S1P receptor modulators and the cardiovascular autonomic nervous system in multiple sclerosis: a narrative review

Victor Constantinescu, Rocco Haase, Katja Akgün and Tjalf Ziemssen

Abstract: Sphingosine 1-phosphate (S1P) receptor (S1PR) modulators have a complex mechanism of action, which are among the most efficient therapeutic options in multiple sclerosis (MS) and represent a promising approach for other immune-mediated diseases. The S1P signaling pathway involves the activation of five extracellular S1PR subtypes (S1PR1–S1PR5) that are ubiquitous and have a wide range of effects. Besides the immunomodulatory beneficial outcome in MS, S1P signaling regulates the cardiovascular function via S1PR1–S1PR3 subtypes, which reside on cardiac myocytes, endothelial, and vascular smooth muscle cells. In our review, we describe the mechanisms and clinical effects of S1PR modulators on the cardiovascular system. In the past, mostly short-term effects of S1PR modulators on the cardiovascular system have been studied, while data on long-term effects still need to be investigated. Immediate effects detected after treatment initiation are due to parasympathetic overactivation. In contrast, long-term effects may arise from a shift of the autonomic regulation toward sympathetic predominance along with S1PR1 downregulation. A mild increase in blood pressure has been reported in long-term studies, as well as decreased baroreflex sensitivity. In most studies, sustained hypertension was found to represent a significant adverse event related to treatment. The shift in the autonomic control and blood pressure values could not be just a consequence of disease progression but also related to S1PR modulation. Reduced cardiac autonomic activation and decreased heart rate variability during the long-term treatment with S1PR modulators may increase the risk for subsequent cardiac events. For second-generation S1PR modulators, this observation has to be confirmed in further studies with longer follow-ups. The periodic surveillance of cardiovascular function and detection of any cardiac autonomic dysfunction can help predict cardiac outcomes not only after the first dose but also throughout treatment.
Introduction

Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system (CNS) with still an unpredictable, often progressive course.1 As the introduction of oral disease-modifying therapies over the past several years, treatment options for people with multiple sclerosis (PwMS) have expanded. First targeted as a treatment for MS, sphingosine 1-phosphate (S1P) receptor (S1PR) modulators have a complex mechanism of action and represent a promising therapeutic strategy for immune-mediated diseases.2 S1P is a membrane-derived lysophospholipid signaling molecule that is involved in a broad spectrum of physiological and pathophysiological processes, primarily through the activation of five extracellular S1PR subtypes (S1PR1–S1PR5).3 These receptors are located at various sites, like the immune, cardiovascular, and nervous systems.4

In 2010, fingolimod, an S1PR modulator, was the first oral therapy to be approved for the treatment of the relapsing forms of MS. Since 2019, three second-generation S1PR modulators have been approved: siponimod for relapsing and secondary progressive MS as well as ozanimod and ponesimod for relapsing MS.5,6 Other second-generation selective S1PR modulators that have been tested for MS are likely to come in the near future: ceralifimod (ONO-4641) and amiselimod (MT-1303).3 The new S1PR modulators have also been evaluated for the treatment of other immune-mediated diseases including inflammatory bowel disease, rheumatoid arthritis, systemic lupus erythematosus, and psoriasis. In 2021, ozanimod received Food and Drug Administration (FDA) approval as the first S1PR modulator for patients with moderately to severely active ulcerative colitis.6

In MS, the primary clinical efficacy of S1PR modulators is achieved through their coupling with subtype S1PR1, thus regulating immune cell trafficking through sequestration of autoreactive lymphocytes in the lymph nodes and presumably reducing migration and subsequent infiltration into the CNS.5,7-9 Further immunomodulatory effects have also been described.10 The S1P signaling pathway is involved in airway hyper-reactivity and pulmonary eosinophil sequestration.11 Also, S1P modulated signal transduction in renal glomerular and tubular cells could intervene in the
pathogenesis of acute and chronic kidney disease.12 PwMS may benefit from S1PR modulation in terms of less annual relapses, a lower magnetic resonance imaging (MRI) activity, and the stabilization of disability outcome.13 Neuroprotective effects of S1PR regulation in CNS cells including neurons, astrocytes, oligodendrocytes, and microglia have also been described.14 There are data that S1PR modulators may support astrocyte activation, blood–brain barrier integrity at the level of endothelial cells, and may impact remyelination.15 In addition, an effect of S1PR modulation on S1P concentration itself was proved.7 Moreover, S1P signaling modulates the cardiovascular function, with S1PR1, S1PR2, and S1PR3 subtypes residing on cardiac myocytes, endothelial, and vascular smooth muscle cells.1 S1PR1 plays a prominent role in the regulation of the heart rate16,17 and is responsible for temporary bradycardia and, less commonly, for a delay in atrioventricular (AV) conduction after the initiation of S1PR modulators therapy. These transitory parasympathomimetic effects usually last up to 6h, which is why cardiac monitoring is required when PwMS are treated with S1PR modulators. The particular changes reveal that S1PR modulators act initially as agonists, but play an antagonist role after down-regulation of S1PR at the cell surface.18 Furthermore, heart rate changes are accompanied by a transient reduction in blood pressure after the first dose of S1PR modulators, which is then followed by a slight increase in blood pressure that reaches a stable plateau after 6months of treatment.18

After prolonged treatment with S1PR modulators, a decrease in the baroreflex sensitivity and the capability to regulate sinus node activity as well as a global impairment in the cardiac autonomic control have been reported.19,20 Our review presents the short- and long-term effects of S1PR modulators on the cardiovascular autonomic function in PwMS. Considering the recent approval of the second generation of S1PR modulators by the regulatory authorities, further research on the cardiovascular impact of these new therapies is needed.

S1PR modulators: mechanism of action
S1P is a bioactive lysophospholipid resulting from the phosphorylation of sphingosine by sphingosine kinase-1 or sphingosine kinase-2.15 S1P activates a family of plasma membrane G protein-coupled receptors, generating selective and variable responses at the same time for at least two reasons. First, there is specificity in the coupling of different S1PR subtypes to various G proteins, such as Gi, Gq, or G12/13 proteins.21 The exception is the S1PR1 that binds exclusively to the Gi protein.22–24 For other S1PR subtypes, different cross-combinations are possible. G protein signaling downstream involves different divergent pathways: cyclic adenosine monophosphate (cAMP), phospholipase C, or RhoA. Therefore, the coupling to particular G proteins as well as the activation of downstream effectors determines the type of cellular feedback, emphasizing the selectivity of S1P signaling.21 Second, distinctive patterns for the S1PR subtypes regulate variable cellular responses25 in different systems inducing a wide range of effects (Table 1). The S1PR subtypes S1PR1, S1PR2, and S1PR3 are expressed at numerous sites in the body, but first and foremost in the cardiovascular, central nervous, and immune systems. At the same time, the expression of S1PR4 and S1PR5 is limited to the immune and nervous systems.18

Fingolimod is distinct from other S1PR modulators as it binds with high affinity to all receptor subtypes, except S1PR2. The second, more selective, generation of S1PR modulators exhibits improved pharmacokinetics and less side effects.

Methods
We systematically reviewed the literature available on the MEDLINE database using the PubMed search engine and entered the following keywords or a combination thereof: ‘multiple sclerosis’ AND (‘S1P receptor modulator’ OR ‘fingolimod, FTY-720’ OR ‘ponesimod, ACT-128800’ OR ‘siponimod, BAF-312’ OR ‘ozanimod, RPC-1063’ OR ‘ceralifimod, ONO-4641’ OR ‘amiselimod, MT-1303’) AND (‘cardiac’ OR ‘cardiovascular’ OR ‘autonomic’). We considered only peer-reviewed articles that had been published or had been officially accepted for publication by January 2022. One hundred seven articles were initially identified by title and abstract. Original articles, reviews, editorials, case series, and cohort studies were assessed. From those we excluded any articles that lacked essential data or were not available in full text. Eventually, thirty-nine articles with relevance to S1PR in PwMS remained. References of retrieved articles were also evaluated and considered for further clarification.
especially cardiovascular ones. While fingolimod and amiselimod act as pro-drugs, which undergo phosphorylation, second-generation S1PR modulators like siponimod, ozanimod, ponesimod, and ceralifimod do not require prior phosphorylation to bind to S1PR.

**Physiological effects of S1P and S1PRs on cardiovascular function**

S1P and the S1PRs have important regulatory functions in the physiologic and pathophysiologic processes of the cardiovascular system. The S1P–S1PRs interaction impacts the heart rate, blood pressure, and cardiac autonomic regulation. Animal models served to understand how specific receptor subtypes contribute to the cardiac function and complemented observations from human studies.

**Animal studies**

**SIP modulation on heart rate.** Available information concerning the S1P signaling pathways was enhanced by studies with S1P receptor subtype–specific knockout mice and sphingosine kinase knockout mice. G protein-gated inwardly rectifying potassium channels (GIRK) are activated after the initial binding of S1PR modulator to S1PR1 (and S1PR3 for fingolimod) on cardiac myocytes. The atrial GIRK channels, which are often attributed to the atrial muscarinic–gated potassium channels (IKACh), intervene in the cholinergic agonists’ modulation on action potential duration and the excitability of ventricular myocytes.

In gene deletion animal studies, the transient decrease in heart rate after S1PR modulation was mediated by activated GIRK channels triggering a signaling cascade via the Gβγ subunit. Bradycardia was caused by S1PR3 activation in mice. This finding motivated the search for S1PR agonists lacking S1PR3 signaling.

A prolonged exposure of atrial myocytes to S1P renders them resistant to recurrent S1P activation of IKACH that are expressed in sinoatrial, AV
nodal cells, and the atrial muscle. Apart from the activation of IKACH, there is also a regulation of other pacing-related currents, such as the hyperpolarization-activated inward current \( I_f \) and the voltage-gated calcium current \( I_{Ca,L} \). Inhibition of \( I_f \) represents the more minor but more sensitive component of the vagally mediated decrease in heart rate. Indeed, under basal conditions, S1P activates the IKACH in sinoatrial nodal cells in animal models, but has no effect on \( I_{Ca,L} \) and \( I_f \) unless these currents are pre-stimulated by the \( \beta \)-adrenergic agonist isoproterenol.

The degree of activation of the \( I_f \) current determines the frequency of action potential firing at the end of an action potential. Because \( I_f \) is controlled by intracellular cAMP and is thus activated by \( \beta \)-adrenergic and inhibited by muscarinic M2 receptor stimulation, it represents a primary physiological mechanism mediating autonomic regulation of the heart rate.

S1P modulation on blood pressure. An important mediator of S1P signaling for vasoreactivity and vascular permeability is the nitric oxide (NO) produced by endothelial NO synthase (eNOS), which affects different cellular mechanisms including cell survival, proliferation, and migration. Endothelium-released NO, as the primary mediator of flow-induced vasorelaxation, is also critical for maintaining normal blood pressure values.

Blood flow exerts shear stress that activates the S1P endothelial pathway signaling through the release of S1P by the endothelium, a mechanism studied in mice. This generates a physiological activation of S1PR1 in the vasculature, acting through G protein–coupled S1P receptors. With the role of S1P–S1PR1–NO signaling as part of a regulatory pathway of vascular vasodilation and blood pressure homeostasis, S1PR1 acts as mechanotransducer in response to flow.

In animal studies, an important protective effect of autocrine S1PR1/eNOS signaling was identified that prevented the onset of hypertension and pathological cardiac hypertrophy. S1PR3 is expressed in vascular smooth muscle cells of various vascular beds and along with S1PR2 induces vasoconstriction via the \( G_\text{q} \)-coupled \( \text{Ca}^{2+}/\text{IP}_3 \) and \( G_{13} \)-coupled \( \text{RhoA/Rho-kinase pathway} \). Oppositely, endothelial S1PR3 mediates vasodilation via eNOS-derived NO production.

At low concentrations, S1P signaling induces vasodilation through S1PR1 and S1PR3 activation on endothelial cells. At higher concentrations, S1P causes vasoconstriction through S1PR2 and S1PR3 activation on vascular smooth muscle cells.

Pharmacological inhibition of S1PR1 can decrease the eNOS activation in response to shear stress, which will reduce NO’s plasma levels and increase S1PR2 and S1PR3 expressions in vascular smooth muscle cells (Figure 1). Therefore, S1PR modulators can induce a shift to a higher-than-normal blood pressure value due to impaired endothelial-dependent vasodilation.

In animal studies, long-term fingolimod treatment seemed to impair not only the functions of endothelial but also of smooth muscle cell by reducing the NO-induced activation of guanylyl cyclase and cyclic guanosine monophosphate (cGMP)–mediated vasodilation. Fingolimod induces vasoconstriction via stimulation of S1PR on vascular smooth muscle cells in coronary and basilar arteries, which can be explained by the variable expression levels of S1PR1–S1PR3.

Besides the S1PR–eNOS signaling, it was assumed that there were also other mechanisms that might explain the blood pressure variation after S1PR modulators initiation. S1PR1–S1PR3 are also found throughout the renal cortex, outer and inner medulla of the kidney, and mediate natriuresis. In animal studies, after the administration of supratherapeutic doses of fingolimod, sodium excretion was found to be reduced, which appeared not to be caused by altered renal functions or structure.

Human studies

S1P modulation on heart rate. A transient heart rate decrease is a known consequence after the first dose of S1PR modulators. Besides the S1PR1 subtype being involved in heart rate control, it was assumed that S1PR3 agonism could also cause bradycardia. A detailed analysis of in situ hybridization and immunohistochemistry of the human cardiovascular tissue have shown that S1PR3 mRNA and protein are weakly expressed in human atrial, septal, and ventricular cardiomyocytes, whereas S1PR1 was present on these cells, being the predominant receptor subtype.

Siponimod, an S1PR1 and S1PR5 selective agonist, induced rapid onset but transient bradycardia
in humans, however, although it has no action on S1PR3. Measurements of the GIRK activity in human atrial myocytes supported the assumption that they were involved in the cellular mechanism inducing bradycardic effects.

The AV node is controlled by both the parasympathetic and sympathetic nervous system. The parasympathomimetic impact of S1PR modulators on the nodal function results from the agonistic effect they have on S1PR, which is similar to acetylcholine activation of muscarinic receptors. In some PwMS, this may lead to an initial prolongation of AV impulse conduction and, albeit very rarely, to second-degree AV blocks. The transient reduction in heart rate and, less commonly, the temporary delay in AV conduction observed in some patients when initiating S1PR modulators result from the activation of S1PR1 on cardiac myocytes prior to internalization and desensitization of the receptor by the S1PR agonist (Figure 2). Fingolimod initially acts as an unselective agonist. As it also induces receptor downregulation, fingolimod is considered a selective functional antagonist of the S1PR1 subtype. This impact on S1PR1 is unique and not seen with the endogenous ligand S1P, which correspondingly internalizes S1PR1 upon binding but afterward dissociates in endosomes while the receptor recycles back to the plasma membrane. Similarly, S1PR3, S1PR4, and S1PR5 are internalized upon fingolimod-phosphate binding, and then presented to the cell surface. This shift to a higher-than-normal blood pressure value due to impaired endothelial-dependent vasodilation and predominant S1PR2- and S1PR3-mediated vasoconstriction.

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**Figure 1.** Short- and long-term effects of S1P receptor modulators on blood pressure regulation. S1P signaling pathway plays an essential role in vasoactivity and vascular permeability. Blood flow exerts shear stress on the vessel’s endothelial wall, which activates the S1P endothelial pathway signaling through G protein–coupled S1P receptors. The nitric oxide (NO) produced by endothelial NO synthase (eNOS) after S1P/S1PR1 activation cascade is critical for blood pressure homeostasis by generating vasorelaxation. Initially, S1P receptor modulators (S1PR-Ms) act as potent agonists on S1PR1 that induce transient endothelium-dependent vasodilation. S1PR1 downregulation consecutive to S1PR overstimulation converts S1PR-M in functional antagonists. Pharmacological inhibition of S1PR1 decreases the eNOS/NO activation in response to shear stress and reduces flow-mediated vasodilation. Concomitantly, there is an increase in S1PR2 and S1PR3 expressions in vascular smooth muscle cells that generates sustained vasoconstriction via PKC/Rho/ROCK signaling pathway activation. Therefore, S1PR modulators can shift to a higher-than-normal blood pressure value due to impaired endothelial-dependent vasodilation and predominant S1PR2- and S1PR3-mediated vasoconstriction.

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Akt, Akt kinase; eNOS, endothelial nitric oxide (NO) synthase; PKB, protein kinase B; PKC, protein kinase C; ROCK, rho-associated kinase; S1PR-M, sphingosine 1-phosphate receptor (S1PR) modulator; SPNS2, spinster homolog 2, S1P transporter; stress fibers, contractile actomyosin bundles.
function that may occur in the course of treatment with S1PR modulators. The agonistic effect of fingolimod on S1PR3, S1PR4, and S1PR5 is responsible for additional biological effects with a less well-known outcome in the long run.84

*S1P modulation on blood pressure.* Like endogenous S1P, S1PR modulators may activate the eNOS/NO pathway leading to short-term vasodilation and a temporary decrease in blood pressure as seen in some patients after treatment initiation.18 A slight increase in blood pressure was continuously observed after several months of treatment.18 Vascular regulation in response to S1PR modulators seems to depend on the drug dose and the length of drug exposure.86,87 The functional antagonism after continuous treatment explains the paradoxical loss of receptor signaling without compensatory receptor upregulation,86 especially for S1PR1, which perhaps could also be responsible for the rise in blood pressure values.

*S1P modulation on cardiac autonomic control.* Heart rate variability (HRV) is a marker of cardiac autonomic regulation underlying the interplay between parasympathetic and sympathetic components.88 As the negative chronotropic effect on the sinus node was associated with a parasympathetic activation,89–93 the impact of S1PR modulators initiation on cardiac autonomic regulation has captured much interest. The negative dromotropic effect on the AV junctional region is related to a decreased sympathetic function.94 The chronotropic and dromotropic effects are interdependent in physiological conditions.95 A significant increase in indices of parasympathetic activity in the time domain analysis of HRV, such as RMSSD (root mean square of successive RR interval differences), or pNN50 (percentage of successive RR intervals that differ by more than 50 ms),96 was correlated with the moment of maximal heart rate drop which was reached about 4–5 h after S1PR modulator initiation.89–93 Frequency domain analysis of the HRV underlined that parasympathetic modulation, expressed by HF (high frequency) power, increased after fingolimod intake, while LF (low frequency) power and LF/HF ratio decreased after medication.90 This result highlights the fact that the acute bradycardic effect observed after the initiation of fingolimod mostly occurs as part of a wider parasympathetic activation.97,98 These acute effects were accompanied by a slight and transient decrease in systolic and diastolic blood pressures.99,100 Further research on the second-generation S1PR modulators is needed as most of the studies conducted so far have relied on fingolimod data. Owing to the direct stimulation of

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**Figure 2.** Transient vagomimetic effect of S1P receptor modulators on cardiac cells after treatment initiation. S1P receptor modulators are competitive antagonists with S1P binding to S1P receptor subtype 1 (S1PR1) generating via GIRK channels activation and downstream signaling a transient vagomimetic effect. This is represented by a decrease in heart rate and delay in atrioventricular conduction. Overstimulation of S1PR1 is transient due to β-arrestins activation, which generates receptor internalization and S1PR1 downregulation. S1PR desensitization determines the functional antagonist effect of S1PR modulators, which imposes a reduction of S1PR1 on the cell’s surface and leads to a change in S1P homeostatic signaling.

Source: Created with BioRender.com.

GIRK, G protein–coupled inwardly rectifying potassium channel; M2 receptor, muscarinic type-2 receptor; S1PR-M, sphingosine 1-phosphate receptor (S1PR) modulator.
S1PR1, the negative chronotropic effect at dose initiation seemed to be less intense in patients treated with amiselimod.93

Among other cardiovascular parameters, baseline heart rate is a strong predictor for nadir heart rate after S1PR modulators initiation.80 It was shown that results from parasympathetic activation tests like the Valsalva maneuver or deep breathing could serve as predictors for the bradycardic effect after the first fingolimod dose and its possible prolongation over time.18,94,101 A prominent parasympathetic and diminished sympathetic cardiac modulation indicated a higher risk for a more evident decrease of heart rate at nadir94 underlining the relevance of the individual autonomic profile prior to treatment.

It seems as although different components of the autonomic nervous system are activated chronologically once S1PR modulators have been administered. An enhancement in cardiac parasympathetic regulation was found already 1 h post-treatment initiation with peaks after 3–5 h.89,90,94,98,102 Lower blood pressure values and decreased sympathetic cardiac regulation were reported 2 h after S1PR modulator initiation.89,97,98 LF and LF/HF ratio had the lowest values toward the fifth post-dose hour suggesting a decrease in the sympathetic component of the sympathovagal balance.98,102

After the nadir point for the heart rate and HRV, the recovery toward baseline values starts at 5 h post-dose98,102,103 which continues for the next several hours, corresponding to the downregulation of S1PR in the myocytes.18 Blood pressure values also start to return to baseline after 5 h98,102 with systolic blood pressure recovering faster than diastolic blood pressure.98 The slight decrease in systolic blood pressure within the first 5 h after S1PR modulator intake most likely results from the parasympathetic activation, in which the complementary decrease of the heart rate reduces cardiac output.102,104 The significant concomitant decrease in diastolic blood pressure may be due to additional arterial vasodilation resulting from endothelium-dependent NO release triggered by S1PR activation.18,102 Furthermore, considering the blood pressure changes as an input and heart rate variation as output for a particular interval, valuable data of the baroreflex feedback system could be provided.

Fingolimod not only changes the heart rate through its direct pharmacologic S1PR1 effects mimicking parasympathetic activity18,98,102 but also has an impact on blood pressure, overall cardiac autonomic modulation, and baroreflex sensitivity.102 Regular baroreflex feedback was described in the maintenance of blood pressure values despite the immediate heart rate decrease after initiation of treatment with fingolimod.102 The initial parasympathomimetic effect of the S1PR modulator covers the baroreflex attempt to reduce cardiovagal stimulation, which leads to a decrease in heart rate and blood pressure after 2 h.102 The parasympathomimetic effect of S1PR modulator outweighs any centrally mediated increase in sympathetic activity102 in the first few hours after administration of the initial dose. In addition, the vagomimetic effect can further alter centrally mediated or baroreflex-dependent cardiovascular responses and thus increase the risk of syncope in PwMS that may already present a compromised central autonomic modulation. Hilz et al.102 reported a significant increase in resting baroreflex sensitivity starting only 1 h after fingolimod initiation and found that the values remained increased even at 6 h post-dose. Baroreflex sensitivity at rest was the highest when fingolimod had shifted the heart rate and blood pressure values to their nadir in response to the steadily increasing vagomimetic effects.102 During the Valsalva maneuver, fingolimod did not impair baroreflex-mediated rapid cardiovascular adjustment to baroreceptor unloading and subsequent baroreceptor loading. Those cardiovascular effects, however, seemed to be mitigated by sympathetic activation105 as the heart rate nadir significantly increased the baroreflex gain. Further studies on the effects of S1PR modulators on sympathovagal modulation and consecutive changes in baroreflex sensitivity as a measure of cardiovascular prognosis still need to be conducted.102,105,106

**Short-term effects of S1PR modulators in clinical trials**

Immediately after S1PR modulator initiation, apart from bradycardia, a slight decrease in blood pressure was reported, which was found likely to have been mediated by endothelium NO release.18,102 There was also a significant increase in total HRV and enhanced cardiac vagal autonomic activity in subjects with bradycardia, which
is deemed to have a cardioprotective effect. Four hours after S1PR modulator initiation, the overall cardiac autonomic modulation reaches the maximum value of parasympathetic and minimum value of sympathetic modulation.

PwMS with specific risk profiles may encounter adverse effects like extreme bradycardia or AV blocks in consequence of an excessive parasympathetic effect. A vigilant screening for cardiovascular risk factors and exclusion criteria for S1PR modulators initiation should be considered. Absolute contraindications for S1PR modulators include myocardial infarction within the last 6 months, unstable angina, stroke, transient ischemic attack, decompensated heart failure requiring inpatient treatment, and the New York Heart Association class III/IV heart failure. Cardiac contraindications for S1PR modulators also include history or presence of Mobitz type II second-degree or third-degree AV block, sinoatrial heart block or sick sinus syndrome (unless the patient has a functioning pacemaker), symptomatic bradycardia or recurrent syncope, uncontrolled hypertension, or severe untreated sleep apnea. In addition, fingolimod is contraindicated in patients with baseline QTc intervals of at least 500 ms on electrocardiogram (ECG) or patients treated with class Ia or III antiarrhythmic drugs.

In three pivotal phase III studies – FREEDOMS, FREEDOMS II, and TRANSFORMS – the transitory effects of fingolimod 0.5 or 1.25 mg taken once a day were analyzed. Short-term major cardiovascular events related to S1PR modulators initiation were rare (Table 2). Blood pressure presented the most significant decrease 4 h after fingolimod initiation, with average values of −3.1 mmHg for systolic blood pressure and −3.9 mmHg for diastolic blood pressure, tending toward normalization within 6 h of treatment initiation.

Table 2. Short-term major cardiovascular events related to S1PR modulators therapy.

| S1PR modulator | Mean HR reduction from baseline | Mean HR <40 bpm 0–6 h post-dose, number of cases (%) | Symptomatic bradycardia (%) | Day 1 Mobitz type I second-degree AV blocks (%) | Day 1 Mobitz type II or higher AV blocks (%) |
|----------------|--------------------------------|--------------------------------------------------|----------------------------|----------------------------------|----------------------------------|
| Fingolimoda (0.5 mg) | −8.1 bpm | 1 (0.3) | 0.6 | 3.7 | 0 |
| Siponimodb (up to 2 mg) | −5.3 bpm | 0 | 0.001 | 0 | 0 |
| Ozanimodc (0.25 mg) | −1.9 bpm | 0 | 0.001 | 2 | 0 |
| Ponesimodd (up to 20 mg) | −8.7 bpm | 3 | 0 | 0 | 0 |
| Amiselimode (0.4 mg) | 0 bpm (−4.4 bpm)e | 0 (1)f | 0 (0) | 0 (1)f | 0 (0) |
| Ceralifimodg (0.10 mg) | −12 bpm | 0 | 0 | 0 | 0 |

AV, atrioventricular; bpm, beats per minute; ECG, electrocardiogram; HR, heart rate. Italics indicate the healthy subjects.

cPooled cardiac and Holter ECG findings from three fingolimod phase III studies [TRANSFORMS, FREEDOMS, and FREEDOMS II] (n = 1212).111

dSiponimod versus placebo in secondary progressive multiple sclerosis [EXPAND]: a double-blind, randomized, phase III study [n = 1099].

Safety and efficacy of ozanimod versus interferon beta-1a in relapsing multiple sclerosis [RADIANCE]: a multicenter, randomized, 24-month, phase III trial [n = 1320],112 and safety and efficacy of the selective sphingosine 1-phosphate receptor modulator ozanimod in relapsing multiple sclerosis [RADIANCE]: a randomized, placebo-controlled phase II trial.113

ePonesimod compared with teriflunomide in patients with relapsing multiple sclerosis in the active-comparator phase III OPTIMUM study [n = 565].

Safety and efficacy of amiselimod in relapsing multiple sclerosis [MOMENTUM]: a randomized, double-blind, placebo-controlled phase II trial [n = 312].113

fCardiac effects of amiselimod compared with fingolimod and placebo: results of a randomized, parallel-group, phase I study in healthy subjects [n = 81 healthy subjects].

gEffect of ceralifimod [ONO-4641] on lymphocytes and cardiac function: randomized, double-blind, placebo-controlled trial with an open-label fingolimod arm [n = 144 healthy subjects].
The START study assessed cardiac safety of fingolimod first dose in relapsing-remitting MS using continuous the Holter ECG monitoring.\textsuperscript{116} Bradycardia [<45 beats per minute (bpm)] was reported in 0.9% of patients, and second-degree Mobitz I (Wenckebach) block and 2:1 AV block in 1.7\%\textsuperscript{117} No Mobitz II AV block was noted. Asymptomatic third-degree AV block occurred in one patient.\textsuperscript{117} Given the upper limit QTc values of 450 and 470 ms for men and women, respectively, 13 PwMS had QTc intervals above those thresholds after 7 days post-dose.\textsuperscript{117} No patient had a QTc interval exceeding 500 ms.\textsuperscript{117} During the follow-up 1 week after initiation, no second-/third-degree AV block was observed confirming that cardiac events after fingolimod initiation are rare and mostly benign.\textsuperscript{116,117} Interestingly, the incidence of second- or third-degree AV blocks was significantly higher in older PwMS (\textgtrapprox 50 years) and in females (87.5\% of all patients with a second- or third-degree AV block), while bradycardia occurred more often in males (58.7\% of all bradycardia events).\textsuperscript{117} Moreover, a higher body mass index (BMI) was associated with a lower risk for bradycardia and PwMS with a BMI below 25 presented a higher incidence of second- or third-degree AV block.\textsuperscript{117} Therefore, people with a lower BMI might experience a higher dose effect. Thus, extra caution in patients with low BMI might be advised. The correlation between bradycardia and low BMI in fingolimod-treated patients should be investigated in more detail in future studies.

In the phase IIIb, open-label FIRST study, the short-term safety and tolerability of fingolimod 0.5 mg in patients with relapsing-remitting MS were evaluated in a real-world setting. The population included PwMS with certain pre-existing cardiac conditions or baseline cardiac findings and also PwMS who were receiving beta-blockers and calcium channel blockers.\textsuperscript{118} Bradycardia occurred in 0.6\% of PwMS and had a higher incidence in patients receiving beta-blockers and calcium channel blockers. Most of the PwMS were asymptomatic and recovered without any pharmacological intervention.\textsuperscript{118} In the first 6 h after fingolimod initiation, asymptomatic Mobitz type I second-degree AV block and 2:1 AV block were more frequently reported in PwMS with pre-existing cardiac conditions than in those without such conditions.\textsuperscript{118} FIRST study results confirmed the cardiac safety profile of short-term fingolimod treatment in a broader population of patients with relapsing-remitting MS, even for study participants with pre-existing cardiac conditions and specific cardiac treatment.\textsuperscript{118}

With the second generation of S1PR modulators, the concept of dose titration was introduced considering the rapid tachyphylaxis of the bradycardic effect.\textsuperscript{119} In the phase II BOLD study, the bradycardic effect that had previously been observed with the therapeutic dose initiation of siponimod was attenuated using a dose titration strategy.\textsuperscript{120} Following the Fibonacci sequence,\textsuperscript{121,122} the concept of dose titration aims to induce the internalization (or downregulation) of S1PR at low doses that are known to have a mild cardiac effect.\textsuperscript{119} The titration scheme for S1PR modulators is illustrated in Table 3.

### Table 3. S1PR modulators initiation therapy recommendations.

| S1PR modulator       | Treatment initiation (days) | Recommendation for re-initiating therapy after treatment interruption |
|----------------------|----------------------------|---------------------------------------------------------------|
| Fingolimoda GILENYA® 0.5 mg | Day 1: 0.5 mg  | The same first-dose monitoring as for treatment initiation is recommended when treatment is interrupted for: |
| Target dose: 0.5 mg/day | Day 2: 0.5 mg  | □ 1 day or more during the first 2 weeks of treatment; |
|                      | Day 3: 0.5 mg  | □ more than 7 days during weeks 3 and 4 of treatment; |
|                      | Day 4: 0.5 mg  | □ more than 2 weeks after at least 1 month of treatment. |
|                      | Day 5: 0.5 mg  |                                               |
|                      | Day 6: 0.5 mg  |                                               | (Continued)
In the extension of the BOLD study, the safety and efficacy of siponimod for up to 24 months were assessed, using five different treatment doses (10, 2, 1.25, 0.5, and 0.25 mg) in patients with relapsing-remitting MS. Within 10 days, dose titration effectively counterbalanced the negative chronotropic effect of siponimod that had been noted at higher doses in the BOLD Study. Eight PwMS, who had undergone washout prior to the extension study, required extended cardiac monitoring during the first 8 days of dose titration. Overall, only two PwMS had transient second-degree AV block on 24-h Holter ECG during the dose titration period. These transient events were not considered clinically significant, and no symptomatic bradycardia was observed. In the BOLD study, siponimod initiation at higher doses (2 and 10 mg) was

Table 3. (Continued)

| S1PR modulator | Treatment initiation (days) | Recommendation for re-initiating therapy after treatment interruption |
|----------------|-----------------------------|---------------------------------------------------------------------|
| Siponimod<sup>a</sup>  
MAYZENT®  
0.25 mg,  
2 mg  
Target dose:  
2 mg/day, | Day 1  
0.25 mg | The same dose escalation regimen is recommended in case of treatment discontinuation for four or more consecutive days. |
|                | Day 2  
0.25 mg |                                                                 |
|                | Day 3  
0.25 mg |                                                                 |
|                | Day 4  
0.75 mg |                                                                 |
|                | Day 5  
1.25 mg |                                                                 |
|                | Day 6  
2 mg |                                                                 |
|                | Day 7  
0.25 mg |                                                                 |
|                | Day 8  
0.25 mg |                                                                 |
|                | Day 9  
0.25 mg |                                                                 |
|                | Day 10 
0.75 mg |                                                                 |
|                | Day 11 
0.75 mg |                                                                 |
|                | Day 12 
1 mg |                                                                 |
| Ozanimod<sup>c</sup>  
ZEPOSIA®  
0.23 mg,  
0.46 mg,  
0.92 mg  
Target dose:  
0.92 mg/day | Day 1  
0.23 mg | The same dose titration is recommended when treatment is interrupted for: |
|                | Day 2  
0.23 mg | □ 1 day or more during the first 14 days of treatment; |
|                | Day 3  
0.23 mg | □ more than seven consecutive days between day 15 and day 28 of treatment; |
|                | Day 4  
0.23 mg | □ more than 14 consecutive days after day 28 of treatment. |
|                | Day 5  
0.46 mg | The next planned dose could be taken if the drug holiday is shorter than the duration mentioned above. |
|                | Day 6  
0.46 mg |                                                                 |
| Ponesimod<sup>d</sup>  
PONVORY®  
2–10 mg,  
20 mg  
Target dose:  
20 mg/day | Day 1  
2 mg | The same dose titration is recommended in case of drug holiday: |
|                | Day 2  
2 mg | □ if less than four consecutive doses are missed, treatment is continued with the first missed dose; |
|                | Day 3  
3 mg | □ if four or more consecutive doses are missed, treatment is reinitiated with day 1 (2 mg) of the titration regimen (new treatment initiation pack). |
|                | Day 4  
3 mg | The same first-dose monitoring is recommended as for treatment initiation if four or more consecutive doses of ponesimod are missed during the titration or maintenance periods. |
|                | Day 5  
4 mg |                                                                 |
|                | Day 6  
4 mg |                                                                 |
|                | Day 7  
5 mg |                                                                 |
|                | Day 8  
6 mg |                                                                 |
|                | Day 9  
7 mg |                                                                 |
|                | Day 10 
8 mg |                                                                 |
|                | Day 11 
9 mg |                                                                 |
|                | Day 12 
10 mg |                                                                 |
|                | Day 13 
10 mg |                                                                 |
|                | Day 14 
10 mg |                                                                 |
|                | Day 15 
20 mg |                                                                 |

<sup>a</sup>Gilenya [fingolimod].<sup>123</sup>  
<sup>b</sup>Mayzent [siponimod].<sup>124</sup>  
<sup>c</sup>Zeposia [ozanimod].<sup>125</sup>  
<sup>d</sup>Ponvory [ponesimod].<sup>126</sup>
associated with a reduction in heart rate in all patients, while the decrease in heart rate was much less pronounced or absent at lower doses (0.25, 0.5, and 1.25 mg).120

In the EXPAND trial, cardiovascular monitoring was performed in people with secondary progressive MS.20 In contrast to the BOLD study, patients were older (mean age 48.0 versus 37.4) and on average had a higher Expanded Disability Status Score (EDSS) (5.4 versus 2.4).20,127 Initiation dosing protocol comprised 6 days of progressive titration starting with 0.25 mg until 2 mg/day.20 Limited cardiovascular side effects were observed during treatment initiation (Table 2). Symptomatic sinus bradycardia was reported in two cases on day 7. In consequence thereof, treatment was not continued in one case.20

In the RADIANCE phase III trial, the safety and efficacy of ozanimod versus interferon beta-1a were analyzed in people with relapsing MS.112 A titration regimen was progressively continued from ozanimod HCl 0.25 mg (equivalent to ozanimod 0.23 mg) to the assigned dose (0.5 or 1.0 mg, equivalent to ozanimod 0.46 or 0.92 mg, respectively). The same protocol of dose titration was applied in the RADIANCE randomized, placebo-controlled, phase II trial.91 During the first 6 h, the maximum reduction in heart rate occurred at a dose of 0.25 mg.91,112 Holter monitoring in the first 24 h revealed a second-degree AV block type I in four patients (2%) and symptomatic bradycardia was reported in one patient after administration of the initial 0.25 mg dose91,112 (Table 2).

In the phase III OPTIMUM study, the efficacy and safety of ponesimod in comparison with that of teriflunomide were tested in people with relapsing MS.5 A gradual titration protocol was followed starting with a dose of 2 mg that was then gradually increased up to a 20-mg maintenance dose on day 15.5 Three PwMS had an asymptomatic post-dose heart rate of less than 40 bpm, while all of them had a pretreatment heart rate of less than 55 bpm.5 The initiation of ponesimod treatment was associated with transient and asymptomatic AV conduction abnormalities5 (Table 2). Those resolved within 24 h without any medical intervention.

In people with relapsing-remitting MS that were treated with amiselimod 0.1 mg (n = 105), 0.2 mg (n = 103), and 0.4 mg (n = 104) during the MOMENTUM phase II study, there were no clinically relevant cardiac events113 (Table 2). One asymptomatic episode of second-degree AV block in the amiselimod 0.1 mg group (1%) and one asymptomatic nonsustained ventricular tachycardia in the 0.2 mg group (1%) were reported as severe treatment-emergent adverse events.113

In a study on healthy subjects that were equally randomized to receive amiselimod 0.4 mg, amiselimod 0.8 mg, placebo or fingolimod 0.5 mg once a day for 28 days in total, no negative chronotropic effects were recorded for either amiselimod dose within the first 6 h after initiation.114 The lowest mean nadir heart rate was detected on day 14 in the amiselimod 0.4 mg group114 (Table 2). In this latter study on healthy subjects, a case of bradycardia with a minimum heart rate of ≤40 bpm that lasted for ≥30 s and a Mobitz type I (Wenckebach) second-degree AV block in the first 24 h were described in the group receiving amiselimod 0.4 mg.114

Healthy subjects were given ceralifimod (0.01, 0.025, 0.05, or 0.10 mg), fingolimod (0.5 mg), or a placebo once a day for a period of 14 days in a randomized, double-blind study.115 A similar diurnal pattern of heart rate decline was observed in all treatment regimens, reaching the peak of decline 5 h after initiation.115 In comparison with people on ceralifimod (−12.0 bpm for the 0.10 mg dose and −6.2 bpm for the 0.05 mg dose), people on fingolimod (−14.9 bpm) exhibited a higher heart rate decrease.115 A second-degree AV block, consisting of a single blocked P wave, was described for four subjects (one each in the placebo, 0.01 mg, 0.025 mg, and 0.05 mg ceralifimod groups) but not for any patients in the ceralifimod 0.10 mg or fingolimod groups.115

Therefore, cardiac effects, that is, a transient decrease in heart rate in the first hours after the initiation of S1PR modulators, were present in PwMS and healthy persons.115,128 Overall, almost half of the PwMS present with cardiovascular dysregulation that increases with longer disease duration.129,130 In light of the recent approval of second-generation S1PR modulators, it might be worth reconsidering more potent drugs for the early treatment of PwMS.

As cardiac autonomic dysregulation is most likely caused by the impaired central modulation of sympathetic and parasympathetic outflow,131 a dysfunction...
in the autonomic nervous system may contribute to long-term disability in PwMS.\textsuperscript{132} The length and severity of the impairment might impact the magnitude of the cardiac autonomic response after the initiation of S1PR modulators. The cardiovascular deconditioning caused by MS-related disability and overall plaque burden throughout the brain and spinal cord interrupting central autonomic pathways might explain the impairment of autonomic activity.\textsuperscript{133} Moreover, cerebral lesions in specific areas of the central autonomic network might also be responsible for the imbalance in sympathetic and parasympathetic cardiovascular modulation. For example, voxel-based lesion symptom mapping was used to reveal associations between cardiovascular autonomic dysfunction and MS-related cerebral lesions in different sites. It was found that several brain regions were involved in the shift toward increased cardiac sympathetic modulation, such as the left insula, hippocampus, right posterior parietal white matter, and right inferior frontal operculum.\textsuperscript{134}

Another essential factor that ought to be taken into account when analyzing cardiac autonomic responses in PwMS is the phenotype of the disease.\textsuperscript{132,135–137} Considering the likely differences in disease burden, the greater chronicity of secondary MS, and, in case of spinal cord involvement, a disability that is likely to be more significant, it seems that autonomic imbalance is more pronounced in the progressive variant of MS than in relapsing forms.\textsuperscript{110,132,133}

**Long-term effects of S1PR modulators in clinical trials**

PwMS show all the common age-related changes in autonomic cardiovascular modulation with an increase in sympathetic and a decrease in parasympathetic cardiac activity that have also been described for healthy individuals.\textsuperscript{102,138,139}

Initially, sympathetic hyperactivity is a compensatory mechanism in aging people, but, unfortunately, this chronic stimulation becomes detrimental to the cardiovascular system and β-adrenergic receptors, thus causing dysfunction in their signaling.\textsuperscript{140} β-adrenoceptor desensitization caused by the phosphorylation of receptors, followed by internalization and changes in G protein and kinase activity were described in connection with aging.\textsuperscript{141} In cardiomyocytes, a reciprocal downregulation occurs between β1-adrenoceptors and the cardioprotective S1PR1.\textsuperscript{142} Endothelial senescence is associated with increased levels of S1PR2 expression, while young endothelial cells express low levels of S1PR2.\textsuperscript{143} In contrast to S1PR2, S1PR1 proved to have vasculoprotective effects.\textsuperscript{143} Overexpression of S1PR2 in immature cells induces senescence-associated endothelial impairments, albeit its inhibition seems to restore endothelial function.\textsuperscript{143,144}

Defective S1P signaling and prolonged sympathetic overactivation in association with impaired β-adrenergic receptor sensitivity increase the cardiovascular risk.\textsuperscript{145,146} Cardiac autonomic dysfunction was described in PwMS.\textsuperscript{131,147} Sympathetic dysregulation could be attributed to the long-term clinical activity of the disease and might also be related to the inflammatory mechanisms of MS in the bidirectional communication between the autonomic and immune systems.\textsuperscript{129,147} It was stated that it thereby may also play a role in the pathogenesis of MS.\textsuperscript{129,148,149} Meanwhile, parasympathetic dysfunction correlated with the progression of disability as reflected by the EDSS, and this autonomic impairment could be the consequence of MS.\textsuperscript{147} Therefore, parasympathetic control is more frequently disrupted in advanced stages of the disease.\textsuperscript{120,133,147,150}

With regard to the long-term effects of S1PR modulators on the sympathovagal balance, here is only limited data available. Most studies were on fingolimod that highlights the need for extended follow-up studies on second-generation S1PR modulators. While some authors described a persisting change in HRV and heart rate which lasted 12.9 ± 7.1 months under treatment with fingolimod, thus emphasizing a continued parasympathetic predominance on the cardiac autonomic modulation,\textsuperscript{151} other results proved the contrary. Simula et al.\textsuperscript{97} described a shift toward a predominantly sympathetic heart rate modulation after 3 months of fingolimod treatment. An important decrease in the baroreflex sensitivity at 6 months after fingolimod initiation together with an impairment of the parasympathetic and sympathetic cardiac control was also reported.\textsuperscript{152} Indices referring to the parasympathetic modulation of the heart rate such as pNN50, RMSSD, and HF power decreased significantly.\textsuperscript{152} Interestingly, at the same time, LF power was also decreasing\textsuperscript{152} which may be due to the impaired central autonomic regulation and dysfunction of β-adrenergic receptor sensitivity and is associated with an increased risk for cardiovascular events.\textsuperscript{147,153,154}

Chronic sympathetic activation may lead to HRV depression and reduced global cardiac autonomic modulation.\textsuperscript{155} In PwMS treated with S1PR
modulators, an antithetical status between the increased autonomic activation in the acute phase of drug initiation and the reduced autonomic activation in the chronic phase of treatment was identified.\(^{18}\)

This might indicate that the prolonged treatment with S1PR modulators produces a global autonomic dysfunction independently from the acute response to the first drug dose. Long-term treatment with S1PR modulators seems to impact the blood pressure control in PwMS, inducing a mild blood pressure increase.\(^{13,156,158}\) In MS clinical trials, patients receiving fingolimod 0.5 mg presented an average increase of approximately 3 mmHg in systolic blood pressure and approximately 1 mmHg in diastolic pressure after 1 month of treatment, which was maintained over 6 months of treatment.\(^{18}\)

In a 24-month follow-up of placebo-controlled trial on fingolimod, hypertension was affirmed in PwMS\(^{156}\) (Table 4). Starting in the second month, the mean systolic and diastolic blood pressures exceeded the recorded baseline values; after 24 months, the mean increase was 1.9 and 0.7 mmHg with 0.5 mg of fingolimod, and 3.6 and 2.1 mmHg, respectively, with 1.25 mg of fingolimod possibly indicating a dose-dependent effect.\(^{156}\) Except for the first 24 hours after fingolimod initiation, it was found that the continued use of fingolimod had no clinically notable effect on the heart rate or AV conduction (Table 4).

The LONGTERMS study\(^{13}\) included patients who completed the phase II and III/IIb core studies FREEDOMS,\(^{156}\) FREEDOMS II,\(^{158}\) and TRANSFORMS\(^{159}\) and their extension studies. Hypertension was identified as an adverse event in 11% of patients treated with fingolimod.\(^{13}\) During the follow-up, the number of patients experiencing hypertension as an adverse event steadily decreased from Year 1 to Year 11 reaching a ratio of 0.6%.\(^{13}\) Bradycardia-related serious adverse events (mostly related to drug initiation) were present in 0.4% of patients in the first year but absent from the second year onwards. No patient reported transient bradycardia post reintroduction of fingolimod after temporary interruption.\(^{13}\)

### Table 4. Long-term major cardiovascular events related to S1PR modulators therapy.

| S1PR modulator | Follow-up period/number of patients | Hypertension number of cases (%) | Mean systolic blood pressure change | Mean diastolic blood pressure change | Major cardiovascular events after day 1 |
|----------------|-----------------------------------|----------------------------------|------------------------------------|-------------------------------------|--------------------------------------|
| Fingolimoda (0.5 mg) | 24 months (n = 425) | 26 (6.1%) | +1.9 mmHg | +0.7 mmHg | 1 (0.2%) second-degree AV block |
| Siponimodb (up to 2 mg) | 24 months (n = 1099) | 115 (10%) | +2–5 mmHg\(^c\) | +2–5 mmHg\(^c\) | 0 |
| Ozanimood (1 mg) | 24 months (n = 434) | 24 (5.5%) | +5.2 mmHg\(^e\) | +2.3 mmHg\(^d\) | 0 |
| Ponesimod\(^{f}\) (up to 20 mg) | 24 months (n = 565) | 57 (10.1%) | +2.9 mmHg | +2.8 mmHg | 1 (0.2%) hypertension-related AE |
| Amiselimo (0.1–0.4 mg) | 24 months (n = 322) | 0 | +0–5 mmHg | +0–2 mmHg | 0 |
| Ceralifimo (up to 0.15 mg) | 6 months (n = 343) | 0 | – | – | 0 |

AE, adverse event; AV, atrioventricular; bpm, beats per minute; HR, heart rate.

\(^{a}\)Placebo-controlled trial of oral fingolimod in relapsing multiple sclerosis, 1272 patients included, fingolimod 0.5 mg compared with 1.25 mg and placebo.\(^{156}\)

\(^{b}\)Siponimod versus placebo in secondary progressive multiple sclerosis (EXPAND): a double-blind, randomized, phase III study (n = 1099).\(^{20}\)

\(^{c}\)European Medicines Agency assessment report for siponimod (Mayzent) (n = 1148).\(^{15}\)

\(^{d}\)Safety and efficacy of ozanimod versus interferon beta-1a in relapsing multiple sclerosis (RADIANCE): a multicenter, randomized, 24-month, phase III trial.\(^{112}\)

\(^{e}\)European Medicines Agency assessment report for ozanimod (Zeposia) (n = 1313).\(^{125}\)

\(^{f}\)Ponesimod compared with teriflunomide in patients with relapsing multiple sclerosis in the active-comparator phase III OPTIMUM study (n = 565).\(^{5}\)

\(^{g}\)Two-year results from a phase II extension study of oral amiselimo in relapsing MS (MOMENTUM Extension study) (n = 322).\(^{13}\)

\(^{h}\)Safety of ONO-4641 in patients with relapsing-remitting multiple sclerosis: results from a 6-month interim analysis of the DreaMS extension study (n = 343).\(^{157}\)
In the phase II BOLD study extension, one patient (3.0%; \( n = 33 \); 10 mg siponimod) was diagnosed with a first-degree AV block at month 12.\(^{127}\) Another patient from the same group presented with a Mobitz type I second-degree AV block recorded during the screening phase and months 3, 6, and 12 of the dose-blinded extension phase. No symptomatic bradycardia was reported in any dose group, and none of the patients had a QTc interval of 480 ms or more. There were no clinically significant changes in blood pressure, except for one patient (2%, \( n = 50 \)) in the 0.25 mg group, who presented with hypertension.\(^{127}\)

In the EXPAND trial, 10% of the siponimod-treated PwMS presented with hypertension during the 2 years of follow-up in comparison with 8% in the placebo group\(^{20}\) (Table 4). Only few data are available on the increase of blood pressure during long-term treatment with siponimod. A small but consistent increase of 2–5 mmHg in systolic and diastolic blood pressures was mentioned in the European Medicines Agency assessment report of siponimod, however.\(^{124}\) In another study with a limited number of patients, a slight increase of 4 and 2 mmHg in mean systolic and diastolic blood pressure values, respectively, was described.\(^{90}\) In the EXPAND trial, long-term follow-up revealed no negative chronotropic or dromotropic effect of siponimod.\(^{20}\)

In the randomized, double-blind phase III SUNBEAM trial, the safety and efficacy of ozanimod \textit{versus} intramuscular interferon beta-1a were assessed in patients with relapsing MS.\(^{160}\) In the follow-up period of at least 12 months, hypertension was identified in 2.4% (\( n = 453 \)) and 1.3% (\( n = 448 \)) of patients treated with 0.5 and 1.0 mg of ozanimod HCl, respectively.\(^{160}\) In the RADIANCE phase III trial in which the follow-up was longer (24 months), hypertension was identified in 4.6% (\( n = 439 \)) and 5.5% (\( n = 434 \)) of patients treated with 0.5 and 1.0 mg of ozanimod HCl, respectively\(^{112}\) (Table 4). No clinically meaningful cardiac findings such as bradycardia or conduction abnormalities were reported in these two studies during follow-up.\(^{112,160}\) It, however, should be noted that after 3 months on ozanimod HCl 1 mg, the systolic and diastolic blood pressures had increased by 4.1 and 1.8 mmHg, respectively, while after 24 months, the mean change from baseline amounted to 5.2 and 2.3 mmHg, respectively.\(^{125}\) A study with a longer follow-up period is required to validate this observation of stabilizing blood pressure values. It is worth mentioning that orthostatic hypotension was reported as an adverse early dose-related effect with a slightly higher incidence in the ozanimod HCl 1 mg than in the 0.5 mg treatment group.\(^{125}\) Long-term results from clinical trials, however, did not raise any significant cardiovascular concerns regarding orthostatic hypotension.\(^{125}\)

In the OPTIMUM study follow-up, hypertension was reported as an adverse reaction in 10.1% of ponesimod-treated PwMS after 108 weeks of treatment.\(^{5}\) After approximately 1 month of treatment, a slight increase in blood pressure was described and persisted throughout the follow-up as long as treatment was continued\(^{126}\) (Table 4). In case of discontinuation of ponesimod treatment, blood pressure values decreased to baseline values.\(^{126}\) Three patients discontinued the treatment because of hypertension-related adverse events; one patient presented with serious hypertensive adverse events.\(^{5}\) No cardiac conduction abnormalities or arrhythmias occurred during follow-up.\(^{126}\)

The MOMENTUM Extension\(^{93}\) was a 72-week continuation to the MOMENTUM phase II study.\(^{113}\) Over a period of 96 weeks, blood pressure levels were stable in all treatment groups (amiselimod 0.2 mg, 0.4 mg, and placebo). In patients treated with amiselimod 0.2 mg, a slight variation of 0–2 mmHg in systolic blood pressure was seen during follow-up, while for the amiselimod 0.4 mg group, an increase of up to 5 mmHg was reported at week 48, which had decreased gradually by week 84\(^{93}\) (Table 4). In both the MOMENTUM phase II study and the MOMENTUM Extension study, hypertension did not emerge as treatment-related adverse event. No arrhythmia of clinical concern was observed during those studies.\(^{93}\) Post-dose hourly heart rates slightly diminished during the daytime period in amiselimod treated patients and did not return to baseline values within 24 weeks of dosing, but were consistently higher than 74 bpm in all groups and not considered clinically significant.\(^{93}\) During nighttime, no significant reduction in heart rate was observed, and there was also no clinically relevant difference in heart rate between these groups.\(^{93}\) A normal pattern of the circadian rhythm of heart rate was reported.\(^{93}\)

In the DreaMS Extension phase II study, PwMS received different ceralifimod dose regimens
The short-term effects of S1PR modulators on the cardiovascular system were most frequently studied. Most of the studies investigating the adverse effects of S1PR modulators focused on their impact on heart rate. We, however, observe that the incidence of bradycardia has become insignificant, and that cardiac arrhythmia and conduction abnormalities have been considered isolated events in the long-term follow-up.

Another point worth noting is the temporary interruption of treatment during long-term follow-up. S1PR modulators present different pharmacokinetics profiles, specifically regarding their elimination half-life. While fingolimod has a relatively long half-life (6–9 days following single or multiple dose administration), siponimod and ponesimod exhibit a shorter half-life (30–120 and 33 h, respectively). Ceralifimod has an elimination half-life of 82 up to 89 h. Ozanimod also has a relatively short elimination half-life, ranging from 17 to 21 h, but its metabolites (CC112273, the major active metabolite, and other minor active metabolites RP101988 and RP101075) have specific pharmacokinetic profiles. The active metabolite CC112273 has a high potency on S1PR1 and a longer half-life of 10–13 days. Both amiselimod and amiselimod-phosphate have a long elimination half-life (about 380–420 h) that is characterized by their gradual accumulation to a steady state over a period of about 10 weeks. Therefore, this pharmacokinetic profile is favorable in the initiation phase of amiselimod treatment, exerting a moderate effect on cardiovascular function and rendering amiselimod the only second-generation S1PR modulator that can be administered without the need of a dose titration regimen. The magnitude of re-initiation associated with heart rate reduction amplifies with the duration of the drug holiday, and, correspondingly, with the decline of S1PR modulator’s seric concentrations corresponding to the elimination half-life. Therefore, in contrast to fingolimod that has a longer half-life and concentration profile over time, cardiac monitoring is needed in case of re-initiation after a shorter drug holiday for second-generation S1PR modulators (siponimod, ponesimod, ozanimod) (Table 3). The shorter half-lives of the selective S1PR modulators, however, may allow for a more rapid discontinuation which can pose an advantage in case of treatment-related complications or pregnancy and also offer more flexibility for retreatment with other agents.

Most of the concerns raised in the long-term follow-up studies regarding the treatment with S1PR modulators are related to blood pressure elevation (Figure 3), as the chronotropic and dromotropic effects are limited over time, and no serious adverse events were reported. Furthermore, it was stated that blood pressure values in fingolimod-treated PwMS tended to stabilize after 2 years of treatment. Similar studies are required for the second generation of S1PR modulators.

The precise mechanism of long-term elevation of blood pressure values in patients treated with S1PR modulators remains unclear. Hypertension, however, could be related to the effect of S1PR in regulating the endothelial barrier but also to an imbalance in the autonomic nervous system. Moreover, a shift of the central autonomic modulation toward higher sympathetic and less parasympathetic control on cardiac activity was described in PwMS under S1PR modulator treatment. In consequence, long-term effects of intensified sympathetic regulation of the cardiac activity in PwMS under S1PR modulators remain unpredictable taking into account the fact that age and other vascular risk factors may add to the total burden of cardiovascular diseases.

**Conclusion**

S1PR modulators exert complex effects on S1PRs and the S1P signaling pathway, depending on the receptor’s subtype specificity, functional antagonism, and bioavailability of active agonists. Understanding the physiological process and the mechanism of action of S1PR modulators in PwMS, besides the proven beneficial effects on the disease’s course, their interference with the autonomic nervous system regulation could be explained. The short-term effects of S1PR modulators on the cardiovascular system were most frequently studied as the initial parasympathomimetic effect related to dose initiation required cardiac monitoring of patients over the first 6 h post-dose. Based on these findings, most of the precautions taken at treatment...
initiation with S1PR modulators initiation consider cardiac conduction abnormalities, bradyarrhythmias, and pre-existing cardiovascular diseases. Initial dose titration over several days in the second generation of S1PR modulators mitigates parasympathomimetic effects. Depending on the individual clinical profile, biomarkers like the pretreatment heart rate or the cardiac autonomic balance in connection with parasympathetic predominance are useful indicators of possible cardiac adverse events that may occur during the initiation of treatment with S1PR modulators. In contrast to the immediate effects on the cardiovascular function that may arise from treatment with S1PR modulators, long-term effects of treatment on the cardiac autonomic regulation and blood pressure homeostasis are still largely unknown.

In the long run, the intake of S1PR modulators seems to shift autonomic regulation toward sympathetic predominance, while S1PR1 downregulation is deprived of its cardioprotective role. All of the S1PR modulators exert their therapeutic effects in MS mainly through the functional antagonism of S1PR1, but this can also interfere with endogenous S1P signaling by over-activating other

Figure 3. Short- and long-term changes of heart rate, blood pressure, and cardiac autonomic modulation during fingolimod treatment. Immediately after fingolimod initiation, a negative chronotropic effect on the sinus node is observed, with a maximal drop of the heart rate after the fifth hour post-dose, associated with a slight and transient decrease in systolic and diastolic blood pressures. A significant parasympathetic activation revealed by increasing values for RMSSD (root mean square of successive RR interval differences), or pNN50 (percentage of successive RR intervals that differ by more than 50 ms), was correlated to the moment of maximal heart rate drop after fingolimod initiation. A reduction in the LF/HF ratio, based on the augmented HF (high frequency) values, another parasympathetic marker, is also observed. After the nadir point for the heart rate and heart rate variability parameters displaying the cardiac autonomic regulation, there is a recovery toward baseline values starting after 5 h, which continues for the next several hours, corresponding to the downregulation of S1P receptors in the myocytes. Diastolic and systolic blood pressure values also recover to baseline starting after 5 h. Long-term treatment is associated with a mild blood pressure increase and a decrease in parasympathetic cardiac modulation, reflected in RMSSD and pNN50 values. The LF/HF ratio reduction is due to the impairment of both sympathetic and parasympathetic modulations, which is correlated to a decrease in baroreflex sensitivity.

BL, baseline; H1, H2, H3, H4, H5, and H6, the first, second, third, fourth, fifth, and sixth hour after fingolimod intake.
receptor subtypes leading to a change in S1P homeostatic signaling in the vascular tissue. Blood pressure values may increase slightly during treatment. Still, the risk for hypertension should not be neglected as it represents a significant adverse event related to treatment in most studies.

The shift in autonomic control and blood pressure values might not just be a consequence of disease progression but could also be the result of S1PR modulation. Therefore, from a clinical perspective, periodic surveillance of the cardiovascular function and autonomic balance can help predict cardiac outcomes during treatment continuation and should thus be extended after the first initiating dose.

Reduced cardiac autonomic activation and decreased HRV during the long-term treatment with S1PR modulators may lead to an increased risk of subsequent cardiac events. Thus, recognizing autonomic dysfunction in PwMS is relevant from both the clinical and pathophysiological perspectives to better understand the role of S1P signaling and identify potential targets for treatment.

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ORCID iDs
Rocco Haase https://orcid.org/0000-0003-2465-4909
Tjalf Ziemssen https://orcid.org/0000-0001-8799-8202

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Author contributions
Victor Constantinescu: Conceptualization; Methodology; Writing – original draft; Writing – review & editing.
Rocco Haase: Conceptualization; Methodology; Supervision; Writing – review & editing.
Katja Akgün: Conceptualization; Supervision; Writing – review & editing.
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References
1. Ziemssen T, Akgün K and Brück W. Molecular biomarkers in multiple sclerosis. J Neuroinflammation 2019; 16: 272.
2. Pérez-Jeldres T, Alvarez-Lobos M and Rivera-Nieves J. Targeting sphingosine-1-phosphate signaling in immune-mediated diseases: beyond multiple sclerosis. Drugs 2021; 81: 985–1002.
3. Roy R, Alotaibi AA and Freedman MS. Sphingosine 1-phosphate receptor modulators for multiple sclerosis. CNS Drugs 2021; 35: 385–402.
4. Peyrin-Biroulet L, Christopher R, Behan D, et al. Modulation of sphingosine-1-phosphate in inflammatory bowel disease. Autoimmun Rev 2017; 16: 495–503.
5. Kappos L, Fox RJ, Burcklen M, et al. Ponesimod compared with teriflunomide in patients with relapsing multiple sclerosis in the active-comparator phase 3 OPTIMUM study: a randomized clinical trial. JAMA Neurol 2021; 78: 558–567.
6. Zeposia Prescribing Information. Zeposia U.S. product information. Princeton, NJ: Bristol-Myers Squibb Company, https://packageinserts.bms.com/pi/pi_zeposia.pdf (2021, accessed 7 February 2022).
7. Sehr T, Akgün K, Proschmann U, et al. Early central vs. peripheral immunological and neurobiological effects of fingolimod – a longitudinal study. *J Mol Med* 2019; 97: 1263–1271.

8. Sehr T, Akgün K, Haase R, et al. Fingolimod leads to immediate immunological changes within 6 h after first administration. *Front Neurol* 2020; 11: 391.

9. Fischer S, Proschmann U, Akgün K, et al. Lymphocyte counts and multiple sclerosis therapeutics: between mechanisms of action and treatment-limiting side effects. *Cells* 2021; 10: 3177.

10. Thomas K, Sehr T, Proschmann U, et al. Fingolimod additionally acts as immunomodulator focused on the innate immune system beyond its prominent effects on lymphocyte recirculation. *J Neuroinflammation* 2017; 14: 41.

11. Yang Y and Uhlig S. The role of sphingolipids in respiratory disease. *Thor Adv Respir Dis* 2011; 5: 325–344.

12. Koch A, Pfeilschifter J and Huwiler A. Sphingosine 1-phosphate in renal diseases. *Cell Physiol Biochem* 2013; 31: 745–760.

13. Cohen JA, Tenenbaum N, Bhatt A, et al. Extended treatment with fingolimod for relapsing multiple sclerosis: the 14-year LONGTERMS study results. *Thor Adv Neurol Disord* 2019; 12: 1756286419878324.

14. Luchtmann D, Gollan R, Ellwardt E, et al. In vivo and in vitro effects of multiple sclerosis immunomodulatory therapeutics on glutamatergic excitotoxicity. *J Neurochem* 2016; 136: 971–980.

15. Cohen JA and Chun J. Mechanisms of fingolimod’s efficacy and adverse effects in multiple sclerosis. *Ann Neurol* 2011; 69: 759–777.

16. Mazurais D, Robert P, Gout B, et al. Cell type-specific localization of human cardiac S1P receptors. *J Histochem Cytochem* 2002; 50: 661–670.

17. Forrest M, Sun SY, Hajdu R, et al. Immune cell regulation and cardiovascular effects of sphingosine 1-phosphate receptor agonists in rodents are mediated via distinct receptor subtypes. *J Pharmacol Exp Ther* 2004; 309: 758–768.

18. Camm J, Hla T, Balshy R, et al. Cardiac and vascular effects of fingolimod: mechanistic basis and clinical implications. *Am Heart J* 2014; 168: 632–644.

19. Racca V, Rovaris M, Cavarretta R, et al. Acute fingolimod effects on baroreflex and cardiovascular autonomic control in multiple sclerosis. *J Cereb Blood Flow Metab* 2019; 11: 1179573519849945.

20. Kappos L, Bar-Or A, Cree BAC, et al. Siponimod versus placebo in secondary progressive multiple sclerosis (EXPAND): a double-blind, randomised, phase 3 study. *Lancet* 2018; 391: 1263–1273.

21. Means CK and Brown JH. Sphingosine-1-phosphate receptor signalling in the heart. *Cardiovasc Res* 2009; 82: 193–200.

22. Lee MJ, Evans M and Hla T. The inducible G protein-coupled receptor edg-1 signals via the G(i)/mitogen-activated protein kinase pathway. *J Biol Chem* 1996; 271: 11272–11279.

23. Lee MJ, Van Brocklyn JR, Thangada S, et al. Sphingosine-1-phosphate as a ligand for the G protein-coupled receptor EDG-1. *Science* 1998; 279: 1552–1555.

24. Zondag GC, Postma FR, Etten IV, et al. Sphingosine 1-phosphate signalling through the G-protein-coupled receptor Edg-1. *Biochem J* 1998; 330: 605–609.

25. Ishii I, Fukushima N, Ye X, et al. Lysophospholipid receptors: signaling and biology. *Annu Rev Biochem* 2004; 73: 321–354.

26. Pham TH, Baluk P, Xu Y, et al. Lymphatic endothelial cell sphingosine kinase activity is required for lymphocyte egress and lymphatic patterning. *J Exp Med* 2010; 207: 17–27.

27. Xu M, Waters CL, Hu C, et al. Sphingosine 1-phosphate rapidly increases endothelial barrier function independently of VE-cadherin but requires cell spreading and Rho kinase. *Am J Physiol Cell Physiol* 2007; 293: C1309–C1318.

28. Novgorodov AS, El-Alwani M, Bielawski J, et al. Activation of sphingosine-1-phosphate receptor S1P5 inhibits oligodendrocyte progenitor migration. *FASEB J* 2007; 21: 1503–1514.

29. Harada J, Foley M, Moskowitz MA, et al. Sphingosine-1-phosphate induces proliferation and morphological changes of neural progenitor cells. *J Neurochem* 2004; 88: 1026–1039.

30. Poulsen RR, McClaskey CM, Rivkees SA, et al. The Sphingosine-1-phosphate receptor 1 mediates S1P action during cardiac development. *BMC Dev Biol* 2011; 11: 37.
31. Lee JF, Gordon S, Estrada R, et al. Balance of S1P1 and S1P2 signaling regulates peripheral microvascular permeability in rat cremaster muscle vasculature. *Am J Physiol Heart Circ Physiol* 2009; 296: H33–H42.

32. Skoura A and Hla T. Regulation of vascular physiology and pathology by the S1P2 receptor subtype. *Cardiovasc Res* 2009; 82: 221–228.

33. Herr DR, Grillet N, Schwander M, et al. Sphingosine 1-phosphate (S1P) signaling is required for maintenance of hair cells mainly via activation of S1P2. *J Neurosci* 2007; 27: 1474–1478.

34. Singleton PA, Moreno-Vinasco L, Sammani S, et al. Attenuation of vascular permeability by methylnaltrexone: role of mOP-R and S1P3 transactivation. *Am J Respir Cell Mol Biol* 2007; 37: 222–231.

35. Chaudhry BZ, Cohen JA and Conway DS. Sphingosine 1-phosphate receptor modulators for the treatment of multiple sclerosis. *Neurotherapeutics* 2017; 14: 859–873.

36. Graeler M and Goetzl EJ. Activation-regulated expression and chemotactic function of sphingosine 1-phosphate receptors in mouse splenic T cells. *FASEB J* 2002; 16: 1874–1878.

37. Schulze T, Golfer S, Tabeling C, et al. Sphingosine-1-phosphatase receptor 4 (S1P4) deficiency profoundly affects dendritic cell function and TH17-cell differentiation in a murine model. *FASEB J* 2011; 25: 4024–4036.

38. Ota H, Beutz MA, Ito M, et al. S1P4 receptor mediates S1P-induced vasoconstriction in normotensive and hypertensive rat lungs. *Palm Circ* 2011; 1: 399–404.

39. Mayol K, Biajoux V, Marvel J, et al. Sequential desensitization of CXCR4 and S1P5 controls natural killer cell trafficking. *Blood* 2011; 118: 4863–4871.

40. Jaillard C, Harrison S, Stankoff B, et al. Edg8/S1P5: an oligodendroglial receptor with dual function on process retraction and cell survival. *J Neurosci* 2005; 25: 1459–1469.

41. Park SJ and Im DS. Sphingosine 1-phosphate receptor modulators and drug discovery. *Biomol Ther* 2017; 25: 80–90.

42. Bordet R, Camu W, De Seze J, et al. Mechanism of action of s1p receptor modulators in multiple sclerosis: the double requirement. *Rev Neurol* 2020; 176: 100–112.

43. Lee SW, Anderson A, Guzman PA, et al. Atrial GIRK channels mediate the effects of vagus nerve stimulation on heart rate dynamics and arrhythmogenesis. *Front Physiol* 2018; 9: 943.

44. Posokhova E, Wydevne N, Allen KL, et al. RGS6/Gβ5 complex accelerates IKACH gating kinetics in atrial myocytes and modulates parasympathetic regulation of heart rate. *Circ Res* 2010; 107: 1350–1354.

45. Liang B, Nissen JD, Laursen M, et al. G-protein-coupled inward rectifier potassium current contributes to ventricular repolarization. *Cardiovasc Res* 2014; 101: 175–184.

46. Kooyrakh L, Roman MI, Brinkmann V, et al. The heart rate decrease caused by acute FTY720 administration is mediated by the G protein-gated potassium channel I. *Am J Physiol Heart Circ Physiol* 2005; 5: 529–536.

47. Sanna MG, Liao J, Jo E, et al. Sphingosine 1-phosphate (S1P) receptor subtypes S1P1 and S1P3, respectively, regulate lymphocyte recirculation and heart rate. *J Biol Chem* 2004; 279: 13839–13848.

48. Gonzalez-Cabreraz PJ, Jo E, Sanna MG, et al. Full pharmacological efficacy of a novel S1P1 agonist that does not require S1P-like headgroup interactions. *Mol Pharmacol* 2008; 74: 1308–1318.

49. Drici MD, Diochot S, Terrenoire C, et al. The bee venom peptide tertiapin underlines the role of I(KACh) in acetylcholine-induced atrioventricular blocks. *Br J Pharmacol* 2000; 131: 569–577.

50. Yamada M. The role of muscarinic K+ channels in the negative chronotropic effect of a muscarinic agonist. *J Pharmacol Exp Ther* 2002; 300: 681–687.

51. Guo J, MacDonell KL and Giles WR. Effects of sphingosine 1-phosphate on pacemaker activity in rabbit sino-atrial node cells. *Pflugers Arch* 1999; 438: 642–648.

52. DiFrancesco D. The onset and autonomous regulation of cardiac pacemaker activity: relevance of the f current. *Cardiovasc Res* 1995; 29: 449–456.

53. DiFrancesco D. Cardiac pacemaker: 15 years of ‘new’ interpretation. *Acta Cardiol* 1995; 50: 414–427.

54. Di Lorenzo A, Lin MI, Murata T, et al. eNOS-derived nitric oxide regulates endothelial barrier function through VE-cadherin and Rho GTPases. *J Cell Sci* 2013; 126: 5541–5552.
55. Rikitake Y, Hirata K, Kawashima S, et al. Involvement of endothelial nitric oxide in sphingosine-1-phosphate-induced angiogenesis. *Arterioscler Thromb Vasc Biol* 2002; 22: 108–114.

56. Igarashi J, Miyoshi M, Hashimoto T, et al. Hydrogen peroxide induces S1P1 receptors and sensitizes vascular endothelial cells to sphingosine 1-phosphate, a platelet-derived lipid mediator. *Am J Physiol Cell Physiol* 2007; 292: C740–C748.

57. Davies PF. Flow-mediated endothelial mechanotransduction. *Physiol Rev* 1995; 75: 519–560.

58. Bevan JA and Henrion D. Pharmacological implications of the flow-dependence of vascular smooth muscle tone. *Annu Rev Pharmacol Toxicol* 1994; 34: 173–190.

59. Venkataraman K, Lee YM, Michaud J, et al. Vascular endothelium as a contributor of plasma sphingosine 1-phosphate. *Circ Res* 2008; 102: 669–676.

60. Cantalupo A, Zhang Y, Kothiya M, et al. Nogo-b regulates endothelial sphingolipid homeostasis to control vascular function and blood pressure. *Nat Med* 2015; 21: 1028–1037.

61. Cantalupo A, Gargiulo A, Dautaj E, et al. S1PR1 (sphingosine-1-phosphate receptor 1) signaling regulates blood flow and pressure. *Hypertension* 2017; 70: 426–434.

62. Zhang Y, Huang Y, Cantalupo A, et al. Endothelial Nogo-b regulates sphingolipid biosynthesis to promote pathological cardiac hypertrophy during chronic pressure overload. *JCI Insight* 2016; 1: e85484.

63. Salomone S, Yoshimura S, Reuter U, et al. S1P3 receptors mediate the potent constriction of cerebral arteries by sphingosine-1-phosphate. *Eur J Pharmacol* 2003; 469: 125–134.

64. Coussin F, Scott RH, Wise A, et al. Comparison of sphingosine 1-phosphate-induced intracellular signaling pathways in vascular smooth muscles: differential role in vasoconstriction. *Circ Res* 2002; 91: 151–157.

65. Nofer JR, van der Giet M, Tolle M, et al. HDL induces NO-dependent vasorelaxation via the lysophospholipid receptor S1P3. *J Clin Invest* 2004; 113: 569–581.

66. Dantas AP, Igarashi J and Michel T. Sphingosine 1-phosphate and control of vascular tone. *Am J Physiol Heart Circ Physiol* 2003; 284: H2045–H2052.

67. Igarashi J and Michel T. S1P and eNOS regulation. *Biochim Biophys Acta* 2008; 1781: 489–495.

68. Cantalupo A and Di Lorenzo A. S1p signaling and de novo biosynthesis in blood pressure homeostasis. *J Pharmacol Exp Ther* 2016; 358: 359–370.

69. Nixon GF, Mathieson FA and Hunter I. The potential roles of sphingolipids in vascular smooth-muscle function. *Biochem Soc Trans* 2007; 35: 908–909.

70. Salomone S, Potts EM, Tyndall S, et al. Analysis of sphingosine 1-phosphate receptors involved in constriction of isolated cerebral arteries with receptor null mice and pharmacological tools. *Br J Pharmacol* 2008; 153: 140–147.

71. Zhu Q, Xia M, Wang Z, et al. A novel lipid natriuretic factor in the renal medulla: sphingosine-1-phosphate. *Am J Physiol Renal Physiol* 2011; 301: F35–F41.

72. Intapad S. Sphingosine-1-phosphate signaling in blood pressure regulation. *Am J Physiol Renal Physiol* 2019; 317: F638–F640.

73. Tawadrous MN, Mabuchi A, Zimmermann A, et al. Effects of immunosuppressant FTY720 on renal and hepatic hemodynamics in the rat. *Transplantation* 2002; 74: 602–610.

74. Budde K, Schmouder RL, Brunskhorst R, et al. First human trial of FTY720, a novel immunomodulator, in stable renal transplant patients. *J Am Soc Nephrol* 2002; 13: 1073–1083.

75. Schmouder R, Serra D, Wang Y, et al. FTY720: placebo-controlled study of the effect on cardiac rate and rhythm in healthy subjects. *J Clin Pharmacol* 2006; 46: 895–904.

76. Bigaud M, Guerini D, Billich A, et al. Second generation S1P pathway modulators: research strategies and clinical developments. *Biochim Biophys Acta* 2014; 1841: 745–758.

77. Sugahara K, Maeda Y, Shimano K, et al. Amiselimod, a novel sphingosine 1-phosphate receptor-1 modulator, has potent therapeutic efficacy for autoimmune diseases, with low bradycardia risk. *Br J Pharmacol* 2017; 167: 1035–1047.
79. Olshansky B, Sabbah HN, Hauptman PJ, et al. Parasympathetic nervous system and heart failure: pathophysiology and potential implications for therapy. Circulation 2008; 118: 863–871.

80. Brinkmann V. Sphingosine 1-phosphate receptors in health and disease: mechanistic insights from gene deletion studies and reverse pharmacology. Pharmacol Ther 2007; 115: 84–105.

81. Kurachi Y. G protein regulation of cardiac muscarinic potassium channel. Am J Physiol 1995; 269: C821–C830.

82. Vanoli E, Pentimalli F and Botto G. Vagomimetic effects of fingolimod: physiology and clinical implications. CNS Neurosci Ther 2014; 20: 496–502.

83. Saccà F, Puorro G, Marsili A, et al. Mobitz type I and II atrioventricular blocks during fingolimod therapy. Neurol Sci 2016; 37: 1557–1559.

84. Huwiler A and Zangemeister-Wittke U. The sphingosine 1-phosphate receptor modulator fingolimod as a therapeutic agent: recent findings and new perspectives. Pharmacol Ther 2018; 185: 34–49.

85. Riddy DM, Stamp C, Sykes DA, et al. Reassessment of the pharmacology of Sphingosine-1-phosphate S1P3 receptor ligands using the DiscoveRx PathHunter™ and Ca2+ release functional assays. Br J Pharmacol 2012; 167: 868–880.

86. Chun J, Giovannoni G and Hunter SF. Sphingosine 1-phosphate receptor modulator therapy for multiple sclerosis: differential downstream receptor signalling and clinical profile effects. Drugs 2021; 81: 207–231.

87. Tölle M, Levkau B, Keul P, et al. Immunomodulator FTY720 Induces eNOS-dependent arterial vasodilatation via the lysophospholipid receptor S1P3. Circ Res 2005; 96: 913–920.

88. Ziemssen T and Siepmann T. The investigation of the cardiovascular and sudomotor autonomic nervous system – a review. Front Neurol 2019; 10: 53.

89. Li K, Konofalska U, Akgün K, et al. Modulation of cardiac autonomic function by fingolimod initiation and predictors for fingolimod induced bradycardia in patients with multiple sclerosis. Front Neurosci 2017; 11: 540.

90. Habek M, Crnošija L, Junaković A, et al. Autonomic nervous system abnormalities predict cardiovascular changes after initiation of siponimod in secondary progressive multiple sclerosis. Clin Neuropathophysiol 2021; 132: 581–585.

91. Cohen JA, Arnold DL, Comi G, et al. Safety and efficacy of the selective sphingosine 1-phosphate receptor modulator ozanimod in relapsing multiple sclerosis (RADIANCE): a randomised, placebo-controlled, phase 2 trial. Lancet Neurol 2016; 15: 373–381.

92. Juif PE, Hoch M, Vaclavkova A, et al. Mitigation of initial cardiodynamic effects of the S1P1 receptor modulator ponseimod using a novel up-titration regimen. J Clin Pharmacol 2017; 57: 401–410.

93. Kappos L, Arnold DL, Bar-Or A, et al. Two-year results from a phase 2 extension study of oral amiselimod in relapsing multiple sclerosis. Mult Scler 2018; 24: 1605–1616.

94. Rossi S, Rocchi C, Studer V, et al. The autonomic balance predicts cardiac responses after the first dose of fingolimod. Mult Scler 2015; 21: 206–216.

95. Alipov NN, Sergeeva OV, Smirnov VM, et al. Chronotropic and inotropic components of cardiac reflexes in cats. Bull Exp Biol Med 2009; 147: 385–389.

96. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Circulation 1996; 93: 1043–1065.

97. Simula S, Laitinen T and Laitinen TM. Effect of fingolimod on cardiac autonomic regulation in patients with multiple sclerosis. Mult Scler 2016; 22: 1080–1085.

98. Simula S, Laitinen TP and Laitinen TM. Sequence of cardiovascular autonomic alterations after fingolimod initiation. Ann Noninvasive Electrocardiol 2017; 22: e12443.

99. DiMarco JP, O’Connor P, Cohen JA, et al. First-dose effects of fingolimod: pooled safety data from three phase 3 studies. Mult Scler Relat Disord 2014; 3: 629–638.

100. Kovarik JM, Riviere GJ, Neddermann D, et al. A mechanistic study to assess whether isoproprenol can reverse the negative chronotropic effect of fingolimod. J Clin Pharmacol 2008; 48: 303–310.

101. Hilz MJ, Intravooth T and Moeller S. Central autonomic dysfunction delays recovery of...
fingolimod induced heart rate slowing. *PLoS ONE* 2015; 10: e0132139.

102. Hilz MJ, Wang R, de Rojas Leal C, et al. Fingolimod initiation in multiple sclerosis patients is associated with potential beneficial cardiovascular autonomic effects. *Ther Adv Neurol Disord* 2017; 10: 191–209.

103. Simula S, Laitinen T, Laitinen TM, et al. Effects of three months fingolimod therapy on heart rate. *J Neuroimmune Pharmacol* 2015; 10: 651–654.

104. Hainsworth R. Heart rate and orthostatic stress. *Clin Auton Res* 2000; 10: 323–325.

105. Hilz MJ, Roy S, de Rojas Leal C, et al. Cardiovascular fingolimod effects on rapid baroreceptor unloading are counterbalanced by baroreflex resetting. *Neurol Sci* 2021; 42: 111–121.

106. La Rovere M, Bigger J, Marcus F, et al. Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction. ATRAMI (autonomic tone and reflexes after myocardial infarction) investigators. *Lancet* 1998; 351: 478–484.

107. McLachlan CS, Ocsan R, Spence I, et al. Increased total heart rate variability and enhanced cardiac vagal autonomic activity in healthy humans with sinus bradycardia. *Proc (Baylor Univ Med Cent)* 2010; 23: 368–370.

108. Singer BA. Initiating oral fingolimod treatment in patients with multiple sclerosis. *Ther Adv Neurol Disord* 2013; 6: 269–275.

109. Scott LJ. Siponimod: a review in secondary progressive multiple sclerosis. *CNS Drugs* 2020; 34: 1191–1200.

110. Kaplan TB, Berkowitz AL and Samuels MA. Cardiovascular dysfunction in multiple sclerosis. *Neurologist* 2015; 20: 108–114.

111. DiMarco JP, O’Connor P, Cohen JA, et al. Fingolimod treatment initiation experience: cardiac and Holter electrocardiogram findings from three phase 3 studies. *Mult Scler* 2012; 18: 227.

112. Cohen JA, Comi G, Selmaj KW, et al. Safety and efficacy of ozanimod versus interferon beta-1a in relapsing multiple sclerosis (RADIANCE): a multicentre, randomised, 24-month, phase 3 trial. *Lancet Neurol* 2019; 18: 1021–1033.

113. Kappos L, Arnold DL, Bar-Or A, et al. Safety and efficacy of amiselimod in relapsing multiple sclerosis (MOMENTUM): a randomised, double-blind, placebo-controlled phase 2 trial. *Lancet Neurol* 2016; 15: 1148–1159.

114. Harada T, Wilbraham D, de La Borderie G, et al. Cardiac effects of amiselimod compared with fingolimod and placebo: results of a randomised, parallel-group, phase I study in healthy subjects. *Br J Clin Pharmacol* 2017; 83: 1011–1027.

115. Krösser S, Wolna P, Fischer TZ, et al. Effect of ceralimod (ONO-4641) on lymphocytes and cardiac function: randomized, double-blind, placebo-controlled trial with an open-label fingolimod arm. *J Clin Pharmacol* 2015; 55: 1051–1060.

116. Limmroth V, Ziemssen T, Lang M, et al. Electrocardiographic assessments and cardiac events after fingolimod first dose – a comprehensive monitoring study. *BMC Neurol* 2017; 17: 11.

117. Limmroth V, Ziemssen T, Kleiter I, et al. A comprehensive monitoring study on electrocardiographic assessments and cardiac events after fingolimod first dose-possible predictors of cardiac outcomes. *Front Neurol* 2020; 11: 818.

118. Gold R, Comi G, Palace J, et al. Assessment of cardiac safety during fingolimod treatment initiation in a real-world relapsing multiple sclerosis population: a phase 3b, open-label study. *J Neurol* 2014; 261: 267–276.

119. Legangneux E, Gardin A and Johns D. Dose titration of BAF312 attenuates the initial heart rate reducing effect in healthy subjects. *Br J Clin Pharmacol* 2013; 75: 831–841.

120. Selmaj K, Li DK, Hartung HP, et al. Siponimod for patients with relapsing-remitting multiple sclerosis (BOLD): an adaptive, dose-ranging, randomised, phase 2 study. *Lancet Neurol* 2013; 12: 756–767.

121. Penel N and Kramar A. What does a modified-Fibonacci dose-escalation actually correspond to? *BMC Med Res Methodol* 2012; 12: 103.

122. Omura GA. Phase 1 dose-finding trials and Fibonacci. *Clin Cancer Research* 2006; 12: 321.

123. Gilenya (fingolimod). Summary of product characteristics, https://www.ema.europa.eu/en/documents/product-information/gilenya-epar-product-information_en.pdf (2011, accessed 12 January 2022).

124. Mayzent (siponimod). Summary of product characteristics, https://www.ema.europa.eu/en/documents/assessment-report/mayzent-epar-public-assessment-report_en.pdf (2019, accessed 12 January 2022).
125. Zeposia (ozanimod). Summary of product characteristics, https://www.ema.europa.eu/en/documents/assessment-report/zeposia-epar-public-assessment-report_en.pdf (2020, accessed 12 January 2022).

126. Ponvory (ponesimod). Summary of product characteristics, https://www.ema.europa.eu/en/documents/product-information/ponvory-epar-product-information_en.pdf (2021, accessed 12 January 2022).

127. Kappos L, Li DK, Stüve O, et al. Safety and efficacy of siponimod (BAF312) in patients with relapsing-remitting multiple sclerosis: dose-blinded, randomized extension of the phase 2 BOLD study. JAMA Neurol 2016; 73: 1089–1098.

128. Schmouder R, Hariry S and David O. Placebo-controlled study of the effects of fingolimod on cardiac rate and rhythm and pulmonary function in healthy volunteers. Eur J Pharmocol 2012; 68: 355–362.

129. Flachenecker P, Reiners K, Krauser M, et al. Autonomic dysfunction in multiple sclerosis is related to disease activity and progression of disability. Mult Scler 2001; 7: 327–334.

130. Habek M. Immune and autonomic nervous system interactions in multiple sclerosis: clinical implications. Clin Auton Res 2019; 29: 267–275.

131. Merkelbach S, Haensch CA, Hemmer B, et al. Multiple sclerosis and the autonomic nervous system. J Neurol 2006; 253 (Suppl. 1): I21–I25.

132. Zawadka-Kunikowska M, Rzepiński Ł, Newton JL, et al. Cardiac autonomic modulation is different in terms of clinical variant of multiple sclerosis. J Clin Med 2020; 9: 3176.

133. Mahovic D and Lakusic N. Progressive impairment of autonomic control of heart rate in patients with multiple sclerosis. Arch Med Res 2007; 38: 322–325.

134. Winder K, Linker RA, Seifert F, et al. Cerebral lesion correlates of sympathetic cardiovascular activation in multiple sclerosis. Hum Brain Mapp 2019; 40: 5083–5093.

135. Racosta J, Sposato L, Morrow S, et al. Cardiovascular autonomic dysfunction in multiple sclerosis: a meta-analysis. Mult Scler Relat Disord 2015; 4: 104–111.

136. Hilz M. Cardiac stunning as first manifestation of multiple sclerosis: a case report reminding us not to overlook cardiovascular autonomic dysfunction in multiple sclerosis. Mult Scler 2016; 22: 847–848.

137. Razazian N, Hedayati N, Moradian N, et al. P wave duration and dispersion and QT interval in multiple sclerosis. Mult Scler Relat Disord 2014; 3: 662–665.

138. Fluckiger L, Boivin J, Quilliot D, et al. Differential effects of aging on heart rate variability and blood pressure variability. J Gerontol A Biol Sci Med Sci 1999; 54: B219–B224.

139. Brown C, Hecht M, Weih A, et al. Effects of age on the cardiac and vascular limbs of the arterial baroreflex. Eur J Clin Invest 2003; 33: 10–16.

140. de Lucia C, Eguchi A and Koch WJ. New insights in cardiac β-adrenergic signaling during heart failure and aging. Front Pharmacol 2018; 9: 904.

141. Chadda KR, Ajjilova OA, Vaseghi M, et al. Ageing, the autonomic nervous system and arrhythmia: from brain to heart. Ageing Res Rev 2018; 48: 40–50.

142. Cannavo A, Rengo G, Liccardo D, et al. β1-blockade prevents post-ischemic myocardial decompensation via β3AR-dependent protective sphingosine-1 phosphate signaling. J Am Coll Cardiol 2017; 70: 182–192.

143. Trayssac M, Hannun YA and Obeid LM. Role of sphingolipids in senescence: implication in aging and age-related diseases. J Clin Invest 2018; 128: 2702–2712.

144. Estrada R, Zeng Q, Lu H, et al. Up-regulating sphingosine 1-phosphate receptor-2 signaling impairs chemotactic, wound-healing, and morphogenetic responses in senescent endothelial cells. J Biol Chem 2008; 283: 30363–30375.

145. Galinier M, Pathak A, Fourcade J, et al. Depressed low frequency power of heart rate variability as an independent predictor of sudden death in chronic heart failure. Eur Heart J 2000; 21: 475–482.

146. Alewijnse AE and Peters SL. Sphingolipid signalling in the cardiovascular system: good, bad or both? Eur J Pharmacol 2008; 585: 292–302.

147. Findling O, Hauer L, Pezawas T, et al. Cardiac autonomic dysfunction in multiple sclerosis: a systematic review of current knowledge and impact of immunotherapies. J Clin Med 2020; 9: 335.

148. Racosta JM, Kimpinski K, Morrow SA, et al. Autonomic dysfunction in multiple sclerosis. Auton Neurosci 2015; 193: 1–6.

149. Shirbani F, Barin E, Lee YC, et al. Characterisation of cardiac autonomic function...
in multiple sclerosis based on spontaneous changes of heart rate and blood pressure. *Mult Scler Relat Disord* 2018; 22: 120–127.

150. Tombul T, Anlar O, Tuncer M, et al. Impaired heart rate variability as a marker of cardiovascular autonomic dysfunction in multiple sclerosis. *Acta Neurol Belg* 2011; 111: 116–120.

151. Akbulak RO, Rosenkranz SC, Schaeffer BN, et al. Acute and long-term effects of fingolimod on heart rhythm and heart rate variability in patients with multiple sclerosis. *Mult Scler Relat Disord* 2018; 19: 44–49.

152. Racca V, Rovaris M, Vaini E, et al. 6-month effects of fingolimod on indexes of cardiovascular autonomic control in multiple sclerosis. *J Am Coll Cardiol* 2016; 68: 2027–2029.

153. Cooley RL, Montano N, Cogliati C, et al. Evidence for a central origin of the low-frequency oscillation in RR-interval variability. *Circulation* 1998; 98: 556–561.

154. Van de Borne P, Montano N, Pagani M, et al. Absence of low frequency variability of sympathetic nerve activity in severe heart failure. *Circulation* 1997; 95: 1449–1454.

155. Laederach-Hofmann K, Mussgay L and Ruddel H. Autonomic cardiovascular regulation in obesity. *J Endocrinol* 2000; 164: 59–66.

156. Kappos L, Radue E, O’Connor P, et al. A placebo-controlled trial of oral fingolimod in relapsing multiple sclerosis. *N Engl J Med* 2010; 362: 387–401.

157. Bar-Or A, Zipp F, Vollmer T, et al. Safety of ONO-4641 in patients with relapsing remitting multiple sclerosis: results from a six-month interim analysis of the DreaMS extension study. In: *P997 ECTRIMS 2013*, https://onlinelibrary.ectrims-congress.eu/ecitrims/2013/copenhagen/34477/doctor.amit.bar-or.safety.ofONO-4641in.patientswith.relapsing.remitting.html (2013, accessed 5 January 2022).

158. Calabresi PA, Radue EW, Goodin D, et al. Safety and efficacy of fingolimod in patients with relapsing-remitting multiple sclerosis (FREEDOMS II): a double-blind, randomised, placebo-controlled, phase 3 trial. *Lancet Neurol* 2014; 13: 545–556.

159. Cohen JA, Barkhof F, Comi G, et al. Oral fingolimod or intramuscular interferon for relapsing multiple sclerosis. *N Engl J Med* 2010; 362: 402–415.

160. Comi G, Kappos L, Selmaj KW, et al. Safety and efficacy of ozanimod versus interferon beta-1a in relapsing multiple sclerosis (SUNBEAM): a multicentre, randomised, minimum 12-month, phase 3 trial. *Lancet Neurol* 2019; 18: 1009–1020.

161. Juif PE, Kraehenbuehl S and Dingemanse J. Clinical pharmacology, efficacy, and safety aspects of sphingosine-1-phosphate receptor modulators. *Expert Opin Drug Metab Toxicol* 2016; 12: 879–895.

162. Tran JQ, Hartung JP, Peach RJ, et al. Results from the first-in-human study with ozanimod, a novel, selective sphingosine-1-phosphate receptor modulator. *J Clin Pharmacol* 2017; 57: 988–996.

163. Rasche L and Paul F. Ozanimod for the treatment of relapsing remitting multiple sclerosis. *Expert Opin Pharmacother* 2018; 19: 2073–2086.

164. Subei AM and Cohen JA. Sphingosine 1-phosphate receptor modulators in multiple sclerosis. *CNS Drugs* 2015; 29: 565–575.

165. Brinkmann V, Billich A, Baumruker T, et al. Fingolimod (FTY720): discovery and development of an oral drug to treat multiple sclerosis. *Nat Rev Drug Discov* 2010; 9: 883–897.

166. Habek M, Junaković A, Karić A, et al. Short- and long-term effects of siponimod on autonomic nervous system in secondary progressive multiple sclerosis. *Mult Scler Relat Disord* 2022; 64: 103966.