SUPPLEMENTARY METHODS

Study design

As typically done in the clinical scenario, before starting treatments, we tested serology against JC virus. The result of this test was one of the criteria used to select either NAT (generally chosen if negative) or FTY (more frequently chosen if positive). Accordingly, ten patients, who were positive for JC virus serology before starting treatment, received FTY.

During the follow-up, none of the patients treated with NAT became seropositive for JC virus, thus nobody discontinued treatment for this reason.

In case of patients previously treated with another second line treatment, FTY was started after an adequate wash-out period (previous treatment was discontinued for at least six weeks), an evaluation of blood tests and the execution of a brain MRI to exclude progressive multifocal leukoencephalopathy. Similarly, NAT was started after an adequate wash-out period (previous treatment was discontinued for at least six-eight weeks) and the normalization of white blood cells and lymphocytes counts.

MRI acquisition and post-processing

Using a 3.0 Tesla scanner (Intera, Philips Medical Systems, Best, The Netherlands) under a regular maintenance program (no major scanner hardware or software upgrade occurred during the study), the following brain images were acquired from all participants: a) dual-echo turbo spin-echo (repetition time [TR]/echo time [TE]=2599/16,80 ms, echo train length [ETL]=6; flip angle [FA]=90º, matrix size=256×256, field of view [FOV]=240 mm×240 mm, 44 contiguous, 3mm-thick axial slices); b) 3D T_1-weighted fast field echo (TR/TE=25/4.6 ms; FA=30°; matrix size=256×256; FOV=230 mm×230 mm; 220 contiguous axial slice, voxel size=0.89 mm ×0.89 mm ×0.8 mm); c) post-contrast (0.1 mmol/kg of Gd-DTPA; acquisition delay: 5 minutes) T_1-weighted inversion recovery sequence (TR/TE/inversion time [TI]=2000/10/800 ms, ETL=5; FA=90º, matrix size=400×320,

FOV=230 mm×195.5 mm, 44 contiguous, 3mm-thick axial slices). For all the MRI acquisitions, subjects were positioned aligning scanner light beam with the canthus of the eyes and moving the table so that it ends up in the magnet isocenter. Moreover the inferior border of the genu and splenium of the corpus callosum was used as reference for the localization procedure, with careful repositioning at follow-up. Correction for deformations due to gradient non linearity was also active. During the post-processing a landmark was set on the midsagittal slice, where the anterior commissure can be univocally identified with a single coordinate, since SPM first aligns all images so that the origins match.

Regional brain atrophy assessment

Voxel-based morphometry was used to map between-group regional differences in volumes at M0. Tensor-based morphometry,[1] as implemented in SPM12 (www.fil.ion.ucl.ac.uk/spm), was used to map regional volume changes over time within and between patient groups and healthy controls (HC). Serial longitudinal registration was used to align scans of the four study time-points of relapsing-remitting multiple sclerosis (RRMS) patients and of the two time-points of HC.[2] The method is based on group-wise alignment among each of the subject's scans, and incorporates a bias field correction. It produces a mid-point average template image. Prior to performing the registration, the scans should already be in very rough alignment. The alignment assumes that all scans have similar resolutions and dimensions and have been collected on the same MRI scanner using the same pulse sequence.

The rate of volume change was quantified from the determinant of the Jacobian deformation matrix. Each map of the Jacobian determinants encodes the relative volume (at each spatial location) between the scan and the median time-point average. For our analysis, we evaluated the evolution of the Jacobian determinants at the different time-points. To this end, the differences between pairs of Jacobian determinants (dj) were calculated and scaled for the follow-up duration. The values represent the ratio of the volume differences to the volume in the mid-point average template, used as reference;

negative values indicate volume loss, positive values volume increase. The mid-point average template was used for group-wise alignment among subjects: first, the mid-point average template images were segmented into different tissue types via the Segmentation routine in SPM12.[3] Then, gray matter (GM) and white matter (WM) segmented images of all subjects, in the closest possible rigid-body alignment with each other, were used to produce GM and WM templates and to drive the deformation to the templates. At each iteration, the deformations, calculated using the Diffeomorphic Anatomical Registration using Exponentiated Lie algebra (DARTEL) registration method,[4] were applied to GM and WM, with an increasingly good alignment of subject morphology, to produce templates. Finally, an affine transformation that maps from the population average (DARTEL Template space) to Montreal Neurological Institute (MNI) space was calculated, and dj images were spatially normalized and smoothed with an 8 mm Gaussian kernel. These last 3 steps are incorporated in a tool, called "Normalise to MNI Space". The steps described for groupwise alignment were repeated for baseline 3D T_1-weighted images to run a voxel-based morphometry analysis. The only variation in the procedure described above was that the normalization to MNI space was applied to GM and WM maps, and that, after transformation, these were intensity modulated to ensure that the overall amount of each tissue class was not altered by the spatial normalization procedure.

SUPPLEMENTARY REFERENCES

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