Favaretto et al. Multiple Sclerosis and Demyelinating Disorders (2018) 3:1
DOI 10.1186/s40893-017-0033-3

Multiple Sclerosis and Demyelinating Disorders

REVIEW Open Access

Effects of disease modifying therapies on brain and grey matter atrophy in relapsing remitting multiple sclerosis

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Abstract

Background: Progressive brain atrophy is a major feature of multiple sclerosis (MS) pathology and is actually considered a major determinant of the progressive accumulation of physical and cognitive disability in MS patients. Although brain atrophy may have different pathological substrates, several lines of evidence suggest that in disease modifying drug (DMD)-treated MS patients, the higher is the anti-inflammatory effect of the DMD the lower is the progression of brain volume loss, grey matter atrophy and the accumulation of disability.

Areas covered: Magnetic resonance imaging (MRI)-based measurements of inflammation (focal white matter and grey matter lesions) and neurodegeneration (decrease in brain volume, cortical and deep grey matter atrophy) are currently included among the primary or secondary end-points of Phase II and III randomized clinical trials (RCT). This review summarizes literature data on the effects of DMDs on either whole brain or grey matter atrophy emerged from RCT and from post-marketing studies.

Commentary: Taken all together, literature data show that DMDs are capable to reduce significantly brain inflammation and, although with different degrees of effectiveness, to slow down global brain and/or grey matter atrophy progression. Moreover, the comparison between early and delayed treatments clearly points out that the most relevant effects on brain and grey matter atrophy are observed when DMDs are initiated in the very early disease phases.

Keywords: Multiple sclerosis, Brain atrophy, Grey matter atrophy, Disease-modifying drugs, Magnetic resonance imaging

Background

The average rate of brain volume loss per year is significantly higher in multiple sclerosis (MS) patients (*range* 0.5–1.3%, *median 0.7–0.8%*) than in healthy gender- and age-matched controls (*range* 0.1–0.3%, *median 0.2%*) [1–4] and it's currently accepted that brain volume decline in MS mainly reflects grey matter (GM) atrophy rather than white matter (WM) loss. Indeed, while WM volume shows a mild reduction during the course of disease, GM volume significantly and progressively decreases [5–13]. Recently, a parenchymal loss of ≥0.4% per year was proposed as "pathological atrophy rate" in MS patients [14].

GM atrophy, especially cortical atrophy, can be early demonstrated in patients with clinically isolated syndrome suggestive of MS (CIS) and rapidly progresses during the relapsing remitting (RRMS) disease course, becoming particularly evident in the frontal, temporal, and parietal lobes [5, 7, 10, 11, 15–19] although in different degrees and with significant regional variation among patients [19–24]. Moreover, deep GM nuclei are site of relevant atrophy as well, i.e., compared to matched healthy subjects, up to 25% loss of the thalamus volume has been demonstrated in RRMS [24–28].

GM atrophy becomes more widespread and severe in secondary (SPMS) and primary progressive MS (PPMS) [29–37] and, in some patients, may undertake a dramatic acceleration, i.e., up to 14-fold compared to age-matched healthy individual [16, 36]. Although PPMS and SPMS show similar extent of total GM atrophy,

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different regional atrophy distribution was described in these two types of MS [34–37] and some correlations between regional WM lesion load and GM atrophy were more often observed in SPMS compared to PPMS, a finding somehow expected given the differences in the WM lesion load that characterize these two MS types. On the other hand, a very weak relationship between WM pathology and GM atrophy is generally found in patients with progressive forms of MS [38], a finding that may be explained with the different rates of evolution of inflammation and neurodegeneration in the more advanced phases of the disease.

All the data above summarized strongly indicate that brain atrophy and cortical and deep GM volume measurements may be useful magnetic resonance imaging (MRI) parameters in predicting MS disease course and progression.

MRI imaging of brain atrophy

MRI measures of global brain atrophy lack pathological specificity, since they may reflect several changes of WM and GM components that are associated to inflammation and neurodegeneration, such as demyelination, axonal damage, neuronal death, astrocyte and microglia proliferation, as well as physiological fluctuations of the water content. Moreover, the anti-inflammatory DMDs currently used for the treatment of MS differentially act on WM inflammation, thus determining an acceleration of brain volume loss, a phenomenon commonly referred to as "pseudo-atrophy" and assumed to reflect the resolution of inflammation and oedema. This phenomenon may complicate the interpretation of DMD effects, especially in the first two years of therapy.

Measurements of percentage brain volume change (PBVC) and brain parenchymal fraction (BPF) over time are among the best-studied and more used methods for quantifying neurodegeneration in MS. Indeed, the majority of the clinical trials have incorporated BPF or PBVC as a secondary outcome measure of disease progression. PBVC can be obtained by means of the Structural Image Evaluation Using Normalization of Atrophy (SIENA) [39] and its cross-sectional version SIENAX [40], both included in FSL suite [41, 42], i.e., the most used methods to extract brain and skull images from the single whole-head input data. These softwares allow tissue-type segmentation, with partial volume estimation, to calculate the total volume of brain tissue and separate estimates of total GM, deep and cortical GM, and WM volumes [43]. BPF is measured as the ratio between the volume of WM + GM and the total intracranial volume WM + GM + ventricular CSF. A meta-analysis of 13 clinical trials - including more than 13.500 RRMS patients and in which brain atrophy was measured as PBVC [44] or BPF [45] - showed that treatment effects on disability progression over two years correlated with the effects observed on both brain atrophy ($p = 0.001$) and T2 lesion volume ($p < 0.001$) [46].

Other automated methods are currently available for WM and GM segmentation, that allow the assessment of the total cerebral GM volume, the volumes of deep and cortical GM, as well as the estimation of regional cortical GM volumes [40, 47, 48]. The results of these automated methods are quite reproducible [40, 49]. BPF can be also obtained from Freesurfer and ANTs pipelines or any software providing brain four-tissue segmentation as FSL [43] or SPM [50]. Cortical thickness (CTh) can be calculated with different software tools as Freesurfer, which includes a surface-based method [51], or the more recent ANTs with its registration-based DiReCT algorithm [52, 53].

GM atrophy and disability

A strong association between physical disability (as measured by means of Expanded Disability Status Scale (EDSS) or timed 25-ft walk (T25FW)) and total, regional and cortical GM volume has been described with consistent agreement among studies. These associations become more remarkable with disease progression from CIS to SPMS. [54–63]. Particularly worth of interest are studies that have demonstrated an association between cerebellar GM loss and the degree of disability in the cerebellar functional system of EDSS [60, 64]. Furthermore, physical disability correlates with both cortical thinning of the frontal, temporal and parietal lobes [29, 30, 65, 66] and with thalamic atrophy [26–28]. In addition, global GM [61, 62, 65–67] and thalamic atrophy [28, 55] have been found to predict EDSS score up to 15–20 years after diagnosis.

Several studies have pointed out a correlation between GM loss in the spinal cord and physical disability and disease duration [68–78]. Indeed, spinal cord atrophy was described in the early MS stages [69, 70, 74] but was much more pronounced in PPMS and SPMS [76, 77], whereas spinal GM volume inversely correlated with physical disability (measured with EDSS, T25FW, and 9-Hole Peg Test scores) in patients with CIS suggestive of MS, and was more predictive for EDSS or Multiple Sclerosis Functional Composite (MSFC) scores that others MRI parameters [68–70, 75–78].

Correlations between cognitive impairment and regional, cortical and total GM volume have also been demonstrated with significant agreement among studies [79–87]. Moreover, associations between specific cognitive domains and various quantitative GM measures (such as thalamic volume, cortical thickness and total GM volume) were found to be strong independent predictors of cognitive decline [23, 26, 34, 84, 87, 88].

In summary, brain atrophy and GM (cortical and deep) loss constitute a clinically relevant aspect of MS pathology since the very early disease phases, and, given their association with physical and cognitive disability, should be definitely considered not only a surrogate marker of treatment efficacy, but rather a primary target of DMDs, especially when the treatment is aimed at reducing disability progression.

Effects of disease-modifying drugs on brain and GM atrophy

Glatiramer acetate

The effect of early versus delayed glatiramer acetate (GA) treatment on brain atrophy was analyzed in the open-label phase of the PreCISe trial [89, 90], in which CIS patients with unifocal manifestations and ≥2 WM T2-lesions were randomized to receive glatiramer acetate 20 mg/day (early-treatment, $n = 198$) or placebo (delayed-treatment, $n = 211$) for 36 months or until conversion to clinically definite MS, followed by open-label GA treatment for two years. Early GA treatment was associated to less brain atrophy: PBVC from baseline to the last observed values, adjusted for study exposure, was significantly lower with early GA treatment compared with delayed treatment (−0.99% vs. −1.28%, $p = 0.02$), with a treatment effect of 28%. Mean PBVC in early GA treated patients ranged between −0.41 in the first year and −0.50 in the fifth year, i.e., values only slightly higher compared to PBVC observed in healthy subjects [91].

These findings are particularly worth of interest since a previous multicentre, randomized, double blind, placebo controlled study in RRMS patients having longer disease duration, failed to demonstrate any impact of GA on brain atrophy [92]. This apparent discrepancy further points out the importance of early treatment. However, other possible explanations of these findings might be the short duration of the follow-up and the application of a not normalized, semi-automated MRI technique (OLD) to analyse brain atrophy. Indeed, a subsequent reanalysis of the same data by means of the fully automated, normalized SIENA method [93], while disclosing similar PBVC average value for both OLD and SIENA techniques, disclosed standard deviations much lower with SIENA. These observations highlight the risk for low reproducibility of not normalized and semi-automated MRI measures for the assessment of brain atrophy and suggest caution when interpreting brain atrophy data.

More recently, in a three year study aimed at analysing efficacy and safety of 40 mg GA three-times-weekly in RRMS, patients that were early treated with GA showed significantly smaller changes in GM volume ($p = 0.015$) compared to patients that were treated with placebo for one year before starting active treatment [94].

Interferon beta

RRMS patients treated for two years with once weekly intramuscular interferon beta (IFNβ) in the MSCRG phase III trial showed significantly less GM atrophy compared with placebo, during year two after treatment initiation [45]. No change in WM atrophy was observed. In this study, the risk of sustained disability progression in IFNβ-treated patients was significantly associated with GM, but not WM, atrophy [95]. Data about the effects of high-dose high-frequency interferon beta 1a (IFNβ1a) on brain parenchymal fraction (BPF) loss can be deduced from the CARE-MS-I and CARE-MS-II trials. In the study CARE-MS-I (alemtuzumab versus IFNβ1a as first-line treatment for RRMS) the effect of alemtuzumab on BPF was compared to that of high-dose high-frequency sub-cutis IFNbβ1a. Median brain volume loss during the two-years of the core study was −1.49 in the IFNb1a cohort (−0.94 in the first year) and −0.87 in the alemtuzumab cohort (−0.59 in the first year) [96]. In the CARE MS-II study (a randomized, controlled, 2-years study on alemtuzumab for patients with RRMS after unsuccessful disease-modifying therapy) [97] the effect of both drugs in slowing down brain volume loss was more pronounced. Indeed, patients treated with high-dose high-frequency IFNbβ1a had a − 0.81% reduction in BPF (−0.54 in the first year) versus −0.61% of alemtuzumab treated patients. Thus, only in the CARE-MS-II IFNβ1a was found to positively impact on brain atrophy. The differences observed between these two studies can be explained with the substantial differences in clinical and demographic characteristics of the patient populations.

To reduce the confounding effect of pseudo-atrophy phenomenon in the evaluation of brain atrophy, in a four-years longitudinal study, we investigated the progression of cortical atrophy in RRMS patients treated with IFNβ1a or GA. Although both drugs were found to decrease the rate of cortical atrophy, a trend in favour of high-dose, high-frequency sub-cutis IFNβ1a was noticed [98].

In a study conducted in a small number of RRMS patients, IFNβ-1a 44 μg SC tiw determined a reduction in whole brain and GM tissue volume during the first three months of therapy (mean change; −0.95%; $p = 0.030$, −1.52%; $p = 0.004$, respectively), suggesting a short-term treatment-induced pseudoatrophy effect, further confirming that the pseudoatrophy effect appears very early as the result of the resolution of inflammation following treatment initiation with interferon β-1a 44 μg SC tiw [99].

More recently, we have further investigated the effect of IFNβ1a on cortical thickness (CTh) in RRMS patients. We performed a four year follow-up study of CTh in a group of 30 IFNβ1a (high-dose high-frequency)-responders RRMS patients (19 females, 11 males) treated

for a mean period of 4 years with low and stable degrees of disability (mean EDSS = 1.5 ± 1.0) and very low annualized relapse rate (mean 0.2 ± 0.47 year) before study entry. MRI was done at enrolment and then yearly for 4 years. CTh was measured by means of Freesurfer on 3D−T1-MPRAGE images. The mean loss of global CTh at the end of the 4 year follow-up was 1.06 ± 2.05%, a value equal to that observed in age-matched normal control individuals, thus confirming that a significant slow-down in cortical GM loss can be observed in patients having a good clinical (anti-inflammatory) response to high-dose high-frequency IFNβ1a [100].

Teriflunomide

RRMS patients treated with teriflunomide in the TEMSO Study [101] showed a significant decrease in %BPF compared to placebo, independently from prior DMD treatments, with no significant pseudoatrophy effect. Indeed, after two years of therapy, teriflunomide-treated naïve patients lost 0.93% BPF against 1.12% of placebo-treated subjects ($p = 0.01$), while patients coming from previous DMD therapies lost 0.73% vs 1.51% ($p = 0.001$).

In a subsequent blinded independent analysis of TEMSO MRI data, the median annualized PBVC from baseline to week 48 was calculated by means of SIENA. PBVC was lower for both teriflunomide groups versus placebo at month 12 (reductions of 34.4% with teriflunomide 7 mg, $p = 0.0011$, and 36.9% with teriflunomide 14 mg, $p = 0.0001$) and month 24 (reductions of 27.6% with teriflunomide 7 mg, $p = 0.0019$, and 30.6% with teriflunomide 14 mg, $p = 0.0001$) [102], thus confirming a significant effect of this drug in slowing-down PBVC loss. These findings are particularly interesting considering the relative modest effect of teriflunomide on the MRI parameters of WM inflammation that suggests a possible direct effect of teriflunomide on grey matter atrophy.

Dimethyl fumarate

Reductions in brain atrophy with dimethyl fumarate (DMF) in comparison to placebo did not reach statistical significance in the CONFIRM study [103], while in the DEFINE study a relative reduction in the progression of brain atrophy from baseline to year 2 (21% reduction; $p = 0.0449$) and from month 6 to year 2 (30% reduction; $p = 0.0214$) was observed and was statistically significant [104].

Recently, a retrospective analysis [105] in a small group of 20 RRMS patients followed for up to 1 year, the DMF group showed a lower rate of whole brain atrophy compared to no-DMT group (PBVC: −0.37 ± 0.49% vs. -1.04 ± 0.67%, $p = 0.005$). Although the DMF-treated group had less change in putamen volume (−0.06 ± 0.22 vs. -0.32 ± 0.28 ml, $p = 0.02$), no significant on-study differences between groups in caudate, globus pallidus, thalamus, total deep gray matter volume, T2 lesion volume, EDSS, or T25FW (all $p > 0.20$) were demonstrated, probably because of the very low number of patients enrolled in the study.

Fingolimod

In the two Phase III studies FREEDOMS and FREEDOMS II [106, 107], fingolimod was found to reduce the PBV loss in RRMS patients by 36% ($p < 0.001$) and 33% ($p = 0.0002$), respectively. Whether this effect on PBV was mediated through the anti-inflammatory effects of the drug on WM focal inflammatory damage or was primarily a direct effect on the diffuse neurodegenerative damage, has been investigated in a post-hoc analysis in which patients with no evidence of focal disease activity from the two studies were pooled [108]. This analysis disclosed that fingolimod was capable to reduce significantly PBV change by 65.5% over 12 months (fingolimod vs. placebo: −0.16 vs −0.45; $p = 0.001$) and by 48.2% over 24 months (−0.42 vs. -0.81; $p = 0.004$). An absolute difference in PBV change of −0.27% ($p < 0.001$) in favour of fingolimod vs. placebo over 24 months was still evident in the pooled intention to treat (ITT) population, after adjusting for active lesions and on-study relapses. The regression model suggested that 54% (−0.27%/−0.51%) of the effect of fingolimod on PBV change was independent of its effects on visible focal WM damage. Thus, the effect of fingolimod on diffuse damage seems partly independent of its anti-inflammatory effect, suggesting that this drug positively affects both the inflammatory and the neurodegenerative components of MS.

In a post hoc analysis of studies assessing fingolimod efficacy, brain volume loss was found to correlate with disease severity at baseline and disease activity during the course of the study [109]. This supports the concept that inflammatory activity, lesion load, and number of relapses contribute to atrophy, and that treatment of this activity has a beneficial effect on atrophy rates.

Natalizumab

The controversial available data on the effects of natalizumab on brain atrophy can be primarily explained with the strong anti-inflammatory effect (i.e., marked pseudoatrophy effect) of this monoclonal antibody and by the clinical and radiological features of the majority of the patients treated with this drug (i.e., RRMS patients with very high disease activity, frequently coming from first-line treatment failure).

Indeed, in a recent study on sixty-two MS patients [110], having a mean age of 34.7 ± 8.3 and a mean disease duration of 10.4 ± 6.6 years, the presence of gadolinium enhancing lesions at baseline was associated

with a strong pseudoatrophy effect. Indeed, the larger PBV decrease ($p = 0.005$) were observed in the first ($p = 0.024$) and second year ($p = 0.019$) but not in the third year ($p = 0.863$). On the contrary, the decrease in PBV in patients having no evidence of active brain inflammation was about 1% in the two years, a figure very close to that of normal subjects. Thus, baseline inflammation of RRMS patients with very high disease activity strongly affects brain volume measures up to 24 months after natalizumab initiation.

A marked pseudoatrophy effect of natalizumab therapy was confirmed in a 18-month study where the PBV of natalizumab-treated patients was found to dramatically decrease especially in the first 12 months of treatment [111].

In a recently published study aimed at investigating whether cerebrospinal fluid (CSF) markers of inflammation or neurodegeneration were associated with PBV change in natalizumab-treated MS and whether this change was reflected in non-lesional WM metabolites (analysed by proton magnetic resonance spectroscopy, ^{1}H–MRS), twenty-five natalizumab-treated RRMS were followed for 3 years. The mean decline in PBV was 3% at the 3-year follow-up, but mean ^{1}H–MRS metabolite levels in non-lesional WM were unchanged. Interestingly, CSF levels of neurofilaments and tau at baseline correlated negatively with PBV change over 3 years ($r = -0.564$, $p = 0.012$, and $r = -0.592$, $p = 0.010$, respectively), suggesting their possible use for evaluating treatment response in MS [112].

In order to study the effect of natalizumab on GM atrophy avoiding the marked pseudo-atrophy effect of the drug on brain parenchymal volume, we analysed the cortical thickness of RRMS patients treated with natalizumab for up to four years. Thirty patients non-responder to IFNβ, having had a mean annualized relapse rate of 1.5 ± 1.0 (range 1–4) in the year prior to natalizumab initiation, evidence of disease activity at brain MRI and mean EDSS score of 3.0 ± 1.6 were enrolled in the study. CTh was measured by Freesurfer on 3DT1 images obtained at study entry and then annually for 4 years. At the end of the study, natalizumab-treated patients had a mean loss of CTh of 2.09 ± 3.3%, corresponding to mean annual CTh loss of 0.5%, a value higher compared to age-matched normal controls, but significantly lower compared to untreated MS patients [100]. Our finding are particularly interesting since they indicate that, once reached EDSS 3.0 (i.e., a disability milestone that marks the entry in the progressive disease phase) the impact of anti-inflammatory therapies on neurodegeneration becomes less pronounced and may be not significant. This further suggests the importance of early therapeutic intervention with second line drugs in patients with poor response to first line therapies.

Alemtuzumab

As above described, in RRMS patients treated with alemtuzumab in the CARE-MS-I RCT the mean percentage of BPF loss after two years was −0.87 against −1.49 of the patients treated with high-dose high frequency sub-cutis IFNβ. At the end of the extension study (core study + extension = 6 years) the BPF loss was −1.43%, corresponding to a mean loss of −0.23%/year, comparable to the mean loss observed in normal individuals. This extraordinary result was confirmed in the CARE-MS-II RCT: after two years the mean percentage of BPF loss was −0.62% in alemtuzumab-treated patients and −0.82 in IFNβ-treated patients. At the end of the extension study, alemtuzumab-treated patients lost a mean percentage of BPF of −0.95, corresponding to a mean annual loss of −0.16% [113].

Daclizumab

In a retrospective analysis aimed at determining the effect of daclizumab on brain atrophy, 26 MS patients treated for a median period of 4.3 years with daclizumab were compared to a control group of 44 patients treated with other DMD (predominantly IFNβ). Supratentorial brain volume declined by 5.17 ml per year (95% confidence limits: 3.58–6.77) in the DMD group and by 3.72 ml ($p = 0.01$) in the daclizumab group. The rate of ventricular enlargement decreased from 1.26 to 0.42 ml per year ($p < 0.001$). Focused analysis suggested that reduction in the rate of GM atrophy could explain the results [114].

Ocrelizumab

In the trial ORATORIO the effect of ocrelizumab (anti-CD20 humanized antibody) on disability progression (primary endpoint) was analysed in the primary progressive form of MS. Among the secondary MRI endpoints, the adjusted mean PBVC from week 24 to week 120 (fourth secondary endpoint) was lower with ocrelizumab than with placebo (−0.90 vs. −1.09) and the difference reached the significance ($p = 0.02$) [115].

In the trials OPERA I and OPERA II (ocrelizumab versus high-dose high frequency sub-cutis IFNβ1a in RRMS), despite the extraordinary effects of the drugs on clinical and MRI-inflammatory endpoints, the differences in the % PBVC from week 24 to week 96 between the ocrelizumab group and the IFNβ group were non-confirmatory in the OPERA I trial (nominal $p = 0.004$) and non-significant in the OPERA II trial (nominal $p = 0.09$) [116]. Although the findings were non-confirmatory as a result of failure of the hierarchical analysis, the percentages of patients who had no evidence of disease activity were higher with ocrelizumab than with IFNβ1a in both trials.

Cladribine

An exploratory analysis on the cladribine tablets treating multiple sclerosis orally (CLARITY) study (phase-3, double-blind, placebo-controlled, multicenter trial in patients with RMS assessing the effects of cladribine tablets given annually over 2 years) [117] disclosed that cladribine significantly reduced brain atrophy in comparison with placebo treatment, with residual rates in treated patients being close to the physiological rates [118]. Indeed, compared with placebo (−0.70% ± 0.79), the annualized percentage brain volume change (PBVC/y) was reduced in patients treated with cladribine tablets 3.5 mg/kg (−0.56% ± 0.68, $p = 0.010$) and 5.25 mg/kg (−0.57% ± 0.72, $p = 0.019$). After adjusting for treatment group, PBVC/y showed a significant correlation with the cumulative probability of disability progression (HR = 0.67, 95% CI = 0.571, 0.787; $p < 0.001$), with patients with lower PBVC/y showing the highest probability of remaining free from disability progression at 2 years and vice versa. Since cladribine crosses the blood brain barrier, it might be hypothesized that this drug acts not only on focal WM inflammation, but also on the diffuse brain, including cortical, tissue damage and neurodegeneration in RRMS.

Conclusions

This review highlights that 1) compared to no treatment, all DMDs reduce the rate of brain volume loss and/or GM atrophy in RRMS, 2) the early treatment generally shows a more relevant impact on brain atrophy measures compared to late treatment, 3) second line therapies generally have a more significant and long lasting impact on MRI metrics of either brain or GM atrophy. Figure 1 reports the brain volume changes observed with some currently used DMDs and clearly shows that DMDs lessen the progression of brain atrophy observed in MS patients to values similar to those observed in normal individuals. Of course, a direct comparison between drugs is hampered by substantial differences among the experimental design of the trials (including patient selection, clinical and MRI parameters).

This review further confirms that the current attitude of treating RRMS with DMD in very early disease phases, possibly since clinical onset, is correct and has a strong rationale also in preventing or slowing down neurodegeneration and disability progression. Moreover, in as much as global brain volume loss and grey matter damage remain MRI parameters not routinely evaluated, the discontinuation of therapy in patients judged to be "clinically" stable for prolonged period of time on the base of the relapse rate and/or rough MRI parameters of WM damage should be considered with caution.

Increasing evidence suggests that MRI metrics of brain atrophy should be definitely considered not only surrogate markers of treatment efficacy, but rather a primary target of DMDs, especially when the treatment is aimed at reducing disability progression. However, further studies aimed at investigating the mechanisms of grey matter damage and how DMDs may positively modify this critical aspect of MS pathology are needed.

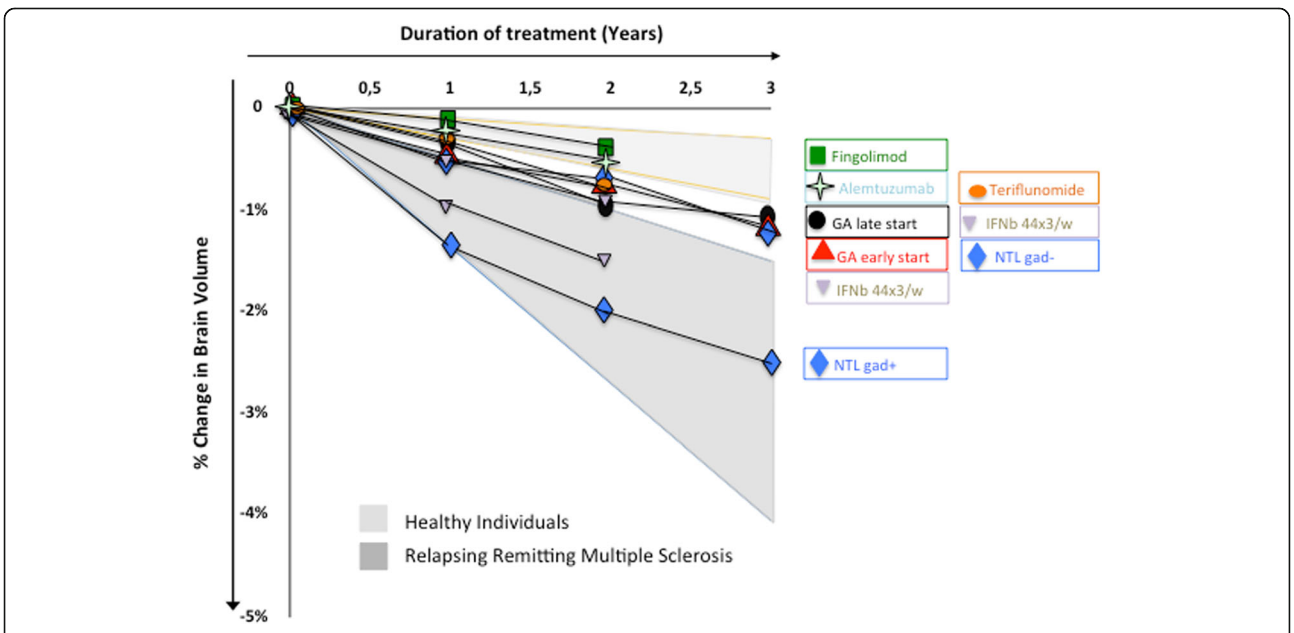

Fig. 1 Percent changes in brain volume in multiple sclerosis patients under treatment for two or three years with disease modifying drugs in Phase III Randomized Clinical Trials or observational studies. The estimated ranges of brain volume loss for healthy individuals and relapsing remitting multiple sclerosis are indicated with different shades of grey

Acknowledgements
None.

Funding
NA

Availability of data and materials
NA

Authors' contributions
All Authors have contributed to manuscript preparation. All authors read and approved the final manuscript.

Ethics approval and consent to participate
NA

Consent for publication
NA

Competing interests
During the past 3 years, Professor P. Gallo has had a financial relationship (lecturer, member of advisory boards and/or consultant or investigator) with Biogen Italy, TEVA, Merck-Serono, Novartis Farma, Sanofi-Genzyme, Roche and Almirall. Dr. A. Favaretto has received funding for travel and speaker honoraria in the last 3 years from Teva, Novartis, Biogen Italy, and Genzyme. A. Lazzarotto, M. Margoni, and D. Poggiali have nothing to disclose.

Publisher's Note

Received: 10 August 2017 Accepted: 14 December 2017
Published online: 29 January 2018

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