SIP signaling: new therapies and opportunities
Pedro J. Gonzalez-Cabrera, Steve Brown, Sean M. Studer and Hugh Rosen*

Abstract
Development of sphingosine-1-phosphate receptor 1 (S1P1) modulators to dampen inflammation and its sequelae is becoming increasingly promising for treating medical conditions characterized by significant immunopathology. As shown by the non-selective S1P receptor modulator FTY720 (fingolimod [Gilenya®]) in the treatment of relapsing-remitting multiple sclerosis (MS), the ability to use S1P1 modulation to precisely block immune cell traffic—immunomodulation—while maintaining immunosurveillance, has opened therapeutic opportunities in various other immune-derived chronic pathologies, including inflammatory bowel disease (IBD), lupus, psoriasis, as well as, potentially, in early acute viral respiratory infection. Proof-of-concept studies across validated animal models with S1P receptor modulators highly selective for S1P1, such as BAF-312 (Siponimod), KRP-203, ONO-4641 (Ceralifimod), ponesimod and RPC-1063, and emerging clinical trials for safety and efficacy in humans, particularly in MS, ulcerative colitis (UC) and psoriasis, have set the stage for us to consider additional testing in various other autoimmune diseases.

Introduction
Four years after Food and Drug Administration (FDA) approval, the first oral treatment for relapsing forms of MS, FTY720, is showing good efficacy for managing morbidity and the progression of active disease [1,2]. Therapeutically, the benefit afforded by FTY720 in managing symptoms of relapsing-remitting MS appears largely dependent on S1P1-dependent modulation on immune blood cells [3], neurons [4], astrocytes [5] and endothelia [6], all mediated by its active phosphorylated FTY720-P (S1P-mimic, Figure 1) product. In 1996, closely following its synthesis, FTY720 demonstrated efficacy in preventing transplant rejection across animal models due to its potent immunosuppressive action. It later became discontinued for that indication based on findings that FTY720 did not afford additional benefit to standard of care therapy. Findings that S1P1 modulation was the driving force behind FTY720’s efficacy and that the FTY720-mediated sequestration of circulating lymphocytes, or immune modulation (correlated with positive therapeutic outcomes), has prompted the search for second-generation compounds. These compounds either have a structural similarity to the FTY720 prodrug backbone, or are newer, directly acting modulators having chemically optimized aromatic backbones and a higher selectivity window for S1P1 over S1P3, while still having S1P3 activity (Figure 2 and Table 1). The other difference between the newer class modulators and FTY720 appears to be in the time course of immunosuppression, with the newer compounds having shorter half-lives and a shorter duration of lymphopenia, in contrast to the long-lasting FTY720 actions [7,8]. In addition, the therapeutic efficacy of the newer compounds appears to correlate well with the lymphocyte reduction mechanism(s) first defined by FTY720. In MS, there are several S1P receptor modulators being tested, such as Siponimod, KRP-203, CS-0777, and RPC-1063. Siponimod is an oral, second-generation S1P1/5 modulator in Phase 3 development for secondary progressive MS. The results from the BOLD Siponimod study, an adaptive dose-ranging Phase 2 study, were published in 2013 [9] and showed that, compared to placebo, Siponimod reduced brain magnetic resonance imaging (MRI) lesions and relapses by up to 80% in
relapsing-remitting MS. Phase 3 development of Siponi-mod in secondary progressive MS started in 2012, and results should be available in 2017. This article will highlight key preclinical findings of immunomodulators with a high selectivity window for S1P1 and/or S1P5 in support of furthering available treatment options in various autoimmune conditions.

Effective S1P-R therapy in murine models of IBD

There is an increasing need for newer and safer therapeutics in IBD. First-line corticosteroidal therapy and sulfasalazine ameliorate intestinal inflammation but have substantial toxicity. Suppressing local inflammation to the intestine by conventional 5-aminosalicylic acids (5-ASA) therapeutics reduces and maintains disease remission but does not truly inhibit relapse rates across all patients. Second-line anti-tumor necrosis factor alpha (TNF-α) monoclonal antibodies, though safer than conventional immunosuppressants, are another option, yet can lose effectiveness over time, and can predispose certain patients to potentially fatal opportunistic infections [10,11]. S1P receptor modulatory strategies have been evaluated in animal models of IBD in the past. Data using FTY720 and KRP-203, and later backed by the ONO-4641 prototype W-061 (S1P1/5 modulator of unpublished structure), show effectiveness for alleviating multiple aspects of chronic intestinal inflammation. KRP-203 is a S1P1/4/5 agonist prodrug with a molecular structure resembling FTY720. Like FTY720, KRP-203 sequesters circulating lymphocytes in secondary lymphoid organs in mice, and was first shown to prolong skin and
The efficacy of KRP-203 was assessed in the validated IL-10-deficient mouse model of human IBD [13,14]. IL-10 knockouts develop distal small bowel inflammation spontaneously and display hallmark IBD histopathological findings due to immune cell recruitment to the mucosa, enhanced production of T helper (Th)-1 cytokines, resulting in the disruption of the mucosal barrier, low body weight and poor survival. Song et al. [15] reported that KRP-203 administration to IL-10 knockout mice reduced mortality once the disease had been established. Both acute, 7-day, and chronic, 28-day, daily KRP-203 protocols reduced histological scores in the colon and prevented weight loss vs. vehicle-treated controls. A decreased recruitment of lymphocytes to the colonic lamina propria in the KRP-203 group correlated with the survival benefit and the immunohistological findings. A separate study using W-061 reported reduced inflammation to the distal colon of mice that underwent chemical dextran sodium sulfate (DSS)-induced colitis [16]. W-061 administration in this model reduced cellular infiltration to the colonic lamina propria, with associated reductions in Th17 and Th1 cytokines as measured in cultured CD4-T cells isolated from the W-061 treated group at study end. W-061 also prevented mucosal thickness and mucin depletion induced by DSS, relative to vehicle treatment. The results with KRP-203 and W-061 are promising and recapitulate some of the findings of FTY720 in animal models of IBD. FTY720 at low 0.3 mg/kg dose was effective in preventing body weight loss in the DSS colitis model and the CD4+ CD62L+ T cell transfer models of colitis. In addition, therapeutic efficacy of FTY720 was reported in the 2,4,6-trinitrobenzenesulfonic acid (TNBS) inflammatory colitis model, whereby drug treatment led to the dampening of IL-12p70 and subsequent Th1 pro-inflammatory cytokines, while simultaneously inducing the functional activity of CD4+CD25+ regulatory T cells. This indicates that, besides migration and the homing of lymphocytes to secondary lymphoid organs via S1P1, FTY720 can also influence cytokine effector function directly, as was shown by the prophylactic and therapeutic efficacy of FTY720 in the Th2-mediated oxazolone-induced colitis model in BALB/c mice.

Another class of S1P1 modulator, RPC-1063 (of unpublished structure, but of similar backbone to RP-001, Figure 2), is being currently tested worldwide as an oral therapeutic in phase 2 clinical trials of UC. In healthy subjects, RPC-1063 has shown no QTc interval alterations, and is additionally being evaluated in phase 3 trials in relapsing-remitting MS.

Figure 2. Chemical structures of S1P-R modulators

![Chemical structures of S1P-R modulators](image-url)
### Advancements of S1P-R modulation therapy in experimental systemic lupus erythematosus (SLE)

FTY720 has been compared against methylprednisolone in the MLR/lpr experimental autoimmunpus mouse model, which has a mutation in T cell-dependent immune dysfuction with human-like SLE phenotype. The clinical goal of therapy relies on suppressing the renal complications, or lupus nephritis that leads the mortality index in SLE. Genetically modified MRL/lpr mice have a mutational deficit in the Fas-mediated apoptosis of lymphoid cells, and spontaneously develop severe glomerulonephritis, vasculitis and tubular atrophy, replicating some of the clinical features of the human disease. Proteinuria, microscopic deposition of anti-double stranded DNA antibodies with complement contribution are strong markers of kidney malfunction in MLR/lpr mice, as well as in patients with SLE. Okazaki et al. [17] reported on survival, disease biomarkers, apoptotic indexes, and immune cellularity in MRL/lpr mice treated with FTY720 or methylprednisolone. FTY720 and the steroid showed survival protection vs. controls, with reduced IgG glomerular complex deposition consistent with the survival advantage. Notably, FTY720 treated MRL/lpr mice had drug-induced apoptotic destruction of a double-negative T cell population that is inherently dysregulated in this model. While the paper did not report on histopathology, Wenderfer et al. [18] showed histological findings after a 12-week 6 mg/kg KRP-203 daily dosing study in MLR/lpr mice. Drug treatment increased survival only at therapeutic dosing, inhibiting glomerulonephritis, vasculitis and tubular atrophy. There was also decreased proteinuria by KRP-203, although no differences in serum anti-double stranded DNA IgGs titers were noted. Findings that KRP-203, like FTY720, promoted dose-dependent apoptosis in double-negative T cells in MLR/lpr mice strongly support the argument that S1P receptor targeting efficacy, in this model, is mostly dependent on Fas-independent pathway apoptosis.

### Psoriasis

The selective S1P<sub>1/3/5</sub> modulator Ponesimod has successfully met the primary endpoint of efficacy and safety in patients with moderate to severe chronic plaque psoriasis. The proportion of patients with at least 75% improvement in Psoriasis Area and Severity Index (PASI) from baseline (PASI75) at week 16 was determined in a double-blind, placebo-controlled study consisting of 326 patients. With Ponesimod 20 mg daily, nearly half of patients improved by at least 75% at week 16 (p<0.0001 vs. placebo) [19]. Doubling the dose improved the outcome by at least 75% in patients at week 16 (p<0.0001 vs. placebo), whereas only 13.4% of the placebo group improved by 75%. Another endpoint of the study included Physician Global Assessment at week 16. Accordingly, patients continued to improve beyond the initial 16-week dosing phase. Safety and tolerability data from this study were consistent with the safety profile of Ponesimod observed in the past, including a Phase 2 study in MS [20]. As expected of S1P receptor modulators, there was a transient bradycardia and, less frequently, a transient effect on atrioventricular conduction. Dyspnea and asymptomatic liver enzyme elevations were two commonly reported side effects. These results suggest that Ponesimod could become a first in its class, oral therapeutic for treating psoriasis, although it has been discontinued as a potential MS therapeutic.

### Dampening the early cytokine storm by local S1P<sub>1</sub> modulation provides a survival advantage to acute lung viral infections

Early studies of acute viral lung pathogenesis, using the WSN strain of the influenza A virus, demonstrated that...
compounds targeting multiple S1P receptor subtypes suppressed the cytokine response during infection. Specifically, the results demonstrated that intratracheal but not intraperitoneal administration of AAL-R [21], an FY720 analog (Table 1), to infected mice reduced mortality and the accumulation and proliferation of activated CD8+ T cells into the lung. Subsequent studies with CYM-5442 and RP-001, compounds designed to target only the S1P1 subtype, also significantly reduced morbidity and mortality in mice infected with the highly virulent human isolate of pandemic 2009 H1N1 [22,23]. The authors proposed that these compounds increased survival by blunting and not abolishing excessive cytokine production often associated with certain virus strains.

A follow up animal study using ferrets, a relevant model used in influenza research due to its human-like mode of airway viral propagation and ability to develop symptoms seen in humans, were performed using the S1P1 specific agonist RP-002. The study demonstrated that agonism of the S1P1 receptor down-regulated and controlled the overly robust innate inflammatory response while minimally altering viral replication. Gavage administration of RP-002 [24] to H1N1:2009 infected ferrets significantly reduced mortality as compared to vehicle-administered controls. Additionally, the survival benefit in the ferret study was improved upon co-administration of RP-002 with the neuraminidase inhibitor oseltamivir. The authors concluded that RP-002 and oseltamivir as combined therapy conferred maximal protection by blunting both the immune pathology and viral replication.

The mechanism used by the S1P1 receptor to modulate cytokine secretion and subsequent morbidity remains unknown. Future studies should examine whether the S1P1 receptor directly modulates Toll-Like receptor-7 (TLR-7). It is feasible that both of these transmembrane proteins reside in the same subcellular compartment and upon S1P1 agonism whether a transient disruption of TLR-7’s microdomain occurs. This disruption may result in the abrogation of TLR-7 signaling through the canonical MyD88-IRF-7 pathway [25]. Since this pathway is chemically tractable, careful dissection of the pathway remains a possibility.

Overall, the data is promising in defining a proof-of-concept mechanism and should be carefully explored as an option to dampen excessive host innate immune collateral damage from highly pathogenic viruses.

**Conclusion**

The remarkable impact of therapeutic modulation of the S1P-S1PR1 axis reflects the multi-point interdiction of autoimmune pathogenesis. By blunting but not abolishing immune protection, these therapies provide unprecedented efficacy in MS and UC with a tolerability window that enhances the possibilities of treating autoimmune diseases with fewer infectious complications. Because disease relapse is clinically unpredictable, and especially difficult to treat in certain patients [26,27], evaluation of S1P receptor modulatory therapies needs to be thoroughly explored in additional pre-clinical studies. Underlying the disease is a chronic and progressive state of local inflammation known to alter the metabolism of pathway(s) regulating S1P levels. For instance, intestinal biopsy samples in patients with UC reveal a deregulated metabolic pathway whereby sphingosine kinase 1 (SphK-1) and S1P phosphohydrolase-1 (Sph-Pase) are upregulated and sphingosine 1-phosphate lyase (SPL) is downregulated (Figure 1), leading to high local tissue S1P concentration [28,29]. SphK-1 upregulation in humans is consistent with a key role of SphK1 in promoting murine intestinal inflammation and colitis-associated cancer via hyperactive intestinal nuclear factor-κB (NF-kB)-signal transducer and activator of transcription 3 (STAT-3) signaling [30,31]. One important question is whether S1P1 modulation alone would be sufficient to clinically reduce fully active intestinal disease, or whether it may be indicated as maintenance therapy. The other question is whether global immunomodulation by S1P1 selective or S1P1/5 selective compounds may be contraindicated with adjunct therapeutics of IBD, as shown with TNF-α blockers and other immunomodulators.

The UC TOUCHSTONE Phase 2 clinical trial results demonstrated the medically significant efficacy of daily 1 mg RPC1063 [32]. The study enrolled 199 patients split into three arms, placebo, low dose (0.5 mg) and high dose (1.0 mg). At 8 weeks of treatment, induction of clinical remission reported by standard Mayo scoring, was 16.4% (p<0.05) of the patients on the 1 mg dose as compared to 6.4% on placebo. The low dose group demonstrated a nonsignificant trend of 13.8% clinical remission. These results provide evidence for the utility of pharmacologically modulating S1P1/5 in UC. Whether human genetic factors are involved that would alter the predisposition for such SphK-1 pathways in UC and colitis-associated cancer needs to be investigated, and whether S1P1 and or S1P1/5 modulator therapy can dampen inflammation-promoting colitis-associated cancer is not known. Nevertheless, S1P1 modulations of lymphocyte trafficking and cytokine production strategies represent a good opportunity to reduce intestinal inflammation in IBD and provide a steroid alternative to chronic use.

**Abbreviations**

DSS, dextran sodium sulfate; IBD, inflammatory bowel
disease; MS, multiple sclerosis; PASI, Psoriasis Area and Severity Index; SLE, systemic lupus erythematosus; SphK1, sphingosine kinase; Th, T helper; TLR, Toll-like receptor; TNF, tumor necrosis factor.

Disclosures
Hugh Rosen is a scientific co-founder and Scientific Advisory Board member of Receptos and has a significant financial interest in the company.

References
1. Radue E, O’Connor P, Polman CH, Hohlfeld R, Calabresi P, Selmaj K, Mueller-Lenne N, Agoropoulou C, Holdbrook F, Vera A de, Zhang-Auberson L, Francis G, Burtin P, Kappos L: Impact of fingolimod therapy on magnetic resonance imaging outcomes in patients with multiple sclerosis. Archives of neurology 2012, 69:1259-69.

2. Kira J, Itoyama Y, Kikuchi S, Hao Q, Kurosawa T, Nagato K, Tsuniyama I, Rosenstiel P von, Zhang-Auberson L, Said T: Fingolimod (FTY720) therapy in Japanese patients with relapsing multiple sclerosis over 12 months: results of a phase 2 observational extension. BMC neurology 2014, 14:21.

3. Chun J, Brinkmann V: A mechanistically novel, first oral therapy for multiple sclerosis: the development of fingolimod (FTY720, Gilenya). Discovery medicine 2011, 12:213-28.

4. Chun J, Hartung H: Mechanism of action of oral fingolimod (FTY720) in multiple sclerosis. Clinical neuropharmacology 2010, 33:91-101.

5. Choi JW, Gardell SE, Herr DR, Rivera R, Lee C, Noguchi K, Teo ST, Yung YC, Lu M, Kennedy G, Chun J: FTY720 (fingolimod) efficacy in an animal model of multiple sclerosis requires astrocyte sphingosine 1-phosphate receptor I (SIP1) modulation. Proceedings of the National Academy of Sciences of the United States of America 2011, 108:751-6.

6. Oo ML, Chang S, Thangada S, Wu M, Rezaul K, Blaho V, Hwang S, Han DK, Hla T: Engagement of S1P-degradative mechanisms leads to vascular leak in mice. The journal of clinical investigation 2011, 121:2290-300.

7. Gonzalez-Cabrera Pj, Cahalan SM, Nguyen N, Sarkisyan G, Leaf NB, Cameron MD, Kago T, Rosen H: S1P(1) receptor modulation with cyclical recovery from lymphopenia ameliorates mouse model of multiple sclerosis. Molecular pharmacology 2012, 81:166-74.

8. Roberts E, Guerrero M, Urbano M, Rosen H: Sphingosine 1-phosphate receptor agonists: a patent review (2010-2012). Expert opinion on therapeutic patents 2013, 23:817-41.

9. Selmaj K, Li, David KB, Hartung H, Hemmer B, Kappos L, Freedman MS, Stüve O, Rieckmann P, Montalban X, Ziemssen T, Auberson LZ, Polhamm H, Mercier F, Dahlke F, Wallström E: Siposimod for patients with relapsing-remitting multiple sclerosis (BOLD): an adaptive, dose-ranging, randomised, phase 2 study. The Lancet Neurology 2013, 12:756-67.

10. Alteweg R, Vincent T: TNF blocking therapies and immunomonitoring in patients with inflammatory bowel disease. Mediators of inflammation 2014, 2014:172821.

11. Viget N, Vernier-Massouille G, Salmon-Ceron D, Yuzdanpanah Y, Colombel J: Opportunistic infections in patients with inflammatory bowel disease: prevention and diagnosis. Gut 2008, 57:549-58.

12. Shimizu H, Takahashi M, Kaneko T, Murakami T, Hakamata Y, Kudou S, Kishi T, Fukushima K, Iwami S, Kuriyama K, Yasue T, Enosawa S, Matsumoto K, Takeyoshi I, Morishita Y, Kobayashi E: KRP-203, a novel synthetic immunosuppressant, prolongs graft survival and attenuates chronic rejection in rat skin and heart allografts. Circulation 2005, 111:222-9.

13. Scheinin T, Butler DM, Salwy F, Scallon B, Feldmann M: Validation of the interleukin-10 knockout mouse model of colitis: anti-tumour necrosis factor-antibodies suppress the progression of colitis. Clinical and experimental immunology 2003, 133:38-43.

14. Rennick D, Davidson N, Berg D: Interleukin-10 gene knock-out mice: a model of chronic inflammation. Clinical immunology and immunopathology 1995, 76:517-48.

15. Song J, Matsuda C, Kai Y, Nashida T, Nakajima K, Mizushima T, Kinoshiba M, Yasue T, Sawa Y, Ito T: A novel sphingosine-1-phosphate receptor agonist, 2-amino-2-propanediol hydrochloride (KRP-203), regulates chronic colitis in interleukin-10 gene-deficient mice. The Journal of pharmacology and experimental therapeutics 2008, 324:276-83.

16. Sanada Y, Mizushima T, Kai Y, Nishimura J, Hagya H, Kurata H, Mizuno H, Uejima T, Ito T: Therapeutic effects of novel sphingosine-1-phosphate receptor agonist W-061 in murine DSS colitis. Röss one 2011, 6:e23933.

17. Okazaki H, Hirata D, Kamimura T, Sato H, Iwamoto M, Yoshio T, Masuyama J, Fujimura A, Kobayashi E, Kano S, Minota S: Effects of FTY720 in MRL-lpr/lpr mice: therapeutic potential in systemic lupus erythematosus. The Journal of rheumatology 2002, 29:707-16.

18. Wenderfer SE, Stępowski SM, Braun MC: Increased survival and reduced renal injury in MRL/lpr mice treated with a novel sphingosine-1-phosphate receptor agonist. Kidney international 2008, 74:1319-26.

19. Brossard P, Scherz M, Halabi A, Maatouk H, Krause A, Dingemann J: Multiple-dose tolerability, pharmacokinetics, and pharmacodynamics of ponesimod, an S1P1 receptor modulator: Favorable impact of dose up-titration. Journal of clinical pharmacology 2014.

20. Olsson T, Bosser A, Fernandez O, Freedman MS, Pozzilli C, Bach D, Berkani O, Mueller MS, Sidorenko T, Radue E, Melanson M: Oral ponesimod in relapsing-remitting multiple sclerosis: a randomised phase II trial. Journal of neurology, neurosurgery, and psychiatry 2014, 85:1198-208.

21. Marsolais D, Hahn B, Edelmann KH, Walsh KB, Guerrero M, Hatta Y, Kawaoa Y, Roberts E, Oldstone MB, Michael BA, Rosen H: Local non systematic modulation of dendritic cell S1P receptors in lung blunts virus-specific immune responses to influenza. Molecular pharmacology 2008, 74:896-903.
22. Walsh KB, Teijaro JR, Rosen H, Oldstone MB, Michael BA: Quelling the storm: utilization of sphingosine-1-phosphate receptor signaling to ameliorate influenza virus-induced cytokine storm. Immunologic research 2011, 51:19-25.

23. Teijaro JR, Walsh KB, Cahan S, Frengen DM, Roberts E, Scott F, Martinborough E, Peach R, Oldstone MB, Michael BA, Rosen H: Endothelial cells are central orchestrators of cytokine amplification during influenza virus infection. Cell 2011, 146:980-91.

24. Teijaro JR, Walsh KB, Long JP, Tordoff KP, Stark GV, Eisfeld AJ, Kawasaki Y, Rosen H, Oldstone MB, Michael BA: Protection of ferrets from pulmonary injury due to H1N1 2009 influenza virus infection: immunopathology tractable by sphingosine-1-phosphate 1 receptor agonist therapy. Virology 2014, 452-453:152-7.

25. Teijaro JR, Walsh KB, Rice S, Rosen H, Oldstone MB, Michael BA: Mapping the innate signaling cascade essential for cytokine storm during influenza virus infection. Proceedings of the National Academy of Sciences of the United States of America 2014, 111:3799-804.

26. Pardi DS, Sandborn WJ: Predicting relapse in patients with inflammatory bowel disease: what is the role of biomarkers? Gut 2005, 54:321-2.

27. Luftus EV, Davis KL, Wang C, Dastani H, Luo A: Treatment patterns, complications, and disease relapse in a real-world population of patients with moderate-to-severe ulcerative colitis initiating immunomodulator therapy. Inflammatory bowel diseases 2014, 20:1361-7.

28. Lépine S, Allegood JC, Park M, Dent P, Milstien S, Spiegel S: Sphingosine-1-phosphate phosphohydrolase-1 regulates ER stress-induced autophagy. Cell death and differentiation 2011, 18:350-61.

29. Maceyka M, Spiegel S: Sphingolipid metabolites in inflammatory bowel disease. Nature 2014, 510:58-67.

30. Snider AJ, Kawamori T, Bradshaw SG, Orr KA, Gilkeson GS, Mannan YA, Obeid LM: A role for sphingosine kinase 1 in dextran sulfate sodium-induced colitis. FASEB journal: official publication of the Federation of American Societies for Experimental Biology 2009, 23:143-52.

31. Liang J, Nagahashi M, Kim EY, Harikumar KB, Yamada A, Huang W, Hait NC, Allegood JC, Price MM, Avni D, Takabe K, Kordula T, Milstien S, Spiegel S: Sphingosine-1-phosphate links persistent STAT3 activation, chronic intestinal inflammation, and development of colitis-associated cancer. Cancer cell 2013, 23:107-20.

32. Receptos Reports Positive Phase 2 Results for TOUCHSTONE Trial of RPC1063 in Ulcerative Colitis [http://ir.receptos.com/irfiles/2014-March-18-Press-Release.pdf].

33. Hait NC, Allegood J, Maceyka M, Strub GM, Harikumar KB, Singh SK, Luo C, Marmorstein R, Kordula T, Milstien S, Spiegel S: Regulation of histone acetylation in the nucleus by sphingosine-1-phosphate. Science (New York, NY) 2009, 325:1254-7.

34. Kharel Y, Lee S, Snyder AH, Sheasley-O’neill SL, Morris MA, Setady Y, Zhu R, Zigler MA, Burcin TL, Ley K, Tung KS, Kenneth SK, Engelhard VH, Macdonald TL, Pearson-White S, Lynch KR: Sphingosine kinase 2 is required for modulation of lymphocyte traffic by FTY720. The Journal of biological chemistry 2005, 280:36865-72.

35. Allende ML, Sasaki T, Kawai H, Olivia A, Mi Y, van Echten-Deckert G, Hajdu R, Rosenbach M, Keohane CA, Mandala S, Spiegel S, Proia RL: Mice deficient in sphingosine kinase 1 are rendered lymphopenic by FTY720. The Journal of biological chemistry 2004, 279:52487-92.

36. Calabresi PA, Kieseier BC, Arnold DL, Balcer LJ, Boyko A, Pelleiter J, Liu S, Zhu Y, Seddighzadeh A, Hung S, Deykin A: Pegylated interferon β-1a for relapsing-remitting multiple sclerosis (ADVANCE): a randomised, phase 3, double-blind study. The Lancet Neurology 2014, 13:657-65.

37. Fryer RM, Muthukumarana A, Harrison PC, Nodop Mazurek S, Chen RR, Harrington KE, Dinallo RM, Horan JC, Putnaude L, Modis LK, Reinhart GA: The clinically-tested SIP receptor agonists, FTY720 and BAF312, demonstrate subtype-specific bradycardia (S1P₃) and hypertension (S1P₁) in rat. PloS one 2012, 7:e52985.

38. Moberly JB, Ford DM, Zahir H, Chen S, Mochizuki T, Truitt KE, Vollrath TL: Pharmacological effects of CS-0777, a selective sphingosine 1-phosphate receptor-1 modulator: results from a 12-week, open-label pilot study in multiple sclerosis patients. Journal of neuroimmunology 2012, 246:100-7.

39. Olson A, Hartung J, Timony G, Peach R, Boehm M, Smith H, Gujivathi S: P356 Safety and PK results of a thorough QT/QTc (TQT) study of orally administered RPC1063, a novel, selective S1PI receptor agonist. Journal of Crohn’s and Colitis 2014, 8:S213.