E-METHODS: ADDITIONAL METHODOLOGICAL DETAILS

Study design: patients

Additional inclusion criteria included documented disease activity, with at least one relapse within 12 months prior to randomization, and a prior brain MRI demonstrating lesion(s) consistent with MS, or evidence of Gd+ lesion(s) of the brain on an MRI performed within the 6 weeks prior to randomization. Patients were excluded if they had progressive forms of MS, pre-specified abnormal laboratory parameters, other major disease that would otherwise preclude them from participation in a clinical trial, previous treatment with GA, or recent exposure to other contraindicated medications prior to enrollment.

Magnetization Transfer Ratio Analysis Methodology

MTR data were required to pass the following quality assurance rules; these are a combination of pre- and post-analysis rules and were constructed to be restrictive with regard to the final data accepted, and to be consistent with the methodology used in the analysis of MTR data from the DEFINE study.[1]

1. Data from 1T scanners were excluded.
2. Data from patients with valid scans at only one timepoint were excluded.
3. A small number of sites were judged unable to provide adequate MTR images and were excluded from the dataset.
4. Scans were reviewed for image quality upon receipt for quality assurance purposes. Those scans which failed quality assurance were excluded. Reasons for exclusion included motion artefact, gross image inhomogeneity and low signal-to-noise ratio.

5. When consecutive annual scans showed a change in MTR (increase or decrease) of >5%, the percentage change for this timepoint pair was excluded as this MTR change was considered to be biologically unlikely.
6. When a software upgrade occurred between consecutive timepoints, the relevant timepoint pair was excluded if a consistent pattern was seen in the MTR percentage change across patients with timepoints interrupted by the same software upgrade, or if the images showed obvious differences in MTR values in a stable scan (i.e., in tissue not obviously affected by disease).
7. Images which showed an absolute volume change (based on the number of pixels in the final MTR map) of >2% between baseline and week 48, or an absolute volume change of >4% between baseline and week 96 were excluded as suggestive of subtle segmentation errors.
8. Image review occurred on all patient timepoint pairs with a percentage change in MTR of between 3 and 5% (increase or decrease) and timepoint pairs were excluded if reason to do so was found. Reasons for exclusion included: poor brain extraction in one or both images and differences in subtle image inhomogeniety between timepoints.
9. Data from patients with a total percentage change in MTR over 2 years of >5% were also investigated using the same criteria as point 8.

Details of the MTR analysis pipeline include:

(i) registration of the magnetization transfer (MT)-weighted image (MT-on) to the non-MT-weighted image (MT-off) at each time point using a standard linear registration algorithm (FLIRT from the FSL analysis software)[2,3];

(ii) MTR calculation using the MT-off and MT-on images registered in 1);

(iii) brain-mask estimation using SIENAX[4] applied to the MT-off images for skull stripping and brain extraction;

(iv) brain-MTR map extraction obtained applying the brain-mask from step 3) to the MTR map from step 2);

(v) median whole brain MTR estimation from all voxels contained in the brain-MTR map from step 4).

Quality assessment included several steps, such as visual inspection of the brain mask obtained in step (iii) and the quality of the images.

MTR analysis references

1. Arnold D, Gold R, Kappos L, et al. Effects of BG-12 on magnetization transfer ratio in whole brain and normal-appearing brain tissue: findings from the DEFINE study. Neurology 2012;78:S11.004.
2. Jenkinson M and Smith SM. A global optimisation method for robust affine registration of brain images. Medical Image Analysis 2001;5:143-156.
3. Jenkinson M, Bannister PR, Brady JM, and Smith SM. Improved optimisation for the robust and accurate linear registration and motion correction of brain images. NeuroImage 2002;17:825-841.

4. Smith SM, Zhang Y, Jenkinson M, et al. Accurate, robust, and automated longitudinal and cross-sectional brain change analysis. NeuroImage 2002;17:479-489.

Post hoc PBVC analysis

SIENA was run on pairs of edited images from consecutive time points. The SIENA results and associated images for all time-point pairs were inspected for processing errors, and any such errors were corrected, where possible, by re-running the processing pipeline. Data was excluded when there had been a scanner change, major software upgrade, coil change or significant sequence change during the relevant time period. Data was also normally excluded if the PBVC was outside a range from -3.5% to +2.5%, because such measures were considered biologically unlikely. In occasional instances where visual inspection of the images showed obvious atrophy that could plausibly be >-3.5%, the data was included.

Lesion Count Methodology

Non-enhancing T1-weighted lesions were counted on post-contrast 2-dimensional T1-weighted spin echo sequences and defined by being hypointense in comparison with surrounding white matter.

Gd+ lesions were also counted on post-contrast 2-dimensional T1-weighted spin echo sequences. Gd+ volumes included only the visibly enhancing (T1-hyperintense) part of

an enhancing lesion, except when there was a complete closed ring of enhancement in which case all lesion within the ring was included.