JAMA Neurology | Original Investigation

Safety and Efficacy of Siponimod (BAF312) in Patients With Relapsing-Remitting Multiple Sclerosis

Dose-Blinded, Randomized Extension of the Phase 2 BOLD Study

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IMPORTANCE This dose-blinded extension of the phase 2 BOLD (BAF312 on MRI Lesion Given Once Daily) Study in relapsing-remitting multiple sclerosis provides evidence on disease activity and safety of a range of siponimod doses for up to 24 months.

OBJECTIVE To assess the safety and efficacy of siponimod for up to 24 months during the dose-blinded extension of the BOLD Study.

DESIGN, SETTING, AND PARTICIPANTS At extension baseline in a randomized clinical trial, patients taking siponimod continued at the originally assigned dose and patients taking placebo were rerandomized to the 5 siponimod doses. Initial treatment was titrated over 10 days. A total of 252 eligible patients were treated at specialized multiple sclerosis centers for this study conducted from August 30, 2010, through June 3, 2013.

INTERVENTIONS Siponimod at 10-mg, 2-mg, 1.25-mg, 0.5-mg, and 0.25-mg doses.

MAIN OUTCOMES AND MEASURES Safety assessment included blood tests, documentation of adverse events at regular scheduled visits and Holter monitoring; key efficacy measures were annualized relapse rate and magnetic resonance imaging lesion activity.

RESULTS Among the 252 eligible patients, the mean (SD) ages were 37.2 (8.4) years, 35.2 (9.1) years, 34.0 (7.6) years, 35.1 (9.2) years, and 36.8 (9.1) years in the 0.25-mg, 0.5-mg, 1.25-mg, 2-mg, and 10-mg groups. Of the 252 patients, 184 (73%) entered the extension and received siponimod (10 mg: n = 33; 2 mg: n = 29; 1.25 mg: n = 43; 0.5 mg: n = 29; and 0.25 mg: n = 50); 159 (86.4%) completed the dose-blinded extension. The incidence of adverse events was similar across treatment groups (10 mg: 87.9%; 2 mg: 89.7%; 1.25 mg: 88.4%; 0.5 mg: 96.6%; and 0.25 mg: 84.0%). Nine patients reported serious adverse events (2 mg: 3/29 [10.3%], 1.25 mg: 1/43 [2.3%], 0.5 mg: 4/29 [13.8%], and 0.25 mg: 1/50 [2.0%]; no serious adverse event was reported for more than 1 patient and no new safety signals occurred compared with the BOLD Study. Dose titration mitigated symptomatic bradycardic events. Reductions in mean (95% CI) gadolinium-enhancing T1 lesion counts from the last BOLD assessment were sustained in the 10-mg, 2-mg, 1.25-mg, and 0.5-mg dose groups (0 [0-0], 0.1 [0-1.9], 0.1 [0-2.6], and 0.1 [0-2.8] at month 24, respectively). At the 3 highest vs 2 lowest doses, the estimated new/newly enlarging T2 lesion counts (95% CIs) were lower during months 6 to 12 (0.5 [0.2-1.3], 0.4 [0.2-1.1], and 0.2 [0.1-0.6] vs 1.3 [0.6-2.8] and 1.4 [0.7-2.7]), months 12 to 18 (0.4 [0.1-1.1], 0.4 [0.1-1.3], and 0.4 [0.2-1.0] vs 1.0 [0.4-2.6] and 3.6 [1.7-7.6]), and months 18 to 24 (0 [0-not estimable], 0.9 [0.1-7.6], and 0.1 [0-1.7] vs 1.6 [0.3-7.7] and 2.0 [0.4-9.5]). Annualized relapse rates (95% CIs) up to month 24 were similarly lower for the 3 highest doses: 0.22 (0.12-0.40) for 10 mg, 0.20 (0.10-0.38) for 2 mg, and 0.14 (0.08-0.26) for 1.25 mg vs 0.33 (0.19-0.56) for 0.5 mg and 0.33 (0.21-0.50) for 0.25 mg.

CONCLUSIONS AND RELEVANCE For up to 24 months of siponimod treatment, multiple sclerosis disease activity was low and there were no new safety signals; investigation in phase 3 trials is encouraged.

TRIAL REGISTRATION clinicaltrials.gov Identifier: NCT01185821

JAMA Neurol. 2016;73(9):1089-1098. doi:10.1001/jamaneurol.2016.1451
Published online July 5, 2016.

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Siponimod (BAF312; Novartis Pharma AG) is an oral once-daily, selective sphingosine 1-phosphate receptor ($S1P_{1,5}$) modulator. Compared with fingolimod, an $S1P_{1,3,4,5}$ modulator approved for relapsing forms of multiple sclerosis (MS),[1,2] siponimod has a novel chemotype and does not need to be phosphorylated in vivo. It has a mean half-life of approximately 30 hours and typically washes out within 6.3 days of treatment discontinuation.[3] Modulation of $S1P_1$ on peripheral lymphocytes[3] inhibits their egress from lymph nodes and therefore infiltration of the central nervous system.[4] Siponimod crosses the blood-brain barrier (BBB) to enter the central nervous system.[5,6] Preclinical data have shown reduction of central nervous system inflammation and indicate effects on repair mechanisms via modulation of $S1P_1$ on astrocytes and $S1P_5$ on oligodendrocytes.[3,7-9]

The phase 2, adaptive, dose-ranging BOLD (BAF312 on MRI Lesion Given Once Daily) Study investigated the efficacy and safety of 5 siponimod doses for up to 6 months in patients with relapsing-remitting MS (RRMS). Treatment with siponimod reduced combined unique active lesions (CUALs, defined as gadolinium (Gd)-enhancing T1 lesions and/or new and newly enlarging T2 lesions, without double counting) magnetic resonance imaging (MRI) lesions by up to 80% vs placebo, with a significant dose-response association (82%, 72%, and 50% reductions at 10 mg, 2 mg, and 0.5 mg, respectively; $P < .001$).[10] Although the BOLD Study was not powered to detect a treatment effect on clinical outcomes, the annualized relapse rate (ARR) was reduced for siponimod, 2 mg, vs placebo (0.20 vs 0.58; $P = .04$) after 6 months of treatment.[10] Consistent with the known pharmacodynamic effects of $S1P_1$ modulators,[10,11] a dose-dependent decrease in heart rate (HR) on treatment initiation was observed in BOLD Study patients who did not receive dose titration (0.5 mg, 2 mg, and 10 mg).[10] Siponimod is currently under phase 3 clinical investigation in secondary progressive MS.

Here, we present the safety and efficacy data for 5 siponimod doses during the dose-blinded phase of up to 24 months of the BOLD extension study to understand whether treatment effects in the BOLD Study were maintained and to assess the mitigating effect of siponimod dose titration on HR changes during treatment initiation.

Key Points

Question Are the efficacy and safety profiles of siponimod observed in the 6-month dose-ranging phase 2 BAF312 on MRI Lesion Given Once Daily (BOLD) Study in patients with relapsing-remitting multiple sclerosis sustained for up to 24 months?

Findings In this extension phase of the phase 2 BOLD Study, disease activity was low for up to 24 months, with some evidence of greater benefit associated with siponimod at 10-mg, 2-mg, and 1.25-mg doses than with siponimod at 0.25-mg and 0.5-mg doses. No new safety signals emerged and dose titration at treatment initiation mitigated cardiac effects.

Meaning The clinical and magnetic resonance imaging benefits and absence of new safety signals associated with a range of siponimod doses over 24 months may help to inform dosing in subsequent studies.

Methods

Study Design and Oversight

Study design and oversight and steering committee members have been previously reported; the study included patients from 73 specialized MS centers from across the globe.[10] The dose-blinded phase of the BOLD extension study lasted up to 24 months and ran from August 30, 2010, until June 3, 2013; it was followed by an open-label phase in which all patients were switched to treatment with siponimod, 2-mg daily, but could receive 1 mg daily if at repeat testing, 2 weeks apart, absolute lymphocyte counts (ALC) were less than 200/μL (to convert to $\times 10^9$ per liter, multiply by 0.001). The extension protocol originally anticipated patients moving from the BOLD Study to the extension without study drug interruption. For administrative reasons and after a protocol amendment to implement a dose-titration schedule for patients who had a study drug interruption/washout of more than 7 days between the BOLD and extension studies, most patients in the 0.5-mg, 2-mg, and 10-mg dose groups (cohort 1) in the BOLD Study started the extension more than 3 months after completing the BOLD Study (eFigure in the Supplement). Several patients in the 0.25-mg and 1.25-mg dose groups in the BOLD Study (cohort 2) entered the extension without study drug interruption.

This study was conducted in accordance with the International Conference on Harmonisation Guidelines for Good Clinical Practice and the Declaration of Helsinki.[12,13] The protocol and all amendments were approved by each site's institutional review board or independent ethics committee.

Participants

All patients who completed the BOLD Study were eligible to enter the extension; inclusion criteria for the BOLD Study have been previously described.[10] All patients provided written informed consent before extension study entry. Extension phase exclusion criteria included discontinuation of the study drug during the BOLD Study owing to an adverse event (AE) or noncompliance. Key exclusion criteria related to cardiac function are listed in the eAppendix in the Supplement.

Randomization and Masking

Patients who received siponimod in the BOLD Study continued at the same dose in the dose-blinded phase of the extension (eFigure, A in the Supplement). Patients receiving placebo in cohort 1 of the BOLD Study were randomized (1:1:1) to receive once-daily siponimod, 10 mg, 2 mg, or 0.5 mg. Patients receiving placebo in cohort 2 of the BOLD Study were randomized (1:1) to receive once-daily siponimod, 1.25 mg or 0.25 mg (eFigure, A in the Supplement). A separate medication randomization list was produced by the study sponsor, Novartis Pharma AG, for all patients entering the extension, using a validated system with automated assignment of random numbers to medication packs containing the study drug.

The extension remained dose blinded to all patients and investigators until database lock and unblinding of the BOLD Study (March 2011). Following the implementation of a protocol amendment (June 2012), all patients subsequently switched to open-label siponimod, 2 mg.

Procedures and Assessments

The primary end point was safety and tolerability of siponimod up to 24 months; secondary end points were effects on clinical and MRI outcomes. The standardized MRI obtained at last visit in the BOLD Study served as the extension baseline assessment if patients entered the extension within 4 weeks of completing the BOLD Study (42/50 patients receiving 0.25 mg and 31/43 patients receiving 1.25 mg). A separate standardized brain MRI was required at extension baseline if patients did not enroll within 4 weeks of completing the BOLD Study. This applied to all patients in the 0.5-mg, 2-mg, and 10-mg dose groups, 8 of 50 patients (16%) in the 0.25-mg group, and 12 of 43 patients (28%) in the 1.25-mg group. For details on procedures and schedule of clinical, MRI, and safety assessments, see the eAppendix in the Supplement. Safety was overseen by an independent data and safety monitoring board.

All patients entering the extension received study medication according to a dose-titration schedule (eg, siponimod, 0.25 mg, on day 1 escalating over 10 days to the assigned dose) (eFigure, B in the Supplement). Cardiac monitoring performed during treatment initiation encompassed vital signs, safety electrocardiogram (ECG), 24-hour Holter ECG, and mobile cardiac telemetry (see the eAppendix in the Supplement for cardiac monitoring procedures and discharge criteria).

Statistical Analyses

Extension baseline was defined as the last nonmissing measurement before initiation of extension study drug. The dose-blinded extension full analysis set for both safety and efficacy analyses consisted of all patients who had received at least 1 dose of extension study drug; analyses were performed according to the randomized extension study drug group. Safety data were summarized based on the frequency of AEs, serious AEs (SAEs), discontinuations owing to AEs, and incidence of clinically notable laboratory abnormalities. The washout period between last dose in the BOLD Study and first dose in the extension varied between treatment groups owing to the time taken to receive approval of the dose-titration protocol amendment (**Table 1**). As study drug interruption had not been anticipated when the extension study was planned, no prospective analysis of treatment washout effects on clinical outcomes was conducted; however, safety events and relapses were captured during the washout period.

A negative binomial regression model adjusted for treatment group, age, and baseline Gd-enhancing T1 lesion count was used to estimate Gd-enhancing T1 lesion and new/newly enlarged T2 lesion counts and their associated 95% CIs. Summary statistics described the percentage change from baseline in normalized brain volume and the proportion of patients free from new MRI activity (CUALs). As in the BOLD Study,[10] ARR was estimated using a negative binomial regression model adjusted for treatment, age, BOLD baseline Expanded Disability Status Scale score, Gd-enhancing T1 lesion count, and the number of relapses in the previous 2 years, with log(years in study) as the offset variable using the log link.

Results

Patient Disposition

Of 252 patients who completed the BOLD Study and were eligible to enter the extension, 184 (73.0%) enrolled and received the study drug. Of these, 159 (86.4%) completed the dose-blinded extension phase (**Figure 1**). Patients taking placebo in cohort 1 of the BOLD Study were randomized 1:1:1 to siponimod 10 mg (n = 33), 2 mg (n = 29), and 0.5 mg (n = 29), and those receiving placebo in cohort 2 were randomized 1:1 to siponimod, 1.25 mg (n = 43) and 0.25 mg (n = 50). Disease characteristics at BOLD baseline were generally balanced across groups (Table 1). For all patients in the 10-mg (n = 33), 2-mg, (n = 33), and 0.5-mg (n = 29) groups, MRI assessment of Gd-enhancing T1 lesion count at extension baseline was performed more than 3 months after the last assessment in the BOLD Study. However, for most patients in the 0.25-mg (n = 42/50) and 1.25-mg (n = 31/43) groups, the last MRI assessment in the BOLD Study was used as the extension baseline assessment, which may explain why patients in these groups appear to have less inflammation at extension baseline than those in the 0.5-mg, 2-mg, and 10-mg groups (Table 1).

There were no significant differences in demographics and baseline characteristics between the dose-blinded extension set and patients in the BOLD Study, except for the proportion of women in the 0.5-mg group (BOLD baseline, 70.0%; extension baseline, 62.1%). There were no significant differences in demographics and baseline characteristics between extension treatment groups. During study drug interruption, there was no increase in ARR when comparing patients taking siponimod with those taking placebo during the BOLD Study (eTable 1 in the Supplement). The median duration of exposure to siponimod was similar across treatment groups (Table 1). The most common reasons for study discontinuation during the dose-blinded extension phase were patient-reported unsatisfactory therapeutic effect, withdrawal of consent, and AEs (Figure 1).

Efficacy

Compared with BOLD baseline, mean estimated Gd-enhancing T1 lesion counts at the end of the BOLD Study were reduced in the siponimod, 10-mg, 2-mg, 1.25-mg, and 0.5-mg groups, an effect that was sustained at similar levels in these treatment groups at extension months 12, 18, and 24 (reductions in mean [95% CI] gadolinium-enhancing T1 lesion counts in the 10-mg, 2-mg, 1.25-mg, and 0.5-mg dose groups were 0 [0-0], 0.1 [0-1.9], 0.1 [0-2.6], and 0.1 [0-2.8] at month 24, respectively; **Figure 2**A). Among patients switching from placebo to siponimod (n = 34), Gd-enhancing T1 lesion counts were lower ($P < .01$) at extension months 6 (by 93.6%), 12 (87.9%), and 18 (89.6%) than at extension baseline (eTable 2 in the Supplement). For patients continuing treatment with siponimod, 2 mg and 1.25 mg, there were no significant differ-

Table 1. Patient Demographics and Baseline Characteristics (Dose-Blinded Extension Set)[a]

Characteristic	Siponimod Dose				
	0.25 mg (n = 50)	0.5 mg (n = 29)	1.25 mg (n = 43)	2 mg (n = 29)	10 mg (n = 33)
Age, mean (SD), y	37.2 (8.4)	35.2 (9.1)	34.0 (7.6)	35.1 (9.2)	36.8 (9.1)
Female, No. (%)	41 (82.0)	18 (62.1)	32 (74.4)	18 (62.1)	21 (63.6)
White, No. (%)	49 (98.0)	29 (100.0)	42 (97.7)	28 (96.6)	33 (100.0)
Baseline EDSS score, mean (SD) [range]					
BOLD	2.3 (1.1) [1.5-3.0]	1.9 (1.1) [1.0-2.5]	2.0 (1.1) [1.5-3.0]	2.0 (1.2) [1.5-2.5]	2.2 (0.9) [1.5-2.5]
Extension	2.2 (1.3) [1.5-3.0]	1.9 (1.4) [1.0-2.5]	2.0 (1.1) [1.0-2.5]	2.2 (1.3) [1.5-2.5]	2.0 (1.0) [1.5-2.5]
Baseline Gd-enhancing T1 lesion count, mean (SD)					
BOLD	1.3 (2.8)	3.1 (6.1)	1.8 (2.7)	1.4 (2.3)	1.7 (4.5)
Extension	0.9 (1.5)	3.3 (6.9)	0.5 (1.0)	1.9 (4.6)	2.2 (3.6)
Patients free from Gd-enhancing T1 lesions at baseline, No. (%)					
BOLD	29 (58.0)	10 (34.5)	22 (51.2)	13 (44.8)	18 (54.5)
Extension	32 (64.0)	10 (34.5)	31 (72.1)	14 (48.3)	14 (42.4)
Duration of interruption between the BOLD and extension studies, d[b]					
Mean (SD)	28.2 (44.2)	285.4 (81.8)	28.0 (39.9)	250.3 (52.7)	268.6 (66.9)
Median (range)	13 (0-195)	271 (145-463)	19 (0-195)	235 (195-407)	249 (176-424)
Time interval, No. (%)					
>1 wk	32 (64)	29 (100)	36 (83.7)	29 (100)	33 (100)
>4 wk	12 (24)	29 (100)	9 (20.9)	29 (100)	33 (100)
>3 mo	4 (8)	29 (100)	2 (4.7)	29 (100)	33 (100)
Duration of exposure during dose-blinded extension, d					
Mean (SD)	603.7 (202.7)	704.1 (87.7)	695.1 (63.7)	642.3 (210.5)	647.8 (179.6)
Median (range)	714 (10-744)	729 (285-769)	719 (356-766)	728 (32-743)	716 (70-753)
Patient-years[c]	82.6	55.9	81.8	51.0	58.5

Abbreviations: BOLD, BAF312 on MRI lesion given once daily; EDSS, Expanded Disability Status Scale; Gd, gadolinium; MRI, magnetic resonance imaging.

[a] Extension baseline is the last available MRI measurement before the first dose of the extension study drug. If patients were unable to enter the extension within 4 weeks after completing the BOLD Study, an MRI assessment was performed at extension study baseline. To avoid unnecessary repetition of assessments, if patients entered the extension within 4 weeks of completing the BOLD Study, MRI assessment at the last visit in BOLD was used as an extension baseline (42 patients receiving 0.25 mg and 31 patients receiving 1.25 mg). All patients in the 0.5-mg, 2-mg, and 10-mg dose groups; 8 of 50 patients in the 0.25-mg group; and 12 of 43 patients in the 1.25-mg group had more than 4 weeks of study drug interruption between the end of BOLD and the start of the extension, so an MRI assessment at extension baseline was performed. Owing to variations in study drug exposure during BOLD and in the duration of study drug interruption between BOLD and the extension, patient characteristics are also provided for BOLD baseline.

[b] The duration of study drug interruption was calculated as [Date of First Intake of Study Drug During Extension] − [Date of Last Intake of Study Drug During BOLD] − 1. Patients with washout were defined as those with more than 7 days of study drug interruption between the date of the last intake of BOLD study drug and the date of the first intake of extension study drug.

[c] Patient-years are calculated as the sum of the numbers of days taking the study drug for all patients in the group/365.25.

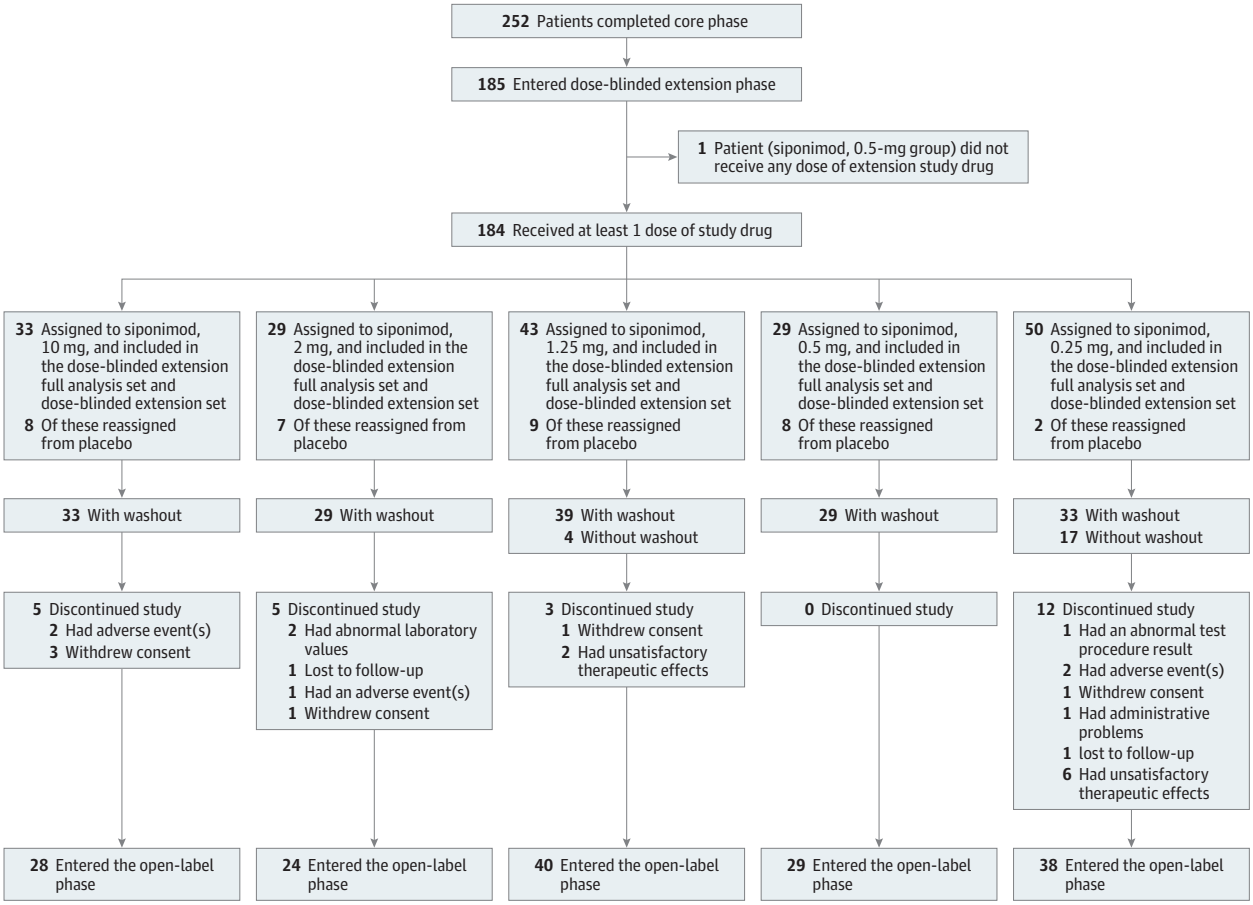
ences in mean estimated Gd-enhancing T1 lesion counts at extension months 6, 12, and 18 compared with the last assessment in the BOLD Study (eTable 2 in the Supplement). At extension month 18, Gd-enhancing T1 lesion counts were lower ($P < .01$) in the 10-mg, 2-mg, and 1.25-mg groups than in the 0.25-mg group (between-group lesion ratio [95% CI], 10 mg vs 0.25 mg: 0.11 [0.03-0.50], $P = .004$; 2 mg vs 0.25 mg: 0.05 [0.01-0.32], $P = .002$; and 1.25 mg vs 0.25 mg: 0.10 [0.03-0.37], $P < .001$; eTable 3 in the Supplement; Figure 2A).

Estimated new/newly enlarging T2 lesion counts during extension months 6 to 12, 12 to 18, and 18 to 24 were numerically lower in the 10-mg, 2-mg, and 1.25-mg groups than in the 0.25-mg group (counts [95% CIs] for 3 highest vs 2 lowest doses, months 6 to 12: 0.5 [0.2-1.3], 0.4 [0.2-1.1], and 0.2 [0.1-0.6] vs 1.3 [0.6-2.8] and 1.4 [0.7-2.7]; months 12 to 18: 0.4 [0.1-1.1], 0.4 [0.1-1.3], and 0.4 [0.2-1.0] vs 1.0 [0.4-2.6] and 3.6 [1.7-7.6]; and months 18 to 24: 0 [0-not estimable], 0.9 [0.1-7.6], and 0.1 [0-1.7] vs 1.6 [0.3-7.7] and 2.0 [0.4-9.5]; Figure 2B). More patients in the 1.25-mg (26/43 [60.5%]) and 2-mg (15/26 [57.7%]) groups were free from new/newly enlarging T2 lesions during the dose-blinded extension phase than in the other dose groups (10 mg: 14/32 [43.8%]; 0.5 mg: 9/29 [31.0%]; and 0.25 mg: 23/48 [47.9%]).

The proportion of patients free from new MRI activity (CUALs) was also higher in the 1.25-mg (25/43 [58.1%]) and 2-mg (15/26 [57.7%]) groups than in the other dose groups (10 mg: 14/32 [43.8%]; 0.5 mg: 9/29 [31.0%]; and 0.25 mg: 23/48 [47.9%]) over the dose-blinded extension phase. There were no clear changes in normalized brain volume within or between any of the treatment groups (data not shown).

Compared with BOLD baseline, ARR remained low at all siponimod doses up to extension month 24. Relapses were less

Figure 1. CONSORT Flow Diagram for the Extension Study



frequent in patients receiving siponimod, 10 mg, 2 mg, and 1.25 mg, than in those receiving siponimod, 0.5 mg and 0.25 mg (ARR [95% CI], 0.22 [0.12-0.40] for 10 mg, 0.20 [0.10-0.38] for 2 mg, and 0.14 [0.08-0.26] for 1.25 mg vs 0.33 [0.19-0.56] for 0.5 mg and 0.33 [0.21-0.50] for 0.25 mg; Figure 2C). There was no treatment-related change in Expanded Disability Status Scale score during the dose-blinded extension phase (data not shown).

Safety

The overall incidence of AEs ranged between 84.0% and 96.6% across treatment groups during the dose-blinded extension phase. The most common AEs (≥10% in any group) were nasopharyngitis, headache, lymphopenia, upper respiratory tract infection, increased alanine aminotransferase, pharyngitis, and insomnia. There were no cases of macular edema. Frequencies of lymphopenia and decreased lymphocyte count were highest in the siponimod, 10-mg, group. Of the 13 patients requiring adjustment or interruption of the study drug owing to an AE, 7 were receiving siponimod, 10 mg, and had lymphopenia or decreased lymphocyte count (**Table 2**).

The incidence of SAEs reported during the dose-blinded extension phase was low; 9 patients reported SAEs (Table 2) and no SAE was reported for more than 1 patient. One patient became pregnant and had an abortion (Table 2). No patients died during the dose-blinded extension phase. Two SAEs were suspected to be drug related (siponimod, 2 mg: gastritis and siponimod, 0.5 mg: cervix neoplasm).

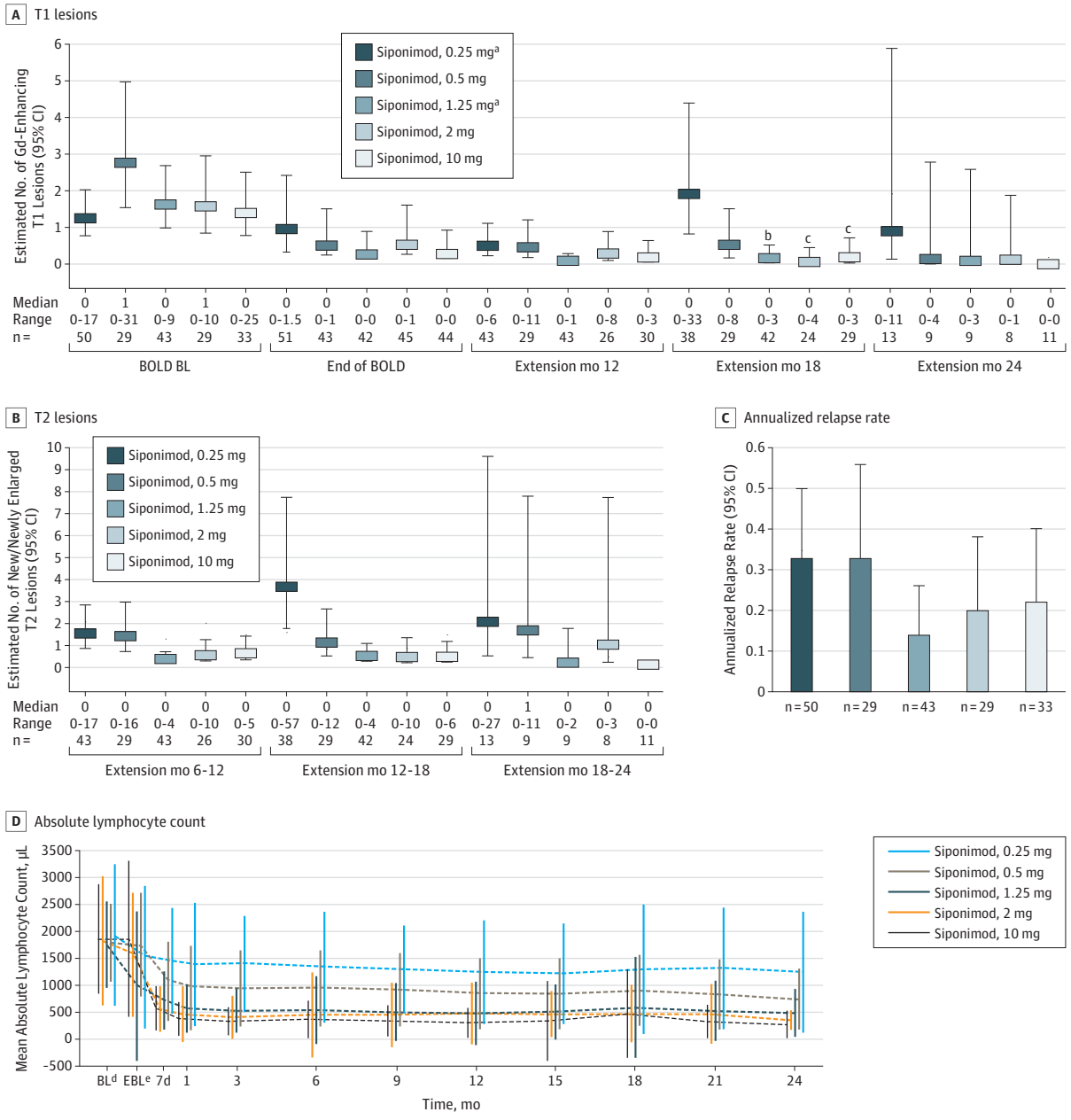
Cardiac Findings During Dose Titration

Dose titration effectively reduced the negative chronotropic effect of siponimod observed at higher doses in the BOLD Study (**Figure 3**). Following dose titration, only minor decreases in HR from predose levels were observed.

Overall, 8 of 163 patients (4.9%) required extended cardiac monitoring; these patients had undergone washout prior to the extension: 3 patients (1.8%) on day 1 (1 patient each in the siponimod, 2-mg, 1.25-mg, and 0.5-mg groups), 2 patients (1.2%) on day 2 (10-mg group), 1 patient (0.6%) on day 7 (2-mg group), and 2 patients (1.2%) on day 8 (10-mg and 0.25-mg groups) (eTable 4 in the Supplement).

Overall, 2 patients had transient second-degree atrioventricular block (AVB) on 24-hour Holter ECG during the dose-titration period. One patient (3.6%; 2-mg group) had Mobitz I second-degree AVB recorded on day 1 more than 6 hours after receiving the dose. During day 7, 2:1 second-degree AVB was recorded for 1 patient (3.3%; 0.25-mg group). This patient had

Figure 2. Efficacy End Points Up to Month 24 (Dose-Blinded Extension Full Analysis Set)



A, Estimated number of gadolinium (Gd)-enhancing T1 lesions. Based on at least 1 scan up to the respective time points. A negative binomial regression model adjusted for treatment group, age, and baseline (BL) Gd-enhancing T1 lesion count was used to estimate Gd-enhancing T1 and new/newly enlarged T2 lesion counts and their associated 95% CIs. For extension month 18 data, a post hoc analysis of Gd-enhancing T1 lesion counts was conducted (see eTable 3 in the Supplement). Pairwise comparisons between siponimod, 0.25 mg, and other doses were made with a negative binomial regression model using the log link, adjusted for BL Gd-enhancing T1 lesion count, age, and treatment group. At extension month 18, there was a greater number of patients with magnetic resonance imaging assessments than at extension month 24 owing to the study design. B, Estimated number of new/newly enlarging T2 lesions. Considering variable durations of study drug interruption between the end of the BOLD (BAF312 on MRI Lesion Given Once Daily) Study and the start of the extension, the 0- to 6-month interval was not considered for analysis. The numbers in parts A and B are the numbers of patients with a magnetic resonance imaging scan. C, The annualized relapse rate was estimated using a negative binomial regression model adjusted for treatment, age, BL Expanded Disability Status Scale score, BL Gd-enhancing T1 lesion count, and number of relapses in the previous 2 years, with log(time in study in years) as the offset variable, using the log link. D, Mean absolute lymphocyte counts per treatment group (dose-blinded extension set). Only data while taking treatment (defined as measurements up to and including 10 days after the date of the last dose of extension study drug) are summarized. Data shown in part D are mean values ±2 SDs.

[a] Patients in the 0.25-mg and 1.25-mg groups received treatment for only 3 months during the BOLD Study.

[b] $P < .001$.

[c] $P < .01$.

[d] Baseline is defined as the last nonmissing pretreatment measurement in the BOLD Study.

[e] Extension BL (EBL) is the last available measurement before the first dose of extension study drug.

Table 2. Adverse Events (≥10% of Patients in Any Group) and SAEs During the Extension (Dose-Blinded Extension Set)[a]

Event	Siponimod Dose, No. (%)				
	10 mg (n = 33)	2 mg (n = 29)	1.25 mg (n = 43)	0.5 mg (n = 29)	0.25 mg (n = 50)
All AEs	29 (87.9)	26 (89.7)	38 (88.4)	28 (96.6)	42 (84.0)
Nasopharyngitis	9 (27.3)	5 (17.2)	14 (32.6)	9 (31.0)	13 (26.0)
Headache	7 (21.2)	4 (13.8)	4 (9.3)	3 (10.3)	7 (14.0)
Lymphopenia	6 (18.2)	2 (6.9)	0	1 (3.4)	0
Lymphocyte count decreased	4 (12.1)	0	1 (2.3)	0	0
γ-Glutamyltransferase increased	3 (9.1)	3 (10.3)	1 (2.3)	1 (3.4)	1 (2.0)
Upper respiratory tract infection	2 (6.1)	5 (17.2)	2 (4.7)	4 (13.8)	5 (10.0)
Alanine aminotransferase increased	2 (6.1)	5 (17.2)	1 (2.3)	1 (3.4)	1 (2.0)
Cough	2 (6.1)	3 (10.3)	0	3 (10.3)	1 (2.0)
Hypercholesterolemia	2 (6.1)	3 (10.3)	0	1 (3.4)	3 (6.0)
Pharyngitis	1 (3.0)	4 (13.8)	1 (2.3)	6 (20.7)	3 (6.0)
Urinary tract infection	1 (3.0)	3 (10.3)	2 (4.7)	2 (6.9)	3 (6.0)
Arthralgia	1 (3.0)	3 (10.3)	0	1 (3.4)	1 (2.0)
Diarrhea	1 (3.0)	2 (6.9)	2 (4.7)	2 (6.9)	5 (10.0)
Pyrexia	1 (3.0)	1 (3.4)	3 (7.0)	2 (6.9)	5 (10.0)
Sinusitis	1 (3.0)	0	3 (7.0)	4 (13.8)	2 (4.0)
Abdominal pain upper	0	1 (3.4)	2 (4.7)	3 (10.3)	2 (4.0)
Insomnia	0	0	7 (16.3)	2 (6.9)	3 (6.0)
Depression	0	0	4 (9.3)	1 (3.4)	6 (12.0)
Any SAE	0	3 (10.3)	1 (2.3)	4 (13.8)	1 (2.0)
Otosclerosis	0	0	0	1 (3.4)	0
Gastritis	0	1 (3.4)	0	0	0
Anaphylactic reaction	0	0	0	1 (3.4)	0
Pyelonephritis acute	0	0	0	0	1 (2.0)
Femur fracture	0	1 (3.4)	0	0	0
Ankle fracture	0	0	0	1 (3.4)	0
Basal cell carcinoma	0	0	1 (2.3)	0	0
Cervix neoplasm	0	0	0	1 (3.4)	0
Abortion	0	1 (3.4)	0	0	0
All AEs leading to study drug adjustment or interruption[b]					
Lymphopenia	4 (12.1)	1 (3.4)	0	0	0
Lymphocyte count decreased	3 (9.1)	0	1 (2.3)	0	0
Neutropenia	1 (3.0)	0	0	0	0
Upper respiratory tract infection	0	1 (3.4)	0	0	0
Hepatic enzyme increased	0	0	1 (2.3)	0	1 (2.0)
Hypertension	0	0	0	0	1 (2.0)

Abbreviations: AE, adverse events; SAE, serious adverse events.

[a] There were no defined limits in the protocol to trigger AE reporting for these events; reporting was based on the judgment of investigators.

[b] Includes all treatment-emergent AEs and all SAEs; patients could have more than 1 AE recorded.

transient sinus tachycardia on day 3. Both events were transient and not recorded as AEs.

Long-term Cardiac Findings

One patient (3.0%; n = 33; 10-mg group) had an AE of first-degree AVB at month 12. One patient (3.0%; 10-mg group) had Mobitz I second-degree AVB recorded during the screening phases of both the BOLD and extension studies, and during months 3, 6, and 12 of the dose-blinded extension phase. No symptomatic bradycardic events or AEs were reported during the extension and there were no clinically significant changes from extension baseline in blood pressure. No patients had a QTc (Fridericia) interval of 480 milliseconds or more.

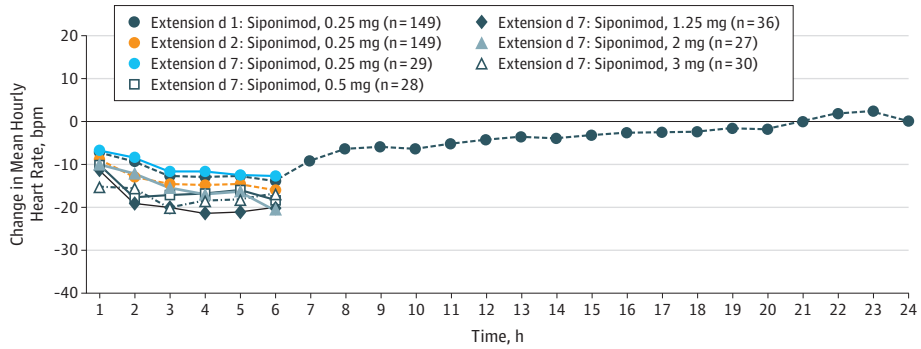
Laboratory Values

Compared with BOLD baseline, a dose-dependent mean decrease in ALC was observed as early as day 7, which persisted throughout the dose-blinded extension phase (Figure 2D); decreases in ALC less than 200/μL at any point were observed in 54.5% of patients taking siponimod, 10 mg; 17.2% taking 2 mg; 9.3% taking 1.25 mg; and none taking 0.5 mg and 0.25 mg.

Discussion

The BOLD Study formally tested the dose response of siponimod and demonstrated a significant effect on MRI lesion activity vs placebo.[10] Post hoc analyses of findings from this

Figure 3. Effect on Heart Rate During Dose-Blinded Titration



Mean change from baseline in Holter electrocardiogram hourly mean heart rate during dose-blinded titration in patients with washout (dose-blinded extension set). The treatment displayed is the dose received on that day, which is derived from the titration schedule in the protocol. According to this schedule, on extension day 1 and day 2, all patients received siponimod, 0.25 mg. In line with the dose titration schedule, on extension day 7, patients received a siponimod dose equivalent to their assigned dose group, except for patients in the 10-mg dose group, who received 3 mg and reached the 10-mg dose on day 10. The values are the number of patients within the subgroup with at least 1 postdose measurement recorded during the visit for which a corresponding baseline measurement was available. Baseline is defined as the last nonmissing pretreatment measurement in the BOLD (BAF312 on MRI Lesion Given Once Daily) Study. Patients with washout are those patients with more than 7 days' study drug interruption between the date of the last intake of study drug in the BOLD Study and the date of first dose of extension study drug, and all patients randomized to placebo in the BOLD Study. bpm indicates beats per minute.

dose-blinded extension phase of the BOLD Study suggest that siponimod, 2 mg and 10 mg, had a sustained effect on MRI and clinical measures, with low disease activity observed over an extended treatment period. These findings support the outcomes of Bayesian longitudinal modeling of BOLD data, which showed that siponimod, 2 mg, yielded near maximum efficacy in reducing CUALs.[10] Improvement in MRI outcomes over time in the lower-dose groups appears less pronounced than that observed in the higher-dose groups, suggesting there was no catch-up effect. The fact that patients in the 0.5-mg, 2-mg, and 10-mg dose groups had more inflammatory activity at extension baseline is likely associated with the longer washout between the BOLD and BOLD extension studies (Table 1). Although this study was not designed to detect treatment effects on relapse outcomes, a low ARR was sustained until extension month 24, with overall fewer relapses in patients receiving higher doses of siponimod compared with those taking lower doses.

The most frequent reason noted for premature study discontinuation was patient-reported unsatisfactory therapeutic effect, and the frequency was highest in the lowest-dose group (siponimod, 0.25 mg). As in the BOLD Study,[10] siponimod was well tolerated during the extension; no new safety signals were observed and the overall incidence of AEs was similar across treatment groups. Serious AEs were not dose related and no patient had more than 1 SAE during the extension. There were no severe or systemic opportunistic infections in any treatment group. Consistent with the pharmacodynamic effects of siponimod,[3] a decrease in ALC persisted throughout the dose-blinded extension phase. Patients receiving siponimod, 10 mg, had higher frequencies of lymphopenia than those taking lower doses.[3] As with any treatment that affects the immune system, long-term vigilance for serious infections is warranted; this is noteworthy given the duration of this extension and the relatively low patient numbers per group. Despite the known dose-dependent risk associated with the approved S1PR modulator fingolimod,[2] there were no instances of macular edema.

In the BOLD Study, siponimod initiation at higher doses (2 mg and 10 mg) was associated with HR reduction in all patients.[10,14] At lower doses (0.25 mg, 0.5 mg, and 1.25 mg), HR reduction was much less pronounced or absent.[10,14] In this extension, dose titration during the first 10 days of treatment mitigated the initial bradycardic and atrioventricular conduction effects at all doses. In patients undergoing dose titration, no symptomatic bradycardic events were observed. The second-degree AVB noted in 2 patients during dose titration was transient and not considered clinically significant. Transient second-degree AVB is an ECG finding often seen in healthy individuals[15,16]; it becomes less common with increasing age, occurring in approximately 11% of teenage boys, 4% of women, and 6% of men.[15,16] Overall, these findings in this extension study, from a large group of patients with RRMS, support initial observations of benefit with dose titration in a subgroup of patients in the BOLD Study[10,14] and in healthy volunteers[17] and provide reassurance that dose titration alleviates the effects of siponimod on HR in patients with RRMS.

The limitations of this study included the relatively low patient numbers per dose and a decreasing proportion of patients with evaluable MRI scans, particularly at later points. Overall, 67 of 252 patients (27%) completing the core BOLD Study did not participate in the extension. Although in many cases this was because of administrative reasons, eg, the long treatment interruption period between the end of the BOLD Study and the start of the extension phase, this loss of patients may have introduced bias that was difficult to assess because we were unable to obtain the individual reasons why patients did not continue to the extension. In addition, because the extension had no placebo-control group (although the

study was dose blinded), we prospectively planned to use descriptive statistics for the study outcomes. Another limitation was the variable duration of treatment interruption between the core and extension studies, which was mainly caused by delayed approval of the protocol amendment allowing implementation of dose titration. The fact that extension baseline activity in patients with longer washout was comparable with that observed at BOLD baseline, and that the median duration of exposure to siponimod was similar across treatment groups, provides some reassurance that any between-group differences relating to the washout period did not introduce important bias.

Conclusions

Notwithstanding some limitations, post hoc analyses of this dose-blinded study indicate that siponimod has a sustained effect on MRI outcomes at both the 2-mg and 10-mg doses. Dose titration mitigated bradycardic first-dose effects in all doses. With similar efficacy but lower rates of lymphopenia relative to the 10-mg dose, siponimod, 2 mg, has been chosen for further development as a treatment for patients with MS and is currently being investigated in a phase 3 study in patients with secondary progressive MS.[18]

ARTICLE INFORMATION

Accepted for Publication: April 5, 2016.

Published Online: July 5, 2016. doi:10.1001/jamaneurol.2016.1451.

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Obtained funding: Wallström.
Administrative, technical, or material support: Li, Hartung, Ziemssen, Wallström.
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Conflict of Interest Disclosures: Dr Kappos' institution (University Hospital Basel) received in the past 3 years and used exclusively for research support the following: steering committee, advisory board, and consultancy fees (Actelion, Addex, Bayer HealthCare, Biogen, Biotica, Genzyme, Lilly, Merck, Mitsubishi, Novartis, Ono Pharma, Pfizer, Sanofi, Santhera, Siemens, Teva, UCB, and XenoPort); speaker fees (Bayer HealthCare, Biogen, Merck, Novartis, Sanofi, and Teva); support of educational activities (Bayer HealthCare, Biogen, CSL Behring, Genzyme, Merck, Novartis, Sanofi, and Teva); royalties (Neurostatus Systems AG); and grants (Bayer HealthCare, Biogen, the European Union, Merck, Novartis, Roche, Roche Research Foundations, Swiss MS Society, and the Swiss National Research Foundation). Dr Li is the director of the UBC MS/MRI Research Group, which has been contracted to perform central analysis of magnetic resonance imaging scans for therapeutic trials with Genzyme, Hoffmann-La Roche, Merck Serono, Nuron Biotech, Perceptive Informatics, and Sanofi. He has also acted as a consultant to Vertex Pharmaceuticals and served on the data and safety advisory board for Opexa Therapeutics and scientific advisory boards for Novartis, Nuron Biotech, and Roche. Dr Li has received research funding from the Canadian Institute of Health Research and Multiple Sclerosis Society of Canada. Dr Stüve has received speaker honoraria from Teva Neuroscience and served as a consultant for EMD Serono, Genzyme, Novartis, Roche, Sanofi, and Teva Neuroscience. Dr Hartung has received speaking fees and honoraria for consulting from Bayer HealthCare, Biogen Idec, EMD Serono, GeNeuro, Genzyme, Novartis, Octapharma, Opexa, Receptos, Roche, Sanofi, and Teva. Dr Freedman has received honoraria or consultation fees from Actelion, Bayer HealthCare, Biogen Idec, Chugai, EMD Canada, Genzyme, Hoffman-La Roche, Novartis, Sanofi, and Teva Canada Innovation; has been a member of a company advisory board, board of directors, or other similar group for Actelion, Bayer HealthCare, Biogen Idec, Chugai, EMD Canada, Hoffman-La Roche, Merck Serono, Novartis, Opexa, Sanofi, and Teva; and has participated in a company-sponsored speakers' bureau for Genzyme. Dr Hemmer has served on scientific advisory boards for Roche, Novartis, Bayer Schering, Merck Serono, Biogen Idec, GlaxoSmithKline, Chugai Pharmaceuticals, Micromet, Genentech, and Genzyme Corp; serves on the international advisory boards of *JAMA Neurology* and *Experimental Neurology*; has received speaker honoraria from Bayer Schering, Novartis, Biogen Idec, Merck Serono, Roche, and Teva Pharmaceutical Industries Ltd; and has received research support from Biogen Idec, Bayer Schering, Merck Serono, Five Prime, Metanomics, Chugai Pharmaceuticals, and Novartis. He has filed patents for the detection of antibodies and T cells against KIR4.1 in a subpopulation of patients with multiple sclerosis and genetic determinants of neutralizing antibodies to interferon-beta. Dr Rieckmann has received honoraria for lectures from Bayer HealthCare, Biogen Idec, Boehringer Ingelheim, Merck Serono, Novartis, Sanofi, and Teva. Dr Montalban has received speaking honoraria and travel expenses for participation in scientific meetings and has been a steering committee member of clinical trials or participated in advisory boards of clinical trials in the past years with Actelion, Almirall, Bayer, Biogen, Genzyme, Merck, Novartis, Octapharma, Receptos, Roche, Sanofi, Teva, and Trophos. Dr Ziemssen has received honoraria and research support from Almirall, Bayer HealthCare, Biogen Idec, Merck Serono, Novartis, Roche, Sanofi/Genzyme, Synthon, and Teva. Dr Selmaj has received honoraria for consultation and invited talks from Biogen Idec, Genzyme, Merck Serono, Novartis, ONO Pharmaceuticals, and Roche. Drs Hunter, Arnould, and Wallström are employees of Novartis Pharma AG.

Funding/Support: This study was funded by Novartis Pharma AG, Basel, Switzerland.

Role of the Funder/Sponsor: Authors employed by Novartis Pharma AG (Drs Arnould, Hunter, and Wallström) were involved in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and the decision to submit the manuscript for publication.

Additional Contributions: We thank the patients and investigators who took part in this study and Sarah Griffiths, PhD (Oxford PharmaGenesis, funded by Novartis Pharma AG), for editorial assistance, collating the comments of authors and editing the manuscript for submission.

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