Supplementary Online Content


eAppendix. eMethods.

eTable. Sequence parameters list.

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

eMethods

Additional methodological details

Outcome measures

MRI assessments were scheduled at screening and months 6, 12 and 24. Inflammatory activity was assessed using numbers of new or enlarged (new/newly enlarged) T2 lesions, and number and volume of Gd-enhancing T1 lesions. Gd-enhancing lesions were identified as hyperintense areas in the post-contrast T1-weighted images compared with the pre-contrast T1-weighted images. Lesions expanding throughout several slices were counted only on the first slice. T2 lesions were identified as areas of hyperintensity compared with the surrounding white and grey matter on proton density–weighted images and T2-weighted images. New/newly enlarged T2 lesions were identified by comparing each T2 lesion on proton density–weighted images with the T2 lesions already observed in the previous examination selected as reference. New lesions were counted if they had a minimum major diameter of 5mm. All new or newly enlarged T2 lesions were counted independently regardless of whether there was contrast enhancement on T1-weighted sequences. New or newly enlarged T2 lesions expanding through several slices were counted only on the first slice.

Burden of disease outcomes were total T2 lesion volume and total T1 hypointense lesion volume. All T2 lesions, old and new, were identified and included in the volume measurement. T1-hypointense lesions were identified as areas of hypointensity compared with the surrounding white matter in T1-weighted images after contrast administration corresponding with a T2 lesion on proton density–weighted images.

Mean percent brain volume change subgroup analyses

In addition to the statistical methods outlined in the main manuscript, several post hoc analyses were performed to assess mean percent change brain volume (PBVC) change in different patient subgroups – the results of these analyses are reported in Table 4.

PBVC during months 0–24 was assessed in the following patient subgroups: treatment-naïve or patients treated with a disease-modifying therapy prior to the study, baseline EDSS score 0–3.5 points or >3.5 points, presence or absence of Gd-enhancing lesions at baseline, and baseline T2 lesion volume of ≤3,300mm³ or >3,300mm³. All subgroup analyses were conducted for patients in the intent-to-treat population with available MRI scans. Comparisons of the differences in PBVC during months 0–24 between the fingolimod groups and the placebo group in each patient subgroup were tested using rank ANCOVA adjusted for treatment, geographic region, and normalized brain volume with a two-sided significance level of 0.05. Differences in treatment effect between each subgroup category were tested for approximate significance of treatment-by-subgroup interaction given that treatment, baseline normalized brain volume, region, subgroup, and nuisance parameters (baseline normalized brain volume-by-subgroup interaction and region-by-subgroup interaction) are already in the rank ANCOVA model (two-sided significance level of 0.10).

Standard MRI protocol

Sequences for main evaluation

For all sequences of the main evaluation, at least 46 slices with 3-mm thickness and no gap were taken to cover the entire brain from vertex to cerebellum.

To obtain at least 46 slices, the use of one series of 46 slices with a slice thickness of 3.0 mm, and an inter-slice gap of 0.0 mm in interleaved measurements was recommended.

Other common parameters for all sequences (Appendix Table 1) were:

- Slice orientation: axial oblique according to (re)positioning methodology
- Square field of vision (FOV): 25 x 25cm, matrix 256 x 256 pixel, or
- Rectangular FOV (¾):18.75 x 25cm, matrix 192 x 256 pixel
- Direction of phase encoding gradient should be left/right
- Flow compensation should be used.
Proton density and T2-weighted images (T2 short/long):
- Dual fast spin echo (FSE)/turbo spin echo (TSE)
- In case FSE/TSE not available use conventional spin echo
- Number of excitations (NEX): 1
- Echo train length: 6–8
- Repetition time (TR) 2,800–3,800ms
- Echo time (TE; short): 14–40ms and TE (long): 80–120ms
- The short echo time should allow for the cerebrospinal fluid to be dark or at least isointense to white matter and the lesions to be bright.

T1-weighted image native (T1):
- Conventional spin echo sequences
- Number of excitations: 2
- TR: 500–650ms
- TE: 10–20ms

T1-weighted contrast-enhanced image (T1Gd):
- Use same parameters as above
- Intravenous Gd-DTPA 0.1mmol/kg (= 0.2ml/kg) is injected over 2 min
- Scanning should begin 5–10 min after the contrast injection is complete; exceptions should be noted on the MRI- Parameter Form.

The sequence parameters approved during the dummy run scan and used at baseline were used for all subsequent scans.
<table>
<thead>
<tr>
<th>Sequence name</th>
<th>Scout/fast localizer (T1-weighted)</th>
<th>Repositioning check (rapid T1)</th>
<th>FSE/TSE (T2 short/long)</th>
<th>Spin echo (T1 native)</th>
<th>Spin echo (T1 Gd-DTPA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TR/ms</td>
<td>100/500–600 (sagittal)</td>
<td>500–600</td>
<td>2,800–3,800</td>
<td>500–650</td>
<td>500–650</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Long: 80–120</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. slices</td>
<td>1–3</td>
<td>1</td>
<td>46</td>
<td>46</td>
<td>46</td>
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<tr>
<td>Slice thick</td>
<td>5mm</td>
<td>3mm</td>
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<tr>
<td>Series</td>
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<td>Interleaved</td>
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</tr>
<tr>
<td>Gap</td>
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<td>0.0mm (0%)</td>
<td>0.0mm (0%)</td>
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<tr>
<td>Matrix</td>
<td>128 x 256</td>
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<td>256 x 256</td>
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<tr>
<td>FOV</td>
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<td>Rectangular matrix</td>
<td>192 x 256</td>
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<tr>
<td>Rectangular FOV (%)</td>
<td>187.5 x 250</td>
<td>187.5 x 250</td>
<td>187.5 x 250</td>
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<tr>
<td>NEX</td>
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<td>1</td>
<td>2</td>
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<td>Phase encoding</td>
<td>Left &gt; right, anterior &gt; posterior (sagittal)</td>
<td>Left &gt; right</td>
<td>Left &gt; right</td>
<td>Left &gt; right</td>
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<td>Flow compensation</td>
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<td>Yes</td>
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<tr>
<td>General remarks</td>
<td>Short echo (proton density) should allow for the cerebrospinal fluid to be darker or at least isointense to white matter and the lesions to be bright</td>
<td></td>
<td>Gd-DTPA 0.1mmol/kg is injected over 2 min, start of scanning 5–10 min after completion of injection, end of scanning 30 min after start of injection</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Sequences could also be performed in one series of 40 slices (3mm with no gap). When two series were planned, the second series had to be shifted by 3mm.

FOV = field of vision; FSE = fast spin echo; Gd-DTPA = gadolinium diethylenetriamine penta-acetic acid; ms = milliseconds; NEX = number of excitations; TE = echo time, TR = repetition time; TSE = turbo spin echo.