medically relevant LP activities are mediated by specific G protein-coupled receptors, implicating them in the etiology of a growing number of disorders. A new class of LP agonists shows promise for drug therapy: the experimental drug FTY720 is phosphorylated *in vivo* to produce a potent S1P receptor agonist (FTY720-P) and is currently in Phase III clinical trials for kidney transplantation and Phase II for multiple sclerosis. Recent genetic and pharmacological studies on LP signaling in animal disease models have identified new areas in which interventions in LP signaling might provide novel therapeutic approaches for the treatment of human diseases.

Lysophospholipid receptors: G protein-coupled receptors with recently validated potential for drug targeting

G protein-coupled receptors (GPCRs) have been identified as molecular targets with proven therapeutic value. GPCRs are the target of >30% of currently marketed drugs (>200) [1]. These cell surface receptors are specifically activated by diverse extracellular stimuli, including classical hormones, neurotransmitters and, as discussed here, lipid mediators. Their activation initiates various second messenger cascades that depend on effector cell identity and function.

The lysophospholipids (LPs) are simple phospholipids that have been recognized for decades as components in the biosynthesis of cell membranes [2]. However, the recent identification and cloning of GPCRs having high affinity for lysophosphatidic acid (LPA; receptors LPA_1–LPA_4) and sphingosine 1-phosphate (S1P; receptors $S1P_1$–$S1P_5$) have enabled a greater mechanistic understanding of their diverse roles in biological processes [2,3]. LPA and S1P regulate the development and function of numerous organ systems, including the cardiovascular, nervous, immune, and reproductive systems. Altered LP signaling has been implicated in the etiology of disorders, such as inflammation, autoimmune diseases, neuropathic pain, atherosclerosis, cancer and obesity [2].

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The recent discovery of the widespread involvement of receptor-mediated LP signaling in physiology and disease has sparked much interest in the potential therapeutic usefulness of modulating LP receptors. Indeed, entry of the novel immunosuppressant and non-selective S1P receptor agonist FTY720 into clinical trials for kidney transplantation and multiple sclerosis (MS) has raised prospects that treatment aimed at LP receptor targets might be therapeutically efficacious in human diseases in the foreseeable future.

This review focuses on the regulation of LP signaling pathways at the level of the receptors; however, downstream effectors and synthetic and metabolic enzymes have also been suggested as therapeutic targets [4–6]. We evaluate the pathophysiological roles of LP receptors in disease models, focusing on clinical data and animal models of disease using genetic and pharmacological approaches. To provide a framework for discussion on LPA and S1P involvement in disease etiology and possible complicating side effects, we first briefly overview LP signaling.

Lysophospholipid synthesis, metabolism and signaling

LP receptor expression, metabolism and downstream signaling pathways have been extensively reviewed elsewhere [2,3]. LPA is synthesized via multiple enzymes, including extracellular autotaxin (ATX, also known as lysophospholipase D or lysoPLD), phospholipase A_2 (PLA_2) and monoacylglycerol kinase (MAG kinase) (Figure 1a). Similarly, extracellular LPA levels are regulated by the activity of numerous enzymes including LPA acyltransferase, at least three lipid phosphate phosphatases (LPPs) and lysophospholipases (Figure 1a). By contrast, the synthesis and degradation of extracellular S1P appear to involve relatively few enzymes [7] (Figure 1b).

LPs are synthesized and released by diverse cell types to regulate and maintain organismal homeostasis (Box 1). During tissue injury in which the vascular–interstitial barrier is breached, LPs originate from activated platelets and other blood-borne cells, including mast cells, and promote wound healing, inflammation and angiogenesis. Furthermore, LPs are derived from cellular components in various other systems (e.g. neural, adipose and reproductive tissues) and they appear to help maintain these systems within normal parameters [2]. LP binding to cognate GPCRs activates diverse intracellular messenger

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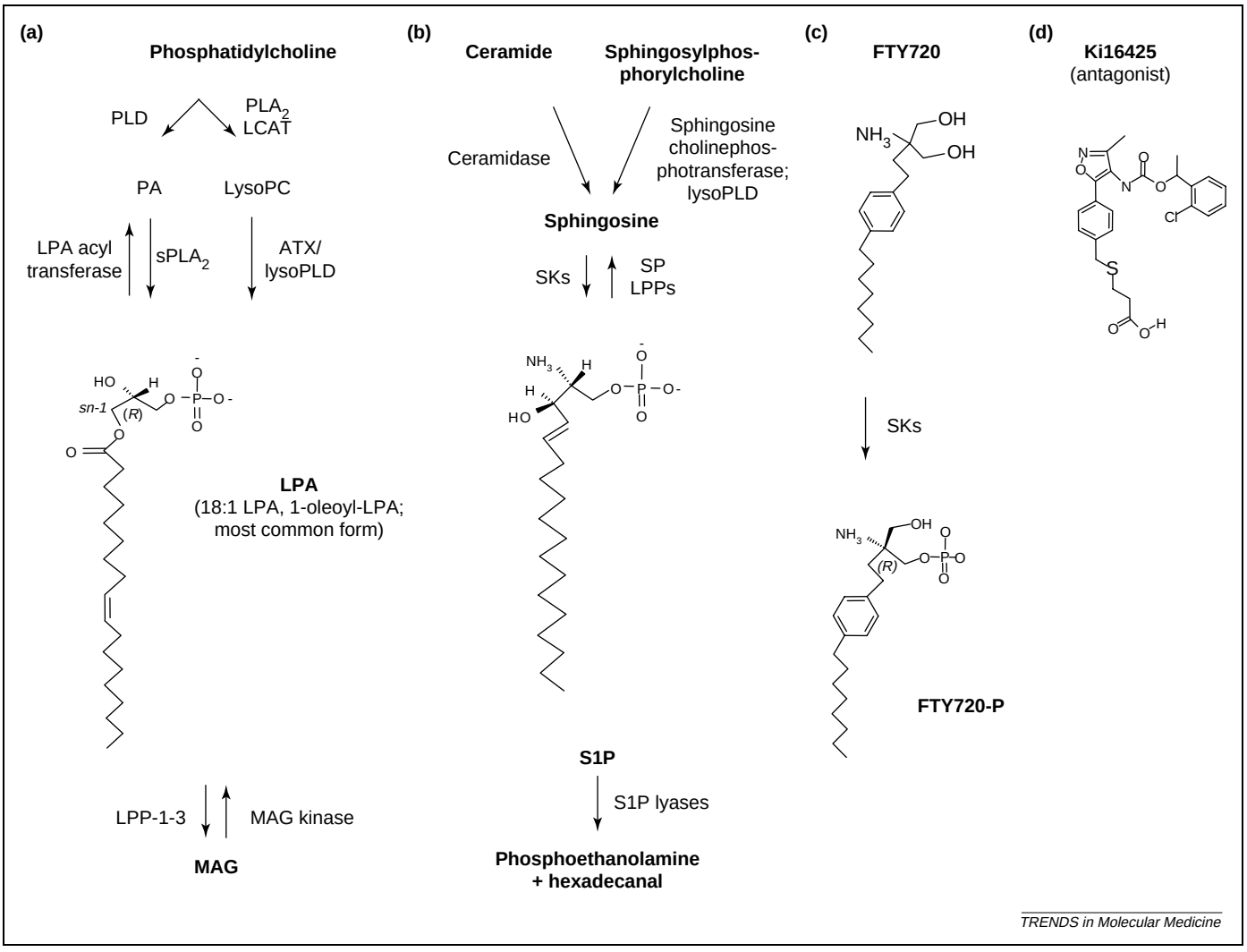

Figure 1. Synthesis and metabolism of major LPs. **(a)** LPA synthetic and metabolic pathways. There are multiple isoforms of LPA; which are generated depends on the backbone carbon from which the alkyl chain is hydrolyzed. The major enzymes involved in the control of 1-*sn*-LPA levels are shown. The synthesis of *sn*-2 LPA occurs when PC or PA are hydrolyzed by PLA_1. Abbreviations: ATX/lysoPLD, autotaxin/lysophospholipase D; LCAT, lecithin cholesterol acyl transferase; LPP, lipid phosphate phosphatase; LysoPC, lysophosphatidylcholine; MAG, monoacylglycerol; PA, phosphatidic acid; PC, phosphatidylcholine; PLA_1, phospholipase A_1; PLA_2, phospholipase A_2; PLD, phospholipase D; $sPLA_2$, secretory phospholipase A_2. **(b)** Major S1P synthetic and metabolic pathways. Abbreviation: SK, sphingosine kinase. **(c)** Phosphorylation of FTY720 to the active S1P receptor agonist FTY720-P occurs via SKs (Table 1). Only the (R) stereoisomer of FTY720-P is active. **(d)** The structure of Ki16425, a specific antagonist at LPA_1 and LPA_3 receptors (Table 1).

systems to produce a variety of biological responses (Box 1; Figure 2).

FTY720: targeting lysophospholipid receptors to prevent transplant rejection and to treat autoimmune disorders

Transplant rejection results from an alloimmune response of the recipient to non-self antigens expressed in donor tissue. Current strategies aimed at preventing acute rejection ultimately target T cells, either directly or indirectly, through inhibitory actions on other immune cell types (e.g. antigen-presenting cells) [8]. Unfortunately, it is not often possible to suppress the immune system sufficiently to prevent rejection while maintaining defense against infections. A breakthrough in the field came from the serendipitous discovery that an S1P receptor agonist is an effective and potent immunosuppressant with a novel mechanism of action.

FTY720 (Figure 1c), a synthetic analog of the natural product myriocin [9], supports pharmacological interventions involving LP signaling. Phosphorylation of FTY720 *in vivo* by sphingosine kinases (SKs) makes it a potent agonist (K_d values in the nanomolar range) for four of the five currently known S1P receptors ($S1P_1$ and $S1P_3$–$S1P_5$) [10,11] (Table 1). It is generally accepted that binding of phosphorylated FTY720 (FTY720-P; Figure 1c) to $S1P_1$ on lymphocytes prevents their exit from secondary lymphoid organs, thus reversibly sequestering them from sites of inflammation [10,11]. However, there is controversy in the literature regarding the cellular mechanism of action of FTY720 on $S1P_1$ receptors. One proposed model is that FTY720 blocks lymphocyte exit via $S1P_1$ agonist activity [10–12]. A second model suggests that FTY720 acts as a 'functional antagonist' to inactivate $S1P_1$ receptors on lymphocytes, and is equivalent to lymphocyte-specific $S1P_1$ deficiency [13–15]. Supporting $S1P_1$ inactivation by FTY720 is the finding that FTY720 itself might induce receptor internalization and subsequent degradation [16].

FTY720 neither overly immunocompromises the patient nor disrupts myelomonocyte-cell function [17]. Results from clinical trials suggest that FTY720 has a

Box 1. Identified lysophospholipid receptor-mediated signaling mechanisms

Six major cellular responses are induced by S1P- and LPA-dependent activation of cell-surface GPCRs, thus producing diverse downstream cellular responses dependent on cell type, developmental stage and environment (Figure 2).

Survival and proliferation

Stimulation of G_i, G_q and $G_{12/13}$ by LPA and S1P confers these receptors with the ability to support cell survival and proliferation [105]. Phosphoinositide 3-kinases (PI3Ks) generate specific inositol lipids implicated in the regulation of cell growth, proliferation, survival, differentiation and cytoskeletal changes through the phosphorylation of specific targets.

Migration

Basic cellular and molecular mechanisms underlie the migration of neoplastic (e.g. migrating gliomas and rectal cancer cells) and non-neoplastic cells (e.g. fibroblasts, keratinocytes, VECs, VSMCs and neurons) [51]. Depending on the expressed surface LP receptors and downstream signaling pathways, LPA and S1P either stimulate or inhibit cell migration by initiating reorganization of the actin cytoskeleton. In particular, stimulation of $G_{i/o}$ and/or $G_{12/13}$ can cause membrane ruffling (lamellipodia movement), stress-fiber formation and regulation of focal adhesion contacts [2]. The use of null mutant mice and pharmacological tools enables the identification of LPA_1 as mediating LPA and ATX/lysoPLD-induced motility in fibroblasts [106] (Figure 2). Membrane ruffling in mouse skin fibroblasts is mediated largely by LPA_1–G_i–Rac1, with some contribution from LPA_2 [106]. LPA_1 activation of both PI3K–Cdc42–p38MAPK and LPA_1–PI3K–Rac1–JNK pathways simultaneously mediates LPA-induced migration of glioma cells [107]. LPA-induced stress-fiber formation includes activation of both LPA_1 and LPA_2 and the downstream $G_{12/13}$–RhoA pathway. Importantly, the LPA_1 coupling to both G_i and $G_{12/13}$ and LPA_2 coupling predominantly to $G_{12/13}$ in endogenous cell systems was also observed in heterologous expression systems for each of these receptors, indicating that recombinant-system studies can be translated, at least in part, to endogenous processes [106]. The complexities of cell-type-dependent activation of particular intracellular signaling pathways are underscored by the observation that LPA stimulates migration of ovarian cancer cells via novel recruitment of Ras–MEKK1-dependent FAK to surface adhesion contact sites in ovarian cancer cell lines [108]. S1P effects on cellular migration depend on receptors and cell context as well [109].

Tumor-cell invasion

Degradation of extracellular matrix components enables the invasion of discrete cell populations by foreign (e.g. metastatic) cells [110]. Activation of LPA_1 in ovarian cancer cells, but not in normal ovarian surface epithelial cells, stimulates synthesis of the extracellular protease urokinase plasminogen activator (uPA) via a novel G_i–Ras–Raf–NF-κB pathway [111]. LPA enhances the invasiveness of several tumor cell types [51].

Cell aggregation

LP-induced platelet aggregation involves both LPA and S1P in thrombus formation and progression of atherosclerosis. Incubation of platelets with dioctyl glycerol pyrophosphate, an antagonist of LPA_1 and LPA_3, blocks mildly oxidized low-density lipoprotein (LDL)- and plaque core-induced platelet shape change and aggregation, indicating a role of these receptors in this response [30]. Moreover, mildly oxidized LDL and LPA activate Src and Syk tyrosine kinases, known to mediate the exposure of fibrinogen-binding sites on integrin $\alpha_{IIb}\beta_3$ during shape change, which is a prerequisite for platelet aggregation [112].

De-differentiation

Unsaturated LPAs induce de-differentiation of VSMCs, as evidenced by conversion to a fibroblastic morphology, and loss of contractility and differentiation marker gene expression [113]. De-differentiation depends on activation of extracellular signal-regulated kinase and p38 mitogen-activated protein kinase.

Smooth-muscle contraction and relaxation

Smooth muscle cells and myofibroblasts contract upon stimulation by LPA and S1P primarily by modulating intracellular Ca^{2+} signaling through $G_{i/o}$, G_q and $G_{12/13}$ [114].

lower toxicity profile than the currently used cyclosporine and might be effective as an adjunct in polytherapy when used together with lower doses of cyclosporine. Thus, the risks associated with classical immunosuppressants (e.g. osteoporosis, pathologic fractures, diabetes, renal dysfunction or hypertension) might be minimized. One potential liability is bradycardia associated with $S1P_3$ agonism [18]; however, the prolonged exposure of human subjects in transplantation-related trials suggests that this might be a tolerable liability.

FTY720 also reveals promising efficacy in the treatment of autoimmune disorders including myasthenia gravis, autoimmune-induced diabetes, arthritis, autoimmune myocarditis, uveoretinitis and systemic lupus erythematosus [19]. One disorder for which there is a paucity of treatment options is MS, an often slowly-progressing disease of the central nervous system (CNS) that is characterized by demyelinating plaques in the spinal cord and brain, and results in numerous sensory and motor neurological deficits. Although the etiology of MS remains unidentified, it is thought to involve immunological abnormalities, particularly in T-cell and microglial function. FTY720 reduces symptoms of both monophasic and relapsing–remitting episodes in experimental autoimmune encephalomyelitis (EAE), a murine model of MS [19–21], and is currently in Phase II clinical trials for MS (Table 2). Prevention of lymphocyte recirculation might contribute to the mechanism of action of FTY720 in MS, but a temporal mismatch between clinical signs of EAE and lymphopenia indicates the involvement of additional mechanisms [21]. Protective effects of LP signaling might have particular relevance in human MS where the prolonged course of the disease appears to produce progressive neurodegeneration [22].

Targeting lysophospholipid receptors for treatment of other pathological disorders

The therapeutic usefulness of FTY720 provides the best, validated example of LP receptor agonist action (via FTY720-P) for the treatment of transplant rejection and human autoimmune diseases. Importantly, several other disorders involving LP signaling have been identified through the study of animal models (Table 2).

Cardiovascular disorders

Cardiovascular phenotypes observed after targeted deletion of LP receptors reveal important roles for LP signaling in the development and maturation of blood vessels, formation of atherosclerotic plaques and maintenance of heart rate [2]. Angiogenesis, the formation of

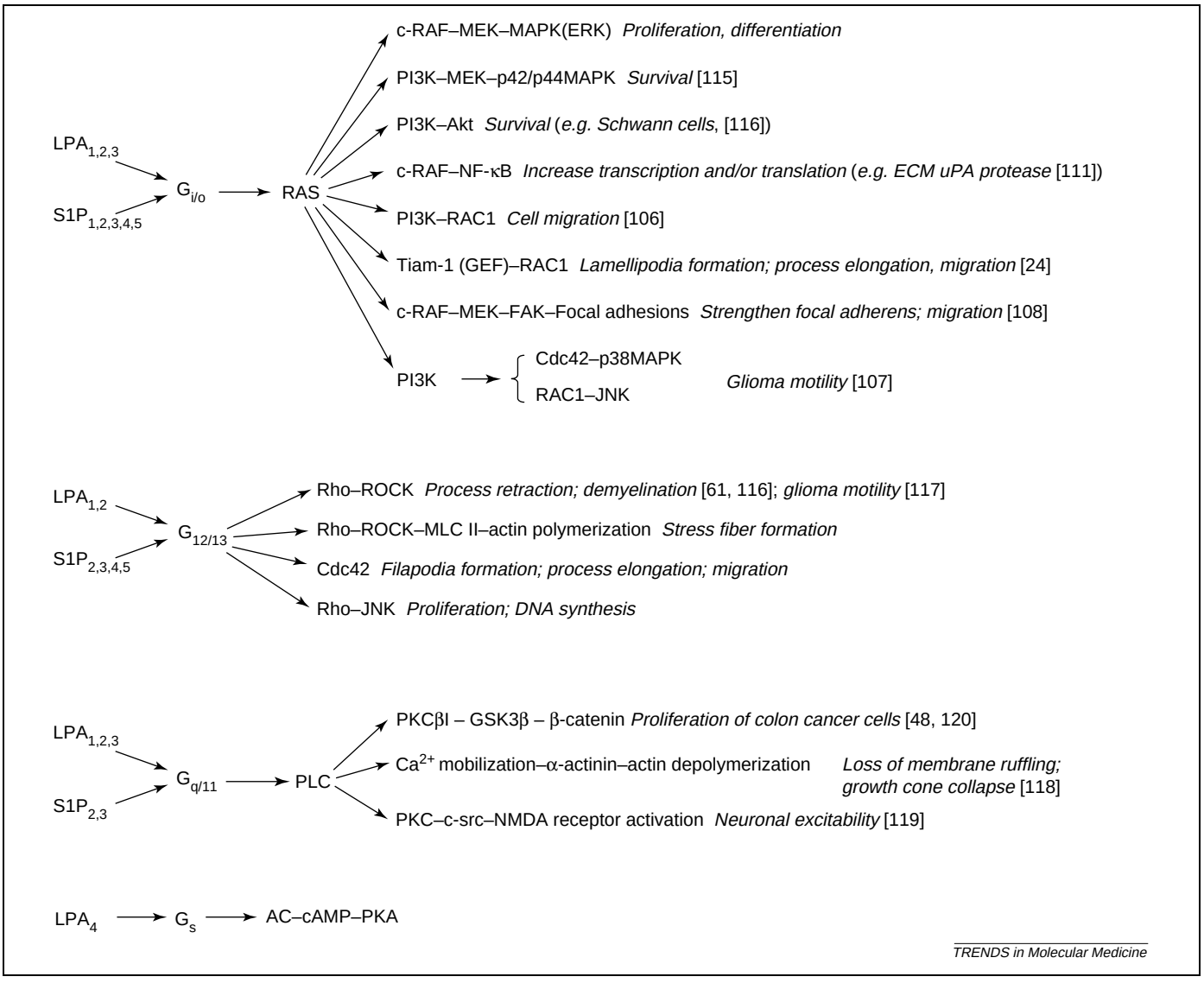

Figure 2. LP receptor-mediated signaling pathways. LP receptors (LPA_1–LPA_4 and $S1P_1$–$S1P_5$) activate diverse second messenger pathways. Examples of intracellular pathways reported for LPA and S1P receptor activation and the subsequent effect on cell function are shown (italics). References are indicated in brackets. General references for this figure are [2,51]. Abbreviations: AC, adenylyl cyclase; Akt, serine-threonine protein kinase B; cAMP, cyclic adenosine monophosphate; Cdc42, cell division cycle 42/GTP-binding protein; c-RAF, proto-oncogene serine/threonine-protein kinase Raf; c-src, proto-oncogene tyrosine-protein kinase Src; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; GEF, guanine nucleotide exchange factor; GSK3β, glycogen synthase kinase 3β; JNK, c-jun amino-terminal kinase; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase; MLC II, myosin light chain II; NF-κB, nuclear factor κB; NMDA, N-methyl-D-aspartate; PI3K, phosphatidylinositol 3-kinase; PKA, protein kinase A; PKC, protein kinase C; RAC1, ras-related C3 botulinum toxin substrate 1; Rho, small GTPase protein; ROCK, Rho-associated kinase.

new capillary networks from pre-existing vasculature, is normally invoked in wound healing, tissue growth and myocardial angiogenesis after ischemic injury. Peptide growth factors [e.g. vascular endothelial growth factor (VEGF)] and LPs control coordinated proliferation, migration, adhesion, differentiation and assembly of vascular endothelial cells (VECs) and surrounding vascular smooth-muscle cells (VSMCs) [2]. Dysregulation of the processes mediating angiogenesis can lead to atherosclerosis, hypertension, tumor growth, rheumatoid arthritis and diabetic retinopathy [23,24].

A crucial role for LP receptors in angiogenesis has recently been elucidated by targeted deletions of $S1P_1$–$S1P_3$ and LPA_1 receptors. Constitutive deletion of $S1P_1$ in mice leads to embryonic lethality due to the impaired ability of VSMCs to migrate, cover and stabilize the nascent vascular network [25,26]. Furthermore, double- and triple-knockout mice showed that $S1P_1$–$S1P_3$ function coordinately to stabilize vessel formation [27]. Downstream signaling pathways evoked by LP receptors include Rac-dependent lamellipodia formation (e.g. $S1P_1$ and LPA_1) and Rho-dependent stress-fiber formation (e.g. $S1P_2$, $S1P_3$ and LPA_1), which is important in cell migration and adhesion [24] (Box 1). Dysfunction of the vascular endothelium can shift the balance from vasodilatation to vasoconstriction and lead to hypertension and vascular remodeling, which are risk factors for atherosclerosis [28].

LPA and S1P contribute to both the early phase (barrier dysfunction and monocyte adhesion of the endothelium) and the late phase (platelet activation and intra-arterial thrombus formation) of atherosclerosis, in addition to its overall progression [29]. In the early phase, LPA from numerous sources accumulates in lesions and activates its

Table 1. Modulator selectivity at lysophospholipid receptors

Modulators	Potency[a]	Refs
Agonists		
S1P	**$S1P_1 \cong S1P_5 > S1P_4 > S1P_2 \cong S1P_3$**	[3]
LPA	**$LPA_1 > LPA_4 \geq LPA_2 > LPA_3 \gg \gg S1P_1$**	[3,99]
FTY720-P[b]	**$S1P_3 > S1P_1 \cong S1P_5 > S1P_4 \gg \gg S1P_2$**	[10]
Antagonists		
Ki16425	**$LPA_1 \cong LPA_3 \gg LPA_2 \gg \gg S1P_2$, $S1P_3$, $S1P_4$**	[100]
JTE-013[c]	**$S1P_2$ (IC_{50} 20 nM) $\gg \gg S1P_3$, LPA_1 ($IC_{50} > 10\,000$ nM)**	[101]

[a] $\gg \gg$ denotes there was no effect of the ligand at the highest concentration tested (10 000 nM).
[b] $_PEC_{50}$ values.
[c] JTE-013 (pyrazolopyridine) is an $S1P_2$-selective antagonist [101].

cognate GPCRs (LPA_1 and LPA_3) expressed on platelets [30]. This triggers platelet shape change and aggregation, leading to intra-arterial thrombus formation and, potentially, myocardial infarction and stroke [29]. In support of its atherogenic activity, LPA can also be a mitogen and motogen to VSMCs and an activator of endothelial cells and macrophages [29]. Thus, patients with cardiovascular disease might benefit from LPA receptor antagonists that prevent thrombus and neointima plaque formation.

Table 2. Effects of lysophospholipids and potential clinical indications for LP receptor modulators

Endogenous ligand	Cell, organ or behavioral effect	Target	Pharmacological modulator	Potential clinical indication (clinical trial stage)	Refs
S1P	Lymphocyte sequestration (lymphopenia); prolonged survival of transplants with FTY720 administration	$S1P_1$	Agonist	Suppressed transplant rejection (FTY720, Phase III)	[102]
S1P	Lymphocyte sequestration; *in vivo* attenuation of relapsing–remitting symptoms of EAE with FTY720 administration	$S1P_{1,?}$	Agonist	Multiple sclerosis (FTY720, Phase II)	[21]
LPA	Plaque-induced platelet activation and thrombus formation	LPA	Antagonist	Late-stage atherosclerosis	[30]
S1P	Coronary artery smooth-muscle contraction; JTE-013 efficacy	$S1P_2$	Antagonist	Coronary artery vasospasm; sinus tachycardia	[36]
S1P	$p70^{s6kinase}$-induced proliferation of endothelial cells and VSMCs	$S1P_1$	Antagonist	Restenosis after coronary artery angioplasty	[31]
S1P	Lymphocyte sequestration; *in vivo* inhibition of airway inflammation, induction of bronchial hyperresponsiveness and goblet cell hyperplasia with FTY720 administration	$S1P_{1,3,4,5}$	Agonist	Asthma	[38]
S1P	Enhanced vascular permeability via tight-junction disruption on alveolar epithelial cells; targeted deletion of $S1P_3$	$S1P_3$	Antagonist	Pulmonary edema; acute respiratory distress syndrome	[45]
S1P	Mast-cell degranulation	$S1P_2$	Antagonist	Inflammation	[41]
S1P	Enhanced pulmonary epithelial integrity by increasing transepithelial resistance with FTY720 administration in conjunction with CTLA4IgG	$S1P_1 >>>>> S1P_3$	Antagonist	Obliterative bronchiolitis after lung transplant	[40]
LPA	Xenografts with LPA_1 overexpressing cells; enhanced mitogenesis, tumor growth and bone metastasis	LPA_1	Antagonist	Breast cancer	[103]
LPA	Proliferation of colon cancer cell lines; xenografts in nude mice (siRNA)	$LPA_{2,3}$ (not LPA_1)	Antagonist	Colon cancer	[48]
S1P	*In vivo* cancer cell apoptosis; downregulation of Atk; prevention of tumor metastasis with FTY720 administration	$S1P_{1,3,4,5}$	Agonist	Breast cancer; liver cancer	[46,47]
LPA	Enhanced survival of peripheral Schwann cells and promotion of myelinogenesis; targeted deletion of LPA_1)	LPA_1	Antagonist	Neuropathic pain	[104]
LPA	Altered amino acid levels in the forebrain and the hippocampus; defective behavior in pre-pulse inhibition assay	LPA_1	Agonist	Schizophrenia	[76]
LPA	Spacing and timing of blastocyst implantation; targeted deletion of LPA_3	LPA_3	Agonist	Female infertility	[81]
S1P	Suppression of oocyte apoptosis *in vivo*; S1P efficacy	S1P	Agonist	Chemotherapy- or radiation-induced infertility	[84]
LPA	Suppression of pre-adipocyte differentiation into adipocytes; targeted deletion of LPA_1	LPA_1	Agonist	Obesity	[96]

S1P is released from thrombin-stimulated platelets and promotes further platelet aggregation and thrombus formation. $S1P_1$ is upregulated in rat neointimal VSMCs close to the developing plaque [31] where it mediates proliferation and migration of VSMCs. Furthermore, $S1P_1$ is induced in neointimal lesions of human in-stent restenosis [32]. S1P activation of the downstream $p70^{S6kinase}$ pathway in VSMCs causes induction of cyclin D1 and a subsequent increase in cell proliferation. Correspondingly, rapamycin, an immunosuppressant that inhibits the $p70^{S6kinase}$ pathway, inhibits the restenotic response after angioplasty, suggesting that blockade of $S1P_1$ might be of therapeutic benefit (Table 2).

However, S1P causes vasoconstriction and exerts cardioprotective effects in some systems, depending on the type of receptor and downstream pathways expressed in the endothelium [2]. For example, the vasoconstrictive effect of S1P on basal arterial tone in isolated cerebral arteries, although partially mediated by $S1P_3$ [33,34], might also be due to activation of $S1P_2$ because this effect is not observed during application of FTY720 [35]. In human VSMCs *in vitro*, the effect of S1P on basal tone seems to depend on $S1P_2$ [36]. Hence, along with its immunosuppressant activity, FTY720 might stimulate $S1P_3$-mediated nitric oxide (NO) production and preserve vascular structure, aiding in the survival of transplant recipients. Additionally, high-density lipoprotein (HDL), which is anti-atherogenic, is a carrier of LPs, especially S1P. It targets S1P to the endothelium via activation of $S1P_3$ and subsequent Akt-mediated activation of endothelial cell NO synthase (eNOS). This signaling increases the levels of the endothelial-derived relaxing factor NO, thus causing vasodilatation [37]. S1P-receptor-specific modulators will be required to determine whether therapeutic benefits can be observed without adverse side effects.

Respiratory disorders

Chronic inflammatory diseases of the airways are often characterized by the infiltration of effector T cells into airway tissue following antigen challenge. FTY720 can reduce bronchial hyper-responsiveness and goblet-cell hyperplasia [38], suggesting therapeutic benefit in some inflammatory airway diseases (e.g. asthma) (Table 2). S1P receptor agonists might also effectively treat respiratory complications associated with lung transplant because $S1P_1$ enhances pulmonary epithelial integrity [39] and FTY720, when in combination with CTLA4IgG, preserves the respiratory epithelium and prevents obliterative bronchiolitis associated with lung transplantation in mice [40] (Table 2).

Proinflammatory effects of S1P and LPA include degranulation of mast cells (in part caused by activation of $S1P_2$), contraction of smooth-muscle cells and release of cytokines from dendritic cells [41]. Furthermore, the broncho-alveolar lavage fluid of allergen-challenged asthmatics contains enhanced S1P levels compared with control subjects, and S1P receptors are present on the airway smooth muscle of these patients [42]. The lack of affinity of FTY720-P for $S1P_2$ might explain its overall therapeutic benefit in inflamed airways [38]. Whether $S1P_2$-specific expression on smooth muscle cells and dendritic cells accounts for the lack of effect of FTY720-P on airway contraction and cytokine release requires further study, and this would benefit from selective $S1P_2$ modulators. Alternatively, extracellular levels of FYT720-P and S1P might not contribute equally to the dynamic balance of intracellular S1P, sphingosine and ceramide levels, which influence S1P receptor downstream signaling [43].

Acute respiratory distress syndrome (ARDS) is characterized by inflammation of the lungs and accumulation of fluid (edema) in air sacs, and can degenerate into complete respiratory failure. Activation of $S1P_1$ receptors on pulmonary endothelial cells increases transendothelial resistance and decreases fluid exchange [44]. By contrast, consistent with the pleiotropic nature of LP responses and differential LP-receptor-subtype distribution, Gon *et al.* [45] showed that S1P activates $S1P_3$ receptors on lung epithelial cells, leading to pulmonary edema. Significantly, $S1P_3$-null mice are protected against S1P-induced pulmonary edema. Electron microscopy revealed that $S1P_3$ signaling can cause disruptions in epithelial cell tight-junction integrity, and that proteins normally present in tight junctions (ZO-1 and claudin) are lost [45]. These findings suggest that $S1P_3$ receptor antagonists might protect against pulmonary edema and lead to a therapy for ARDS (Table 2).

Cancer

The initiation, progression and metastasis of cancer involve several concurrent and sequential processes including cell proliferation and growth, survival and anti-apoptosis, migration of cells, penetration of foreign cells into defined cellular layers and/or organs, and promotion of angiogenesis. The control of each of these processes by LPA and S1P signaling in physiological and pathophysiological conditions underscores the potential therapeutic usefulness of modulating LP signaling pathways for the treatment of cancer, especially at the level of the LP receptors or ATX/lysoPLD (Figure 1).

Recent studies using pharmacological and genetic tools implicate LP receptor signaling in the etiology of cancer. Pro-apoptotic activity of FTY720 markedly prevented tumor growth and metastasis in *in vivo* mouse breast [46] and liver [47] cancer models. The size of tumors established by colon-cancer-cell xenografts in the flank of nude mice was reduced when expression of LPA_2 or LPA_3, but not LPA_1, was knocked down by small-interfering RNA (siRNA) targeting of specific LPA-receptor expression before xenograft injection [48].

Cell transformation *in vitro* and tumor growth *in vivo* depend on SK activity [49,50] and S1P functions as a pro-survival factor in some cancers [51]. ATX/lysoPLD was originally identified for its role in the progression of cancer [52,53] and is upregulated in several malignancies (e.g. breast and lung cancers [54]). Enhanced ATX/lysoPLD expression from ATX-recombinant tumorigenic cell lines injected into (athymic) nude mice stimulates invasion, metastasis and angiogenesis [55,56]. LPA receptors mediate both migration of and invasion by pancreatic cancer cell lines: an antagonist of LPA_1 and LPA_3 (Ki16425) and LPA_1-specific siRNA effectively blocked

in vitro migration in response to LPA and peritoneal fluid (ascites) from pancreatic cancer patients; in addition, Ki16425 blocked the LPA-induced and ascites-induced invasion activity of a highly peritoneal metastatic pancreatic cancer cell line [57]. The role of LPA in cancer is described in more detail elsewhere [2,51].

LPA levels are high in ovarian cancer ascites and breast cancer effusions [51,58], and the levels of LPA and the specific complement of LPA isoforms in ascites can both be useful biomarkers for ovarian cancer [59]. However, although it has been reported that serum LPA levels are elevated in >90% of stage I or II ovarian cancer patients and that LPA might serve as a biomarker for the disease [60], the levels of LPA in serum or plasma might be overestimated because of the activation of platelets during sample preparation [58]. Therefore, the use of LPA as a prognostic marker for certain cancers requires further investigation. Clinical studies are required to evaluate the therapeutic potential of LP signaling interventions in ovarian and other cancers. The diversity of LP signaling pathways among cancer types will probably necessitate specific disease-targeted therapies.

Neuropathic pain

Although pain felt immediately after nerve injury is protective (acute pain), chronic neuropathic pain (NP) that sometimes follows is maladaptive, and treatment of NP represents an unmet medical need. Little is known about the events subsequent to tissue injury that trigger NP. However, recent evidence indicates that LPA signaling, early after nerve injury, can trigger the onset of NP and suggests that early intervention at the level of LPA receptors could decrease the progression and/or severity of NP [61].

Major cellular components of nerve tissue, such as central and sensory neurons, Schwann cells, and oligodendrocytes, are responsive to LPA. LPA is pro-nociceptive when administered locally to the hindpaw through its activation of LPA_1 [62] and it directly stimulates peripheral nociceptor endings via $G_{i/o}$ and subsequent release of the pro-nociceptive agent substance P [63].

A crucial role for LPA_1 and downstream Rho–ROCK activation in the initiation of NP signaling was recently demonstrated [61]. Intrathecal injection of LPA into wild-type mice elicited long-term sensitivity to both non-noxious and noxious stimuli, a response that is consistently observed after nerve injury [61]. Intrathecal administration of LPA (but not S1P) produced hyperalgesia and tactile allodynia in wild-type but not in $lpa_1^{(-/-)}$ mice; this effect was blocked by the Rho inhibitor *Clostridium botulinum* C3 exoenzyme BoToxC3 and by the Rho-kinase (ROCK) inhibitor Y27632, consistent with the involvement of Rho and ROCK. Furthermore, $lpa_1^{(-/-)}$ mice were resistant to NP produced by partial sciatic nerve ligation [61]. The mechanism underlying the NP induced by LPA injection and nerve injury might involve demyelination because myelination in dorsal roots was disrupted in a RhoA-dependent manner [61].

When vascular integrity is disrupted by injury, increased interstitial LPA levels might affect both neuronal and glial functions. In fact, aberrant myelination correlates with some forms of NP (e.g. trigeminal neuralgia), where it is thought that unensheathed injured axons increase the excitability of uninjured fibers [64]. Additionally, $lpa_1^{(-/-)}$ mice were resistant to nerve-injury-induced and LPA-induced demyelination. The precise role of LPA in nerve-injury-associated demyelination and NP remains to be determined. The identification of LPA and LPA_1–Rho–ROCK signaling as initiators of NP suggests that targeting the LPA_1 signaling pathway pharmacologically might provide new therapeutic options for the treatment of NP (Table 2).

Psychiatric disorders

Several psychiatric diseases, such as schizophrenia, are thought to have a developmental etiology [65]. Aberrant LP signaling might contribute to the progression of diseases characterized by increased cell number and/or inappropriate circuitry because LPs markedly influence neural progenitor cell (NPC) morphology (e.g. process retraction, cell rounding and growth cone collapse), survival or apoptosis, cell-cycle progression (e.g. proliferation) and migration during embryogenesis [2]. The physiological and pathological importance of both LPA and S1P in the nervous system is underscored by their biologically relevant concentrations in the developing brain [66,67], the source of which includes postmitotic neurons and extracellular ATX/lysoPLD isoforms [68,69]. The spatial–temporal regulation of LP receptors during embryogenesis [70,71] indicates a developmental role for LP signaling in neurogenesis, neuronal migration and neuritogenesis [2]. The comparatively high level of expression of LPA_1 in the ventricular zone (VZ), the neurogenic region of the embryonic cerebral wall, reflects the density of NPCs. Furthermore, LPA_2 is expressed in postmitotic cells of the embryonic cortex [70,72]. Exogenous LPA dramatically induces thickening and abnormal folding of the cortical wall in *ex vivo* embryonic rodent hemisphere cultures; this is attributable to increased terminal mitosis of NPCs and decreased cell death within the VZ, and depends on LPA_1 and LPA_2 [73]. It is noteworthy and somewhat surprising that few gross neuroanatomical and neurological defects are observed in surviving $lpa_1^{(-/-)}$ [74], $lpa_2^{(-/-)}$ and $lpa_1^{(-/-)}lpa_2^{(-/-)}$ mice [74,75]. Surviving LPA_1 null mice revealed a higher incidence of hematomas and impaired suckling [74]; LPA_2 null mice exhibited no obvious CNS related phenotype [75]. $Lpa_1^{(-/-)}lpa_2^{(-/-)}$ mice revealed a phenotype similar to that observed in $lpa_1^{(-/-)}$ mice albeit with increased incidence of perinatal frontal hematoma and detectable signaling deficits in primary mouse embryonic fibroblasts [75]. It is likely that compensatory signaling systems are crucial for ensuring appropriate neural development. However, subtle neurological phenotypes are likely to be identified when null mice are investigated for defects in particular behavioral paradigms.

Harrison *et al.* independently generated $lpa_1^{(-/-)}$-C57Bl/6J mice and reported a behavioral phenotype reminiscent of schizophrenia [76] (Table 2). Significant and region-specific decreases in levels of amino acids (e.g. aspartate, GABA and taurine) in the hippocampus prompted an electrophysiological investigation of

hippocampal function that revealed no gross abnormalities in synaptic activity. However, $lpa_1^{(-/-)}$ mice were less responsive than control littermates in the acoustically driven prepulse-inhibition behavioral test, suggesting impaired sensorimotor gating, a hallmark of schizophrenia [77]. The defects responsible for these changes might include myelination dysregulation, which has been observed in some cases of human schizophrenia [78,79]. Notably, LPA and S1P receptor expression in oligodendrocytes follows the process of myelination in the rodent CNS [71,80] and might contribute to glial differentiation, maturation and myelination during development, in addition to pathological processes (e.g. gliosis).

LPA_1, LPA_2 and $S1P_1$ activation is likely to act redundantly in brain development because these receptors reveal overlapping embryonic expression patterns, activate many of the same downstream pathways and produce similar cellular effects *in vitro*. Such redundancy would underscore the importance of these pathways in neural development. It remains possible that aberrant LP signaling contributes to the pathophysiology of neurological diseases; a combination of genetic, behavioral and pharmacological approaches will probably elucidate the roles of LPs in these diseases.

Reproductive disorders

The pleiotropic roles of LPs in mammalian reproductive physiology and pathophysiology are demonstrated by: (i) the regulated expression of LP receptors [81]; (ii) the presence of LPA and ATX/lysoPLD in the follicular fluid [82]; (iii) the pro-survival effects of S1P in oocytes, including protection from apoptosis during chemotherapy and radiotherapy [83–85]; (iv) the temporally regulated increase of ATX/lysoPLD, PLA_2 and LPA levels in females in a pregnancy-dependent manner [52,60]; (v) the activation of parturition-dependent cellular processes by LPs *in vitro* [86] and *in vivo* [87]; and (vi) the delayed embryo implantation and aberrant embryo spacing revealed in LPA_3 null female mice [81] (Table 2).

Expression of LPA_3 is observed in the oviducts, placenta and uterus, but not in the ovaries, oocytes or embryos up to the pre-implantation blastocyst stage [81]. The delay in implantation phenotype observed in $lpa_3^{(-/-)}$ mice depends on maternal LPA_3 expression. This phenotype can be abrogated by the exogenous administration of prostaglandin E2 (PGE_2) or a stable analog of prostaglandin I2 (PGI_2) into $lpa_3^{(-/-)}$ pregnant mice, consistent with a role of cyclooxygenase 2 (COX_2) activity [88,89] downstream of LPA_3 activation [81]. The tight regulation of blastocyst-implantation rate and embryo spacing are also important concerns in human fertility, suggesting that LPA_3 activation in the uterine wall during implantation enhances fertility rate.

In males, LP receptor-activated signaling might also be important in fertility [90]. LP generation during the acrosomal reaction is crucial for spermatozoon-regulated exocytosis and membrane fusion with the oocyte in the fertilization process [60,91]. The therapeutic benefit of LP modulation of downstream receptors will depend on cell type and context. In mice with targeted deletions of LPP-1, an enzyme that dephosphorylates LPs, male genitalia (testes and Leydig cells) were severely atrophied, and spermatogenesis was severely disrupted [92]. These observations point to possible therapeutic avenues related to fertility involving LP signaling.

Obesity

Excessive adipose tissue accumulation is a key factor leading to type 2 diabetes and an increased risk of cardiovascular diseases [93]. Adipocytes regulate lipid and lipoprotein metabolism through several secreted products including LPA and ATX/lysoPLD [94]. The ratio of adipocyte precursors cells to differentiated adipocytes is tightly controlled in individuals of normal weight, and the proliferation and differentiation of pre-adipocytes is modulated by numerous factors including LPA [95–97]. A recent report suggests that peroxisome proliferator-activated receptor γ (PPARγ) is an intracellular receptor for LPA [98] that can bind to peroxisome proliferator response elements (PPREs) to activate gene expression. LPA displaced the PPARγ modulator rosiglitazone in binding studies using recombinant $PPAR\gamma_1$, but binding to $PPAR\gamma_2$ (another product from the same gene) was not evaluated in these studies [98]. The specificity and biological relevance of this observation remains unclear [2], particularly in view of the recently reported LPA_1-mediated downregulation of $PPAR\gamma_2$ in adipocytes [96]. Future studies might clarify possible direct or indirect interactions between LPA and PPARγ subtypes. In a pre-adipocyte cell line, LPA_1 activation downregulated PPARγ gene expression and inhibited triglyceride accumulation and adipocyte-specific gene expression [96], which are markers of adipogenesis. Furthermore, despite a lower body weight, LPA_1 null mice had more perigonadic adipose tissue than wild-type littermates and their adipose tissue contained more pre-adipocytes that could be differentiated in culture. These observations suggest that LPA_1 activation is anti-adipogenic. The therapeutic benefit of enhancing LPA_1 signaling in adipose tissue will depend on the redundancy of existing mechanisms to modulate adipose-tissue composition and the need to limit potentially deleterious side effects on other organ systems (Table 2).

Potential limitations associated with lysophospholipid receptor targeting

LP receptor modulation might enable therapeutic intervention in one or more diseases (Table 2). To provide the greatest clinical benefit, therapies must achieve selectivity of the pharmacological response and avoid side effects. This is a challenge for LP receptors, considering their widespread distribution in many cell types. Issues that need to be addressed in developing a drug to target LP receptors include: (i) determining whether efficacy involves single or multiple receptor subtypes; (ii) determining whether agonist or antagonist activities are desired; (iii) understanding hierarchies of LP receptor function both within this receptor family and among different activators of shared signaling pathways; and (iv) understanding the details of receptor function in different cells and tissues. The likely existence of new receptors adds to these challenges but the fact that all of the *bona*

fide LP receptors are GPCRs increases the likelihood that one or more of them might represent legitimate targets for therapeutic intervention.

Concluding remarks

A growing body of scientific literature and the entry of FTY720 into clinical trials support the idea of LP receptors and their signaling pathways as novel targets for therapeutic intervention (Table 2). Modulation of S1P receptors to reduce transplant rejection has been validated in humans using FTY720, and its usefulness in treating autoimmune diseases such as MS is under evaluation in Phase II clinical trials. In pharmacological studies using animal models considered to be clinically relevant, FTY720 and other specific modulators of LP receptors (e.g. Ki16425; Figure 1d) have revealed the potential usefulness of LP agonists and antagonists (Table 2). Targeted deletion of LP receptors, particularly in conjunction with the use of receptor agonists and/or antagonists in mice, has revealed roles for individual receptors in many disease models. Thus, loss of LPA_1 signaling has been reported to block the initiation of nerve injury-induced pain, produce a schizophrenic-like phenotype and alter the balance of adipocytes and their precursors in adipose tissue. Loss of LPA_3 has underscored the importance of LPA signaling in the timing and spacing of embryo implantation in fertility [81]. It should be noted, however, that LP signaling is widespread, at times redundant, and depends on cell type and context, and therefore the path to clinical development of LP-related compounds should be considered in its infancy. Nevertheless, the efficacy of the S1P agonist FTY720-P in humans raises the real possibility that LP receptors and their upstream and downstream signaling pathways might provide new approaches to the treatment of a range of human ailments.

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