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REVIEW

Sphingosine-1-phosphate antibodies as potential agents in the treatment of cancer and age-related macular degeneration

Roger A. Sabbadini

Lpath, Inc., and Department of Biology, San Diego State University, San Diego, CA, USA

Correspondence
Roger A. Sabbadini, PhD, Lpath, Inc., 6335 Ferris Square, Suite A, San Diego, CA 92121, USA.
E-mail: rsabbadini@lpath.com

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Sphingosine-1-phosphate (S1P) is a pleiotropic bioactive lipid thought to be dysregulated in a variety of disease conditions. In this review, we discuss the roles of S1P in cancer and in wet age-related macular degeneration. We also explore potential treatment strategies for these disorders, including the utility of anti-S1P antibodies acting as molecular sponges to neutralize dysregulated S1P in relevant tissues.

Abbreviations
AMD, age-related macular degeneration; CNV, choroidal neovascularization; GLP, good laboratory practices; NHP, non-human primate; PED, pigmented epithelial detachment; RPE, retinal pigmented epithelium; S1P, sphingosine-1-phosphate; SphK, sphingosine kinase

Sphingosine-1-phosphate is an attractive target for drug discovery

Bioactive lipids are important signalling mediators that are becoming attractive targets for drug discovery because of their roles in cancer, inflammation and other pathological conditions. Examples of such bioactive lipids include: (i) eicosanoids (such as the thromboxanes and leukotrienes); (ii) phospholipids and their lysophospholipid derivatives such as platelet activating factor (PAF) and lysophosphatidic acid; and (iii) sphingolipids such as sphingosine-1-phosphate (S1P). In recent years, receptors and other targets have been discovered for many of these bioactive lipids, suggesting extracellular signalling roles for these lipid mediators and growth factors (Im, 2009). One difficulty in targeting proteins that are responsible for their dysregulation in disease is that there are commonly several biosynthetic pathways for a particular bioactive lipid. Equally challenging is that there are commonly several receptors, ion channels or other proteins responsible for the action of a particular bioactive lipid and, in some cases, not all of the receptors that have been elucidated as novel receptors continue to appear in the literature (Im, 2009).

Of the 1000 or so bioactive lipids, sphingolipids are recognized as important intercellular and intracellular signalling molecules participating in physiological and pathological processes associated with cellular survival, proliferation, differentiation and adhesion function (Moolenaar, 1999; Goetzl *et al.*, 2002; Birgbauer and Chun, 2006; Gardell *et al.*, 2006). The key elements of the sphingomyelin-associated signalling pathway include the bioactive lipid mediators, ceramide (CER), sphingosine (SPH) and S1P.

The most celebrated sphingolipid mediator is S1P whose well-documented pleiotropic biological activities are mediated via a family of G protein-coupled cell surface receptors (GPCRs) belonging to the family of endothelial differentiation genes (EDG). These high-affinity receptors are $S1P_{1\text{-}5}$/EDG-1,3,5,6 and 8 and are coupled to heterotrimeric G-proteins ($G_{i/o}$, $G_{q\text{-}}$, $G_{i\text{-}}$, $G_{12\text{-}13}$) and small GTPases of the Rho family (Anliker and Chun, 2004). Most of the

growth-promoting actions of S1P described in multiple organ systems, including immune, inflammation and cardiovascular, are exerted by S1P's action on its cognate GPCRs.

The major source of S1P is that produced from SPH through the action of sphingosine kinase (SphK) (Taha *et al.*, 2006). Two isoforms of the kinase have been identified, SphK1 and SphK2. Sphingosine kinase controls a 'ceramide-S1P rheostat' that decides whether a cell is sent into the death pathway (via CER or SPH) or is protected from apoptosis by S1P (Cuvillier *et al.*, 1996). For example, increasing ceramide/S1P ratios makes cancer and other hyperproliferative cells more sensitive to apoptosis. Similarly, decreasing the S1P level, either by inhibiting SphK or removing S1P, diminishes cell proliferation and tumour-associated angiogenesis and makes cancer cells more susceptible to apoptotic cell death (Anliker and Chun, 2004; Colombaioni and Garcia-Gil, 2004; Hla, 2004; Hait *et al.*, 2006).

There is a reservoir of S1P stored in and potentially released from red blood cells, platelets and mast cells to create a local pulse of free S1P sufficient enough to exceed the K_d of the S1PRs (e.g. K_d for $S1P_1$ = 20 nM), whereby wound healing and inflammation are promoted (Murata *et al.*, 2000a). Under normal conditions, the total S1P in the human plasma is high (300–500 nM), far exceeding the K_d for the receptors. However, it has been hypothesized that most S1P may be 'buffered' by serum proteins, particularly lipoproteins [e.g. high-density lipoprotein (HDL) > low-density-lipoprotein (LDL) > very low-density lipoprotein] and albumin, so that the bio-available S1P (1–2% of total) is not sufficient to activate S1P receptors (Murata *et al.*, 2000a,b). If this were not the case, inappropriate cardiovascular effects, angiogenesis and inflammation would result as suggested by others (Murata *et al.*, 2000b; Yatomi, 2006).

While we and others have initially focused on the importance of plasma S1P levels as mirroring what might be happening in the tumour microenvironment, it is clear from the work with FTY-720 and lyase inhibitors that interstitial fluid tissue levels of S1P might be the culprit. For example, SphK1 knockout mice have increased vascular permeability while experiencing no alterations in plasma S1P levels (Li *et al.*, 2008b). One view is that plasma S1P is mostly 'buffered' and not biologically active unless S1P-binding proteins such as HDLs are able to present S1P to S1P receptors, possibly via ligand passing via Srb1-S1PR contact (Lucke and Levkau, 2010). It is also not known whether or not substantial amounts of S1P are associated with cell membranes other than RBCs (Bode *et al.*, 2010) or in tissue lipid microenvironments. While SphK is ubiquitously expressed in tissues, the concentration of biologically active S1P in the interstitial compartment in local tissues is not known. It is generally believed that bioactive or free S1P can be dysregulated in cancer and inflammatory disorders such that the tissue levels of biologically active S1P can then exert autocrine and paracrine actions. In such cases, S1P can become harmful and can contribute to disease processes such as cancer and inappropriate angiogenesis (such as in exudative age-related macular degeneration or AMD). The next two sections of this review will concentrate on the role of dysregulated S1P in cancer and AMD as examples. Further, the merits of using anti-S1P antibodies to neutralize dysregulated extracellular S1P will be addressed for these two diseases, as anti-S1P antibodies are currently being tested in clinical trials.

S1P and cancer

Sphingosine kinase and the S1P receptors: roles in cancer

Because of the important role of sphingolipids in cancer progression, it has been argued that sphingolipid-based therapeutics will be the next generation of cancer treatments (Milstien and Spiegel, 2006; Fyrst and Saba, 2010; Pyne and Pyne, 2010). This view comes from findings that cancer cells exploit the sphingolipid rheostat by promoting conditions that favour the production of S1P through an up-regulation of SphK1, the isoform that is thought to be responsible for the release of S1P into the extracellular compartment (see comprehensive review by Pyne and Pyne, 2010). The ability of cancer cells to release S1P into the tumour microenvironment promotes the infiltration of platelets, fibroblasts, mast cells, endothelial cells (ECs) and neutrophils, resulting in an inflammatory response and tumour angiogenesis. The infiltrating cells cause further release of S1P into the tumour microenvironment, with the resulting manifestation of tumourigenic and pro-angiogenic effects of S1P.

The S1P produced by SphK1 has been established as a general growth-like factor and a potent protector against apoptosis caused by cytotoxics. The S1P produced promotes cellular proliferation, migration and protection from apoptosis (An *et al.*, 1998; Maceyka *et al.*, 2002; Radeff-Huang *et al.*, 2004). S1P is also able to promote angiogenesis, the formation of new blood vessels, which are presumed to feed the growing tumour (Sabbadini, 2006; Teicher, 2011). Furthermore, it has been shown that the *sphk1* gene is over-expressed in several tumour types and, as a result, directs attention to the kinase as a protein target for anti-cancer drug discovery (Milstien and Spiegel, 2006; Shida *et al.*, 2008b).

NIH-3T3 fibroblasts and HEK 293 cells transfected with the kinase exhibited enhanced cell proliferation and protection from apoptosis accompanied by increased cellular S1P production (Olivera *et al.*, 1999). In addition, the SphK1 over-expressers escaped contact inhibition, a property commonly exhibited by transformed cells. This observation is consistent with recent reports showing that S1P enhances metastatic potential of selected human cancer cell lines (Takuwa, 2002). Moreover, the SphK1 transfectants produced tumours when injected subcutaneously into non-obese diabetic/severe combined immune deficiency (SCID) mice (Xia *et al.*, 2000). These results were recently confirmed in two studies showing that small molecule inhibitors of SphK1 could reduce tumour volumes in SCID mice grafted with either subcutaneous injections of JC mammary adenocarcinoma cells (French *et al.*, 2003) or of human histocytic leukaemia U937 cells (Paugh *et al.*, 2008). In addition, it has been demonstrated that several human tumour-derived cell lines became apoptotic when treated with the SphK1 small molecule inhibitors, and that their effectiveness could be accounted for by their abilities to reduce S1P levels (Bektas *et al.*, 2005a). SphK1 has been shown to be over-expressed in many solid tumours, such as those of the breast, colon, lung, ovary, stomach, uterus,

kidney and rectum (French *et al.*, 2003; Johnson *et al.*, 2005; Kawamori *et al.*, 2006; Shida *et al.*, 2008a). Increased expression of the SphK1 in tumour samples has been correlated with a significant decrease in survival rate in patients with several forms of cancer (Van Brocklyn *et al.*, 2005; Ruckhaberle *et al.*, 2008; Li *et al.*, 2008a; 2009; Facchinetti *et al.*, 2010; Long *et al.*, 2010). However, the situation is predictably more complicated in breast cancer in that the expression and/or activity or SphK1 and its correlation with patient survival can depend on ER/HER status such that SphK1 can reverse its role and become protective (Long *et al.*, 2010). For example, when one stratifies breast cancer patients, one finds that ER+ patients who have low HER1-3/SphK1 expression ratios, survive longer. This is in contrast to the inverse correlation between SphK1 and survival in unstratified breast cancer patient populations (Long *et al.*, 2010). Certainly, there are other factors, including a potential role for SphK2, which conspire to influence time to disease progression.

SphK1 can confer resistance to cytotoxic agents and other therapies

It is widely appreciated that cancer cells are particularly successful in escaping therapy by adapting themselves to the tumour microenvironment and by mutating and evolving such that they can become resistant to cytotoxics or anti-angiogenic agents. The up-regulation of the oncogene, *sphk1*, and the resulting release of S1P into the tumour microenvironment could represent an important way cancer cells become resistance to treatment (recently reviewed by Raguz and Yague, 2008; Cuvillier et al., 2010). There is a growing consensus that individual patient genomics/proteomics profiling and biomarker evaluation will eventually identify which drug resistance mechanism is responsible for a patient's tumour progression. Thus, it may eventually be possible to identify some patients whose resistance can be attributed to an overproduction of S1P**.** While over-expression of SphK1 has been demonstrated in many cancer types and is correlated with patient survival (see above), a direct connection between SphK1 up-regulation and drug resistance has only recently been established, as reviewed by Cuvillier et al. (2010).

One potential mechanism explaining how chemoresistance can be established is the case of the S1P/hypoxia-inducible transcription factor (HIF) axis where the response of cancer cells to hypoxia involves the up-regulation of the S1P/SphK system. The S1P/HIF axis was the subject of a recent review by Ader *et al.* (2009).

Targeting extracellular S1P might be an optimum strategy for cancer

The overwhelming body of evidence so far implicates the type 1 isoform of SphK in chemoresistance and in the enhanced tumourigenicity adaptations to hypoxia, all of which correlate with poor patient outcomes in several cancer types. Thus, SphK1 is a very attractive target for drug discovery. Along the path towards the full validation of SphK1 as a cancer target, several papers have recently appeared demonstrating the efficacy of various SphK inhibitors in delaying the progression of xenografted human tumours in mice. For example, the kinase inhibitor, SK1-II, showed significant retardation of tumour growth of JC mammary adenocarcinoma cell allografts (French *et al.*, 2003); the SK1-I (BML-258) SphK1-specific inhibitor reduced tumour growth and improved survival in glioblastoma xenograft models employing orthotopically placed LN229-H2B-GFP cells (Kapitonov *et al.*, 2009) and in AML xenograft models using human U-297 cells (Paugh *et al.*, 2008); the SphK1 inhibitor, B-5354c, reduced tumour progression in orthotopically implanted hormone-resistant PC3-GFP cells (Pchejetski *et al.*, 2008). SphK1 is thought to be translocated to the surface membrane and to produce/release the extracellular S1P that is tumourigenic and angiogenic. There is also evidence that targeting SphK2 with a small molecule inhibitor, ABC294640, specific for this isoform can delay tumour progression in xenograft models of human renal cell cancer using A498 cells implanted subcutaneously in SCID mice (Beljanski *et al.*, 2010) and in JC mammary allografts (French *et al.*, 2006). The latter two findings with SphK2 inhibitors complicate the otherwise simple story that pointed to SphK1 as the single appropriate cancer target (Maceyka *et al.*, 2005).

It has been argued that the two isoforms, SphK1 and SphK2, may have opposite actions with regard to cancer cell growth (Maceyka *et al.*, 2005). If this is the case, an SphK-targeted anti-cancer therapeutic would have to selectively inhibit SphK1 and not SphK2. This may prove to be difficult, as both enzymes have five highly conserved domains and high-percent identity in their amino acid sequences. In addition, several splice variants of both isoforms have been characterized. Additionally, an acylglycerol kinase with homology to SphK1 has been recently identified (Bektas *et al.*, 2005b), thus further complicating target selectivity of SphK-directed agents. Furthermore, other SphK-independent sources of S1P have been suggested. One such player is the ectoenzyme, autotaxin (ATX), which is capable of producing S1P from sphingosylphosphoryl choline (Clair *et al.*, 2003). Potential additional mechanisms to generate S1P, like ATX or SphK2, may explain why *sphk1*-null mice exhibited no significant changes in tissue S1P levels even though tissue SphK1 activity was nearly eliminated (Allende *et al.*, 2004). Consequently, selectively blocking only SphK1 activity may not mitigate S1P production by other routes.

Another intervention point in the sphingolipid pathway is to prevent S1P from interacting with its G-protein coupled receptors. The majority of pro-tumourigenic, angiogenic and metastatic actions of S1P are mediated through these receptors; however, intracellular roles for S1P have recently been demonstrated such as histone deacetylases 1 and 2 (Hait *et al.*, 2009) as well as TRAF2 (Alvarez *et al.*, 2010). There are five isoforms of the S1P receptor named $S1P_{1\text{-}5}$ (Sanchez and Hla, 2004). $S1P_{1\text{-}3}$ are usually expressed ubiquitously, while $S1P_4$ and $S1P_5$ are mainly expressed in the lymphatic system and the central nervous system respectively (Sanchez and Hla, 2004). Treatment with the Novartis compound, FTY720 (fingolimod), a functional antagonist (i.e. reverse agonist) of S1P receptors, reduces tumour growth and tumour-associated angiogenesis in a variety of animal models of human cancer (Ho *et al.*, 2005; Schmid *et al.*, 2005; Neviani *et al.*, 2007; Liu *et al.*, 2008; Lucas da Silva *et al.*, 2008; Silva, 2008). It is believed that FTY720 acts as an antagonist of $S1P_1$, $S1P_3$, $S1P_4$ and $S1P_5$ but not $S1P_2$. In particular, FTY720 causes internalization of the receptors, thus acting as reverse agonists/functional antagonist. As such, FTY720 has been shown to

induce apoptosis in multiple myeloma cells (Yasui and Palade, 1995), inhibit angiogenesis and reduce tumour vascularization (Lamontagne *et al.*, 2006).

It must also be appreciated that some S1P receptors have opposite actions. For example, $S1P_1$ and $S1P_3$ are primarily responsible for the pro-migratory and angiogenic effects of S1P (Chae *et al.*, 2004; Langlois *et al.*, 2004). Conversely, $S1P_2$ has been demonstrated to have the opposite effects on cell migration and angiogenesis (Goparaju *et al.*, 2005; Skoura and Hla, 2009). In the B16-F10 melanoma cell line, which exclusively expresses $S1P_2$, S1P stimulation has no effect on proliferation but inhibits cell migration *in vitro* (Arikawa *et al.*, 2003). S1P-mediated inhibition of migration by U118 and U138 cells correlated with $S1P_2$ expression (Lepley *et al.*, 2005). The over-expression of $S1P_2$ in these cell lines further enhanced the suppression of migration upon S1P stimulus, while a down-regulation of $S1P_2$ by RNA interference reversed this inhibitory effect (Lepley *et al.*, 2005). The literature shows that each cancer cell lineage has a unique pattern of S1PR expression that varies inconsistently not only among cancer types but also among patients within a cancer type. So, targeting an individual S1P receptor becomes problematic and is not likely to be a viable anti-S1P drug discovery strategy for cancer unless all receptors are blocked.

A more direct approach, which avoids all of the said limitations, is the prevention of ligand binding to all cognate receptors using an anti-S1P monoclonal antibody (mAb). Preclinical data with the murine variant of the anti-S1P mAb (Sphingomab™ or LT1002) demonstrate that this approach deprives growing tumour cells of important growth and survival factors and largely prevents tumour angiogenesis (Visentin *et al.*, 2006). Considerable experimental data suggest that preventing the action of extracellular S1P could be an effective therapeutic approach for targeting tumour cells and tumour-associated vasculature.

It remains to be determined whether or not absorbing extracellular S1P with a mAb can influence intracellular pools such that the mAb can pull S1P from the cell. This is unlikely as aggressive dosing of both murine and humanized mAbs in good laboratory practice (GLP) toxicology studies failed to elicit serious adverse events such as those one might expect to see if all sources of S1P (including intracellular) were neutralized by the mAb. If that had happened, mice and monkeys would have experienced serious adverse events stemming from increased vascular permeability. Mice with conditional deletion of both SphK1 and SphK2 [*Sphk1fl/–:Sphk2–/–:Mx1Cre+* (pS1Pless mice)] experience profound increases in vascular permeability (and death) after PAF and histamine challenge (Camerer *et al.*, 2009).

It is not known what role, if any, membrane-bound S1P might have in the tumour microenvironment or in other disorders where S1P is dysregulated. It is clear that the mAbs are able to extract/release membrane-associated S1P from RBCs *in vitro* (Bode *et al.*, 2010) as they do from plasma proteins such as HDLs, LDLs and serum albumin (R. Sabbadini, unpubl. obs.). Using surface plasmon resonance, the affinities of full-length anti-S1P mAbs were found to be high, in the 0.03–0.06 nM range (O'Brien *et al.*, 2009) or 1.1 nM for the Fab'(Wojciak *et al.*, 2009), while HDLs LDLs and albumin were not measurable under identical conditions (R. Sabbadini, unpubl. obs.).

The anti-S1P approach has distinct advantages over 'singly-targeted' therapeutics in that S1P not only has its own dual effects (tumorigenic and angiogenic) which should be neutralized, but is also permissive in promoting the actions and/or release of other important growth factors [e.g. IL-8, IL-6, matrix metalloproteinases, platelet-derived growth factor (PDGF), transforming growth factor β (TGFβ), vascular endothelial growth factor (VEGF), etc.]. An additional advantage of targeting S1P is that, unlike protein targets that can exist in multiple isoforms and/or splice variants, S1P has a single molecular structure that is conserved among species, making it both an attractive therapeutic target and one in which animal data may be more translatable to human diseases.

Anti-S1P antibody as a potential therapeutic

The murine anti-S1P, Sphingomab, was humanized for clinical development by grafting the complementarity determining regions onto a human IgG1κ framework and was then optimized to retain the specificity and affinity characteristics of the murine mAb (O'Brien *et al.*, 2009). This humanized, optimized antibody is referred to as sonepcizumab ('S-one-P' cizumab) or LT1009. The crystal structure of the Fab' fragment was resolved to 1.9 Angstrom resolution, demonstrating that the hypervariable domains of the Fab' interact with the ligand, S1P, in a manner predicted by site-directed mutagenesis studies (Wojciak *et al.*, 2009).

In anticipation of the Phase I clinical studies, the strength and nature of the binding of sonepcizumab to S1P was determined in order to determine the performance characteristics of the antibody and to evaluate its safety and efficacy in humans. Sonepcizumab exhibited favourable characteristics with respect to kinetic, stoichiometric and binding specificity, as well as thermal stability, making it suitable as a clinical candidate. Binding kinetics of sonepcizumab to S1P as measured by BiaCore showed that sonepcizumab bound well to the S1P-tethered surfaces. The kinetics on the lowest density surface fit well to a simple 1:1 interaction model and yielded an affinity of <0.1 nM under these conditions (Wojciak *et al.*, 2009).

The specificity of anti-S1P binding was tested in a competitive ELISA for cross-reactivity against over 60 bioactive lipids and other molecules of interest (O'Brien *et al.*, 2009). Sonepcizumab did not recognize lipids if the phosphate group of the polar head was absent or substituted (S1P vs. sphingosine and D-galactosyl-sphingosine); it did not recognize lipid structures if a fatty acid was added to the amino group on the sphingoid base (S1P vs. ceramide-1-phosphate); sonepcizumab recognized a form of S1P with a reduction of the double bond in the sphingoid base (dihydro S1P); and phosphate ester group added to the sphingoid base forming sphingosylphosphoryl choline. Epitope mapping revealed that sonepcizumab recognized preferentially the phosphate group and the amino-alcohol carried by the polar head of the sphingosine base. This structure has been confirmed by X-ray diffraction of the crystallized Fab' of sonepcizumab and was recently published (Wojciak *et al.*, 2009). The binding of sonepcizumab and Sphingomab towards an extracellular bioactive lipid target such as S1P affords a potential advantage for therapeutic efficacy *in vivo*, as S1P is highly conserved

across species and therefore not subject to the drug-resistant mutations in response to therapy in the same manner as protein targets.

Thermal stability of sonepcizumab was determined to be greater than the murine mAb, Sphingomab: the thermal unfolding transitions (Tm) of sonepcizumab is 73 ± 2°C compared with 55 ± 2°C for Sphingomab (O'Brien *et al.*, 2009).

Thereby, sonepcizumab displayed performance characteristics that made it suitable as a clinical candidate. Sonepcizumab was formulated into two separate drug candidates: (i) ASONEP™, the oncology formulation which was investigated in a recently completed Phase I trial in cancer patients (see next for details); and (ii) iSONEP™, the ocular formulation of which was also investigated in a recently completed Phase I trial for wet-AMD patients (see next for details).

Sonepcizumab is possibly the first humanized monoclonal antibody against a bioactive lysolipid, and it is certainly the first to be advanced into the clinic.

Preclinical pharmacology and efficacy

Preclinical studies with sonepcizumab and its murine counterpart, Sphingomab, administered every 2–3 days at doses of 10–80 mg·kg^{-1}, demonstrated the ability of anti-S1P mAbs to reduce tumour volumes and metastatic potential, probably the result of inhibiting tumour-associated angiogenesis. In mouse models of murine and human cancer, the murine anti-S1P mAbs significantly retarded the progression of several orthotopic and subcutaneous human tumours implanted in nude mice (Visentin *et al.*, 2006), including breast MDA MB 231 and 468, ovarian SKOV3 and lung A549, as well as melanoma B16/F10 allograft tumours. The anti-S1P mAbs inhibited S1P-induced tumour cell migration, proliferation and protection from apoptosis induced by chemotherapeutic agents (Visentin *et al.*, 2006).

The murine anti-S1P mAb dramatically reduced tumour-associated angiogenesis in subcutaneous murine melanoma B16-F10 allograft, human lung A549 and ovarian SKOV3 xenograft models (Visentin *et al.*, 2006). Both murine and humanized antibodies neutralized bFGF- and VEGF-induced angiogenesis in the murine Matrigel plug assay (Visentin *et al.*, 2006; O'Brien *et al.*, 2009). Consistent with *in vivo* anti-angiogenic properties, the antibodies neutralized S1P-induced EC tube formation, migration and protection from cell death in various *in vitro* assays (Visentin *et al.*, 2006). S1P-induced release of pro-angiogenic growth factors such as IL-8, IL-6 and VEGF from tumour cells was also demonstrated *in vitro* and *in vivo* (Visentin *et al.*, 2006).

In completely different preclinical animal models of ocular angiogenesis, Sphingomab and sonepcizumab blocked choroidal and retinal neovascularizations (Caballero *et al.*, 2009; O'Brien *et al.*, 2009; Xie *et al.*, 2009).

The efficacy in animal xenograft and angiogenesis studies with the anti-S1P mAbs was demonstrable despite the fact that plasma S1P levels are ~threefold higher in mice compared with humans (He *et al.*, 2009) such that, in mice, the antibody molecular sponge has more antigen to neutralize. The efficacy of anti-S1P mAbs in these models suggests that treatment with an anti-S1P antibody may provide an innovative and useful approach to cancer treatment in humans. Antibody-mediated neutralization of extracellular S1P could result in a marked decrease in cancer progression in humans as a result of inhibition of new blood vessel formation with concomitant loss of the nutrients and oxygen needed to support tumour growth. In addition, the remaining blood vessels would be expected to be normalized to allow for more efficient delivery of cytotoxic drugs. In fact, many angiogenesis inhibitors may also act as anti-invasive and anti-metastatic compounds, which could also aid in the mitigation of the spread of cancer to sites distant from the initial tumour (Teicher, 2011).

Preclinical safety and toxicity

GLP-quality safety/toxicology evaluations in mice and non-human primates (NHPs) were performed for sonepcizumab. A battery of preclinical studies with anti-S1P mAb complying with the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines was performed to support the initial clinical development of the antibody in the proposed clinical indication showing no dose-limiting toxicities. Two GLP toxicology studies with sonepcizumab in NHPs were completed to support the initial clinical development of sonepcizumab. In addition, three tissue cross-reactivity studies were conducted with sonepcizumab and three mouse tissue cross-reactivity studies were completed with the murine antibody, Sphingomab. Neither the murine nor the humanized mAbs recognized any protein epitopes in the more than 30 tissues studied.

C57BL6 mice were generally tolerant of large, single doses of Sphingomab, with no evidence of toxicity up to 240 mg·kg^{-1} administered intravenously. The administration of this drug to mice over 28 days produced a decrease in absolute lymphocyte counts. At 30 mg·kg^{-1} (cumulative dose over 28 days of 840 mg·kg^{-1}), the lymphopoenia observed in mice was more pronounced with Sphingomab than the very mild, reversible decrease in lymphocyte counts (but not lymphopoenia) in monkeys treated with a similar 100 mg·kg^{-1} dose of sonepcizumab (cumulative dose of 1000 mg·kg^{-1}). Decreased lymphocytes were an expected pharmacological response to systemic anti-S1P mAb treatment as S1P has been shown to control trafficking of lymphocytes out of primary and secondary lymphoid tissues into the peripheral circulation (Cyster, 2004; Graler and Goetzl, 2004). In NHP studies, lymphocyte counts were reduced in a dose-dependent fashion after neutralization of systemic S1P by sonepcizumab in agreement with published accounts of mice treated with Sphingomab (O'Brien *et al.*, 2009) as well as the GLP toxicology studies performed in mice cited previously. As a result, decreased lymphocyte counts were used as a surrogate marker of anti-S1P pharmacological activity *in vivo* for a Phase I sonepcizumab trial in cancer patients (see next). While this signal may not necessarily represent an effect of sonepcizumab on tumour progression *per se*, it does demonstrate pharmacological activity of the antibody. Additionally, the ability of anti-S1P mAbs to reduce tumour progression in xenografted nude mice that are not immunocompetent suggests that anti-S1P actions are independent of effects on lymphocyte trafficking.

Besides the mild decreases in absolute lymphocyte counts shown in the mice and monkey studies, no consistent adverse events were noted in either species. For both sonepcizumab

and Sphingomab, no effects were seen in animal mortality, body weight, clinical pathology (haematology except for lymphocytes, clinical chemistry, coagulation and urinalysis) and lymphocyte phenotyping. Mild histopathological changes were noted in mice only in the spleen. No histopathological changes were noted in monkeys dosed with sonepcizumab.

A battery of other safety and pharmacological studies were performed with both sonepcizumab and Sphingomab. The findings, with respect to vascular permeability and wound healing, suggest little effect of the antibody treatment on these systems. No toxicologically relevant effects of sonepcizumab administration were observed in cytomologous monkeys in GLP ophthalmological studies (intravitreal administration, including measurement of electroretinogram), or in cardiovascular function as measured by ECG. The data also indicated that sonepcizumab does not induce antibody-dependent cellular cytotoxicity, cytokine release or blood haemolysis.

Taken together, the safety and pharmacology studies supported an Investigational New Drug application with the Food and Drug Administration and warranted moving sonepcizumab into clinical development with the oncology formulation, ASONEP.

Phase I clinical trial with sonepcizumab

Lpath has recently conducted a Phase I clinical trial in cancer. The Phase I study was a multi-centre, open-label, single-arm, Phase I dose escalation study of sonepcizumab administered as a single agent weekly to subjects with refractory advanced solid tumours. The objectives of this study were to characterize the safety, tolerability and dose-limiting toxicities (DLTs) for sonepcizumab. Doses between 1 and 24 $mg \cdot kg^{-1}$ were tested. Other than infusion-related reactions observed at the highest dose of 24 $mg \cdot kg^{-1}$, sonepcizumab was well tolerated across the range of doses that was tested, and no DLTs were observed.

Of the 21 patients who completed the initial four-treatment evaluation period, 12 showed stable disease at the end of the first cycle and 11 had stable disease for 2 months or longer. One patient with an extremely aggressive metastatic melanoma showed stable disease through 8 months; another patient with an adenoid cystic tumour was treated for over a year without disease progression. Yet another patient – with carcinoid tumour – is still being treated (as of the writing of this article) with sonepcizumab 26 months after the initial dose, without disease progression. This patient has also shown significant symptomatic improvement (near elimination of the diarrhoea and flushing that makes carcinoid so debilitating). These data were presented at a recent ASCO meeting (Gordon *et al.*, 2010).

Because sonepcizumab was well tolerated by cancer patients in the Phase I clinical study and because of the potential to see an efficacy signal in cancer patients in first or second-line treatment settings, sonepcizumab is being considered for Phase II clinical trials.

S1P and exudative AMD

Exudative (i.e. wet) AMD is characterized by choroidal neovascular (CNV) lesions that eventually lead to the degeneration of the macula, the area of the retina responsible for central vision. Macular damage is caused collectively by: (i) new and leaky blood-vessel growth from the choroid layer into the retinal region; (ii) sub-retinal fibrosis; and (iii) inflammation in the retinal area. The most widely used drugs for wet AMD are Lucentis® and off-label use of Avastin®, both of which target the protein, VEGF, a well-established promoter of the vascular permeability experienced by wet-AMD patients. These drugs exert most of their beneficial effect via an anti-permeability action that results in the resolution of intra- and sub-retinal oedema, and reduces the progression of further neovascularization. Although anti-VEGF drugs improve vision in about a third of patients, the majority do not experience visual improvement over the long term. Anti-VEGF therapies do not cause regression of already-established lesions and the treatments have only a modest effect in reducing pigmented epithelial detachments (PEDs) in patients experiencing this common complication of wet AMD (Ach *et al.*, 2010). Thus, despite the impressive clinical success of anti-VEGF therapies, there is room for a second generation of agents to treat AMD.

Growing evidence suggests that S1P modulates exudative-AMD-associated neovascularization, inflammation and fibrosis. S1P's effects on cell migration, proliferation and protection from cell death have been observed in multiple cell types including fibroblasts, ECs, pericytes and inflammatory cells, all implicated in the pathogenesis of exudative AMD. S1P is also implicated in the trans-activation and production of VEGF, FGF, PDGF and other growth factors that play a major role in the pathogenesis of CNV and are targets of other CNV therapeutics (Spiegel and Milstien, 2003). As the next paragraphs will demonstrate, considerable experimental data suggest that preventing the action of S1P could be an effective therapeutic approach for exudative AMD with distinct non-overlapping mechanisms of action *vis a vis* current anti-VEGF based therapies.

S1P and pathological neovascularization and vascular permeability in the retina

Several lines of evidence suggest that S1P, along with its complement of receptors, plays a major regulatory role in the angiogenic process (Argraves *et al.*, 2004). S1P stimulates DNA synthesis and chemotactic motility of local and bone marrow-derived vascular EC to sites of vascularization and induces differentiation of multicellular structures consistent with early blood vessel formation (Lee *et al.*, 1999b). Also, S1P stimulates the formation and maintenance of vascular EC assembly and integrity by activating $S1P_1$, $S1P_3$ and S1P-induced EC adherens junction assembly mediated by Rho and Rac GTPases (Lee *et al.*, 1999a; Paik *et al.*, 2001). Interestingly, S1P promotes N-cadherin junction formation between ECs and mural cells (Paik *et al.*, 2004). In these studies, cocultures of 10T1/2 pericytes with ECs produced junctions which were eliminated when EC $S1P_1$ receptors were knocked down with siRNA. Normal connections were restored upon expression of the $S1P_1$ receptor. All of these effects were completely independent of VEGF and angiopoietin 1 and 2.

Importantly, S1P induces significant capillary tube formation and is thought to be at least as pro-angiogenic as basic fibroblast growth factor and VEGF in promoting the development of vascular networks *in vivo* (Lee *et al.*, 1999a,b; Visentin

et al., 2006). In addition to the direct angiogenic effects of S1P, there is some cross-talk between S1P and other pro-angiogenic growth factors. For example, S1P transactivates epidermal growth factor and $VEGF_2$ receptors (Tanimoto *et al.*, 2002) and VEGF up-regulates $S1P_1$ receptors (Igarashi *et al.*, 2003). S1P as well as VEGF independently activate the endothelial isoform of nitric oxide synthase (eNOS), which has essential roles in angiogenesis (Igarashi *et al.*, 2003).

A pivotal paper implicating a role of S1P in ocular angiogenesis was published by Skoura *et al.* (2007). Using a $S1P_2$ knockout mouse and a retinopathy of prematurity (ROP) model, they demonstrated that the retinal neovascularization characteristic of this model could be retarded by the genetic deletion of $S1P_2$. Unfortunately, very few studies have directly demonstrated the presence of S1P in the retina. One study reported a biochemical determination of S1P that revealed substantial levels of S1P in rabbit vitreous fluid (Xie *et al.*, 2009), and it has also been demonstrated that S1P can be localized by immunohistochemistry in retinal tissue (Caballero *et al.*, 2009). These data are supported by findings of SphK expression in relevant primary human retinal cell types (Swaney *et al.*, 2008) or by cell lines (Zhu *et al.*, 2009).

Consistent with a role for S1P in ocular angiogenesis and vascular permeability, a recent report in the rat steptozotocin-induced diabetic retinopathy model showed that the inhibition of S1P production using a SphK inhibitor, SK1-II, attenuated VEGF-induced retinal EC proliferation and migration and reduced retinal vascular leakage (Maines *et al.*, 2006). The systemic administration of SK1-II also inhibited subcutaneous VEGF-induced vascular permeability in the Miles assay, suggesting that leakage from the peripheral vascular bed can be reduced by anti-S1P interventions.

Recently, two papers demonstrated the efficacy of anti-S1P mAbs in preventing choroidal and retinal angiogenesis in standard murine CNV and ROP models. Xie *et al.* (2009) found that single intravitreal injection of sonepcizumab showed a dose-dependent decrease in retinal neovascularization and leakiness in the ROP model of ischaemia-induced angiogenesis. These findings were consistent with role of $S1P_2$ in promoting angiogenesis in ROP mice (Skoura *et al.*, 2007). It was also demonstrated that both murine and humanized anti-S1P mAbs substantially reduced choroidal neovascularization after laser disruption of Bruch's membrane (the CNV model of wet AMD) (Caballero *et al.*, 2009; O'Brien *et al.*, 2009). Importantly, immunostaining of CNV lesions revealed a dramatic up-regulation of S1P levels in what appeared to be produced by the retinal pigmented epithelium (RPE) layer after laser-induced injury (Caballero *et al.*, 2009). This is consistent with the finding that human RPE cells express both isoforms of SphK, including SphK1 (Swaney *et al.*, 2008; Zhu *et al.*, 2009), the isoform thought to be responsible for the extracellular release of S1P. Significantly, both human choroidal and retinal ECs also express SphKs and S1P receptors (Maines *et al.*, 2006; Swaney *et al.*, 2008). The S1P receptors expressed by endothelia are probably the targets for extracellular S1P's actions in promoting angiogenesis and vascular permeability.

S1P and fibrosis

S1P is increasingly being recognized for its ability to promote pro-fibrotic function by several cell types. S1P promotes the transformation of human lung fibroblasts and human dermal fibroblasts to the profibrotic, myofibroblast phenotype (Keller *et al.*, 2007). Also TGFβ, a well-known profibrotic factor, stimulates the expression of SphK1 (Yamanaka *et al.*, 2004). Recent reports have also shown that S1P exhibits cross-talk with other profibrotic signalling pathways such as TGFβ (Xin *et al.*, 2004) and PDGF (Alderton *et al.*, 2001; Hobson *et al.*, 2001), and S1P stimulates the expression of connective tissue growth factor (Katsuma *et al.*, 2005), a protein implicated in numerous fibrotic disorders as well as in wet AMD (He *et al.*, 2003). A fibrogenic role for S1P is also suggested by findings that transgenic mice over-expressing SphK1 exhibit profound cardiac remodelling associated with myocardial fibrosis (Takuwa *et al.*, 2009).

Using primarily $S1P_2$ and Rho signalling, we demonstrated that S1P stimulates the expression of collagen in primary fibroblasts coincident with promoting differentiation of fibroblasts to the myofibroblast phenotype (Gellings Lowe *et al.*, 2009). Substantial cross-talk between S1P and TGFβ was also demonstrated in the cardiac fibroblast in that study. Importantly, the murine anti-S1P mAb, Sphingomab, blocked the profibrotic effects of S1P in murine primary cardiac fibroblasts (Gellings Lowe *et al.*, 2009). Of particular relevance to ocular fibrosis, we recently showed that human primary retinal RPE cells express S1P receptors and that S1P's action on these GPCRs promotes the differentiation of RPE cells into a myofibroblast phenotype capable of expressing collagen as well as other fibrotic mediators such as PAI-1 and HSP-47 (Swaney *et al.*, 2008). We also have published work demonstrating a role of S1P in ocular and other fibrotic processes, including data showing the efficacy of anti-S1P mAbs in mitigating fibrotic responses after laser-induced injury of Bruch's membrane (Caballero *et al.*, 2009).

Combined, these data suggest that S1P may serve as a novel mediator of fibrosis, and in particular, the ocular fibrosis (e.g. disciform scarring) associated with advanced stages of wet AMD. Anti-S1P antibodies may interfere with extracellular S1P actions and this might be a useful therapeutic strategy in disorders such as wet AMD where dysregulated fibrosis and/or scarring plays a role in the pathogenesis or progression of disease.

S1P and inflammation

There is growing evidence that S1P is an important mediator of inflammatory events (Olivera and Rivera, 2005), contributing to both wet and dry AMD. Activated platelets and mast cells serve as rich sources of S1P during coagulation and inflammation (Yatomi *et al.*, 2000). S1P receptors are expressed by macrophages from a variety of sources and these GPCRs mediate S1P-dependent activation of macrophage function and protection against cell death, as well as having a stimulating effect on macrophage trafficking (Gude *et al.*, 2008; Weigert *et al.*, 2009). One notable exception is a recent paper showing increased macrophage trafficking in a $S1P_2$ KO mouse model (Michaud *et al.*, 2010).

Macrophages are important mediators of the dysfunctional inflammatory response associated with AMD. The presence of macrophages in histological studies of CNV implicates these cells as a contributing factor in the progression of wet AMD (Tsutsumi *et al.*, 2003). In addition, generalized macrophage depletion has been shown to reduce the

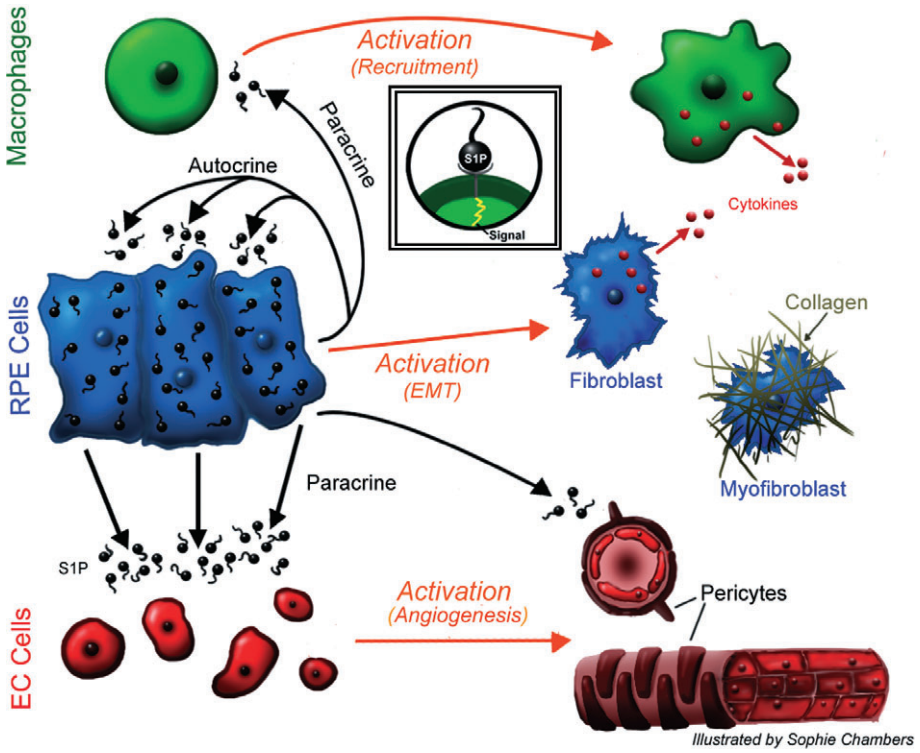

Figure 1

Proposed central role of RPE cells in wet AMD. We speculate that the RPE cells are a major source of S1P in the posterior segment of the eye and that the S1P stored and released from RPEs is responsible for the pathological angiogenesis, vascular permeability, fibrosis and inflammatory responses associated with wet AMD. A positive feedback loop is proposed whereby S1P released from the RPE layer acts in an autocrine fashion to further activate RPE cells and promote their differentiation to a myofibroblast, pro-fibrotic phenotype capable of expressing collagen. Released S1P also serves a paracrine function to promote choroidal endothelial cells and pericytes to form new blood vessels that will eventually create a CNV. S1P also promotes the inflammatory component of wet AMD by either directly activating macrophages or indirectly promoting their survival. RPE, retinal pigmented epithelium; AMD, age-related macular degeneration; S1P, sphingosine-1-phosphate; CNV, choroidal neovascularization.

size and leakage of laser-induced CNV (Sakurai *et al.*, 2003). Because mast cells, neutrophils, platelets and macrophages are important components in the inflammatory response and tissue loss, S1P may regulate these inflammatory events via the control of inflammatory cell function in ocular disorders such as wet and dry AMD as well as diabetic retinopathy.

As an initial demonstration that ocular S1P may be involved in retinal inflammation, Xie *et al.* (2009) have published work demonstrating that intravitreal injection of Sphingomab could substantially reduce the infiltration of F4/80 positive macrophages in a murine model of ROP. It has been argued that this effect may have been due to reduced survival of macrophages caused by antibody neutralization of the anti-apoptotic S1P, rather than a direct effect on macrophage trafficking (Weigert *et al.*, 2009).

The alternative complement pathway has also been implicated in CNV based on the finding that inhibitors of the alternate complement pathway reduce the size of CNV lesions (Bora *et al.*, 2006; Rohrer *et al.*, 2009). Dysregulated complement has also been implicated in the pathogenesis of dry AMD (Petrukhin, 2007). Recently, a connection between complement and S1P in a C5a/S1P axis of inflammation has been established. Specifically, the activation of macrophages by the alternative complement pathway has been attributed to SphK1 where S1P is implicated as the major downstream mediator of C5a action (Melendez *et al.*, 2000; Melendez and Ibrahim, 2004; Vlasenko and Melendez, 2005; Pushparaj *et al.*, 2008; Puneet *et al.*, 2010). Collectively, these papers demonstrate that the inflammatory action of complement C5a on the relevant GPCR expressed on macrophages is mediated by an up-regulation of SphK1 (Melendez and Ibrahim, 2004), thus proving support for the C5a/S1P axis mechanism. S1P-dependent C5a actions include degranulation, migration and release of TNFα, IL-8 and IL-6. Interestingly, it is well established that C5a receptors are also expressed by RPE cells of the eye (Fukuoka and Medof, 2001). Thus, one may speculate that the alternative complement pathway could be a stimulus for S1P production and release by RPE cells, as well as leading to C5a-dependent macrophage activation, recruitment and cytokine release in the posterior segment of the eye. The C5a/S1P axis could involve a positive feedback system for promoting an exaggerated inflammatory cascaded in the eye through an interaction between RPE cells and macrophages (see Figure 1).

Central role for RPE cells in ocular pathology

We hypothesize that RPE cells play a central role in the angiogenic, inflammatory and fibrotic responses to injury or

in wet AMD (Figure 1). While human choroidal and retinal ECs express SphK1 as a potential source for S1P in the posterior segment of the eye, we propose that the RPE layer could be the major source of S1P in injured eyes and that the release of S1P from the RPE cells acts in a paracrine manner to stimulate choroidal ECs to participate in pathological neovascularization. Additionally, the postulated local pulse of S1P may serve to activate choroidal fibroblasts and promote the infiltration and activity of macrophages, and other inflammatory cells as discussed previously. Similarly, we propose that the S1P pulse could serve in an autocrine fashion to promote the epithelial-to-mesenchymal transition (EMT) of RPE cells towards the hypercontracile myofibroblast phenotype, an effect that has been described as one of S1P's actions on human RPE cells *in vitro* (Swaney *et al.*, 2008).

RPEs play an important role in wet AMD as many of these patients experience PEDs where the RPE layer is detached from the basement membrane. Sub-retinal oedema is often the cause but one must consider that the hyper-contractile nature of the RPE cells could also be responsible for the detachment. This was elegantly demonstrated by Agrawal *et al.* (2007) who demonstrated in a model of peripheral vitreoretinopathy (PVR) that human RPE cells injected into rabbit vitreous promoted retinal detachment. We speculate that PED could be due to the effect of S1P in promoting the EMT and the resultant hyper-contractile state of the RPE layer (Figure 1).

The profound pro-fibrotic effects of S1P on cells from multiple regions of the eye and the anti-fibrosis data in mice suggest that sonepcizumab or other anti-S1P therapies could be efficacious in disorders such as PED, PVR and various anterior-segment diseases, including intraocular pressure disorders such as glaucoma. Regarding glaucoma, S1P has been shown recently to reduce outflow facility in *ex vivo* perfused human eyes probably through actions on trabelcular cells and inner wall Schlemm's canal cells expressing $S1P_1$ and $S1P_3$ receptors (Stamer *et al.*, 2009). Thus, anti-S1P-based therapy could correct S1P-mediated intraocular hypertension in glaucoma. As discussed previously, the systemic administration of the SphK inhibitor, SK1-II, attenuated retinal vascular leakage in the STZ rat model of diabetic retinopathy (Maines *et al.*, 2006), suggesting that S1P may play a role in this ocular disorder as well.

Sonepcizumab as a potential treatment for wet AMD

Taken together, the data suggest that inhibiting the action of S1P with anti-S1P mAbs could be an effective and novel therapeutic treatment for wet AMD as well as other ocular disorders. As a consequence, the anti-S1P mAbs were shown to markedly reduce CNV lesion volume, sub-retinal fibrosis and pericyte recruitment in a murine model of laser-induced rupture of Bruch's membrane. These findings were the first demonstration that a non-protein (specifically, a lipid) is a biological mediator of CNV formation. In addition, S1P is present in vitreous fluids and several ocular cell types express S1P receptors and SphK isoforms. In preclinical animal studies, anti-S1P mAbs exhibited a favourable safety and pharmacokinetic profile following both systemic and intravitreal administrations.

It is therefore possible that iSONEP, the ocular formulation of sonepcizumab, could deprive fibroblasts, pericytes, endothelial and immune cells of important growth factors. The ability of sonepcizumab/iSONEP to neutralize S1P-mediated trans-activation of VEGF and PDGF could prove effective in mitigating macular oedema associated with these growth factors (Vinores *et al.*, 2000; Sanchez *et al.*, 2003). Pericytes play a critical role in the development and maintenance of vascular tissue, and their presence seems to confer a resistance to anti-VEGF agents and compromise their ability to inhibit CNV (Ishibashi *et al.*, 1995; Yamagishi and Imaizumi, 2005). S1P promotes adherens junction formation between pericytes and ECs, and promotes maturation of blood vessels during angiogenesis (Paik *et al.*, 2004). By interfering with pericyte signalling, sonepcizumab could strip pericytes from existing lesions and could promote lesion regression by depriving CNV lesions of supportive mural cells. Finally, S1P produced locally by ischaemic/damaged cells could, in part, be responsible for the maladaptive wound healing associated with remodelling and scar formation. By inhibiting S1P, sonepcizumab could diminish the degree of fibroblast infiltration and collagen deposition associated with remodelling and scar formation. A therapeutic agent like sonepcizumab that simultaneously targets the vascular and extravascular components of exudative AMD has the potential to be a more effective treatment than 'singly-targeted' therapies such as anti-VEGF agents. Importantly, the success of Lucentis and Avastin in the treatment of wet AMD has demonstrated that antibodies have long half-lives, biodistribution and stability characteristics suited for intravitreal injection.

Thus, considerable experimental data have been generated to support the hypothesis that inhibiting the action of S1P could be an effective therapeutic approach for treating wet AMD, and this approach may have distinct non-overlapping mechanisms of action compared with current anti-VEGF therapies that solely target one vascular component of wet AMD. Because of the pleiotropic nature of S1P's actions in inflammation, angiogenesis and fibrosis, it is possible that anti-S1P treatment in wet AMD could have beneficial long-term outcomes including lesion regression and prevention of RPE detachments (PED or pigmented epithelial detachments). In fact, preliminary anecdotal findings from our Phase I clinical trial supports this contention (see next).

Phase I clinical trial in wet AMD with sonepcizumab

A multi-centre, open-label, single-arm, Phase I, dose escalation study of sonepcizumab administered as an intravitreal injection to subjects with CNV secondary to AMD was initiated. Five dose levels were evaluated: 0.2, 0.6, 1.0, 1.4 and 1.8 mg per eye. Subjects received a single intravitreal injection of sonepcizumab in one eye. The objectives were to determine the safety, tolerability, maximum tolerated dose (MTD) and DLT of sonepcizumab, and to characterize the systemic pharmacokinetics of sonepcizumab, determine doses for future clinical studies and investigate preliminary efficacy on retinal lesion thickness by optical coherence tomography, size and extent of CNV and lesion area and visual acuity. Results of this study were presented at a recent ophthalmics meeting (Stoller *et al.*, 2010).

The patients in the cohort were largely those who were refractory to previous anti-VEGF treatments. The most significant benefit observed was with the five patients diagnosed with some form of occult CNV (vs. classic CNV). All of these five patients exhibited an apparent strong biological response. Specifically, three of the four occult patients with an active lesion exhibited a significant regression (>75%) in their CNV lesion, which is the underlying cause of the disease that eventually leads to the degeneration of the macula, the area of the retina responsible for central vision. This type of clinical benefit is not typical, as the published data (Heier *et al.*, 2006) suggest that, even with repeated Lucentis dosing, the total physical size of CNV lesion does not show substantial reduction, especially with a single dose. Another distinctive benefit for the patients with occult disease was the resolution of RPE detachment (PED or pigmented epithelial detachment), a potentially serious condition that is often a part of the pathology of wet AMD. Of the two occult patients diagnosed with RPE detachment in the Phase I trial, both experienced complete or near-complete resolution of the condition. Neither Lucentis nor Avastin commonly produces this type of clinical benefit with a single dose (Ach *et al.*, 2010). Because of these anecdotal signals of potential efficacy combined with strong safety data, sonepcizumab is now being advanced into Phase II clinical trials.

Conclusions

When considering the roles of dysregulated S1P levels in the pathogenesis of such diverse diseases as cancer and wet AMD, one is struck by the many commonalities these two disorders share. For example, both pathologies suffer from exaggerated and pathogenic elements of the inflammatory and wound-healing responses. Dysfunctional angiogenesis is also common to cancer (i.e. tumour angiogenesis) and to wet AMD (i.e. CNV). In addition, both are hyperproliferative disorders; the cancer being one of transformed, mitotically active primary cells and AMD being complicated by hyperproliferative ECs. Serendipitously, the pleiotropic nature of S1P in promoting angiogenesis, inflammation, wound healing and cell survival explain the reason why anti-S1P therapy could be useful in controlling both of these disorders. The anti-S1P antibody molecular sponge approach is one of several therapeutic strategies that are being explored but holds promise as a novel, first-in-class treatment.

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