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Review Article

Lysophospholipid receptors in drug discovery

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ABSTRACT

Lysophospholipids (LPs), including lysophosphatidic acid (LPA), sphingosine 1-phospate (S1P), lysophosphatidylinositol (LPI), and lysophosphatidylserine (LysoPS), are bioactive lipids that transduce signals through their specific cell-surface G protein-coupled receptors, LPA_{1-6}, $S1P_{1-5}$, LPI_1, and $LysoPS_{1-3}$, respectively. These LPs and their receptors have been implicated in both physiological and pathophysiological processes such as autoimmune diseases, neurodegenerative diseases, fibrosis, pain, cancer, inflammation, metabolic syndrome, bone formation, fertility, organismal development, and other effects on most organ systems. Advances in the LP receptor field have enabled the development of novel small molecules targeting LP receptors for several diseases. Most notably, fingolimod (FTY720, Gilenya, Novartis), an S1P receptor modulator, became the first FDA-approved medicine as an orally bioavailable drug for treating relapsing forms of multiple sclerosis. This success is currently being followed by multiple, mechanistically related compounds targeting S1P receptor subtypes, which are in various stages of clinical development. In addition, an LPA_1 antagonist, BMS-986020 (Bristol-Myers Squibb), is in Phase 2 clinical development for treating idiopathic pulmonary fibrosis, as a distinct compound, SAR100842 (Sanofi) for the treatment of systemic sclerosis and related fibrotic diseases. This review summarizes the current state of drug discovery in the LP receptor field.

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Contents

Abbreviations: LP, Lysophospholipid; LPA, lysophosphatidic acid; S1P, sphingosine 1-phospate; LPI, lysophosphatidylinositol; LysoPS, lysophosphatidylserine; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; LPG, lysophosphatidylglycerol; ATX, autotaxin; Sphk, sphingosine kinases; GPCR, G protein-coupled receptor; MS, multiple sclerosis; EAE, experimental autoimmune encephalomyelitis; NMO, neuromyelitis optica; IPF, idiopathic pulmonary fibrosis; FDA, Food and Drug Administration; EMA, European Medicines Agency; NLM ID, National Laboratory of Medicine Identifier.

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Introduction

Lysophospholipids (LPs) such as lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S1P) are a class of bioactive lipids [1,2], which have a phosphate head group and a single fatty acyl chain attached to a 3-carbon backbone in their chemical structures. LPs have a fairly long history dating back to the early 20th century, but the field has shown markedly accelerated growth in recent decades (Fig. 1A). LPA and S1P are the best studied LPs and play pivotal roles in physiological events including cell proliferation, survival, motility, cytoskeletal changes, and electrophysiological changes as well as pathophysiological processes that include autoimmune disease, fibrotic disease, cancer, inflammation, bone diseases, pain, metabolic syndrome, infertility, and hair loss. LPA is produced through several enzymatic pathways. Lysophospholipase D (known as autotaxin, ATX) liberates a choline group from lysophosphatidylcholine (LPC), whereas phospholipase A_1 and A_2 deacylate phosphatidic acid to produce 2-acyl and 1-acyl LPA, respectively [2]. Importantly, LPA is also *de novo* synthesized from glycerol-3-phosphate by the action of acyltransferases [3]. By comparison, S1P is produced by phosphorylation of sphingosine *via* the sphingosine kinases, Sphk1/2 [4].

LPs exert their effects by binding to specific G protein-coupled receptors (GPCRs) [5] that are the largest membrane receptor family in the human genome and contain nearly 800 receptors (including olfactory receptors) [6]. There are currently six LPA (LPA_{1-6}) and five S1P receptors ($S1P_{1-5}$) [2,7,8]. Recently, lysophosphatidylinositol (LPI) and lysophosphatidylserine (LysoPS) have been also shown to activate cognate GPCRs [9]. About 40 receptors within the 350 non-olfactory GPCRs have been identified as lipid GPCRs to date, of which ~40% are LP receptors (Fig. 1B). It is likely that additional receptors for LPLs may

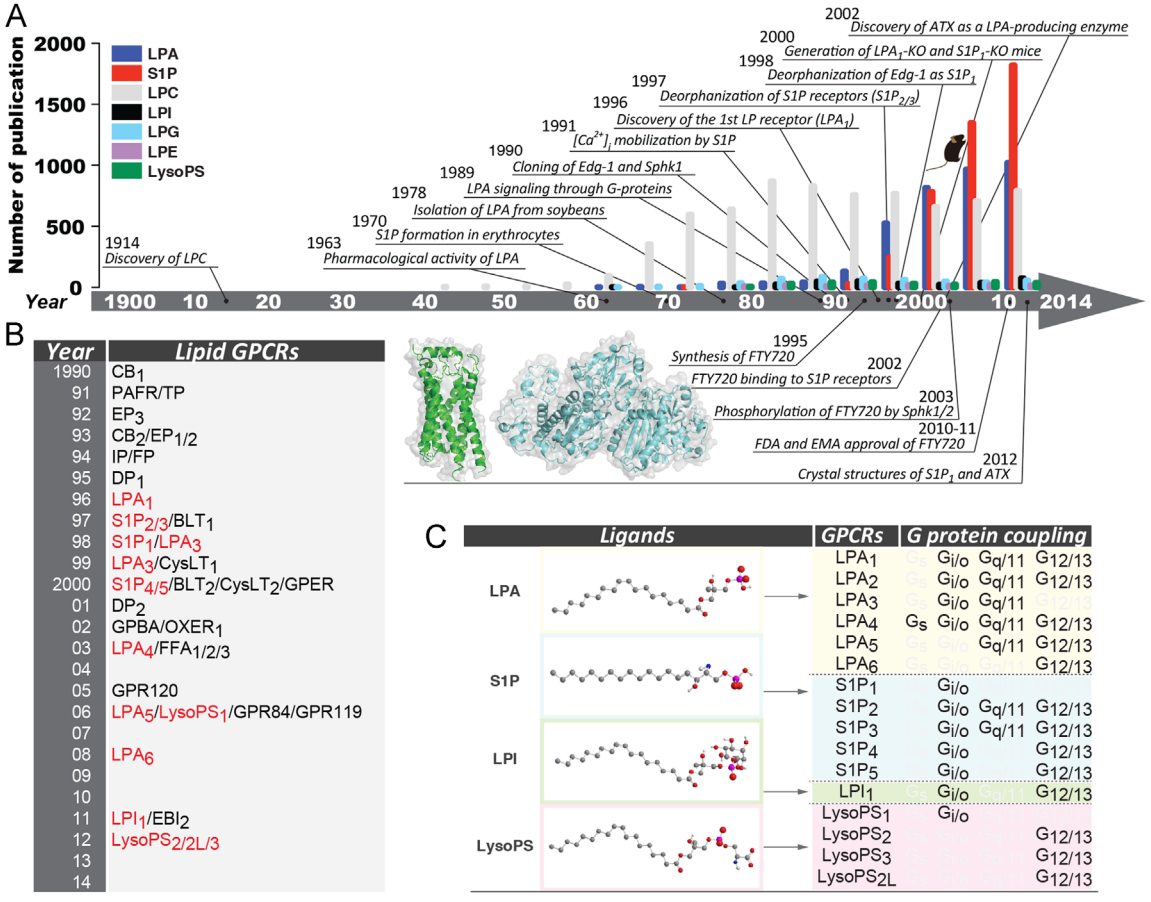

Fig. 1 – Chronology of the LP field, LP and other lipid receptors, and overview of proximal LP signaling features. (A) Chronology of the LP receptor field. Vertical bars indicate the number of publications within 5-year bins, which were searched in PubMed with unabbreviated names of the indicated keywords. (B) Chronological table for identification of lipid GPCRs. LP receptors are noted in red. (C) The chemical structures of LPs, GPCR names, and associated heterotrimeric G-proteins as defined by their Gα subunits.

be identified that interact with not only known receptor ligands but also other LPs such as lysophosphatidylethanolamine (LPE) and lysophosphatidylglycerol (LPG).

GPCRs remain a major target for drug discovery [10–12], and progress in the LP GPCR field has led to drug discovery efforts targeting LP receptors for a wide range of disorders, including the development of an actual medicine: fingolimod (FTY720, Gilenya) that was approved by the FDA for the treatment of multiple sclerosis (MS), and follow-up compounds targeting S1P receptors are being developed. Similarly, LPA signaling pathways are being explored and LPA_1 antagonism is currently being evaluated clinically as a novel and effective drug for treating fibrotic disease. Here, we briefly review the LP receptors and recent advances of drug discovery efforts to target LP receptors; recent reviews on additional aspects of LP receptors have been published for LPA receptors [2,7], S1P receptors [4,13], and other LP receptors [9,14].

Lysophospholipid receptors and signaling

The International Union of Basic and Clinical Pharmacology (IUPHAR) receptor nomenclature currently lists two LP receptor groups as class A GPCRs that are the LPA receptors (LPA_{1-6}) and the S1P receptors ($S1P_{1-5}$) [8]. Recent studies identifying other LP receptors for LPI (LPI_1) and LysoPS ($LysoPS_{1-3}$) [8] will add to the LP receptor family (provisional IUPHAR names are noted). Fig. 1C summarizes the G proteins that couple with LP GPCRs.

LPA receptors (LPA_{1-6})

A first LP receptor that recognizes LPA was reported in 1996 and is now called LPA_1 [15], and subsequently LPA_2 and LPA_3 were found by homology as reported in 1998 by Goetzl's group and in 1999 by Aoki's group, respectively (Fig. 1A) [16,17]. In 2003, Shimizu and co-workers discovered a fourth LPA receptor, LPA_4, from an orphan GPCR, GPR23/p2y9, that showed less than 20% amino acid sequence identity with LPA_{1-3} [18]. In 2006, we and others identified GPR92, which was another orphan GPCR closely related to LPA_4, as a fifth LPA receptor, LPA_5 [19,20]. In 2008, a P2Y family orphan GPCR, p2y5, was deorphanized as the latest LPA receptor, LPA_6 [21].

LPA_1 couples to $G_{\alpha i/o}$, $G_{\alpha q/11}$, and $G_{\alpha 12/13}$, resulting in LPA_1-dependent cellular responses such as cytoskeletal change, cell migration, adhesion, and Ca^{2+} mobilization [2,7,8]. Studies of mice lacking LPA_1 showed pathophysiological roles of LPA–LPA_1 axis in neural development, bone homeostasis, pain, hydrocephalus, and autoimmune disorders [2,5,7,22]. LPA_1 antagonists have been developed and are entering Phase 2 clinical trials to treat idiopathic pulmonary fibrosis and fibrotic diseases (discussed below). LPA_2 couples to multiple G-proteins including $G_{\alpha i/o}$, $G_{\alpha q/11}$, and $G_{\alpha 12/13}$ and is associated with cell migration and survival, cancer metastasis, neural development, and immune function [2,7,8]. LPA_3 transduces signaling through $G_{\alpha i/o}$ and $G_{\alpha q/11}$ and prefers 2-acyl-LPA containing unsaturated fatty acids. LPA_3 knockout mice showed a reproductive defect whereby embryo implantation was abnormal [23]. In addition, LPA_3 also regulates chemotaxis of immature dendritic cells and levels of pain [2,7,8]. LPA_4 couples to $G_{\alpha s}$, $G_{\alpha si/o}$, $G_{\alpha q/11}$ and $G_{\alpha 12/13}$ and is involved in neurite retraction, cell motility, cell aggregation and adhesion, osteoblast differentiation, and blood and lymphatic vessel formation [2,7,8]. A Japanese patent (2012-239450) indicates that LPA_4 mediates adipocyte differentiation and its removal improves insulin resistance, based upon LPA_4 knockout mice. LPA_5 can couple to $G_{\alpha q/11}$ and $G_{\alpha 12/13}$, and increases cAMP accumulation *via* a non-$G_{\alpha s}$ mechanism [20]. LPA_5 regulates neuropathic pain [24] and immune function [25,26]. LPA_6 utilizes $G_{\alpha 12/13}$ for transducing signals through the Rho pathway and it has some preference for 2-acyl-LPA rather than 1-acyl-LPA [27]. LPA_6 was discovered as an autosomal dominant genetic factor from patients with hypotrichosis simplex that is characterized by familial hair loss [21]. Genetics and *in vitro* studies support the idea that PA-$PLA_{1\alpha}$ was required to activate LPA_6 by generating 2-acyl-LPA [28].

S1P receptors ($S1P_{1-5}$)

Hla and Macaig discovered an orphan GPCR, endothelial differentiation gene-1 (Edg-1), by a differential display method in 1990 [29], and Hla and co-workers identified it as an S1P receptor in 1998 based on the sequence homology with LPA_1/Edg-2/Vzg-1 [30], which is now designated as $S1P_1$. In 1997, two orphan GPCRs (Edg-5 and Edg-3, now called $S1P_2$ and $S1P_3$, respectively) were deorphanized as S1P receptors by Goetzl and co-workers [31]. In 2000, the other two orphan GPCRs (Edg-6 and Edg-8), which show a highly conserved amino acid sequence with $S1P_{1/2/3}$, were reported as S1P receptors $S1P_4$ and $S1P_5$, respectively [32,33].

$S1P_1$ couples to $G_{\alpha i/o}$ and induces intracellular signaling such as inhibition of cyclic AMP accumulation, intracellular Ca^{2+} increase, MAPK activation, small GTPase Rac activation, and likely other signals. $S1P_1$ knockout mice [34] identified important roles of $S1P_1$ in vascular and cardiac morphogenesis. Studies using genetic and pharmacological approaches have established that $S1P_1$ regulates cell migration and lymphocyte trafficking in both homeostatic and disease settings [35], and is a major target of drug discovery as evidenced by fingolimod, which is discussed below. $S1P_2$ preferably utilizes $G_{\alpha 12/13}$ for activating Rho signaling, while coupling to multiple G-proteins including $G_{\alpha i/o}$ and $G_{\alpha q/11}$. $S1P_2$ abrogates motility by inhibiting Rac activity. Indeed, Cyster and co-workers demonstrated that B cells are retained in the germinal centers of lymphoid follicles through the S1P–$S1P_2$ axis [36]. $S1P_2$ is reported to play important roles in vascular development and remodeling, cardiovascular function, bone maintenance, inner ear function, and metastasis [4,7,8,13]. $S1P_3$ couples to $G_{\alpha i/o}$, $G_{\alpha q/11}$, and $G_{\alpha 12/13}$. No obvious phenotype or developmental defects were observed in $S1P_3$ knockout mice [37]. $S1P_{2/3}$ double knockout mice have reduced fertility [38], indicating the existence of redundancy among S1P receptors. S1P–$S1P_3$ signaling may be relevant to several diseases including breast cancer, sepsis, and liver fibrosis [4,7,8,13]. $S1P_4$ transduces signaling through $G_{\alpha i/o}$ and $G_{\alpha 12/13}$. This receptor is most prominent in adult hematopoietic tissues and is thought to promote cell migration. Studies in $S1P_4$ knockout mice showed pivotal roles in thrombopoiesis, neutrophil migration, and T_H17 differentiation [4,7,8,13]. $S1P_5$ can couple to $G_{\alpha i/o}$ and $G_{\alpha 12/13}$ and considering its common roles in inhibition of cell migration and promotion of cell process retraction, it may preferentially couple to $G_{\alpha 12/13}$. $S1P_5$ plays important roles in lymphocyte trafficking and NK cell differentiation [4,7,8,13]. $S1P_5$ has yet-to-be determined functions in the central nervous system (CNS) in view of its expression in oligodendrocytes [39,40].

Other lysophospholipid receptors (LPI_1 and $LysoPS_{1-3}$)

GPR55 was first reported as an LPI receptor in 2007 [41], although it was thought at the time to be a cannabinoid receptor. Other studies support its identity as an LPI receptor [14], leading to its provisional IUPHAR name [8]. LPI_1 couples with $G\alpha_{13}$ and transduces signals to the RhoA–ROCK pathway [9,14]. Studies of mice lacking *Lpir1* gene identified effects on bone homeostasis, pain, and autoimmune disorders [9,14].

The first LysoPS receptor, GPR34, was reported in 2006 [42] and confirmed by Aoki and co-workers who identified 2-acyl-LysoPS as a preferred ligand over 1-acyl-LysoPS [43]. They developed a novel TGFα shedding assay that detects GPCR signaling [44]. This assay validated the first LysoPS receptor and identified additional members, leading to the proposed IUPHAR names designed to avoid confusion between the well-known abbreviation LPS for lipopolysaccharides [8]: GPR34 as $LysoPS_1$, P2Y10 as $LysoPS_2$, A630033H20 as $LysoPS_{2L}$, and GPR174 as $LysoPS_3$. Further studies are necessary for understanding the roles of LysoPS–LysoPSx axis.

Drug discovery targeting S1P receptors

The most important success of drug discovery in the LP receptor field has been fingolimod (also known as FTY720) (Fig. 2A) [45]. In 1995, fingolimod was first synthesized from an immunosuppressive natural product, myriocin (ISP-I), that was isolated from culture broths of *Isaria sinclairii* [45]. As expected, oral administration of fingolimod prevents rodents from exhibiting symptoms in the allograft model [45]. In addition, fingolimod sequesters circulating lymphocytes, resulting in decreased peripheral blood lymphocyte count and increased numbers of lymphocytes in the secondary lymphoid organs [45]. However, the molecular basis of fingolimod activities was not demonstrated until 2002, when two groups independently proposed that fingolimod interacts with S1P receptors [46,47] through a phosphorylated metabolite of FTY720, FTY720-P, which binds to $S1P_1$, $S1P_3$, $S1P_4$, and $S1P_5$ as a high affinity agonist. Fingolimod was reported to be phosphorylated *in vivo* by S1P-generating enzyme, Sphk1 or particularly Sphk2 [48]. Paradoxically, fingolimod activity on lymphocytes involves not agonist but rather functional antagonist activity against $S1P_1$, revealed by phenocopying $S1P_1$ loss in lymphocytes [49]. FTY720-P downregulates $S1P_1$ on the cell surface by sustained internalization and subsequent degradation of the receptor [50,51], explaining reductions of lymphocyte egress from secondary lymphoid organs, thymus, and bone marrow [45]. In 2002, Brinkmann et al. first reported the prophylactic effects of fingolimod on an animal model of MS, experimental autoimmune encephalomyelitis (EAE) [46]. Moreover, Webb et al. demonstrated the therapeutic use of fingolimod on relapsing–remitting EAE in SJL mice [52]. These studies indicated that fingolimod had the potential for treating autoimmune diseases like MS. Fig. 2B illustrates the possible actions of fingolimod in MS.

Because of its hoped-for immunosuppressive effects, fingolimod was first tested in humans as an agent to improve renal transplantation [53]. However, it failed to reach its clinical endpoints even as an adjunctive agent at high dosage combined with cyclosporine for preventing renal allograft rejection in two Phase 3 studies, and was accompanied by adverse events [53], resulting in termination of the renal transplantation program. However, evidence from animal models of MS suggested fingolimod utility in MS, and in 2006, Phase 2 clinical data supported fingolimod use in MS [54]. In 2010, fingolimod (Gilenya, Novartis) received Food and Drug Administration (FDA) approval in the U.S. as a first-line agent for the treatment of relapsing forms of MS, followed by European Medicines Agency (EMA) approval in 2011 [55]. A 2014 Phase 3 extension study (FREEDOMS II) report confirmed the beneficial effects of fingolimod on relapse rates in MS patients and showed a consistent safety profile [56]. Fingolimod was ranked in the top 100 prescribed medicines by U.S. National Sales in the 4th quarter 2013 [57]. On the other side of the coin, fingolimod has potential side effects [58] that will need to be assessed, as well as in other agents targeting S1P receptors.

S1P receptor-targeted drugs, including fingolimod, are in clinical trials for treatment of several diseases including MS (Fig. 2A), ulcerative colitis, dermatomyositis, along with proof-of-concept evaluations in other disorders like amyotrophic lateral sclerosis (ALS) and stroke. Newer compounds are designed to have receptor subtype selectivity, such as $S1P_1/S1P_5$ specificity (siponimod, ponesimod, and RPC1063) and $S1P_1$ specificity (KRP-203 and GSK2018682). Considering the success of fingolimod, these next-generation compounds may exhibit improved efficacy and/or fewer side effects for a range of possible therapeutic indications.

Drug discovery targeting LPA receptors

Currently, no drugs targeting LPA receptors have been approved by any regulatory agency, although advances in the LP field support therapeutic relevance for a range of diseases including fibrotic diseases of the skin, lung, kidney, and liver (Fig. 2C). One disease that may benefit from LPA receptor modulation is idiopathic pulmonary fibrosis (IPF) which is characterized by interstitial infiltrates in lung bases, progressive dyspnea, and worsening of pulmonary function [59], and is associated with high mortality rates and thus represents an unmet medical need. In 2008, Tager et al. identified receptor-mediated LPA signaling as an important mechanism in lung fibrosis [60]. In a mouse model of bleomycin-induced lung fibrosis, LPA concentrations in bronchoalveolar lavage rose to 400 nM at 14 days after challenge. LPA_1 knockout mice were significantly protected from bleomycin-induced fibrosis based upon reduced mortality, fibroblast and leukocyte recruitment, and vascular leakage. In 2010, Amira Pharmaceuticals reported that a potent and orally bioavailable LPA_1 antagonist, AM966, was beneficial in treating bleomycin-induced lung fibrosis [61]. In 2011, Bristol-Myers Squibb acquired Amira Pharamaceuticals and had completed Phase 1 clinical trials of an LPA_1 antagonist, BMS-986202 (previously AM152). In 2014, another LPA_1 antagonist, BMS-986020, began Phase 2 clinical trials to test its efficacy on IPF (NLM ID: NCT01766817).

Another fibrotic disease is systemic sclerosis (scleroderma), which is a chronic connective tissue disease characterized by vasculopathy, autoimmunity, and extensive fibrosis [62]. Tokumura et al. reported elevated levels of 2-arachidonoyl-LPA (as well as S1P) in sera of systemic sclerosis patients [63]. In 2011, Tager and co-workers revealed that LPA_1 knockout mice were protected in a mouse model of scleroderma [64], suggesting that LPA_1 antagonists may be effective for treating systemic sclerosis. Sanofi

A

Drugs	Targets	Indication	Stage	Companies	NLM ID
SAR100842	LPA_1	Systemic sclerosis	Phase 2	Sanofi	NCT01651143
BMS-986020	LPA_1	IPF	Phase 2	Bristol-Myers Squibb	NCT01766817
Gilenya (Fingolimod)	$S1P_{1/3/4/5}$	Relapsing-remitting MS	Approved	Novartis	
		CIDP	Phase 3		NCT01625182
BAF312 (Siponimod)	$S1P_{1/5}$	Secondary progressive MS	Phase 3	Novartis	NCT01665144
		Polymyositis	Phase 2		NCT01801917
		Active dermatomyositis	Phase 2		NCT02029274
KRP-203	$S1P_1$	Moderately active refractory ulcerative colitis	Phase 2	Novartis	NCT01375179
		Hematological malignancies	Phase 1		NCT01830010
Ponesimod	$S1P_{1/5}$	Relapsing-remitting MS	Phase 2	Actelion	NCT01093326
		Psoriasis	Phase 2		NCT01208090
GSK2018682	$S1P_1$	Relapsing-remitting MS	Phase 1	GlaxoSmithKline	NCT01431937
RPC1063	$S1P_{1/5}$	Relapsing-remitting MS	Phase 3	Receptos	NCT02047734
		Ulcerative colitis	Phase 2		NCT01647516

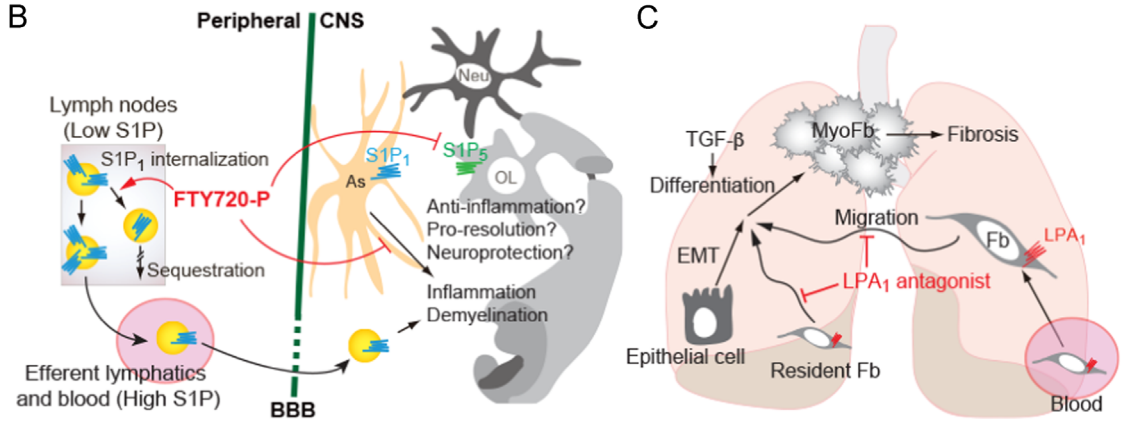

Fig. 2 – Disease mechanisms being accessed by LP-based drug discovery and compounds in clinical development. (A) LP receptor-based compounds currently in clinical trials. NLM, National Laboratory of Medicine; IPF, idiopathic pulmonary fibrosis; CIDP, chronic inflammatory demyelinating polyneuropathy. (B) Multiple sclerosis pathology and actions of FTY720. Lymphocytes expressing $S1P_1$ migrate from lymph nodes to blood based on S1P concentration differences. FTY720 redistributes these lymphocytes into lymph nodes (*i.e.*, secondary lymphoid organs), resulting in reduced entry of pathogenic lymphocytes into the CNS. FTY720 also inhibits astrocyte function, may alter oligodendrocyte function *via* $S1P_5$ and results in reduced inflammation, demyelination, with possibly other beneficial functions. As astrocytes; BBB, blood brain barrier; CNS, central nervous system; Neu, neuron; OL, oligodendrocyte. (C) IPF pathology and accessed mechanisms for an LPA_1 antagonist. Circulating and resident fibroblasts (Fb) are recruited to the injured site, which may be interrupted by LPA_1 antagonists. Epithelial cells differentiate into fibroblasts by epithelial mesenchymal transition (EMT). These fibroblasts differentiate into myofibroblasts (MyoFb), which accelerate fibrosis and may also be inhibited by LPA_1 antagonists.

has entered Phase 2 clinical trials of an $LPA_{1/3}$ inhibitor, SAR100842, for systemic sclerosis (NLM ID, NCT01651143) with results suggesting efficacy but that require validation in larger clinical trials [65].

Conclusions

The LP field has advanced with an expanding repertoire of both receptors and ligands. Fingolimod's entry into the treatment of MS has established lysophospholipid signaling as a clinically validated molecular pathway, raising prospects for novel therapeutics in a number of medically important therapeutic areas. Basic studies on LP receptor signaling mechanisms and their relationship with normal physiological and pathophysiological processes should continue to expand the development of novel therapeutics.

Conflict of interest

Y.K. declares no competing financial interests. H.M. is an employee of Ono Pharmaceuticals.

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