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Modulators of the Sphingosine 1-Phosphate Receptor 1

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Sphingosine-1-phosphate (S1P) is a bioactive lysophospholipid that mediates an array of cellular processes including cell differentiation, migration, survival, calcium homeostasis, as well as angiogenesis, inflammation, endothelium integrity, immunity. S1P acts in an autocrine and paracrine mode through a class A family of G-protein coupled receptors named S1P₁₋₅-Rs.¹⁻⁴ The S1P₁₋₃-Rs are widely expressed in many cell types including those of immune, cardiovascular and CNS. The S1P₄-R is predominantly expressed on lymphoid tissues and lung, while the S1P₅-R is primarily located in the CNS, skin and spleen.¹ Regulation of lymphocyte recirculation and homing is among the most investigated effects of the receptor signal transduction. S1P/S1P₁-R axis promotes egress of T and B cells from primary and secondary lymphoid tissues without inhibiting their activation or proliferation nor their effector function.^{5,6} Due to these properties, the role of the S1P₁-R in autoimmune diseases such as multiple sclerosis (MS) has been the focus of intense research activity in the academic, clinical, and pharmaceutical sectors. A significant achievement was attained in 2010 when FTY720 (Fingolimod, Figure 1) was approved by the FDA as the first orally active drug for the treatment of relapsing-remitting MS (RRMS). FTY720 is metabolized *in vivo* to its bioactive *S*-isomeric monophosphate ester (FTY720-P), which activates four S1P-Rs (S1P_{1,4,5}-R EC₅₀'s = 0.3-0.6 nM, S1P₃-R EC₅₀ ~ 3 nM).⁷ Following the discovery of FTY720 a number of S1P₁-R agonists are undergoing pre-clinical studies or have been advanced to clinical trials for autoimmune diseases.

The efficacy of FTY720 and known S1P₁-R agonists in both animals and humans results from impaired recirculation of autoreactive lymphocytes, their sequestration within secondary lymphoid organs and prevention of their infiltration into the CNS.^{1,6-7} Accordingly, in MS patients receiving FTY720 as monotherapy at daily oral doses of 1.25

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mg and 5 mg, peripheral blood lymphocyte (PBL) counts decreased to 20–30% of baseline values with differential effects on T-cell sub-populations.⁸ A transient reversible peripheral lymphodepletion is a desirable pharmacodynamic effect elicited by FTY720. However, in humans FTY720 has a half-life of 9–10 days owing to high volume of distribution,⁷ and after drug cessation lymphocyte counts only return to baseline after 4–8 weeks.⁹

In regards to the precise mechanisms underlying the induced lymphopenia, binding of FTY720-P to the S1P₁-R on lymphocytes themselves causes receptor internalization accompanied by proteosomal degradation.^{4,10} This is thought to result in functional antagonism creating a temporary pharmacological S1P₁-null state in lymphocytes. This in turn prevents lymphocyte exit from lymphoid tissues into blood or lymph along a gradient of endogenous S1P.^{6,11–12} However, FTY720-P-induced internalized S1P₁-R has also been suggested to signal in a process known as persistent S1P₁-R signaling.¹³ To date, it remains unclear how internalization, degradation, and sustained signaling collectively regulate the S1P₁-R, although the immunosuppressant effects induced by S1P₁-R antagonists, such as NIBR-0213 and TASP0251078 strongly suggest that the therapeutic efficacy of FTY720 may be due to “functional antagonism” rather than persistent signaling at the S1P₁-R.^{14–15}

Moreover, ligand binding to the S1P₁-R expressed on endothelial cells potentiates the effects of S1P in tightening the endothelial barrier, which may indirectly contribute to lymphocyte sequestration in peripheral lymphoid organs.^{1,16}

Although the CNS effects of FTY720 are poorly understood, significant evidence suggests a CNS component to its efficacy. Brain levels of FTY720 and its bioactive form significantly exceed blood concentrations and preferentially localize to the CNS white matter of rodents, suggesting the potential for functional interaction with glial cells expressing the S1P₁-R.¹⁷ Consistently, S1P₁-R expression in the central MS lesions is dramatically increased, and null mouse mutants lacking CNS S1P₁-R phenotypically show reduced disease severity, astrogliosis, demyelination and axonal loss.¹¹ Previous studies already indicated that intracerebroventricular application of FTY720 in an experimental autoimmune encephalomyelitis (EAE), a model of human MS, reduced symptom severity without significantly affecting PBL counts.¹⁷

Bradycardia and electrical conductivity abnormalities are both recognized risks within the first twenty hours after initial dosing of FTY720, and physician treatment instructions by both the FDA and EMEA require monitoring patients for cardiac problems during that time frame.^{18–19} Further observed side effects associated with FTY720 include macular edema and impaired lung function, the latter being linked with the action on S1P-Rs on smooth cells and endothelial barrier cells.¹⁹ Nevertheless, FTY720 provides an important health benefit and remains the first-line treatment for those suffering from RRMS. In addition, FTY720 is currently in phase 3 clinical trials for primary progressive MS and kidney transplantation.

Until recently, the heart rate effect was linked exclusively to the S1P₃-R agonism since treatment with FTY720 resulted in bradycardia in normal but not in S1P₃-R knockout mice.^{21–22} Distinct research indicated that cardiovascular effects of FTY720 in humans are not S1P₃-R mediated as in rodents but rather S1P₁-R dependent *via* activation of the G protein-coupled inwardly-rectifying potassium (GIRK/IK_{ACh}) channel in cardiomyocytes.^{23–25} Nevertheless, extensive research is still focused on identifying S1P₁-R agonists with increased selectivity over the S1P₃-R to improve the safety profile. Human trials of more selective S1P₁-R agonists such as BAF312 (Siponimod) and CS-0777 (Figure 1, Table 1) seem to confirm that the transient effect on heart rate may not be linked with S1P₃-R activation in humans.²⁵ On the other hand, RPC1063, currently in phase 2 clinical

trials for RRMS and ulcerative colitis, successfully completed a study of the heart's electrical conduction system (TQT study), thus stirring up controversy. Indeed, RPC1063 did not cause prolongation of the QT interval or changes of the heart rate in healthy subjects at both therapeutic (1 mg/kg) and supra-therapeutic (2 mg/kg) doses over 14 days of treatment (Table 1).

Medicinal chemistry efforts have also focused on developing S1P₁-R direct-acting agents rather than pro-drugs with shorter half-life and improved brain penetration than FTY720. S1P₁-R agents that are currently in clinical trials and their main features are summarized in Table 1.

Although scarcely pursued until recently, S1P₁-R antagonists have been recently shown to have similar therapeutic potential versus S1P₁-R agonists for the treatment of immune-related diseases.¹⁴ However, so far there have been no drug candidates in clinical trials.

Much progress has been made in the field of structural biology and modeling of the S1P₁-R. The receptor crystal structure has been recently solved using a S1P₁-R antagonist, and various mutagenesis studies have identified specific amino acids essential in ligand recognition, binding and receptor activation.²⁶ It is predicted that the cationic Arg¹²⁰ (TM3), and Arg²⁹² (TM7) both bind with the anionic phosphate moiety of S1P and that the anionic Glu¹²¹ (TM3) interacts with the S1P protonated amino group, via a salt bridge or hydrogen bond. Known S1P₁-R agonists can be chemically classified into two groups: ligands, lipid or non-lipid like molecules, that possess a polar head group mimicking the S1P polar head interactions; and ligands that do not require polar head interactions and rely more on the shape complementarity to the agonist pocket.²⁶

This review covers the primary literature that has been published since 2012 on modulators of the S1P₁-R, with an overview of the chemical structures, structure-activity relationships (SAR), biological and pharmacological properties. The patent literature on S1P₁-5-R modulators has been the subject of recent reviews.²⁷⁻²⁸

S1P₁-R agonists

A limited number of immunomodulators containing oxazolidin-2-one-4-carboxamide as the phosphate surrogate of FTY720 and a phenoxy or alkoxy phenyl as the lipophilic tail (e.g. **7**, Table 2) were studied by the Beijing Institute.²⁹ These molecules were hypothesized to bind to the S1P₁-R via H-bonds between amino acids Arg¹²⁰/Phe²⁹⁵ and the oxazolidin-2-one ring. Only four of the six synthesized molecules displayed some *in vitro* functional activity at the S1P₁-R with EC₅₀'s in the range of 1.1-3.6 μ M, while no selectivity data was reported. When orally administered in mice, the most active compound **7** that had a cytotoxicity 40-fold lower than FTY720, was less effective than FTY720 in decreasing peripheral blood CD³⁺ T cells at doses of 0.1 to 3 mg/kg. Nevertheless, since at a dose of 10 mg/kg **7** showed *in vivo* efficacy comparable to FTY720, this compound was selected as a drug candidate for further development.

A series of 1,2,4-oxadiazole tricyclic indoles, disclosed by Arena, proved to be significantly more potent *in vitro* and *in vivo* than **7**.³⁰ Selected molecules **8** and **9** exhibited S1P₁-R nanomolar agonist activity, poor selectivity at S1P₅-R but excellent selectivity against the S1P₃-R in addition to good oral bioavailability in rats (Figure 2). Both compounds produced in mice a significant PBL lowering 5 h after an oral dose of 6 mg/kg, or even at 1 mg/kg for compound **9**. This property translated into significant efficacy in three different models of autoimmune diseases. In particular, **9** delayed the onset and reduced the severity of disease in an EAE model. In non-obese diabetic mice this compound was effective at delaying the disease progression in a cyclophosphamide-induced Type 1 diabetes model via a reduction

in T-cell mediated β -cell destruction. In a collagen induced arthritis model performed in rats, **9** appeared to exceed the efficacy of the 1 mg/kg dose of FTY720 and was nearly equipotent to the first line therapy methotrexate. Importantly, unlike FTY720 used as a positive control, the compound did not induce bradycardia in rats dosed orally at 3 and 30 mg/kg over a 20 h period.

A structurally related family of S1P₃-R selective 1,2,4-oxadiazole indoles was explored by GlaxoSmithKline (Table 3).³¹ SAR around the indole chemotype **10**, that was previously disclosed by the team,³² suggested that position 7 on the indole was the optimal to attach the oxadiazole and that the 3-chloro-4-isopropoxy phenyl moiety was crucial for the *in vitro* potency (**11a**).³¹ Moreover, bioisosteric replacement of the carboxylic acid with amides (e.g. **11b**) or a tetrazole was tolerable, and methylating the indole *N* (**11c**) as well as adding a fluorine at positions 4 (**11d**) or 5 (**11e**) of the indole was beneficial. In pharmacological studies, PBL counts were reduced to 21% of the control at 4 h and normalized at 24 h following a single oral dose of 1 mg/kg of **11d**. Of note, **11d** showed *in vivo* efficacy comparable to FTY720 in a mouse EAE. The full selectivity profile against the S1P_{2,4,5}-Rs was not described.

Driven by the early hypothesis of S1P₃-R dependent bradycardia of FTY720, the Daichii Sankyo team developed potent, orally active S1P₃-R-sparing S1P₁-R agonists by chemically modifying Merck's 1,3,4-oxadiazole azetidine carboxylic acid **12** (Figure 3).³³⁻³⁴ The left-hand phenylthiophene and the right-hand phenyl rings were successfully replaced with a 4-phenoxyphenyl and a thiophene ring respectively. The selectivity was tuned by adding small alkyl groups as in compound **13** (CS-2100). The high selectivity of CS-2100 was rationalized by docking studies with a homology model of S1P₁-R and S1P₃-R showing that the ethyl group at the thiophene ring was sterically hindered by Phe²⁶³ in the S1P₃-R but not by Leu²⁷⁶ in the S1P₁-R. Remarkably, CS-2100 showed immunosuppressive efficacy in rats on host versus graft reaction (HvGR) with an ID₅₀ of 0.407 mg/kg. However, its central 1,2,4-oxadiazole ring was found to be susceptible to cleavage by enterobacteria in rat and monkey, thus raising concerns about broad variability in pharmacokinetic (PK) and pharmacodynamics profiles across species and individuals.³⁵ Hence, the 1,2,4-oxadiazole was successfully replaced by a 1,3-thiazole. 1,3-thiazoles having a pyridine as the right-hand aryl ring such as **14a** maintained good *in vitro* potency/selectivity profile and *in vivo* immunosuppressive activity, while superior pharmacodynamics were found for the thiophene analog **14b** which showed an ID₅₀ of 0.07 mg/kg in HvGR in rats. **14b** had favorable bioavailability (>90%) and fair half-life (7.2 h) in monkeys, and showed a higher stability against enterobacteria compared to CS-2100. Follow-up studies on this molecule are ongoing as stated by the authors.

Along the same line, GlaxoSmithKline focused on the optimization of the 1,2,4-oxadiazole core of **15a** to reduce the risk of oxadiazole ring opening possibly dependent on its low aromatic index (AI) (Table 4).³⁶ Since FTY720 effectiveness also resides on its neuroprotective influence in the CNS, the team sought to increase the free drug concentration in the brain. Indeed, **15a** had shown efficacy comparable to FTY720 in an animal model of MS and reasonable CNS penetration, but displayed high plasma protein binding (*F_u*) and very low free concentration in the brain. Exploring aromatic rings with higher AI led to the identification of 1,2,4-thiadiazole **15b** and pyrimidine **15c** with higher *F_u*. **15b** and **15c** caused a pharmacologically relevant PBL reduction at 4 h in mice with a rapid recovery at 24 h. Additionally, they demonstrated efficacy comparable to FTY720 in a mouse EAE model. Subsequent replacement of the carboxylic acid for amino acids such as piperidine carboxylic acid resulted in **15d** which showed similar pharmacodynamics in a mouse EAE model, but improved *F_u* (Table 5). In a repeat-dose study in rats **15d** produced rapidly a daily lymphocyte reduction in the range of 20-60% of baseline over 11-day

treatment. Importantly, PBLs recovered to normal range (~70 % of baseline) 3 days after treatment cessation. With regards to the selectivity profile, all compounds were found to be highly selective against the S1P₃-R, while the selectivity against the remaining S1P-R subtypes was not reported. The authors pointed out the importance of pursuing selectivity over the S1P₃-R due to possible related hemodynamic and pulmonary side effects.

Amgen aimed at developing S1P₃-R-sparing S1P₁-R agonists, mostly in light of early cardiovascular findings. A *N*-atom scan SAR strategy consisting in systematically incorporating *N* atoms into the scaffold of quinolinone **17a**, constrained analog of the hit **16**, culminated in the identification of mono-aza-analog **17b** and di-aza-analog **17c** that elicited the ability of reducing PBL in rats (Figure 4, Table 6).³⁷ However, at oral doses up to 100 mg/kg, the exposure of both compounds in rats failed to increase proportionally relative to the 3 mg/kg dose probably due to their sub-optimal solubility. This issue was addressed by installing a polar group at 7-position of the quinolinone.³⁸ Indeed, the 6-aza-7-hydroxymethyl analog **18** achieved dose-proportional exposure in rats up to at least 100 mg/kg, and in male non-human primates (NHP) displayed excellent PK parameters with good oral bioavailability (*F* = 67%) (Figure 4, Table 6). Thus, **18** was selected for further preclinical development.

A distinct chemical template has been also described by Amgen. The benzothiazole hit **19** was chemically modified to achieve lower cLogP and higher tPSA in line with a potentially more favorable safety profile (Figure 5).³⁹ Introduction of a pyridine ring in the tail region of **19** produced moderately potent molecules **21a** and **21b**. Systematic SAR led to the discovery of 2-ethyl thiazolidine amide (+)-**22** which showed nanomolar activity and moderate selectivity versus the S1P₃-R. (+)-**22** displayed significant reduction of PBL counts in rats. Although its lymphopenic effects were reported to be not superior compared to the hit, (+)-**22** was selected for future modifications due to its improved physicochemical properties. Pursuing novel S1P₃-R-sparing S1P₁-R agonists, Amgen concomitantly modified the azetidine carboxylate head-group of AMG369 while keeping its lipophilic tail (Table 7).⁴⁰⁻⁴¹ Of note, 3-amino succinamic acids **23a**, **23b** and α -methyl- α -aminobutyrate (–)-**23c** proved exceptionally potent S1P₁-R agonists with EC₅₀'s in sub-nanomolar range and highly selective against the S1P₃-R. The selectivity at the S1P₅-R was not provided even though AMG369 was previously reported to have a nanomolar S1P₅-R agonist activity.⁴¹ Amongst the explored molecules, **23c** showed the best efficacy in reducing PBL counts following oral dosing in rats at 1 mg/kg, and moderate lymphocyte depletion could be observed even at 0.3 mg/kg. **23c** had acceptable clearance, moderate protein binding in human and rat plasma (*F*_u = 3.7, 3.9%), but low oral bioavailability (16%, 3 mg/kg p.o.). This acyclic amino carboxylate **23c**, was indicated as suitable for further preclinical evaluation.

A different dihydronaphthalene scaffold was reported by Ono Pharmaceutical. A detailed SAR study of the terminal lipophilic coil of the hit **24** was conducted to optimize S1P₁-R agonist activity and *in vivo* PBL lowering efficacy without impairing selectivity over the S1P₃-R (Figure 6).⁴² Fluorinating the terminal phenyl ring of the homolog **25a** led to improved *in vitro* S1P₁-R activity and *in vivo* efficacy (**25b**), and introducing a (*S*)-methyl group at the propylene linker improved selectivity over the S1P₃-R (**25c**) (Table 8). Interestingly, the chlorinated analog **25d** in addition to having sub-nanomolar S1P₁-R activity and high selectivity over the S1P₃-R, showed efficacy at a dose of 0.095 mg/kg at 24 h after oral administration in mice. The improved PK and *in vivo* PBL activity of **25b-25d** compared to **24** was hypothesized to result from the effect of the fluorine or chlorine atom in preventing phenyl oxidative metabolism.

A series of zwitterionic amido-1,3,4-thiadiazoles were identified by Almirall as novel potent S1P₁-R agonists by modifying a micromolar high-throughput screening (HTS) hit, **26**, at the polar head, lipophilic tail and amide substituent (Figure 7).⁴³ (Sub-)nanomolar activity could be achieved introducing in the polar head an amino acid, with the azetidine carboxylic acid giving the best S1P₁-R potency/S1P₃-R selectivity profile (e.g. **27**). The increase of potency was rationalized with a double anionic interaction between the amino acid and both S1P₁-R Arg¹²⁰ and Glu¹²¹, as predicted by an in-house rhodopsin-based S1P₁-R homology model. The butyl at the amide *N* and the 2-fluorobenzamide accounted for the most potent compounds. In particular, **27** showed sub-micromolar activity versus the S1P_{4,5}-R, while no data is provided for the S1P₂-R isoform. At 4 h, a significant effect on rat lymphopenia was achieved at all oral doses tested (1-30 mg/kg), whereas doses of 10-30 mg/kg were required to maintain the effect over a 24 h period. When orally administered once a day for 7 days at 10 mg/kg to rats in an EAE, **27** significantly reduced the progression of the neurological disability. The major drawback was the human hepatic clearance which was predicted to be inadequate for a once a day administration of the compound in humans. Hence, enhancing the DMPK was pointed as the main focus of further optimization of this series.

Strikingly similar compounds **28a** and **28b** were identified by GlaxoSmithKline by screening their in-house compound collection (Figure 8).⁴⁴ Systematic modifications of the polar head and lipophilic tail afforded additional (sub-)nanomolar S1P₁-R agonists, **29a-29b**, highly selective against the S1P₃-R. However, these compounds had no significant lymphopenic activity in mice probably due to the high *in vitro* intrinsic clearance in liver microsomes and low blood exposure.

Previously identified 1,2,4-oxadiazole **30** with picomolar S1P₁-R potency and high S1P₃-R selectivity was modified by GlaxoSmithKline to increase molecular diversity (Figure 9).⁴⁵ First, the oxadiazole ring was cyclized with the indole moiety to produce moderately active oxazolo[5,4-*b*]pyridine analogs, exemplified by **31a-31c**. Introducing the 4-phenyl-3-trifluoromethylphenyl as the left-hand phenyl ring drastically increased the S1P₁-R potency (**32a**). Interestingly, the benzoxazole was identified as the only bioisostere that showed more stability under basic conditions than the oxazolo[5,4-*b*]pyridine core, while maintaining its potency/selectivity profile (**32b**). Based on a homology model the carboxylic acid of **32b** was found to interact with Arg¹²⁰/Arg²⁹². Since Glu¹²¹ is located closely to Arg¹²⁰, the addition of an amine group was hypothesized to enhance ligand-binding affinity. In particular, the piperidine carboxylic acid **33b** was synthesized and found to have the best potency/selectivity profile compared to other explored amino acids such as azetidine **33a**. Within this series, **33b** was selected for further *in vivo* studies in mice. **33b** had good brain to plasma ratio (BBR ratio = 3.73 at 4 h, 2 mg/kg. i.v.) and oral bioavailability (F = 100%), was efficacious at 1 mg/kg in an acute mouse lymphopenia model and had a desirable transient lymphopenic effect within 24 h.

Novartis has reported the identification of a direct-acting S1P_{1,5}-R agonist BAF312 (**3**, Siponimod) derived from modifications of the hydrophobic alkyl chain of FTY720 (Figure 10).⁴⁶ SAR studies, guided by the goal to overcome FTY720 cardiovascular liabilities and long half-life, afforded the biphenylmethyl oxime analog **34** which was as efficacious as FTY720 in inducing lymphocyte redistribution in mice. However, since the half-life of its bioactive form **34-P** (*t*_{1/2} > 30 h) remained sub-optimal, direct-acting agents rather than pro-drugs were pursued and β-alanine, exemplified by **35**, was identified as a suitable function to mimic the amino phosphate of **34-P** and FTY720-P. Molecular modeling based on the S1P₁-R crystal structure indicated a salt bridge between the carboxylic acid of **35** and Lys³⁴/Arg¹²⁰, and between the charged amine and Glu¹²¹. In the lipophilic region of this series, the optimal substitution pattern at the phenyl ring A was a trifluoromethyl in position 2, and either a cyclopentyl or cyclohexyl group was tolerable as the ring B. Introducing on ring C a

small alkyl group (R) as in **36** led to an increase of selectivity against the S1P₃-R. The ethyl azetidine carboxylic acid BAF312 was found to be the best compound in terms of potency and selectivity over the S1P₃-R (Figure 10).^{25,46} Of note, unlike FTY720, BAF312 allows modulation of lymphocyte trafficking without binding to the S1P₃-R and is highly selective against the S1P₄-R isoform.

In preclinical studies BAF312 reduced PBL counts in rats by 88% at 8 h after oral intake of 1 mg/kg, and in contrast to FTY720 after 48 h PBLs returned to normal levels in treated animals.⁴⁶ Interestingly, the lack of activity on the S1P₃-R and the lack of cardiac effects in mice did not translate into humans, as treatment with BAF312 caused GIRK channel activation in human atrial myocytes and transient dose-dependent bradycardia in healthy subjects. A differential pharmacological profile compared to FTY720 was observed regarding lymphocyte recovery. Indeed, the PK profile with a mean half-life of 30 h in humans allowed a relatively rapid recovery of lymphocytes that returned to the normal range within a week on stopping treatment. As emphasized by Novartis, this feature is of considerable clinical benefit in cases where fast lymphocyte recovery is desirable such as during infections.²⁵ BAF312 is presently in phase 3 clinical trials for secondary progressive MS and in phase 2 for RRMS and polymyositis.

S1P₁-R antagonists

As aforementioned, S1P₁-R agonists likely behave as “functional antagonists”. This hypothesis has recently kindled the search for direct antagonists of the target. The arylsulfonamide carboxylic acid **37** showing nanomolar antagonist activity and lymphopenic effects at a high oral dose of 100 mg/kg in rats was selected as the starting point for a S1P₁-R antagonist program by Novartis (Figure 11).¹⁴ This compound could not be used in an EAE model due to its high clearance and poor oral bioavailability leading to sub-optimal duration of action.^{14, 47} Novartis applied two different approaches aimed at reducing the polar surface area and increasing cell permeability with the ultimate goal of improving the PK profile and the *in vivo* efficacy of **37**. The first approach aimed at decreasing the polarity by removing the sulfonamide group led to a highly potent and selective competitive antagonist **38** (NIBR-0213) with a significant oral blood exposure in rodents.¹⁴ NIBR-0213 given orally at 30 mg/kg to rats reduced the PBL counts by 75-85% within 14 h and maintained a significant effect after 48 h. Indeed, NIBR-0213 is the first reported S1P₁-R antagonist with an effect on lymphocyte egress adequate for EAE studies. NIBR-0213 had comparable efficacy than FTY720 in therapeutic mouse EAE, and had similar impact on PBL counts, with no significant effect on monocytes and neutrophils. Remarkably, NIBR-0213 was devoid of any GIRK channel activity in cardiomyocytes which could potentially translate into a safer cardiac profile in humans than S1P₁-R agonists. On the other hand, probably as a result of the inhibition of the S1P₁-R function on vascular endothelial cells, NIBR-0213 induced pulmonary vascular leakage with an ED₅₀ similar to the ED₅₀ for the reduction of PBL counts. Although no adverse effects related to pulmonary function were observed in the course of the EAE study, further safety tests are needed to assess the effects of long-term chronic dosing with NIBR-0213 on lung function.¹⁴

The secondary approach involved the reduction of the H-bond donors of **37** by alkylating the amide N and/or carboxy group and installing a polar amine in the amino acid region.⁴⁷ This allowed the discovery of the highly permeable base-labile pro-drug **39a** that is hydrolyzed *in vivo* to its bioactive metabolite **39b**. Administration of **39a** (30 mg/kg p.o.) promoted persistent reduction of PBLs (to 22% of control) for 24 h and the lymphocyte levels returned to normal 48 h after compound administration. In a repeat-dosing experiment with **39a** (5 days, 30 mg/kg p.o.) PBL counts were reduced to ~ 20% of control values over the entire experiment. Of note, pulmonary leakage with **39a** in rats was described as a transient effect

that decreased after multiple dosing. In a rat heart transplantation model, **39a** in combination with sub-therapeutic dose of sotrastaurin or everolimus significantly prolonged cardiac allograft survival with excellent histology.⁴⁷

Exelixis sought to probe the function of a S1P₁-R antagonist on angiogenesis and tumor growth. SAR analysis was conducted on the HTS-derived hit dipeptide **40** with micromolar S1P₁-R antagonist activity (Figure 12).⁴⁸ This study showed that the 1*H*-imidazol-5-yl and the 4-fluorophenyl were suitable substituents in the glycine and phenyl ether regions. In particular, compound **41** showed some oral exposure in mice (8 μ M at 1 h at 100 mg/kg p.o.) and higher potency at the S1P₁-R while retaining the selectivity against the S1P_{2,3}-Rs. Reducing the number of rotatable bonds by cyclizing the left-hand amide and replacing the imidazole with a triazole yielded the nanomolar antagonist **42** with good oral exposure in rats (108 μ M at 1 h at 100 mg/kg p.o.). This molecule was selective against the S1P_{2,3}-Rs, while no selectivity data for the S1P_{4,5}-Rs was provided. Orally dosed in mice for 14 days at 100 mg/kg, **42** induced a 43% tumor growth inhibition.

An additional class of S1P₁-R antagonists has been published by Fujii *et al.*¹⁵ who discovered the sub-micromolar antagonist TASP0251078 with high selectivity against the S1P_{4,5}-Rs and moderate selectivity versus the S1P₃-R, as determined by binding affinity assays (Figure 13). This molecule was a cherry-pick from a HTS campaign. A single oral administration of TASP0251078 (50 mg/kg) in rats significantly reduced PBLs to less than 30% of the control values within 1-8 h. The authors highlighted that the plasma concentration that effectively induced lymphopenia did not cause any significant changes in the number of neutrophils, monocytes, eosinophils, or basophils in mice. *In vivo* studies in immune-mediated disorders of this chemical class have not yet been reported.

Conclusions

With its clinical benefit FTY720 marked the inception of a new generation of therapeutics in the field of immune-related disorders.

FTY720 offers patients suffering from RRMS an improved therapy and compliance with a daily oral dosing versus injections of conventional drugs. In addition to RRMS, FTY720 may offer some prospects of therapeutic intervention in primary progressive MS or organ transplantation. Cardiovascular liabilities and long half-life are the FTY720 critical pitfalls whose significance will be defined by the long-term drug evaluation in patients.

Following the discovery of FTY720 and the validation of the S1P₁-R as a target for autoimmune diseases, tremendous and sustained efforts have since focused on the development of second-generation S1P₁-R agonists with reduced half-life and improved safety profile, particularly with regards to the transient asymptomatic bradycardia. Drug development approaches have targeted pro-drugs that require bioactivation to a phosphate, and direct-acting agonists designed to reduce the half-life and improve the pharmacodynamics of FTY720. Direct-acting S1P₁-R agonists with a rapidly reversible effect on blood lymphocytes have now entered clinical trials, although it remains to be determined if they do provide a superior therapeutic benefit compared to FTY720.

Despite evidence linking bradycardia with the S1P₁-R-induced activation of potassium channels on cardiomyocytes in humans, earlier pre-clinical studies ascribing the cardiovascular effects to the S1P₃-R binding in rodents became an accepted dogma that has been channeling substantial research work from industry and academia towards agents with high selectivity against the S1P₃-R isoform. To the best of our knowledge, there is only one

S1P₁-R selective clinical candidate with an emerging more favorable cardiovascular profile. Yet there is no certainty about the importance of the S1P₁-R-selectivity on the safety profile.

Rather than agonists with selectivity against the S1P₃-R, recently disclosed S1P₁-R antagonists with a profound inhibition of lymphocyte trafficking and lack of effects on cardiomyocyte potassium channels may offer a valid strategy to decouple the mechanistic heart side-effects of the S1P₁-R agonists. Future clinical studies with S1P₁-R antagonists will assess their therapeutic impact.

Many pharmacological companies recently began to invest their S1P portfolios in therapeutic areas distinct from MS such as psoriasis, polymyositis, lupus erythematosus, transplantation and oncology, thus opening up to novel clinical applications of the target. The precise immunological and CNS mechanisms, through which discovered S1P₁-R modulators elicit their immunosuppressant activity, have yet to be fully elucidated. Nevertheless, the proven clinical importance of the S1P₁-R together with the disease spectrum under investigation and the chemical range of small molecule S1P₁-R modulators in preclinical and clinical trials raise optimism in terms of developing S1P₁-R therapeutics with improved safety profile and broader clinical benefit in autoimmune settings than FTY720.

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Figure 1.
S₁P₁-R agonists.

Figure 2.
1,2,4-oxadiazole tricyclic indoles with efficacy in different rodent models of autoimmune diseases.

Figure 3.
Identification of CS-2100 and its analogs.

Figure 4.
Identification of aza quinolinone-based S1P₁-R agonists.

Figure 5.
 Benzothiazole hit **19**, AMG 369, **21a**, **21b**, (+)-**22**.

Figure 6.
S1P₁-R agonists with a dihydronaphthalene scaffold.

Figure 7.
Amido-1,3,4-thiadiazole azetidine carboxylic acid **27** with activity in rat EAE.

Figure 8.
 Amido-1,3,4-thiadiazole indoles **29a**, **29b** with no significant lymphopenic activity in mice.

Figure 9. Benzoxazole piperidine carboxylic acid **33b** with potent transient lymphopenic effects in mice.

Figure 10.
 Direct-acting $S1P_3\text{-R}$ -selective oxime amino acid BAF312.

Figure 11.
 $S1P_1\text{-R}$ antagonists with efficacy in mouse EAE and rat heart transplantation model.

Figure 12.
Discovery of an S1P₁-R antagonist with efficacy on tumor growth in mice.

43, TASP0251078 PBL depletion, 50 mg/kg p.o. in rats:
S1P₁-R IC₅₀ = 383 ± 108 nM > 70% at 1-8 h
S1P₁-R K_i = 249 ± 25 nM
S1P₂-R K_i N.T.
S1P₃ K_i = 4.88 ± 1.62 μM
S1P₄₋₅ K_i > 10 μM

Figure 13.

Discovery of S1P₁-R antagonist TASP0251078 with lymphopenic activity in rats.

Table 1

Update of S1P₁-R agonists in clinical trials (<http://clinicaltrials.gov/>).

Compound (developing company)	Development status/ Clinical Trials	Compound class	S1P ₁₋₅ -R activity	Remarks
FTY720 (Novartis AG; Mitsubishi Tanabe Pharma) ^a	<ul style="list-style-type: none"> Launched for RRMS Phase 3 for primary progressive MS, kidney transplantation 	Pro-drug	S1P _{1,3-5}	<ul style="list-style-type: none"> First-in-class orally active drug for RRMS Transient bradycardia can occur within 20 h after first dose Macular edema and impaired lung function observed Long t_{1/2} (9-10 days)
BAF312 (Novartis AG)	<ul style="list-style-type: none"> Phase 3 for secondary progressive MS Phase 2 for polymyositis, RRMS 	Direct-acting	S1P _{1,5}	<ul style="list-style-type: none"> Transient bradycardia Shorter t_{1/2} than FTY720 (~ 30 h)
Ponesimod (ACT-128800, Actellion)	Phase 2 for RRMS, plaque psoriasis	Direct-acting	S1P _{1,3,5}	Shorter t _{1/2} than FTY720 (~ 30 h) ^b
ONO-4641 (Ono Pharmaceuticals)	Phase 2 for RRMS	Unknown	Unknown	Transient bradycardia ^c
KRP-203 (Novartis AG/Kyorin Pharmaceutical)	Phase 2 for ulcerative colitis, lupus erythematosus (terminated)	Pro-drug	S1P _{1,4,5}	
CS-0777 (Daiichi Sankyo)	Phase 1 for MS (completed)	Pro-drug	S1P _{1,4,5}	<ul style="list-style-type: none"> Long t_{1/2} (~ 7 days)^d Transient bradycardia^e
RPC1063 (Receptos, Inc.)	Phase 2 for RRMS, ulcerative colitis	Direct-acting	S1P ₁	No QT prolongation in TQT study ^f
APD334 (Arena Pharmaceuticals) ^g	Phase 1 for autoimmune diseases	Unknown	S1P ₁	

^a Mitsubishi Tanabe Pharma licensed out to Novartis the development and marketing rights of FTY720 worldwide, except for Japan (September 1997). <http://www.novartis.com/newsroom/media-releases/en/2011/1555145.shtml>; http://www.mt-pharma.co.jp/e/ir/annual/pdf/annual_2012_en.pdf

^b Brossard P. *et al. Br. J. Clin. Pharmacol.* doi: 10.1111/bcp.12129

^c http://registration.akm.ch/einsicht.php?XNABSTRACT_ID=156130&XNSPRACHE_ID=2&XNKONGRESS_ID=171&XNMASKEN_ID=900

^d Dyckman, A. J. *Annu. Rep. Med. Chem.* **2012**, 47, 195.

^e http://registration.akm.ch/einsicht.php?XNABSTRACT_ID=94798&XNSPRACHE_ID=2&XNKONGRESS_ID=106&XNMASKEN_ID=900

^f <http://ir.receptos.com/releasedetail.cfm?ReleaseID=769291>

^g <http://www.arenapharm.com/apd334>

Table 2

Oxazolidin-2-one-4-carboxamide lead compound.

7S1P₁-R EC₅₀ = 2.84 μM

Dose (mg/kg p.o. in mice)	%CD3 ⁺ cell decrease	
	FTY720	7
0.1	9.7 ± 26.6	-
1	79.0 ± 7.6	20.0 ± 6.1
10	86.6 ± 15.8	84.3 ± 4.0

Table 3

1,2,4-oxadiazole indole **11d** with *in vivo* efficacy in mouse EAE.

Cpd	R ²	R ³	R ⁴	SIP ₁ -REC ₅₀ (nM)	SIP ₃ -REC ₅₀ (μM)
11a	CO ₂ H	H	H	0.0125	> 10
11b	CONHMe	H	H	0.0398	> 10
11c	CO ₂ H	Me	H	0.0063	> 10
11d	CO ₂ H	Me	4-F	< 0.01	> 10
11e	CO ₂ H	Me	5-F	< 0.01	> 10

Table 4

Optimization of 1,2,4-oxadiazole core.

Cpd	Ar	AI	SIP ₁ -R EC ₅₀ (nM)	SIP ₃ -R EC ₅₀ (μM)	F _u (%) (human)	BBR (mouse)	PBL ₅ ^a (vs baseline)
15a		39	0.2	> 10	0.04	0.53 (1 h)	-
						0.68 (4 h)	
15b		72	2.5	> 10	0.21	0.36 (1 h)	22.7% (4 h)
						0.52 (4 h)	87.9% (24 h)
15c		84	1	> 10	0.19	0.47 (1 h)	34.9% (4 h)
						0.48 (4 h)	103.2% (24 h)

^a AI 1 mg/kg p.o. in mice.

Table 5*In vivo* properties of 1,2,4-thiadiazole **15d**.

S1P ₁ -R EC ₅₀ (nM)	S1P ₃ -R EC ₅₀ (μM)	F _u (%) (human)	BBR (mouse)	PBLs ^a (vs baseline)
0.8	< 3.2	0.64	0.23 (1 h) 0.65 (4 h)	23% (4 h) 72% (24 h)

^a At 1 mg/kg p.o. in mice.

Table 6Effects of N-scan on potency, PK, PBL counts of quinolinones **17a-17c, 18**.

Cpd	CH $\rightarrow$ N	SIP ₁ -R EC ₅₀ (nM)	SIP ₃ -R EC ₅₀ (nM)	Cl (L/h/kg) (1 mg/kg i.v., rats)	t _{1/2} (h) (1 mg/kg i.v., rats)	F%	PBL _{sc} ^a (vs baseline)
17a	-	70	1500	0.93	15	60	3%
17b	6	24	> 2500	0.16	41	95	64%
17c	6, 3'	20	> 25000	0.15	24	110	64%
18	-	18	> 25000	0.31	13-16	132	60%

^a After 24 h at 1 mg/kg p.o. in rats.

Table 7

Non-azetidine carboxylate derivatives of AMG369.

Cpd	R	S1P ₁ -R EC ₅₀ (nM)	S1P ₃ -R EC ₅₀ (μM)	PBLs ^a (vs baseline)
AMG369		2	0.888	71%
23a		0.6	0.834 (31%)	0
23b		0.5	1.13 (35%)	0
(-)- 23c		0.2	> 2.5	71%

^a After 24 h at 1 mg/kg p.o. in rats.

Table 8

Optimization of dihydronaphthalene analogs.

Cpd	R	R ¹	SIP ₁ -R EC ₅₀ (nM)	SIP ₃ -R EC ₅₀ (μM)	Mouse PBL ED ₅₀ (mg/kg) p.o. 24 h
25b	F	H	0.84	3.2	0.25
(S)- 25c	F	Me	0.37	9.45	0.22
(S)- 25d	Cl	Me	0.55	8.2	0.095