

Cerebrospinal fluid inflammatory biomarkers predicting interferon-beta response in MS patients

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Abstract

Background and Aims: Interferon beta (IFN β) is a safe first-line drug commonly used for relapsing-remitting (RR)-MS. Nevertheless, a considerable proportion of patients do not respond to IFN β treatment. Therefore, until now, a number of studies have investigated various markers that could predict the patients who would respond to IFN β therapy. The objective of this study was to identify reliable biomarkers to predict the efficacy of IFN β treatment in MS.

Methods: In a group of 116 patients with clinically isolated syndrome (CIS) and RR-MS, we explored the association between CSF detectability of a large set of proinflammatory and anti-inflammatory molecules at the time of diagnosis and response to IFN β after the first year of treatment. The absence of clinical relapses, radiological activity and disability progression (NEDA-3) was assessed at the end of 1-year follow up. The results were compared with those obtained in additional groups of CIS and RR-MS patients treated with other first-line drugs (dimethyl fumarate and glatiramer acetate).

Results: CSF undetectability of macrophage inflammatory protein (MIP)-1 α was the main predictor of reaching NEDA-3 status after 1 year of IFN β treatment. Moreover, detectable platelet-derived growth factor (PDGF) was associated with higher probability of reaching NEDA-3. Conversely, no associations with the CSF molecules were found in the two other groups of patients treated either with dimethyl fumarate or with glatiramer acetate.

Conclusion: MIP-1 α and PDGF could potentially represent suitable CSF biomarkers able to predict response to IFN β in MS.

Keywords: cerebrospinal fluid (CSF), clinically isolated syndrome (CIS), interferon beta (IFN β), macrophage inflammatory protein (MIP)-1 α , NEDA-3, platelet-derived growth factor (PDGF), relapsing-remitting (RR)-MS

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Introduction

Interferon beta (IFN β) is one of the most frequently prescribed first-line disease-modifying therapies (DMTs) in patients with clinically isolated syndrome (CIS) and relapsing-remitting (RR)-multiple sclerosis (MS). IFN β treatment is used commonly in newly diagnosed patients due to beneficial risk-benefit profile, since IFN β

administration slightly improves the course of MS, moderately slowing the long-term progression of disability associated with a 30% reduction of relapse rate.^{1,2} Nevertheless, a significant proportion of patients do not respond to IFN β treatment,³ partly due to the pathogenetic differences between patients, resulting in both non-responders and good clinical responders among them. Consequently,

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numerous attempts have been made to identify possible markers to predict response to IFN β therapy. It has been reported that some disease demographic characteristics, including clinical disability, disease duration, and pretreatment relapse rate could be associated with IFN β response; particularly, higher age before starting IFN β therapy,⁴ and lower annual relapse rate before treatment initiation.⁵ Furthermore, it has been proposed that the expression of various genes involved in inflammatory response and its modulation during IFN treatment could contribute to explain inter-individual variability in IFN β response.^{6,7} However, clear associations have not yet been established, due mainly to differences in the definition of response to treatment, different populations studied, and different statistical power linked to number of patients included in the studies. Therefore, the identification of biomarkers capable to predict the response to IFN β treatment might be extremely useful.

It has been demonstrated that inflammation negatively influences the disease course of RR-MS. Accordingly, elevated cerebrospinal fluid (CSF) concentrations of various proinflammatory molecules at the time of diagnosis have been associated with enhanced disease activity, increased long-term disability and shift to the second-line therapies.⁸ Notably, previous studies have demonstrated that IFN β treatment has immunomodulatory properties and regulates the expression of different proinflammatory molecules. IFN β reduces antigen presentation by microglia and monocytes, decreases T-cell responses,⁹ inhibits T cells migration across the blood-brain barrier, prevents Th17 differentiation reducing the expression of IL-17 and IFN gamma (IFN γ), and enhances the production of IL-10.¹⁰

It is not clear whether pre-treatment expression of CSF inflammatory and anti-inflammatory molecules could play a role in predicting the individual variability of the response to IFN β therapy. We therefore explored in a group of CIS and RR-MS patients, the association between CSF detectability of numerous molecules at the time of diagnosis, and response to IFN β after the first year of treatment. Moreover, the same association was explored in two different groups of patients treated with other first-line DMTs [glatiramer acetate (GA) and dimethyl fumarate (DF)].

Methods

Study protocol and MS patients

A group of 226 patients admitted to the neurological clinics of University Tor Vergata Hospital and Neuromed Institute, diagnosed as CIS or RR-MS, between 2009 and 2019, were enrolled in this retrospective study. The study was approved by the Ethics Committees of the University Tor Vergata Hospital in Rome and Neuromed Research Institute in Pozzilli, Italy, according to the Declaration of Helsinki. All patients gave written informed consent to participate in the study.

In all patients recruited in this investigation, the diagnosis of CIS or RR-MS was made based on the clinical, laboratory, and magnetic resonance imaging (MRI) findings.¹¹ Clinical evaluation, brain and spinal cord MRI, and CSF collection, were performed during hospitalization.

The mean follow-up period was 20.3 ± 82.7 months, median 19.7 month (IQR = 29.6). Follow-up consisted of neurological examination and MRI scans performed every 6 months after diagnosis. Additional clinical evaluation and MRI were performed, when clinical relapses had occurred. DMTs were initiated after the diagnosis. The IFN β group ($n=116$) included people with MS treated with different IFN β preparations (Table 1). Two groups of CIS and RR-MS patients, treated either with DF ($n=59$) or GA ($n=51$) were also included in the study.

Clinical evaluation and MRI parameters

Disability was measured using the Expanded Disability Status Scale (EDSS).¹² Disease duration was calculated as the time interval between the first episode of neurological dysfunction suggestive of MS and the time of confirmed diagnosis during hospitalization at above-mentioned neurological clinics. Clinical relapses have been defined as the development of new or recurrent neurological symptoms persisting at least 24h and not associated with fever or infection. The number of clinical relapses that occurred before lumbar puncture (LP) was recorded for each patient. Disease activity at the time of LP was defined as the presence of clinical relapse or active MRI lesions at that time.

In all patients, MRI of the brain and spinal cord (cervical, thoracic and lumbar) were performed.

Table 1. Interferon-beta preparations in patients with CIS and RR-MS treated with interferon beta.

Interferon-beta preparations	Number (%)
Avonex	11 (9.5)
Rebif 22 mcg	11 (9.5)
Rebif 44 mcg	62 (53.5)
Betaferon	28 (24.1)
Plegridy	4 (3.4)
Total	116 (100)

Treatment duration with interferon-beta ranged from 1 to 12 months (mean 10.4 months); 85/116 (73.3%) were treated for 12 months.
CIS, clinically isolated syndrome; RR-MS, relapsing-remitting multiple sclerosis.

Patients were examined by 1.5 or 3.0 T MRI, including the following sequences: dual-echo proton density, fluid-attenuated inversion recovery, T1-weighted spin-echo (SE), T2-weighted fast SE, and contrast-enhanced T1-weighted SE after intravenous gadolinium (Gd) infusion (0.2 ml/kg). A Gd-enhancing (Gd+) lesion was defined as an area of hyperintense signaling on contrast-enhanced T1-weighted images. An active MRI was defined as one showing new or enlarging T2-weighted lesions and/or contrast-enhanced T1-weighted lesions.

No evidence of disease activity (NEDA) status was assessed for each patient at the end of 1-year follow up. In the present study, NEDA was defined as the absence of radiological and clinical disease activity and the absence of disability progression (NEDA-3).¹³

CSF collection and analysis

CSF was collected by LP. Immediately after LP, CSF was centrifuged and stored at -80°C until being analyzed using a Bio-Plex multiplex cytokine assay (Bio-Rad Laboratories, Hercules, CA, USA), according to the manufacturer's instructions. CSF detectability of the following molecules has been examined: interleukin (IL)-1 β , IL-1 receptor antagonist (IL-1ra), IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, tumor necrosis factor alpha (TNF α), IFN γ , macrophage inflammatory protein (MIP)-1 α /CCL3, MIP-1 β /CCL4, monocyte chemoattractant protein

(MCP)-1/CCL2, interferon gamma-induced protein 10 (IP-10)/CXCL10, eotaxin, granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), vascular-endothelial growth factor (VEGF), and Regulated on Activation, Normal T Cell Expressed and Secreted (RANTES)/CCL5. CSF detectability of all molecules tested in our CIS and RRMS patients, separated in three groups according to DMTs (IFN-beta, GA, DF) used, is presented in the Supplemental Table.

Statistical analysis

To determine the significance of the different proportions, a chi square test was used, and the mean values compared by using ANOVA, if the data distribution was normal. In case of not normal distribution, non-parametric tests were used for testing statistical significance. Predictive value of investigated variables was assessed by logistic regression analysis, with NEDA-3 after 1 year (yes/no) as the dependent variable. All variables with significance levels of 0.05 by univariate analysis were included in the multivariate models (method: enter). The statistical significance of CSF chemokine (MIP-1 α and PDGF) detectability pattern was assessed by a chi square test for trend. Sensitivity, specificity, and accuracy, were calculated. Two-tailed *p* values less than 0.05 were considered as significant. Data were analyzed using the Statistical Package for the Social Sciences (SPSS) software (Advanced Statistics, version 21.0, Chicago, IL, USA).

Results

IFN β cohort

Demographic and clinical characteristics of CIS and RR-MS patients treated with IFN β are shown in Table 2.

In the IFN β group, the proportion of NEDA-3 patients at the end of a 1-year follow up was 61/116 (52.6%). Treatment duration with IFN β ranged from 1 to 12 months (mean 10.4 months), 85/116 patients (73.3%) were treated during 12 months.

Predictors of reaching NEDA-3 in the IFN β group by univariate logistic regression analysis are presented in Table 3. We found that only PDGF

Table 2. Demographic and clinical characteristic of CIS and RR-MS patients.

Variable	Interferon-beta	GA	DF
	Value	Value	Value
Number	116	51	59
Duration of MS, years (mean ± SD)	2.7 ± 5.6	2.7 ± 4.8	4.0 ± 6.8
Gender			
-male (n, %)	41 (35.3)	13 (25.5)	18 (30.5)
-female (n, %)	75 (64.7)	38 (74.5)	41 (69.5)
Age, years (mean ± SD)	34.1 ± 11.1	38.1 ± 11.8	36.8 ± 12.9
EDSS score (median, IQR)	1.5 (1.0)	2.0 (1.9)	2.0 (1.8)
Unique CSF OCB	*	**	***
-positive (n, %)	83 (73.5)	39 (84.8)	38 (66.7)
-negative (n, %)	30 (26.5)	7 (15.2)	19 (33.3)
Previous number of relapses before LP (mean ± SD)	1.6 ± 0.9	1.5 ± 0.9	1.4 ± 0.7

*missing data for three patients (2.6%); **missing data for five patients (9.8%); ***missing data for two patients (3.4%).
CIS, clinically isolated syndrome; CSF, cerebrospinal fluid; DF, dimethyl fumarate; EDSS, expanded disability status scale; GA, glatiramer acetate; IQR, interquartile range; LP, lumbar puncture; MS, multiple sclerosis; OCB, oligoclonal bands; RR-MS, relapsing remitting multiple sclerosis; SD standard deviation.

Table 3. Predictors of reaching NEDA-3 in patients treated with interferon-beta by univariate logistic regression analysis.

Variable	OR	95% CI	p
Disease duration	1.02	0.95–1.10	0.538
Gender	0.69	0.32–1.49	0.344
Age	1.00	0.97–1.04	0.847
Baseline EDSS score	0.73	0.50–1.07	0.110
Unique CSF OCB	0.66	0.28–1.52	0.321
Number of relapses before LP	1.05	0.68–1.61	0.834
CSF detectability of PDGF	3.15	1.22–8.14	0.018
CSF detectability of MIP-1 α	0.24	0.08–0.74	0.013

Non-significant correlations with CSF molecules are not reported; bold values denote statistical significance.
CI, confidence interval; CSF, cerebrospinal fluid; EDSS, expanded disability status scale; LP, lumbar puncture; MIP-1 α , macrophage inflammatory protein-1 α ; NEDA, no evidence of disease activity; OCB, oligoclonal bands; OR, odds ratio; PDGF, platelet-derived growth factor.

and MIP-1 α CSF detectability were associated significantly with NEDA-3 status. MIP-1 α

detectability was associated significantly with reduced probability of reaching NEDA-3 status, 1 year after diagnosis [odds ratio (OR)=0.24; 95% confidence interval (CI)=0.08–0.74; $p=0.013$]. Conversely, PDGF detectability was associated significantly with higher probability of reaching NEDA-3 at a 1-year follow up (OR=3.15; 95% CI=1.22–8.14; $p=0.018$). No significant association emerged between other CSF molecules detectability and NEDA-3. After including both variables significant in univariate analysis at the level of $p<0.05$, in the multivariate model, MIP-1 α CSF detectability was independent predictor for NEDA-3 status in the IFN β group [OR=0.24 (95% CI=0.08–0.74); $p=0.013$].

In order to assess the trend of relative risk to reach NEDA-3 after a 1-year follow-up, we combined three variables: presence/absence of NEDA-3, CSF detectability/undetectability of both MIP-1 α , and PDGF. The analysis was performed in a group 55 of patients in which both MIP-1 α and PDGF had been analyzed. We considered patients who reached NEDA-3 with detectable PDGF and not detectable MIP-1 α , as a reference group (OR=1.00). Patients who

had one of the following settings: a) detectable, both MIP-1 α and PDGF, and b) detectable neither MIP-1 α , nor PDGF, had lower probability to reach NEDA-3 (OR=0.35) in comparison with reference group. Finally, those patients who had detectable CSF MIP-1 α and undetectable PDGF had the lowest probability to reach NEDA-3 (OR=0.16). This trend is statistically highly significant ($\chi^2=7.045$, $p=0.008$) (Figure 1).

We have assessed the statistical validity of these biomarkers. The sensitivity of non-detectability of MIP-1 α was high (98.2%), and specificity was 56.9%. The accuracy of non-detectability of MIP-1 α to correctly classify patients with CIS and MS treated with IFN β according to reaching NEDA-3 after 1-year follow up was high (77.0%). In addition, the sensitivity of detectability of PDGF was 56%, and specificity was high (81.8%). The accuracy of detectability of PDGF to correctly classify patients with CIS and MS treated with interferon-beta according to reaching NEDA-3 after 1-year follow up was also high (68.6%).

GA and DF cohorts

The association between CSF inflammatory molecules detectability and response to treatment was explored in two additional cohorts of patients treated with other first-line DMTs (GA and DF).

Demographic and clinical characteristics of CIS and RR-MS patients treated with GA or DF are shown in Table 2.

In the GA group, NEDA-3 status at the end of a 1-year follow up was achieved in 20/51 patients (40%). We explored predictors of reaching NEDA-3 in patients treated with GA by univariate logistic regression analysis. No significant associations emerged between NEDA-3 status and CSF molecules explored.

In the DF group, 42/59 patients (75%) reached NEDA-3 at the end of a 1-year follow up. Predictors of reaching NEDA-3 in patients treated with DF were explored by univariate logistic regression analysis. Number of clinical relapses before LP was the only variable significantly associated with NEDA-3 in this group: OR=0.27 (95% CI=0.08–0.92), $p=0.036$. No significant associations emerged between CSF molecules and NEDA-3.

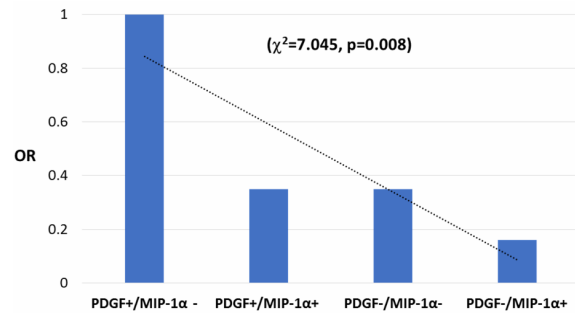


Figure 1. Probability to reach NEDA-3 based on CSF MIP-1 α and PDGF detectability in patients treated with interferon beta after a 1-year follow-up.

CSF, cerebrospinal fluid; MIP-1 α , macrophage inflammatory protein-1 α ; NEDA, no evidence of disease activity; OR, odds ratio; PDGF, platelet-derived growth factor.

Table 4. Predictors of reaching NEDA-3 in patients treated with glatiramer acetate or dimethyl fumarate by univariate logistic regression analysis.

Variable	OR	95% CI	<i>p</i>
Disease duration	0.96	0.89–1.04	0.311
Gender	1.62	0.67–3.92	0.285
Age	1.02	0.99–1.05	0.249
Baseline EDSS score	1.19	0.84–1.70	0.321
Unique CSF OCB	1.42	0.68–2.92	0.521
Number of relapses before LP	0.97	0.59–1.58	0.894
CSF detectability of PDGF	0.57	0.22–1.46	0.243
CSF detectability of MIP-1 α	2.48	0.21–8.61	0.268

*Other non-significant correlations with CSF molecules are not reported.
CI, confidence interval; CSF, cerebrospinal fluid; EDSS, expanded disability status scale; LP, lumbar puncture; MIP-1 α , macrophage inflammatory protein-1 α ; NEDA, no evidence of disease activity; OCB, oligoclonal bands; OR, odds ratio; PDGF, platelet-derived growth factor.

We applied the univariate regression model on patients on GA and DF, which showed that none of variables examined was predictive for reaching NEDA-3 after 1-year follow up (Table 4). Therefore, it was not possible to perform multivariate regression analysis.

Discussion

The increasing number of available DMTs makes clinical management of MS patients more complex. One of the common strategies today for the significant number of RR-MS patients worldwide

is still to start MS treatment as soon as possible with first-line drugs, including IFN β , GA and DF. However, a considerable proportion of these patients continue to exhibit disease activity during such treatments. For them, second-line therapies are available, such as alemtuzumab, natalizumab, or ocrelizumab, have proven to be more effective, but potentially associated with serious adverse events.¹⁴ Therefore, the identification of reliable biomarkers able to predict the efficacy of generally safe IFN β treatment in individual patients with MS might be extremely useful.

In the present study, we explored in a group of newly diagnosed CIS and RR-MS patients treated with IFN β , the association between a large set of proinflammatory and anti-inflammatory molecules at the time of diagnosis, and NEDA-3 status 1-year after treatment. Our results showed that CSF undetectability of the proinflammatory molecule MIP-1 α was the main factor associated with increased probability of reaching NEDA-3 status after 1 year of IFN β treatment. However, also detectable PDGF in CSF was found to predict higher probability of reaching NEDA-3. Therefore, patients who had detectable CSF MIP-1 α and undetectable PDGF had 84% lower probability to reach NEDA-3, and this trend was statistically highly significant.

It has been demonstrated that proinflammatory molecules play a crucial role in the pathogenesis of MS, influencing the disease course. For example, elevated CSF levels of the proinflammatory molecules IL-6 and IL-8, have been associated with increased disease activity and disability in RR-MS and CIS patients.⁸ In addition, CSF expression of IL-1 β , has been also associated with worse disease course in MS.¹⁵ Thus, it has been shown that detectable CSF IL-1 β in RR-MS patients during remission at the time of diagnosis was associated with increased midterm disease progression.¹⁵

MIP-1 α /CCL3 is a proinflammatory molecule released by several immune cells, including monocytes/macrophages, T and B lymphocytes, neutrophils, dendritic, and glial cells.^{16,17} MIP-1 proteins bind to various receptors, including CCR1, CCR3, and CCR5, influencing the activity of numerous immune cells and regulating cell differentiation, chemotaxis, and synthesis of inflammatory mediators.¹⁸ MIP-1 α interacts with G-protein-coupled receptors promoting Th1 differentiation; accordingly, CCR5-deficient mice

show Th2 polarization.¹⁹ Therefore, MIP-1 proteins represent key players involved in the pathogenesis of many autoimmune inflammatory diseases including EAE and MS.

Previous studies have demonstrated that MIP-1 α participates in the CNS inflammatory process observed in EAE and MS. Studies in animal models of MS have shown that expression of MIP-1 α was enhanced during acute inflammation and correlated with the degree of inflammation.²⁰ Experimental data support a role of MIP-1 α in the pathogenesis of EAE. Accordingly, it has been shown that the administration of anti-MIP-1 α antibodies prevented the transfer of EAE.²¹ Moreover, also selective blocking of MIP-1 α receptors strongly reduced clinical and pathological manifestations.²² In MS patients, enhanced expression of MIP-1 α has been evidenced in peripheral T lymphocytes, in the CSF and in post-mortem brain samples.^{17,23} MIP-1 α and its receptors CCR3 and CCR5 are expressed by astrocytes and macrophages within the plaque.¹⁷ These receptors are crucially involved in the pathogenesis of MS regulating the migration of immune cells.²⁴ Accordingly, it has been demonstrated that peripheral T cells from MS patients showed increased migration toward RANTES and MIP-1 α compared with control subjects, which is associated with increased expression of CCR5.²⁵ These findings are in line with our results, which have demonstrated that undetectability of MIP-1 α in CSF is associated with higher probability of reaching NEDA-3 in patients treated with IFN β .

Conversely, anti-inflammatory molecules and neurotrophins could exert beneficial effect, decreasing T cell infiltration, reducing neurodegeneration, and promoting better disease course in EAE and MS. Previous findings have shown that PDGF promotes neuronal growth and remyelination,^{26,27} and may play a protective role in CIS and RR-MS patients.²⁸ It has been proposed that the beneficial effects of PDGF in MS could be related to increased synaptic plasticity expression, as reported in vitro and in RR-MS patients.^{29,30} Accordingly, this molecule could enhance the compensation of clinical deficit caused by newly appearing demyelinating lesions, promoting stable clinical course.²⁸

It has been consistently demonstrated that IFN β has immunomodulatory properties, reducing Th1 cytokines and inducing a shift toward the

production of anti-inflammatory molecules.³¹ Accordingly, it has been evidenced that IFN β reduces the expression of inflammatory cytokines, and enhances expression of several anti-inflammatory molecules and growth factors, including IL-10, BDNF, and PDGF.^{32–34} Studies in EAE have shown that IFN β administration decreases the expression of several chemokines including MIP-1 α and RANTES and their receptors by Th1/Th17 cells, reducing CNS infiltration.³⁵ In addition, it has been reported that IFN β inhibits signaling via Toll-like receptor 9 in plasmacytoid dendritic cells from MS patients, reducing the release of immune mediators including MIP-1 α , MIP-1 β , and RANTES.³⁶ Overall, these results could indicate that the immunomodulatory effects of IFN β imply reduced activation of the innate immune system and that down-regulation of MIP-1 α expression may decrease the inflammatory response and lymphocytic infiltration into the CNS.

Potential inflammatory biomarkers associated with IFN β response in MS have been previously investigated. Some studies explored the role of inter-individual genetic variability investigating whether the expression of certain genes could be associated to response to IFN β therapy.^{6,7} In one study, exploring gene expression in peripheral blood mononuclear cells from RR-MS patients, and its modulation during IFN β treatment, it has been shown that the expression of type I IFN-regulated genes could predict 2-year response to IFN β .⁷ In particular, the basal expression of various type I IFN-regulated genes was found to be increased in non-responders; conversely, in responders low basal expression of these genes increased during treatment. Therefore, it has been hypothesized that non-responder patients to IFN β treatment may represent a pathogenetically different MS phenotype, characterized by intrinsically activated innate immunity and by monocyte dysfunction.⁷

In the present study, after analyzing an ample array of CSF proinflammatory and anti-inflammatory molecules, we found that MIP-1 α and PDGF detectability profile predicted NEDA-3 status in patients treated with IFN β . Short follow-up duration represents a limitation of the present study. Therefore, assessing NEDA-3 status after longer follow up would strongly support the potential clinical application of these biomarkers. Moreover, whether the actual CSF levels of these

molecules could also be useful to predict the response to IFN β should be investigated in a larger cohort of MS patients considering the extremely high variability of CSF cytokine levels. These results suggest that MIP-1 α detectability/PDGF undetectability could represent suitable biomarkers able to predict response to IFN β therapy.

Conflict of interest statement

The authors declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: F.S. acted as Advisory Board members of Novartis, Biogen and Merck Serono. R.Fu. has received honoraria as speaker or for research support from Biogen, Novartis, Merck, Roche, Genzyme. G.A.M. received honoraria for speaking, consultation fees and travel funding from Roche, Almirall, Bayer Schering, Biogen Idec, Merck Serono, Novartis, Sanofi-Genzyme, Mylan and Teva. She is the principal investigator in clinical trials for Actelion, Biogen Idec, Merck Serono, Mitsubishi, Novartis, Roche, Sanofi-Genzyme, Teva. D.C. is an Advisory Board member of Almirall, Bayer Schering, Biogen, GW Pharmaceuticals, Merck Serono, Novartis, Roche, Sanofi-Genzyme, and Teva and received honoraria for speaking or consultation fees from Almirall, Bayer Schering, Biogen, GW Pharmaceuticals, Merck Serono, Novartis, Roche, Sanofi-Genzyme, and Teva. He is also the principal investigator in clinical trials for Bayer Schering, Biogen, Merck Serono, Mitsubishi, Novartis, Roche, Sanofi-Genzyme, and Teva. His preclinical and clinical research was supported by grants from Bayer Schering, Biogen Idec, Celgene, Merck Serono, Novartis, Roche, Sanofi-Genzyme and Teva. F.B. acted as Advisory Board members of Teva and Roche and received honoraria for speaking or consultation fees from Merck Serono, Teva, Biogen Idec, Sanofi, and Novartis and non-financial support from Merck Serono, Teva, Biogen Idec, and Sanofi.

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Data Availability Statement

Datasets are available on request. Please contact corresponding author.

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Supplemental material

Supplemental material for this article is available online.

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