

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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ONLINE APPENDIX

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ADDITIONAL METHODOLOGICAL DETAILS

Study Endpoint and Procedures

In addition to the evaluations outlined in the main manuscript, ophthalmic examinations and pulmonary function tests were performed at screening and months 1, 3, 6, 12, 18, and 24. Ophthalmic examination included eye history, visual acuity, and dilated ophthalmoscopy. All patients underwent optical coherence tomography assessments to evaluate macular thickness at screening and month 24. Following reports of skin malignancies in the fingolimod phase 2 study,^{1,2} the protocol was amended to include regular dermatologic monitoring of all patients: monthly self-examination by patients and examination by a dermatologist at months 12 and 24. All participants had been enrolled when this amendment was implemented, so the initial dermatologist examination occurred for all patients after dosing had commenced. An independent physician monitored patients' vital signs after the first dose of oral medication. Sitting heart rate and blood pressure were assessed before the first dose and every hour for at least 6 hours after the first dose. Electrocardiograms (ECGs) were also assessed at baseline or pre-dose and post baseline. Patients were discharged after administration of the first dose when all the following criteria were met: heart rate of at least 51 bpm, heart rate more than 80% of baseline value, heart rate at discharge not the lowest hourly value of the monitoring period, no symptoms of decreased heart rate, no treatment for bradycardia received, and ECG at 6 hours did not show any significant abnormalities versus pre-dose other than sinus bradycardia. Patients experiencing symptomatic reductions in heart rate were hospitalized overnight.

Relapses were treated with methylprednisolone up to 1000 mg/day for 3–5 days without an oral taper.

Magnetic resonance imaging (MRI) scans were acquired according to a standard protocol. The scans were processed centrally at the MS MRI Evaluation Center (Basel, Switzerland). The central reader checked the scans for completeness

and quality, then all scans were analyzed by blinded readers. Subsequently, the scans were sent electronically to the Contract Research Organization (PPD Inc), which monitored the entire study. T1-weighted images, before and after administration of single-dose gadolinium (Gd) diethylenetriamine penta-acetic acid (0.1 mmol/kg), and dual-echo T2-weighted images were obtained at screening and months 6, 12, and 24. Numbers of new or enlarging T2 lesions, number and volume of Gd-enhancing lesions, total volume of T2 lesions, total volume of T1 hypointense lesions, and brain volume at baseline and change over time were obtained according to a standard protocol. Once lesions were identified and marked by the radiologist following a detailed plaque-marking and -segmenting protocol, volume calculations were performed separately by specially trained technicians (intra-rater variability of $\leq 5\%$) using an interactive segmentation program on the Amira[®] platform (Mercury Computer Systems GmbH). Segmented lesions were reviewed and approved by a neuroradiologist. Brain volume change calculations were performed using the fully-automated structural image evaluation of normalized atrophy (SIENA) software (Function MRI of the Brain Analysis Group, Oxford, England). At baseline, SIENA utilized a single-time-point method (SIENAX) to estimate the normalized brain volume. Details about assessment of relapses and disability progression are provided in the main manuscript.

Statistical analyses (not reported in the Methods)

The primary analysis population for all efficacy analyses was the intent-to-treat (ITT) population. Baseline characteristics were compared for treatment differences using the Cochran-Mantel-Haenszel test stratified by country for gender, proportion of patients with history of disease modifying treatment, and proportion of patients free from Gd-enhancing lesions. An analysis of covariance (ANCOVA) model adjusted for treatment and country was used to assess the remaining baseline characteristics.

For most secondary efficacy analyses, three pair-wise treatment comparisons were made: fingolimod 1.25 mg versus placebo, fingolimod 0.5 mg versus placebo, fingolimod 1.25 mg versus fingolimod 0.5 mg.

Time to first relapse was compared using the log-rank test (main analysis) and Cox's proportional hazards model adjusted for treatment, country, number of relapses in the 2 years before baseline, and baseline Expanded Disability Status Scale (EDSS) score (supportive analysis).

Changes in EDSS and Multiple Sclerosis Functional Composite scores from baseline were compared between treatment groups using ANCOVA on ranks adjusted for treatment group, country, corresponding baseline value, and age.

The MRI efficacy variables (continuous and count) were presented as summary statistics. For the analyses of the proportions of patients free from Gd-enhancing lesions or new or enlarged T2 lesions, treatment comparisons were tested using the logistic regression model adjusting for treatment group, country, and the corresponding baseline number of lesions (when available). For the number of new or newly enlarged T2 lesions, treatment comparisons were tested with a negative binomial model adjusted for treatment group and country.

Rank ANCOVA adjusted for treatment group, country, and corresponding baseline values were used for treatment comparisons of the number of Gd-enhancing lesions, MRI volumes (percent change from baseline in total volume of T2 lesions, percent change from baseline in total volume of T1 hypointense lesions), and percent change from baseline in brain volume.

Safety and tolerability endpoints

Assessment of safety was summarized based primarily on the frequency of adverse events, serious adverse events and notable laboratory abnormalities using the safety population. Other safety data were summarized as appropriate. Data from specific tests (e.g. ECG or vital signs) were listed, notable values were flagged, and any other information collected was listed as appropriate.

ADDITIONAL DETAILS ON ADVERSE EVENTS OF SPECIAL INTEREST AND SELECTED CLINICALLY NOTABLE HEMATOLOGY AND BIOCHEMISTRY VALUES

Multiple sclerosis relapses reported as serious adverse events:

The symptoms observed were typical of multiple sclerosis relapses, except in one case, reported previously.³ In this case (a 27-year-old patient), the neurological examination was normal 6 months after onset of the initial relapse, except for mild cognitive sequelae and slightly reduced drive (EDSS score of 2 points); the patient returned to work on a part-time basis. Wider use of fingolimod, may better inform whether the few severe relapses observed in patients randomized to this drug were a chance phenomenon or are associated with a distinguishable risk profile.

E-Table 1. Selected clinically notable hematology and biochemistry values.

	Fingolimod		Placebo (N=418)
	1.25 mg (N=429)	0.5 mg (N=425)	
White blood cell count ($10^9/L$)			
Baseline, mean \pm SD	6.81 \pm 2.01	6.50 \pm 1.81	6.64 \pm 1.88
Month 24, mean \pm SD	4.03 \pm 1.44	4.24 \pm 1.61	6.47 \pm 1.93
Lymphocyte count ($10^9/L$)			
Baseline, mean \pm SD	1.85 \pm 0.54	1.84 \pm 0.62	1.82 \pm 0.56
Month 24, mean \pm SD	0.42 \pm 0.26	0.49 \pm 0.34	1.76 \pm 0.57
Neutrophil count ($10^9/L$)			
Baseline, mean \pm SD	4.28 \pm 1.64	4.02 \pm 1.45	4.16 \pm 1.54
Month 24, mean \pm SD	3.06 \pm 1.28	3.22 \pm 1.42	4.07 \pm 1.57
Alanine transaminase (U/L)			
Baseline, mean \pm SD	18.85 \pm 10.40	19.81 \pm 11.46	20.60 \pm 13.30
Month 24, mean \pm SD	32.15 \pm 21.24	33.33 \pm 23.13	21.19 \pm 13.98
≥ 3 xULN, no. (%)	53 (12.5)	36 (8.5)	7 (1.7)
≥ 5 xULN, no. (%)	13 (3.1)	8 (1.9)	4 (1.0)

SD, standard deviation; ULN, upper limit of normal

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