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ORIGINAL COMMUNICATION

Effectiveness of Rituximab in rElapsiNg Multiple SclErosis previously treated with hiGhly-Active Disease modifying thErapies (RENEGADE study)

Clara Grazia Chisari[1,2] · Salvatore Lo Fermo[2] · Alessia Di Sapio[3] · Maria Pia Amato[4,5] · Giuseppe Salemi[6] · Ilaria Pesci[7] · Erica Curti[8] · Diana Ferraro[9] · Alessandra Lugaresi[10,11] · Luca Massacesi[12] · Matilde Inglese[13,14] · Paola Gazzola[15] · Sabrina Realmuto[16] · Cristina Fioretti[17] · Umberto Aguglia[18,19] · Sara Montepietra[20] · Massimo Filippi[21,22,23,24,25] · Francesco Patti[1,2] on behalf of the Italian Multiple Sclerosis and Related Disorders Register Group

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Abstract

Background Highly effective disease-modifying therapies (HEDMTs) for relapsing multiple sclerosis (RMS) have changed the landscape of MS treatment. However, their discontinuation may potentially result in recrudescence of the disease activity. We aimed to investigate the effectiveness of Rituximab (RTX) in RMS patients who discontinued HEDMTs because of efficacy or safety reasons.

Methods This observational study analyzed data of RMS patients treated with RTX who discontinued natalizumab [NTZ]; fingolimod [FTY], alemtuzumab [ALM], cladribine [CLD], cyclophosphamide [CYC], and mitoxantrone [MIT]), followed by the MS centers contributing to the Italian Multiple Sclerosis and Related Disorders Register. Disability progression (progression independent from relapse activity [PIRA] and from MRI activity [PIRMA]) and disease activity (annualized relapse rate [ARR], relapse-associated worsening [RAW]) were compared at pre-baseline (last evaluation during HEDMTs), at baseline (at the time of RTX initiation; ± 3 months), at 12 ± 3 (T12), and at 24 ± 3 months (T24) after RTX initiation.

Results Out of 68,621 RMS patients, 599 were treated with RTX. Of them, 362 (119 [67.6%] females, mean age of 44.2 ± 11.6 years) were finally enrolled. A total of 176 (48.6%) patients were previously treated with NTZ, 160 (44.2%) with FTY, 11 (3%) with ALM, 10 (2.8%) with CLD and 5 (1.4%) with CYC-MIT. After RTX initiation, in the NTZ, and CYC-MIT groups, disability outcomes remained stable over the time. In the FTY, ALM and CLD groups, RAW, PIRA, and PIRMA significantly reduced after 2 years of treatment with RTX. Cox analysis showed that higher EDSS before starting HEDMTs and at pre-baseline were associated to higher risk of PIRA, while factors predicting higher risk of PIRMA were older age at RTX initiation and EDSS before starting HEDMTs.

Discussion RTX potentially represents a rescue therapy for those patients requiring the discontinuation of highly active drugs and more vulnerable to relapse or disease progression.

Keywords Relapsing multiple sclerosis · Rituximab · High-efficacy disease-modifying therapies · Disability progression

Introduction

Multiple sclerosis (MS) is an inflammatory and neurodegenerative demyelinating disease of the central nervous system (CNS) causing neurological deficits referable to damage to the spinal cord, brainstem, optic nerves, cerebellum, and cerebrum [1].

The armamentarium of disease-modifying treatments (DMTs) for relapsing–remitting MS (RRMS) has considerably increased over the past 20 years. Older DMTs, such as interferon beta 1a (IFN beta1a) and glatiramer acetate (GA), can reduce relapse rates by about 30% and have an uncertain long-term benefit [2]. Active MS management requires the use of high-efficacy DMTs (HEDMTs), such as fingolimod (FTY), natalizumab (NTZ), cladribine (CLD), or alemtuzumab (ALM), in order to reduce relapse activity, disability accrual and irreversible brain atrophy [3–10].

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However, for some patients exhibiting persistent disease activity despite highly active immunosuppressive DMTs, there is the need to switch to another DMTs [11, 12]. Moreover, some patients may discontinue HEDMTs due to shared decisions regarding safety concerns (i.e., progressive multifocal leukoencephalopathy [PML] risk), pregnancy, and poor tolerability [13]. In addition, the suspension may increase the risk of MS relapses and MRI activity. For instance, a consistent return of underlying pre-treatment disease activity after 4–7 months following NTZ discontinuation was demonstrated [3–7]. A disease rebound, defined as a marked clinical and radiological worsening or as a disease activity well above the level of disease activity before starting NTZ, was reported in 10–30% of patients who suspended NTZ [14–18]. Regarding FTY, several reports highlighted a possible risk of disease exacerbation or rebound after FTY cessation, which could potentially result in debilitating disease progression with the reasons for FTY withdrawal have varied among reports, ranging from pregnancy, adverse events, and minimal efficacy to switching to other DMTs [8–10].

Despite the robust data about the efficacy, some patients treated with ALM may experience clinical and/or MRI activity after completing the treatment course. In this case, treatment options are limited, and few data exist on the possible subsequent use of HEDMTs with different mechanisms of action, i.e., B cell-depleting agents [19–21].

More recently, CLD, a purine nucleoside analog, was approved in tablet form to be administered in two annual treatment courses, each divided into two treatment cycles comprising 4–5 days of treatment [22]. Long-term extension studies provided evidence that oral CLD has a prolonged impact on disease activity [23]. However, available evidence about the suitable sequencing of DMTs in case of disease activity occurring after year 2 of oral CLD therapy is limited.

Thus, in patients who discontinued HEDMTs for several reasons, evidence about the appropriate therapeutic strategies is limited.

During the last 10 years, several studies have highlighted the efficacy of anti-CD20 monoclonal antibodies on inflammatory activity of MS [24]. Particularly, numerous reports have demonstrated that rituximab (RTX) induces a dramatic reduction of inflammatory activity of RRMS [25, 26]. The OLYMPUS study showed that RTX was effective in primary progressive MS (PPMS) with MRI activity [27]. Unlike ocrelizumab and ofatumumab, RTX is a chimeric monoclonal antibody targeting CD20, off-label for MS in several countries. While ocrelizumab and ofatumumab are approved as first- or second-line therapies for RMS, RTX is typically considered third-line due to regulatory status and limited formal trials in MS despite compelling real-world efficacy data [28].

In Italy, RTX is currently approved as off-label therapy for MS and treatment algorithms for relapsing–remitting MS include RTX as a third-line therapy primarily due to its off-label status, absence of Phase III MS-specific approval trials, and reimbursement limitations in several countries [29].

However, little information is currently available concerning the efficacy and safety of RTX as escape strategy in patients who need to discontinue HEDMTs because of efficacy and/or safety reasons in a real-world setting.

In this view, we aimed to evaluate the effectiveness of RTX in RMS patients who discontinued HEDMTs (NTZ; FTY, ALM, CLD, cyclophosphamide [CYC] and mitoxantrone [MIT]) because of efficacy or safety reasons.

Methods

Study design and participants

This retrospective observational multicenter study analyzed data prospectively collected about the effectiveness of RTX in RMS patients who discontinued HEDMTs (NTZ; FTY, ALM, CLD, CYC and MIT) because of efficacy and/or safety reasons in the period between January 2012 and November 2022 and who are followed by the MS centers contributing to the Italian Multiple Sclerosis and Related Disorders Register.

Inclusion criteria were the following: age > 18 years at disease onset; diagnosis of RMS in accordance with the revised McDonald criteria (2010) [30]; previous treatment with highly active DMTs (NTZ, FTY, ALM, CLD, CYC and MIT) continuously administered for at least 1 year and interrupted before starting RTX; at least one course of RTX of 2000 mg (1000 mg at day 1 and at day 14), at least 12 months of follow-up from treatment with RTX (for further details, see "RTX dosing regimen"); availability of clinical (relapse and EDSS evaluations), and MRI data (baseline and at least one follow-up visit), to ensure adequate longitudinal clinical and radiological information for analysis.

We also excluded patients with: diagnosis of primary progressive MS and neuromyelitis optica, previous treatment with RTX for other concomitant medical conditions, and lack of follow-up data.

Ethical statement

The Italian Multiple Sclerosis and Related Disorders Register was approved by the ethical committee at the "Azienda Ospedaliero-Universitaria-Policlinico of Bari" (Study REGISTRO SM001—approved on 08/07/2016) and by the local ethics committees in all participating centers where patients signed an informed consent that allows to use clinical data for research purposes.

Definition of outcomes and variables

In order to enhance the robustness of our results, RTX was considered active from the day of first infusion up to 12 months after the last administration.

Duration of RTX treatment was calculated from the date of the first infusion of RTX and the last clinical evaluation. For patients who discontinued RTX, the treatment duration was considered up to 12 months after the last infusion of RTX in the absence of any other treatment.

Reasons to discontinuation of the previous highly active DMT were categorized as following: PML risk, other safety issues (including adverse events, pregnancy etc.), and efficacy (clinical relapse and/or progression and/or MRI activity).

To evaluate disability progression, EDSS assessments were compared at pre-baseline (last EDSS during the prior highly effective therapy, within 6 months from its discontinuation), at baseline (at the time of RTX initiation; ± 3 months), at 12 ± 3 months (T12), and at 24 ± 3 months (T24) after RTX initiation.

Confirmed disability worsening (CDW) was defined as an increase of ≥ 1.0 point in EDSS from baseline (or ≥ 0.5 points for baseline EDSS ≥ 5.5), confirmed after at least 6 months.

Relapse-associated worsening (RAW) was defined as an EDSS increase (≥ 1.5 points if the previous EDSS was 0, ≥ 1.0 point if < 5.5, or ≥ 0.5 points if ≥ 5.5) occurring within 90 days of a documented relapse and persisting for at least 6 months [31]. To minimize the influence of transient relapse effects, EDSS assessments used for CDW confirmation were required to be performed at least 30 days after a relapse event.

Progression independent of relapse activity (PIRA) was defined as confirmed disability worsening (sustained for ≥ 6 months) not associated with a relapse in the 90 days preceding the EDSS increase. Disability worsening was defined as a ≥ 1.0-point increase from an EDSS baseline score ≤ 5.0 or a ≥ 0.5-point increase from a baseline ≥ 5.5, confirmed after 6 months in the absence of relapse [31, 32].

Progression independent of relapse and MRI activity (PIRMA) was defined as confirmed disability worsening (sustained for ≥ 6 months) occurring without clinical relapse or MRI activity within the same 90-day period. MRI activity was assessed by comparing the most recent MRI obtained within 90 days before the EDSS worsening (reference scan) with the subsequent follow-up scan, and was defined as the appearance of at least one new or enlarging T2 lesion or one gadolinium-enhancing lesion [31, 33]. Time to first PIRA and PIRMA events was also evaluated.

Relapse was considered as the occurrence of neurological signs persisting more than 24 h, in the absence of fever or infections, and confirmed by a neurologist on clinical examination. Dates of each relapse during treatment with the prior highly effective DMT and after RTX initiation were collected. The annualized relapse rates (ARRs) were collected at baseline, at T12 and T24.

All the available data about MRI exams performed before and after RTX were collected. The presence of brain and/or spinal cord contrast-enhanced lesions (CELs) and the number of new brain and/or spinal cord T2 lesions compared to the previous MRI were evaluated. Particularly, we collected MRI data during previous treatment with highly active DMTs (pre-baseline), before RTX initiation (baseline), at T12 and T24. According to the reasons of discontinuation, clinical and MRI data were compared between patients who switched to RTX for efficacy reasons (relapse and/or MRI activity) or for safety concerns (PML risk, adverse events, poor tolerability, or pregnancy).

All adverse events (AEs) after RTX initiation mentioned in the medical records were registered and graded according to the Common Terminology Criteria for Adverse Events (CTCAE) v4.0. The following AEs were recorded: deaths, malignancies, autoimmune disorders, allergic reactions, infusion-related reactions, and infections. Uncomplicated lower urinary tract infections and uncomplicated upper respiratory tract infections were not considered.

RTX dosing regimens

Considering that dosing regimen of RTX may vary among each MS centers, the infusion's protocols were stratified as follows:—1000 mg 2 weeks apart every 6 months;—1000 mg 2 weeks apart repeated after CD19 repopulation;—1000 mg 2 weeks apart followed by 375 mg/m^2 every 6 months.

Statistical analysis

Statistical analysis was performed using the STATA 16.1 software. Analyses were based on available data; no imputation was performed for missing values. Missingness was low for EDSS (< 5%) and moderate for MRI data (~ 22% at T24).

Shapiro–Wilk test was applied to assess the normality of the distribution. Continuous variables were summarized by the number of observations, mean, and standard deviation (SD), while categorical data were presented as absolute and relative frequencies (*n* and %) or in contingency tables. If assumptions for parametric tests (*t* test or ANOVA) were violated, equivalent non-parametric methods were used. Specifically, for non-normally distributed data, Kruskal–Wallis test was applied for multiple-group comparisons and Mann–Whitney *U* test for pairwise analyses. Given that annualized relapse rate (ARR) represents count data, relapse counts were also modeled using Poisson regression;

when overdispersion was detected, a negative binomial model was applied.

Analysis of variance (ANOVA) was used to examine changes across time points (pre-baseline, baseline, T12, and T24), with Bonferroni correction for multiple post hoc pairwise comparisons. When normality assumptions were not met, the corresponding non-parametric tests were applied instead of ANOVA.

Spearman correlation coefficient (Rho) was calculated to assess the strength of correlations between the analyzed variables.

A multivariable Cox regression analysis was performed to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for associations between disease characteristics at pre-baseline and baseline with outcomes over a follow-up period of up to 2 years. Three models were developed for each outcome, considering different clinical predictors: (1) time to first relapse, (2) time to first MRI activity (contrast-enhanced lesions [CELs] or new/enlarged T2 lesions), and (3) time to first PIRA event. The full models were adjusted for baseline demographic and clinical characteristics, including age, sex, number of relapses at baseline and pre-baseline, EDSS at baseline and pre-baseline, previous HEDMT (NTZ, FTY, ALM, CLD, or CYC-MIT), RTX dosage protocol, RTX dosing interval, and reasons for discontinuation (lack of efficacy, safety concerns, or PML risk). Non-significant covariates were removed, and reduced models were fitted using only statistically significant variables. A p-value ≤ 0.05 was considered statistically significant.

Results

Out of 68,621 RMS patients identified in the Italian Multiple Sclerosis and Related Disorders Register at the time of data extraction, 502 were treated with RTX. Of them, 362 (119 [67.6%] females, with a mean age of 44.2 ± 11.6 years, age at onset 27.7 ± 11.8 years, and age at diagnosis of 30.4 ± 11.6 years), met the inclusion/exclusion criteria and were finally enrolled (Fig. 1). A total of 176 (48.6%) patients were previously treated with NTZ, 160 (44.2%) with FTY, 11 (3%) with ALM, 10 (2.8%) with CLD and 5 (1.4%) with CYC-MIT. Given the small sample size, patients treated with CYC and MIT were combined into a single group (CYC-MIT).

Demographical and clinical data at pre-baseline (before starting RTX) of the study cohorts are summarized in Table 1.

Patients were treated with RTX IV every 6–12 months, with a mean interval of 9.9 ± 5.6 (range 6–18) months.

In the NTZ group, the mean number of drug infusions was 37.8 ± 24.6 (median 28, range 6–69), the mean wash-out period was 34.3 ± 12.2 days (median 32, range 28–48).

As for inclusion criteria, all patients previously treated with ALM and CLD have completed the 2-year treatment courses, with a mean wash-out period of 39.1 ± 10.8 and of 32.8 ± 16.4 months, respectively.

Patients treated with ALM were younger compared to CYC-MIT group and showed a higher number of relapses before starting HEDMTs compared to FTY, CLD and CYC-MIT. Moreover, at MS diagnosis, ALM group had a higher number of CELs compared with CLD and CYC-MIT and a higher number of new or enlarged T2-weighted lesions compared to patients treated with CYC-MIT (Table 1).

Overall, the main reason to suspend the HEDMT was PML risk (203 [40.3%]), followed by lack of efficacy (196 [39.0%]) and other safety issues (Table 1). Lack of efficacy was more frequently reported in the ALM and in the CLD groups than in other groups, while PML risk was predominant among patients treated with NTZ. Other safety reasons were more frequently recorded in the CYC-MIT group.

Over all, the first PIRA event occurred at a median (IQR) time of 1.8 (1.2–2.0) years, and 100 of patients (27.6%) developed PIRA within the first 2 years of the RTX initiation. In addition, the first PIRMA event occurred at a median (IQR) time of 2.2 (1.9–3.5) years, and 56 of patients (15.5%) developed PIRMA within the first 2 years of the

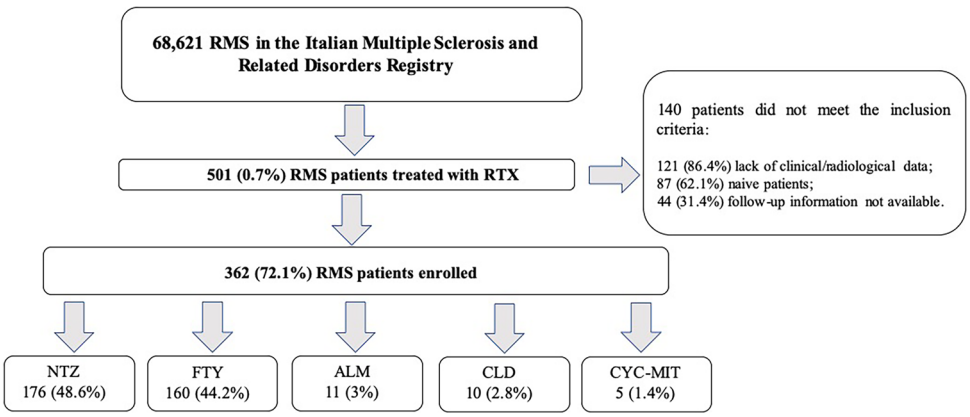

Fig. 1 Flowchart of the enrolled patients. *ALM* alemtuzumab, *CLD* cladribine, *CYC-MIT* cyclophosphamide/mitoxantrone, *FTY* fingolimod, *NTZ* natalizumab, *RMS* relapsing-multiple sclerosis, *RTX* rituximab

Table 1 Demographic and clinical characteristics of the study cohorts during treatment with HEDMTs

Tot. 362 *N* (%)	NTZ 176 (48.6) A	FTY 160 (44.2) B	ALM 11 (3) C	CLD 10 (2.8) D	CYC-MIT 5 (1.4) E	*p* value
Female; *N* (%)	119 (67.6)	98 (61.2)	7 (63.6)	8 (80)	3 (60)	D vs B, E $p<0.05$
Age (years); mean ± SD Median (range)	42.4 ± 10.6 38 (31–56)	44.8 ± 14.2 41 (32–58)	38.8 ± 9.7 36 (28–49)	40.5 ± 3.5 40 (38–43)	52.8 ± 10.5 47 (41–61)	E vs C $p<0.05$
Age at onset (years); mean ± SD Median (range)	34.1 ± 12.2 31 (25–46)	36.8 ± 13.9 32 (28–48)	31.1 ± 8.1 29 (24–41)	33 ± 2.8 34 (28–39)	37.1 ± 17.3 36 (30–50)	0.4
Disease duration (years); mean ± SD Median (range)	7.1 ± 4.3 6.4 (5–12)	7.6 ± 3.8 6.3 (4–12)	7.5 ± 3.7 6.1 (4–11)	8.5 ± 6.0 7.2 (5–18)	15.6 ± 7.8 12.3 (6–21)	E vs A, B C $p<0.05$
EDSS at diagnosis of MS; mean ± SD Median (range)	2.0 ± 1.1 1.5 (0.0–3.5)	2.3 ± 1.6 1.5 (0.0–3.5)	2.5 ± 2.1 2.5 (0.0–4.5)	2.0 ± 1.2 1.5 (0.0–3.0)	2.4 ± 1.9 2.0 (1.0–4.0)	0.2
EDSS before starting HEDMT; mean ± SD Median (range)	2.2 ± 1.6 2.0 (0.0–4.5)	2.5 ± 1.8 2.0 (1.0–4.5)	3.1 ± 2.2 3.0 (1.0–5.5)	3.0 ± 1.9 2.5 (1.5–5.0)	2.8 ± 1.6 2.0 (1.5–4.5)	0.3
N. of relapses at diagnosis of MS; mean ± SD Median (range)	1.9 ± 1.5 1 (1–5)	1.5 ± 1.3 1 (1–4)	1.7 ± 1.8 1 (1–5)	1.5 ± 1.3 1 (1–3)	1.6 ± 1.5 1 (1–3)	0.7
N. of relapses before starting HEDMT; mean ± SD Median (range)	1.9 ± 1.1 1 (1–5)	1.3 ± 1.1 1 (1–5)	2.4 ± 1.8 2 (1–7)	1.3 ± 1.1 1 (1–3)	1.6 ± 1.5 1 (1–3)	C vs B, D, E $p<0.05$
N. of CELs before starting HEDMT; mean ± SD Median (range)	1.4 ± 0.8 1 (0–9)	0.8 ± 0.6 0 (0–4)	1.8 ± 0.9 2 (0–12)	0.5 ± 0.7 0 (0–5)	0.3 ± 0.6 0 (0–4)	C vs D, E $p<0.05$
N. of new or enlarged T2-weighted lesions before starting HEDMT; mean ± SD Median (range)	0.9 ± 0.3 0 (0–6)	0.7 ± 0.6 0 (0–6)	1.2 ± 0.4 1 (0–11)	0.8 ± 0.5 0 (0–7)	0.4 ± 0.2 0 (0–4)	C vs E $p<0.05$
Reason of discontinuation; *N* (%)						
Lack of efficacy	31 (17.6)	92 (57.5)	11 (100)	10 (100)	2 (40)	A vs C, D $p<0.05$
PML risk	144 (81.8)	0	0	0	0	NA
Other safety issues	1 (0.6)	68 (42.5)	0	0	3 (60)	A vs B, E $p<0.05$

ALM alemtuzumab, *CELs* contrast-enhanced lesions, *CLD* cladribine, *CYC-MIT* cyclophosphamide-mitoxantrone, *HEDMT* high-efficacy disease-modifying therapies, *EDSS* Expanded Disability Status Scale, *FTY* fingolimod, *PML* progressive multifocal leukoencephalopathy, *NA* not applicable, *NTZ* natalizumab, *SD* standard deviation

RTX initiation. No differences in terms of time to first PIRA and PIRMA events were found among patients treated with different DMTs.

In the NTZ group, after RTX initiation, EDSS, number of relapses, ARR, RAW, PIRA, and PIRMA remained stable over the time. Similarly, radiological outcomes showed no significantly differences among each time point (Table 2).

Among patients treated with FTY, a substantial decline in the patients reporting PIRA ($p<0.01$), PIRMA ($p<0.01$), in the ARR ($p<0.001$), and in the number of patients with MRI activity ($p=0.02$) was observed between baseline and T24, with a downward trend also found in the number of CELs, in the number of new/enlarged T2 lesions and in the number of patients with MRI activity, even if not statistically significant (Table 3).

In the ALM and in the CLD groups, ARR, and RAW significantly reduced after 2 years of treatment with RTX ($p<0.01$), with a marked decrease in the percentage of patients with MRI activity ($p<0.01$) (Tables 4 and 5).

No changes in the MS activity and disability outcomes were found in patients previously treated with CYC-MIT over 2 years of follow-up (Table 6).

Across a total follow-up of 640.3 patient-years, 36 adverse events (AEs) were documented in 18 patients, corresponding to an incidence rate of 5.6 events per 100 patient-years and 2.8 affected patients per 100 patient-years.

No fatalities or malignancies were recorded. Only 2 AEs have CTCAE grade 3, one hypogammaglobulinemia and one respiratory tract infection. A total of 29 (80.6%) AEs were considered mild. Infusion reactions were the most frequent AEs (28 [77.8%] of 36), followed by infections (16 [44.4%]), and hematological (8 [22.2%]). The most frequent infection AE involved the urinary tract (22 [78.6%] of 28), followed by respiratory (5 [17.8%]), and cutaneous (1 [3.6%]). No cases of PML were recorded. Among the hematological AEs, most of them were hypogammaglobulinemia (6 [75%] of 8) and 2 (25%) were neutropenia. No differences in terms

Table 2 Outcome measures in NTZ-treated patients

N (% of 362)	NTZ 176 (48.6)			
	Pre-baseline	Baseline	T12	T24
EDSS; mean ± SD Median (range)	2.6 ± 1.8 2.0 (1.5–5)	2.5 ± 1.7 2.0 (1.5–5)	2.6 ± 1.5 2.0 (1.5–5.5)	2.7 ± 1.5 2.0 (2.0–5.5)
N. of relapses; mean ± SD Median (range)	0.4 ± 0.1 0 (0–3)	0.4 ± 0.2 0 (0–3)	0	0.4 ± 0.2 0 (0–4)
ARR; mean ± SD	–	0.08 ± 0.1[a]	0	0.2 ± 0.1
RAW; *N* (%)	–	7 (4)[a]	8 (4.5)	8 (4.5)
PIRA; *N* (%)	–	6 (3.4)[a]	7 (4)	6 (3.4)
PIRMA; *N* (%)	–	4 (2.3)	3 (1.7)	5 (2.8)
N. of CELs; mean ± SD Median (range)	0.2 ± 0.1 0 (0–2)	0.2 ± 0.1 0 (0–2)	0.3 ± 0.1 0 (0–1)	0.3 ± 0.1 0 (0–1)
N. of new or enlarged T2-weighted lesions; mean ± SD Median (range)	0.4 ± 0.3 0 (0–5)	0.4 ± 0.3 0 (0–5)	0.5 ± 0.3 0 (0–3)	0.7 ± 0.3 0 (0–4)
N. of patients with MRI activity; *N* (%)	6 (3.4)	7 (4)	7 (4)	6 (3.4)

Pre-baseline: last evaluation during highly active therapies; within 6 months from the discontinuation, baseline: at the time of RTX initiation; ± 3 months; T12: 12 ± 3 months after RTX initiation; T24: 24 ± 3 months after RTX initiation

ARR annualized relapse rate, *CELs* contrast-enhanced lesions, *EDSS* Expanded Disability Status Scale, *MRI* magnetic resonance imaging, *NTZ* natalizumab, *PIRA* progression independent from relapse activity, *PIRMA* progression independent from relapse activity and MRI activity, *RAW* relapse-associated worsening, *SD* standard deviation

[a]These evaluations were performed considering the last year before starting RTX

*$p < 0.05$, assessed by ANOVA with Bonferroni correction or Mann–Whitney U test for non-parametric data

of frequency and severity were observed among each group. The details of the AEs are presented in Table 7.

In an exploratory correlation analysis, no significant association was found between the length of the washout period and relapse occurrence after RTX initiation (Spearman's $\rho = 0.08$, $p = 0.37$).

Cox analysis showed that higher EDSS before starting HEDMTs and at pre-baseline were associated to higher risk of PIRA (HR 1.54, 25% CI 1.06–5.36, $p = 0.03$ and HR 1.41, 25% CI 1.03–5.64, $p = 0.04$, respectively). Similarly, factors predicting higher risk of PIRMA were older age at RTX initiation and EDSS before starting HEDMTs (HR 1.59, 25% CI 1.14–7.88, $p = 0.03$ and HR 1.19, 25% CI 1.03–4.65, $p = 0.04$, respectively). Finally, younger age and longer disease duration were associated with higher relapse risk (HR 2.02, 25% CI 1.65–4.82; $p = 0.008$ and HR 1.87, 25% CI 1.41–4.09; $p = 0.01$, respectively).

Results from the multivariable Cox proportional hazards model were presented in Fig. 2A–C.

Discussion

The main finding of our study is that RTX offers comparable clinical stability to NTZ and superior outcomes to FTY in RMS patients switching from HEDMTs. The findings indicate that RTX, through its B cell depletion mechanism, offers significant clinical benefits in terms of controlling disease progression and reducing relapse rates in a population characterized by treatment resistance and disease activity.

Overall, our data suggest that RTX's mechanism of action extends beyond merely preventing relapses, also contributing to the stabilization of disability progression, as measured by PIRA. This finding is consistent with previous studies showing that relapsing MS patients treated with RTX had a lower risk of confirmed disability progression compared with those treated with other DMTs or untreated cohorts [34–36]. Nonetheless, in our cohort RTX resulted in a reduction of disease activity, such as fewer relapses and less MRI-detected inflammation. However, its impact on disability progression was generally milder. This may be due to the complex mechanisms underlying PIRA which involve chronic and progressive neurodegenerative processes, such as mitochondrial dysfunction, iron accumulation, and

Table 3 Outcome measures in FTY-treated patients

	FTY 160 (44.2)			
N (% of 362)	Pre-baseline (A)	Baseline (B)	T12 (C)	T24 (D)
EDSS; mean ± SD Median (range)	2.8 ± 1.8 2.5 (1.5–5.5)	2.6 ± 1.7 2.0 (1.5–5.5)	3.0 ± 1.8 2.5 (2.0–5.5)	3.0 ± 2.1 2.5 (2.0–5.5)
N. of relapses; mean ± SD Median (range)	1.1 ± 0.6 1 (0–5)	1.2 ± 0.9 1 (0–5)	0.6 ± 0.3 0 (0–3)	0.8 ± 0.2 0 (0–4)
ARR; mean ± SD	–	0.4 ± 0.1[a,*]	0.3 ± 0.2	0.2 ± 0.1*
RAW; *N* (%)	–	11 (6.9)[a]	10 (6.3)	7 (4.4)
PIRA; *N* (%)	–	25 (15.6)[a,*]	20 (12.3)	10 (6.4)*
PIRMA; *N* (%)	–	20 (12.3)*	12 (7.5)	6 (3.8)*
N. of CELs; mean ± SD Median (range)	0.6 ± 0.3 0 (0–4)	0.8 ± 0.5 1 (0–8)	0.5 ± 0.1 0 (0–4)	0.3 ± 0.2 0 (0–3)
N. of new or enlarged T2-weighted lesions; mean ± SD Median (range)	0.8 ± 0.2 1 (0–9)	0.7 ± 0.4 0 (0–6)	0.5 ± 0.4 0 (0–3)	0.4 ± 0.3 0 (0–3)
N. of patients with MRI activity; *N* (%)	21 (13.1)	26 (16.3)*	16 (10)	11 (6.9)*

Pre-baseline: last evaluation during highly active therapies; within 6 months from the discontinuation, baseline: at the time of RTX initiation; ± 3 months; T12: 12 ± 3 months after RTX initiation; T24: 24 ± 3 months after RTX initiation

ARR annualized relapse rate, *CELs* contrast-enhanced lesions, *EDSS* Expanded Disability Status Scale, *FTY* fingolimod, *MRI* magnetic resonance imaging, *PIRA* progression independent from relapse activity, *PIRMA* progression independent from relapse activity and MRI activity, *RAW* relapse-associated worsening, *SD* standard deviation

[a]These evaluations were performed considering the last year before starting RTX

*$p < 0.05$, assessed by ANOVA with Bonferroni correction or Mann–Whitney U test for non-parametric data

chronic oxidative stress, which are not specifically targeted by anti-inflammatory treatments [37].

According to our results, RTX was able to reduce the risk of the fearsome rebound after NTZ discontinuation, allowing for stabilization in terms of relapse activity and disability progression. In line with these findings, a recent retrospective study evaluating a cohort of 100 RRMS patients (50 cases in each group) treated with NTZ and FTY who were switched to RTX, found a significant reduction in clinical relapse and disability progression in both groups after six months of follow-up, with no modifications in the pattern of MRI activity in NTZ-treated patients [38]. Another analysis of patients who switched from NTZ to anti-CD20 therapies demonstrated a significant reduction in ARR. In particular, similar to our study, in the subgroup of rituximab ($n = 23$), after a mean treatment duration of 48.57 months, nearly three-fourths (73.9%) of patients showed no disability progression [39].

As demonstrated in a previous analysis and confirmed in the present study, a significantly higher percentage of MS patients discontinued NTZ because of PML risk rather than to lack of efficacy [13]. Taking into account that NTZ discontinuation has been associated with rebound/return of disease activity [40, 41], several exit strategies were investigated in order to mitigate the risk of disease activity. A multicenter Swedish study of 256 RMS patients who discontinued NTZ because of JCV antibody positivity, demonstrated that RTX was markedly superior to FTY in keeping clinical and MRI activity stable over a period of 18 months [42]. In addition, global post-marketing safety and clinical trial data demonstrated that the occurrence of PML is very rare among RTX-treated patients [43].

Nevertheless, RTX was able to reduce clinical and MRI disease activity in active RMS patients even though previously treated with immunosuppressive DMT [44]. An observational French retrospective study identified 351 off-label RTX-treated patients through a cohort of 15,984 MS patients from the French Observatory of Multiple Sclerosis (OFSEP) register, who exhibited disease activity prior to starting RTX, despite ongoing immunosuppressive therapies (FTY, NTZ, or MIT). The authors found a significantly decrease in ARR from 0.8 during last immunosuppressive drug to 0.18 after RTX, with a marked reduction of percentage of CELs (from 72% during last DMT, to 8% on the first MRI performed after RTX) [44].

Furthermore, several studies have highlighted the importance of minimizing wash-outs after NTZ when switching to other HEDMTs. As demonstrated by other studies, the

Table 4 Outcome measures in ALM-treated patients

***N* (% of 362)**	**ALM 11 (3)**			
	Pre-baseline (A)	Baseline (B)	T12 (C)	T24 (D)
EDSS mean ± SD Median (range)	3.3 ± 2.4 2.5 (2.0–6.5)	3.3 ± 2.6 2.5 (2.0–6.5)	3.5 ± 2.1 2.5 (2.0–7.0)	3.3 ± 2.8 2.5 (2.0–7.0)
N. of relapses; mean ± SD Median (range)	1.5 ± 1.1 1 (0–6)	1.8 ± 1.0* 1 (0–8)	0.6 ± 0.5 0 (0–5)	0.7 ± 0.2* 0 (0–4)
ARR; mean ± SD	–	1.2 ± 1.1[a,*]	0.7 ± 0.3	0.5 ± 0.2*
RAW; *N* (%)	–	5 (45.4)[a,*]	3 (27.3)	2 (18.2)*
PIRA; *N* (%)	–	4 (36.4)[a]	3 (27.3)	3 (27.3)
PIRMA; *N* (%)	–	3 (27.3)	2 (18.2)	3 (27.3)
N. of CELs; mean ± SD Median (range)	0.9 ± 0.4 0 (0–6)	1.0 ± 0.1 1 (0–11)	0.5 ± 0.4 0 (0–6)	0.4 ± 0.1 0 (0–4)
N. of new or enlarged T2-weighted lesions; mean ± SD Median (range)	0.6 ± 0.4 1 (0–7)	0.9 ± 0.4 1 (0–8)	0.5 ± 0.4 0 (0–3)	0.5 ± 0.3 0 (0–4)
N. of patients with MRI activity; *N* (%)	4 (36.4)	6 (54.4)*	5 (45.4)	4 (36.4)*

Pre-baseline: last evaluation during highly active therapies; within 6 months from the discontinuation, baseline: at the time of RTX initiation; ± 3 months; T12: 12 ± 3 months after RTX initiation; T24: 24 ± 3 months after RTX initiation

ALM alemtuzumab, *ARR* annualized relapse rate, *CELs* contrast-enhanced lesions, *EDSS* Expanded Disability Status Scale, *MRI* magnetic resonance imaging, *PIRA* progression independent from relapse activity, *PIRMA* progression independent from relapse activity and MRI activity, *RAW* relapse-associated worsening, *SD* standard deviation

[a]These evaluations were performed considering the last year before starting RTX

*$p < 0.05$, assessed by ANOVA with Bonferroni correction or Mann–Whitney U test for non-parametric data

relatively short wash-out period may have contributed to reduced risk of disease reactivation even in our cohort [45, 46]. In our cohort, the median washout period after NTX discontinuation was approximately 34 days, which may have contributed to minimizing rebound risk. In an exploratory analysis, washout duration did not significantly correlate with relapse occurrence after RTX initiation, suggesting that the favorable outcomes were not solely driven by the short sequencing interval.

Several reports highlighted a possible risk of disease exacerbation or rebound after FTY cessation, which could potentially result in debilitating disease progression with the reasons for FTY withdrawal have varied among reports, ranging from pregnancy, adverse events, and minimal efficacy to switching to other DMDs [8–10]. RTX's efficacy in patients pretreated with FTY could be attributed to its unique mechanism of action, which differs fundamentally from that of other HEDMTs [25]. Notably, the failure of FTY in some patients may be related to the persistence of B cell-driven pathology despite these therapies [47, 48]. Accordingly, a recent analysis of 73 patients, 33 had switched from FTY to CLD and 40 to RTX, showed lower risk of rebound after switching to RTX compared to CLD [49].

RTX's ability to deplete B cells likely addresses this pathological component more directly, offering a mechanistic explanation for its efficacy in patients who have not responded to other HEDMTs, such as CLD and other immunosuppressants [50].

As previously observed in other studies, RTX demonstrated a favorable tolerance profile, with most side effects being mild. No deaths or cases of PML were recorded. Especially when compared to other potentially effective treatments for active RMS, such as MIT and ALM, RTX shows a promising safety profile [44]. Additionally, extensive data on the safety of RTX are available from its use in other diseases over several years [51]. However, the overall number of reported infections was lower than that observed in controlled prospective studies, in which severe infections requiring hospital care have been reported at rates of approximately 20 per 1000 patient-years. This difference likely reflects the retrospective, registry-based data collection, which underestimates minor or outpatient-managed infections.

In accordance with the current literature, our multivariate analysis confirmed that younger patients with longer disease duration were associated with higher relapse risk and

Table 5 Outcome measures in CLD-treated patients

N (% of 362)	CLD 10 (2.8)			
	Pre-baseline (A)	Baseline (B)	T12 (D)	T24
EDSS mean ± SD Median (range)	2.2 ± 1.4 1.0 (0.0–4.5)	2.1 ± 1.6 1.0 (0.0–4.5)	2.2 ± 1.6 1.0 (0.0–4.5)	2.2 ± 1.8 1.0 (0.0–5.0)
N. of relapses; mean ± SD Median (range)	1.9 ± 1.3 1 (0–6)	1.8 ± 1.1* 1 (0–8)	0.6 ± 0.3 0 (0–5)	0.4 ± 0.1* 0 (0–3)
ARR; mean ± SD	–	1.6 ± 1.0[a,*]	0.8 ± 0.3	0.6 ± 0.2*
RAW; *N* (%)	–	4 (40)[a]	3 (30)	2 (20)
PIRA; *N* (%)	–	2 (20)[a]	2 (20)	2 (20)
PIRMA; *N* (%)	–	0	2 (20)	1 (10)
N. of CELs; mean ± SD Median (range)	0.6 ± 0.4 0 (0–3)	0.7 ± 0.3 0 (0–5)	0.6 ± 0.3 0 (0–5)	0.4 ± 0.1 0 (0–4)
N. of new or enlarged T2-weighted lesions; mean ± SD Median (range)	0.8 ± 0.5 1 (0–7)	0.8 ± 0.5 1 (0–8)	0.6 ± 0.4 0 (0–5)	0.4 ± 0.3 0 (0–4)
N. of patients with MRI activity; *N* (%)	6 (60)	6 (60)*	0	0

Pre-baseline: last evaluation during highly active therapies; within 6 months from the discontinuation, baseline: at the time of RTX initiation; ± 3 months; T12: 12 ± 3 months after RTX initiation; T24: 24 ± 3 months after RTX initiation

ARR annualized relapse rate, *CELs* contrast-enhanced lesions, *CLD* cladribine, *EDSS* Expanded Disability Status Scale, *MRI* magnetic resonance imaging, *PIRA* progression independent from relapse activity, *PIRMA* progression independent from relapse activity and MRI activity, *RAW* relapse-associated worsening, *SD* standard deviation

[a]These evaluations were performed considering the last year before starting RTX

*$p < 0.05$, assessed by ANOVA with Bonferroni correction or Mann–Whitney U test for non-parametric data

Table 6 Outcome measures in CYC-MIT-treated patients

N (% of 362)	CYC-MIT 5 (1.4)			
	Pre-baseline	Baseline	T12	T24
EDSS mean ± SD Median (range)	3.4 ± 2.6 3 (1.5–6.5)	3.5 ± 2.7 2.5 (1.5–6.5)	4.0 ± 1.9 2.5 (2.0–6.5)	3.5 ± 2.0 2.5 (1.0–7.5)
N. of relapses; mean ± SD Median (range)	1.5 ± 1.1 1 (0–5)	1.4 ± 1.0 1 (0–6)	1.2 ± 0.5 1 (0–5)	0.7 ± 0.3 0 (0–5)
ARR; mean ± SD	–	1.2 ± 0.8[a]	0.9 ± 0.4	1.0 ± 0.4
RAW; *N* (%)	–	4 (80)[a]	3 (60)	2 (40)
PIRA; *N* (%)	–	3 (60)[a]	2 (40)	2 (40)
PIRMA; *N* (%)	–	2 (40)	2 (40)	1 (20)
N. of CELs; mean ± SD Median (range)	0.5 ± 0.2 0 (0–3)	0.4 ± 0.3 0 (0–3)	0.5 ± 0.1 0 (0–4)	0.4 ± 0.2 0 (0–4)
N. of new or enlarged T2-weighted lesions; mean ± SD Median (range)	0.5 ± 0.2 0 (0–5)	0.6 ± 0.3 0 (0–6)	0.5 ± 0.4 0 (0–5)	0.4 ± 0.2 0 (0–4)
N. of patients with MRI activity; *N* (%)	2 (40)	2 (40)	0	0

ARR annualized relapse rate, *CELs* contrast-enhanced lesions, *CYC-MIT* cyclophosphamide-mitoxantrone, *EDSS* Expanded Disability Status Scale, *MRI* magnetic resonance imaging, *PIRA* progression independent from relapse activity, *PIRMA* progression independent from relapse activity and MRI activity, *RAW* relapse-associated worsening, *SD* standard deviation

[a]These evaluations were performed considering the last year before starting RTX

*$p < 0.05$, assessed by ANOVA with Bonferroni correction or Mann–Whitney U test for non-parametric data

Table 7 Frequency and severity of adverse events (AEs)

Adverse events	Total N. 36
Death	0
PML	0
Severity of adverse effect	
Grade 1	29
Grade 2	5
Grade 3	2
Type of adverse effect	
Infectious	16
Hematological	8
Neoplastic	0
Reactions to infusion	28
Pregnancy	0

PML progressive multifocal leukoencephalopathy

of MRI activity [52]. On the other hand, higher EDSS before starting HEDMTs and at baseline were associated to higher risk of disability accrual, in terms of PIRA, while factors predicting higher risk of PIRMA were older age at RTX initiation and EDSS before starting HEDMTs. According to these findings, the higher baseline disability may reflect a greater degree of irreversible damage, which predisposes them to further progression despite treatment. As patients age, they experience more pronounced neurodegenerative changes, which contribute to overall disease progression. Older patients are likely to have accumulated more neurodegenerative damage by the time they start RTX, which makes it harder to mitigate disability progression despite reducing inflammatory activity. Furthermore, older patients may have a diminished capacity for remyelination and neuroplasticity, leading to a higher risk of worsening disability after relapses [52, 53].

Several limitations should be acknowledged. First, the retrospective nature of the analysis and the lack of a control group limit the ability to draw definitive causal inferences. Variability in MRI evaluations and washout intervals may have affected the precision of outcome measurements. Indeed, because MRI scans were not systematically performed at fixed 12-month intervals, ascertainment of PIRMA events may have been incomplete, potentially underestimating subclinical activity and inflating stability rates. Notably, only patients with both MRI and EDSS data available at two or more time points were included, some missing values occurred at later visits, reflecting real-world follow-up variability. Despite these limitations, the use of standardized data collection within the Italian MS Register strengthens the internal consistency of the findings; however, these observations remain hypothesis-generating and warrant confirmation in prospective studies. Second, variability in the dosing regimen of RTX re-treatment among Italian MS centers may have reduced the generalizability of our results. However, in our study the mean interval dosing was roughly 10 months, in line with recent findings suggesting that anti-CD20 dose interval extension over 12 months could be considered in patients with RMS with stable disease [54]. Third, most patients switching from NTZ to RTX did so for safety reasons (mainly PML risk) and had relatively stable disease and short washout periods, whereas those switching from CLD or ALM generally showed disease reactivation or progression. Although we stratified analyses and adjusted for previous DMT and reason for discontinuation, residual confounding cannot be completely excluded.

This study focused on patients who had discontinued HEDMTs, so the generalizability of these results to other MS populations, such as those naïve to high-efficacy therapies, remains to be determined. Future research should explore the potential role of RTX earlier in the treatment algorithm and identify biomarkers predicting response to B cell depletion, particularly in light of emerging evidence suggesting that early and aggressive treatment can lead to better long-term outcomes in MS [12, 55]. Finally, adverse event reporting may have been influenced by the registry-based nature of data collection, which prioritizes clinically relevant events and may underrepresent mild or transient reactions. Although AE incidence rates were provided per 100 patient-years to improve comparability, the lack of systematic recording of mild events (e.g., transient infusion reactions, mild or outpatient-managed infections) remains a limitation.

One of the key considerations in selecting RTX is its off-label status for the treatment of MS in several countries. Nevertheless, RTX is now considered an accepted off-label alternative, supported by accumulating real-world evidence and clinical trials. Notably, a recent phase 3 randomized trial comparing RTX with dimethyl fumarate in predominantly newly diagnosed RRMS patients demonstrated superior efficacy of RTX in preventing relapses and MRI activity, further reinforcing its therapeutic value in early disease stages [56].

A recent nationwide study using data from Swedish registers investigating the accrued real-world costs for MS DMTs, demonstrated that RTX is much more cost-effective than MS-approved alternatives [57, 58]. The ongoing accumulation of data supporting RTX's efficacy and safety

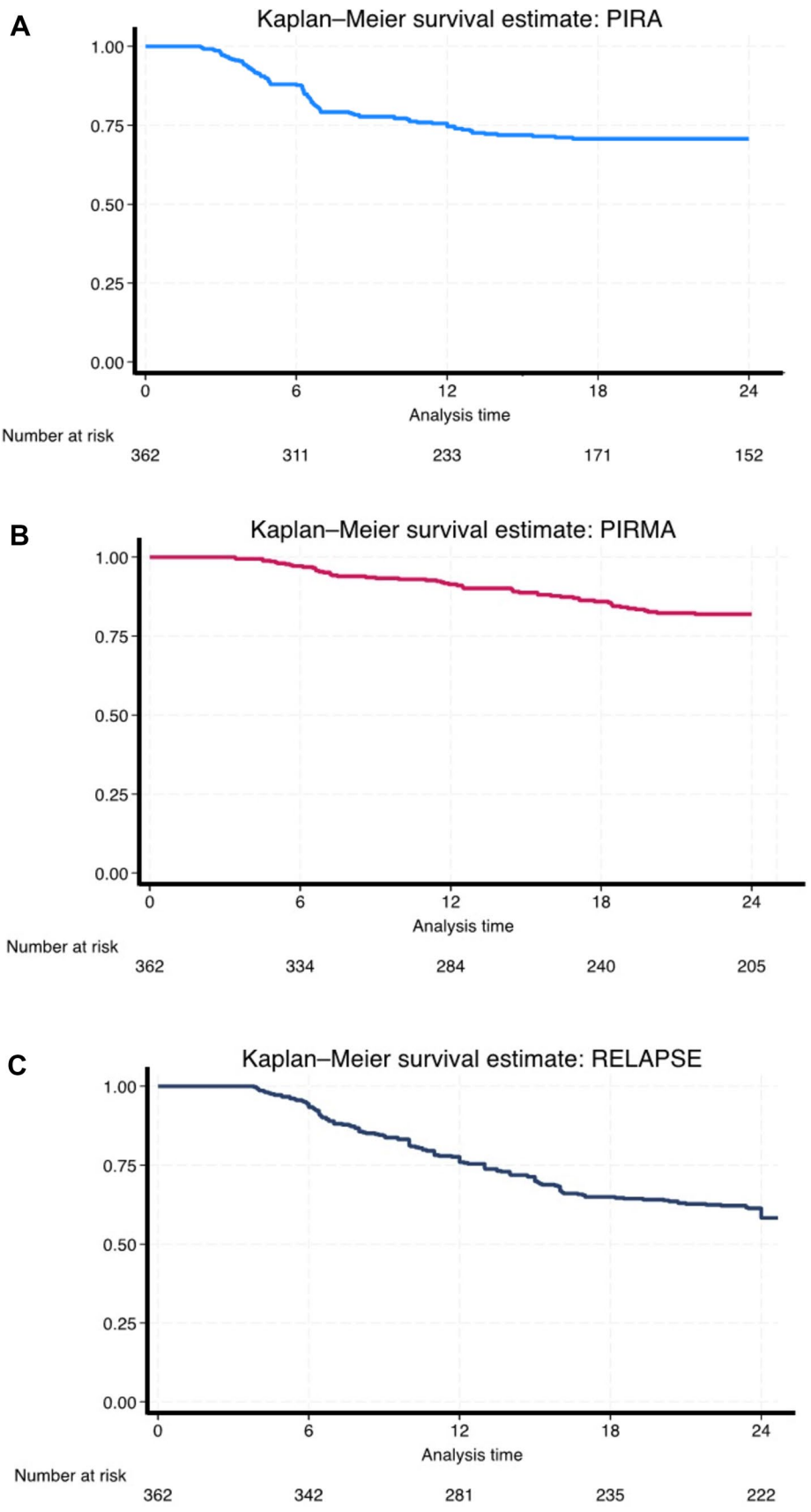

Fig. 2 Cox regression analyses of risk factors for PIRA (**A**), PIRMA (**B**), and first relapse (**C**) during RTX treatment. *PIRA* progression independent from relapse activity, *PIRMA* progression independent from MRI activity, *RTX* rituximab

may eventually influence regulatory and clinical guidelines, potentially leading to more widespread adoption and official approval for use in MS [29].

In conclusion, our findings have important implications for clinical practice. They suggest that RTX represents a viable and effective therapeutic option for patients with MS who have discontinued highly effective DMTs. The significant reduction in relapse rates and stabilization of disability observed in this challenging patient population highlights RTX's potential as a key therapeutic option. As the treatment landscape for MS continues to evolve, RTX offers a promising alternative for patients with refractory disease, warranting further investigation and consideration in clinical practice.

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Author contributions Clara Chisari and Francesco Patti contributed to the study concept and design, to analysis and interpretation of the data and drafted the manuscript. Salvatore Lo Fermo, Alessia Di Sapio, Maria Pia Amato, Giuseppe Salemi, Ilaria Pesci, Erica Curti, Diana Ferraro, Alessandra Lugaresi, Luca Massacesi, Matilde Inglese, Paola Gazzola, Sabrina Realmuto, Cristina Fioretti, Umberto Aguglia, Sara Montepietra, and Massimo Filippi contributed to acquisition and interpretation of the data and approved the final manuscript.

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Availability of data and material Dataset is available under reasonable request to the corresponding author.

Declarations

Conflicts of interest Clara G. Chisari reported receiving grants to attend scientific congresses or speaker honoraria from Biogen, Merck-Serono, Novartis, Roche, Sanofi/Genzyme, Teva. Salvatore Lo Fermo reported no disclosures. Alessia Di Sapio reported no disclosures. Maria Pia Amato reported no disclosures. Giuseppe Salemi reported no disclosures. Ilaria Pesci reported no disclosures. Erica Curti reported no disclosures. Diana Ferraro reported no disclosures. Alessandra Lugaresi has received personal compensation from Alexion, Amgen/Horizon, Biogen, Bristol Myers Squibb/Celgene, Janssen/Johnson&Johnson, Merck Serono, Novartis, Roche, Sanofi/Genzyme and Fondazione Italiana Sclerosi Multipla (FISM). Her institutions received research grants from Novartis, Roche and Sanofi/Genzyme. Luca Massacesi reported no disclosures. Matilde Inglese reported no disclosures. Paola Gazzola reported no disclosures. Sabrina Realmuto reported no disclosures. Cristina Fioretti reported no disclosures. Umberto Aguglia reported no disclosures. Sara Montepietra reported no disclosures. Massimo Filippi received compensation for consulting services from Alexion, Almirall, Biogen, Merck, Novartis, Roche, Sanofi, speaking activities from Bayer, Biogen, Celgene, Chiesi Italia SpA, Eli Lilly, Genzyme, Janssen, Merck-Serono, Neopharmed Gentili, Novartis, Novo Nordisk, Roche, Sanofi, Takeda, and TEVA, participation in Advisory Boards for Alexion, Biogen, Bristol-Myers Squibb, Merck, Novartis, Roche, Sanofi, Sanofi-Aventis, Sanofi-Genzyme, Takeda, scientific direction of educational events for Biogen, Merck, Roche, Celgene, Bristol-Myers Squibb, Lilly, Novartis, Sanofi-Genzyme, he receives research support from Biogen Idec, Merck-Serono, Novartis, Roche, the Italian Ministry of Health, the Italian Ministry of University and Research, and Fondazione Italiana Sclerosi Multipla. Francesco Patti reported receiving honoraria for speaking activities by Almirall, Bayer Schering, Biogen Idec, Merck Serono, Novartis, Roche, Sanofi Genzyme, and TEVA; he also served as advisory board member the following companies: Bayer Schering, Biogen Idec, Merck Serono, Novartis, Roche, Sanofi Genzyme, and TEVA; he was also funded by Pfizer and FISM for epidemiological studies; he received grants for congress participation from Almirall, Bayer Shering, Biogen Idec, Merck Serono, Novartis, Roche, Sanofi Genzyme, and TEVA.

Ethical approval This study protocol was approved by the local Ethical Committee of the University of Catania (Catania 1, Italy) and by the Ethical Committee of the participating centers. The study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Informed consent Each patient participating to the study signed an Informed Consent specifically designed to participate to the study protocol.

References

1. Merkel B, Butzkueven H, Traboulsee AL, Havrdova E, Kalincik T (2017) Timing of high-efficacy therapy in relapsing-remitting multiple sclerosis: a systematic review. Autoimmun Rev 16(6):658–665
2. Kappos L, Bates D, Hartung HP, Havrdova E, Miller D, Polman CH et al (2007) Natalizumab treatment for multiple sclerosis: recommendations for patient selection and monitoring. Lancet Neurol 6(5):431–441
3. Fox RJ, Cree BA, De Seze J, Gold R, Hartung HP, Jeffery D et al (2014) MS disease activity in RESTORE: a randomized 24-week natalizumab treatment interruption study. Neurology 82(17):1491–1498
4. Rammohan KW, Ortega MR, Delgado SR, Tornes L (2011) Disease activity return during natalizumab treatment interruption in patients with multiple sclerosis. Neurology 77(21):1930 (**discussion -1**)
5. Berger JR, Centonze D, Comi G, Confavreux C, Cutter G, Giovannoni G et al (2010) Considerations on discontinuing natalizumab for the treatment of multiple sclerosis. Ann Neurol 68(3):409–411
6. Evdoshenko E, Stepanova A, Shumilina M, Davydovskaya M, Khachanova N, Neofidov N et al (2019) Real-world study of efficacy, risk management and reasons for discontinuation of natalizumab for treatment of multiple sclerosis in Russia. PLoS ONE 14(5):e0217303

7. Patti F, Leone C, Zappia M (2015) Clinical and radiologic rebound after discontinuation of natalizumab therapy in a highly active multiple sclerosis patient was not halted by dimethyl-fumarate: a case report. BMC Neurol 7(15):252
8. Sempere AP, Berenguer-Ruiz L, Feliu-Rey E (2013) Rebound of disease activity during pregnancy after withdrawal of fingolimod. Eur J Neurol 20(8):e109–e110
9. Havla JB, Pellkofer HL, Meinl I, Gerdes LA, Hohlfeld R, Kumpfel T (2012) Rebound of disease activity after withdrawal of fingolimod (FTY720) treatment. Arch Neurol 69(2):262–264
10. Beran RG, Hegazi Y, Schwartz RS, Cordato DJ (2013) Rebound exacerbation multiple sclerosis following cessation of oral treatment. Multiple Scler Relat Disord 2(3):252–255
11. Arrambide G, Iacobaeus E, Amato MP, Derfuss T, Vukusic S, Hemmer B et al (2020) Aggressive multiple sclerosis (2): treatment. Multiple Scler 12:1352458520924595
12. Simpson A, Mowry EM, Newsome SD (2021) Early aggressive treatment approaches for multiple sclerosis. Curr Treat Options Neurol 23(7):19
13. Chisari CG, Comi G, Filippi M, Paolicelli D, Iaffaldano P, Zaffaroni M et al (2022) PML risk is the main factor driving the choice of discontinuing natalizumab in a large multiple sclerosis population: results from an Italian multicenter retrospective study. J Neurol 269(2):933–944
14. Vellinga MM, Castelijns JA, Barkhof F, Uitdehaag BM, Polman CH (2008) Postwithdrawal rebound increase in T2 lesional activity in natalizumab-treated MS patients. Neurology 70(13 Pt 2):1150–1151
15. Miravalle A, Jensen R, Kinkel RP (2011) Immune reconstitution inflammatory syndrome in patients with multiple sclerosis following cessation of natalizumab therapy. Arch Neurol 68(2):186–191
16. Borriello G, Prosperini L, Marinelli F, Fubelli F, Pozzilli C (2011) Observations during an elective interruption of natalizumab treatment: a post-marketing study. Multiple Scler 17(3):372–375
17. Havla J, Gerdes LA, Meinl I, Krumbholz M, Faber H, Weber F et al (2011) De-escalation from natalizumab in multiple sclerosis: recurrence of disease activity despite switching to glatiramer acetate. J Neurol 258(9):1665–1669
18. West TW, Cree BA (2010) Natalizumab dosage suspension: are we helping or hurting? Ann Neurol 68(3):395–399
19. Hartung HP, Aktas O, Boyko AN (2015) Alemtuzumab: a new therapy for active relapsing-remitting multiple sclerosis. Multiple Scler 21(1):22–34
20. Brannigan J, Jones JL, Stacpoole SRL (2020) Severe paradoxical disease activation following alemtuzumab treatment for multiple sclerosis. Neurol Neuroimmunol Neuroinflamm. https://doi.org/10.1212/NXI.0000000000000799
21. Barclay K, Carruthers R, Traboulsee A, Bass AD, LaGanke C, Bertolotto A et al (2019) Best practices for long-term monitoring and follow-up of alemtuzumab-treated MS patients in real-world clinical settings. Front Neurol 10:253
22. Moser T, Ziemssen T, Sellner J (2022) Real-world evidence for cladribine tablets in multiple sclerosis: further insights into efficacy and safety. Wien Med Wochenschr 172:365–372
23. Giovannoni G, Comi G, Rammohan K, Rieckmann P, Dangond F, Keller B et al (2021) Long-term disease stability assessed by the expanded disability status scale in patients treated with cladribine tablets 3.5 mg/kg for relapsing multiple sclerosis: an exploratory post hoc analysis of the CLARITY and CLARITY extension studies. Adv Ther 38(9):4975–4985
24. Frisch ES, Pretzsch R, Weber MS (2021) A milestone in multiple sclerosis therapy: monoclonal antibodies against CD20-yet progress continues. Neurotherapeutics 18(3):1602–1622
25. Chisari CG, Sgarlata E, Arena S, Toscano S, Luca M, Patti F (2022) Rituximab for the treatment of multiple sclerosis: a review. J Neurol 269(1):159–183
26. Salzer J, Svenningsson R, Alping P, Novakova L, Bjorck A, Fink K et al (2016) Rituximab in multiple sclerosis: a retrospective observational study on safety and efficacy. Neurology 87(20):2074–2081
27. Hawker K, O'Connor P, Freedman MS, Calabresi PA, Antel J, Simon J et al (2009) Rituximab in patients with primary progressive multiple sclerosis: results of a randomized double-blind placebo-controlled multicenter trial. Ann Neurol 66(4):460–471
28. Oreja-Guevara C, Martinez-Yelamos S, Eichau S, Llaneza MA, Martin-Martinez J, Pena-Martinez J et al (2024) Beyond lines of treatment: embracing early high-efficacy disease-modifying treatments for multiple sclerosis management. Ther Adv Neurol Disord 17:17562864241284372
29. Brancati S, Gozzo L, Longo L, Vitale DC, Drago F (2021) Rituximab in multiple sclerosis: are we ready for regulatory approval? Front Immunol 12:661882
30. Thompson AJ, Banwell BL, Barkhof F, Carroll WM, Coetzee T, Comi G et al (2017) Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol 17:162–173
31. Kappos L, Wolinsky JS, Giovannoni G, Arnold DL, Wang Q, Bernasconi C et al (2020) Contribution of relapse-independent progression vs relapse-associated worsening to overall confirmed disability accumulation in typical relapsing multiple sclerosis in a pooled analysis of 2 randomized clinical trials. JAMA Neurol 77(9):1132–1140
32. Montobbio N, Cordioli C, Signori A, Bovis F, Capra R, Sormani MP (2024) Relapse-associated and relapse-independent contribution to overall expanded disability status scale progression in multiple sclerosis patients diagnosed in different eras. Ann Neurol 97:95–103
33. Ciccarelli O, Barkhof F, Calabrese M, De Stefano N, Eshaghi A, Filippi M et al (2024) Using the progression independent of relapse activity framework to unveil the pathobiological foundations of multiple sclerosis. Neurology 103(1):e209444
34. Torgauten HM, Myhr KM, Wergeland S, Bo L, Aarseth JH, Torkildsen O (2021) Safety and efficacy of rituximab as first- and second line treatment in multiple sclerosis—a cohort study. Multiple Scler J Exp Transl Clin 7(1):2055217320973049
35. Scotti B, Disanto G, Sacco R, Guigli M, Zecca C, Gobbi C (2018) Effectiveness and safety of Rituximab in multiple sclerosis: an observational study from Southern Switzerland. PLoS ONE 13(5):e0197415
36. Naegelin Y, Naegelin P, von Felten S, Lorscheider J, Sonder J, Uitdehaag BMJ et al (2019) Association of rituximab treatment with disability progression among patients with secondary progressive multiple sclerosis. JAMA Neurol 76(3):274–281
37. Haider L (2015) Inflammation, iron, energy failure, and oxidative stress in the pathogenesis of multiple sclerosis. Oxid Med Cell Longev 2015:725370
38. Fakih AU, Sahraian MA, Paybast S, Naser Moghadasi A (2023) Effectiveness and safety of switching from fingolimod and natalizumab to rituximab in patients with relapsing remitting multiple sclerosis. Multiple Scler Relat Disord 71:104564
39. Meglio M (2023) Anti-CD20 therapies shown to be effective after switch from natalizumab. In: ECTRIMS 2023.
40. Prosperini L, Kinkel RP, Miravalle AA, Iaffaldano P, Fantaccini S (2019) Post-natalizumab disease reactivation in multiple sclerosis: systematic review and meta-analysis. Ther Adv Neurol Disord 12:1756286419837809
41. Papeix C, Vukusic S, Casey R, Debard N, Stankoff B, Mrejen S et al (2016) Risk of relapse after natalizumab withdrawal: results from the French TYSEDMUS cohort. Neurol Neuroimmunol Neuroinflamm 3(6):e297
42. Alping P, Frisell T, Novakova L, Islam-Jakobsson P, Salzer J, Bjorck A et al (2016) Rituximab versus fingolimod after natalizumab in multiple sclerosis patients. Ann Neurol 79(6):950–958

43. Berger JR, Malik V, Lacey S, Brunetta P, Lehane PB (2018) Progressive multifocal leukoencephalopathy in rituximab-treated rheumatic diseases: a rare event. J Neurovirol 24(3):323–331
44. Durozard P, Maarouf A, Boutiere C, Ruet A, Brochet B, Vukusic S et al (2019) Efficacy of rituximab in refractory RRMS. Multiple Scler 25(6):828–836
45. Lo Re M, Capobianco M, Ragonese P, Realmuto S, Malucchi S, Berchialla P et al (2015) Natalizumab discontinuation and treatment strategies in patients with multiple sclerosis (MS): a retrospective study from two Italian MS centers. Neurol Ther 4(2):147–157
46. Naegelin Y, Rasenack M, Andelova M, Von Felten S, Fischer-Barnicol B, Amann M et al (2018) Shortening the washout to 4 weeks when switching from natalizumab to fingolimod and risk of disease reactivation in multiple sclerosis. Multiple Scler Relat Disord 25:14–20
47. Hausser-Kinzel S, Weber MS (2019) The role of B cells and antibodies in multiple sclerosis, neuromyelitis optica, and related disorders. Front Immunol 10:201
48. Comi G, Bar-Or A, Lassmann H, Uccelli A, Hartung HP, Montalban X et al (2021) Role of B cells in multiple sclerosis and related disorders. Ann Neurol 89(1):13–23
49. Nygaard GO, Torgauten H, Skattebol L, Hogestol EA, Sowa P, Myhr KM et al (2022) Risk of fingolimod rebound after switching to cladribine or rituximab in multiple sclerosis. Multiple Scler Relat Disord 62:103812
50. Claverie R, Perriguey M, Rico A, Boutiere C, Demortiere S, Durozard P et al (2023) Efficacy of rituximab outlasts B-cell repopulation in multiple sclerosis: time to rethink dosing? Neurol Neuroimmunol Neuroinflamm. https://doi.org/10.1212/NXI.0000000000200152
51. van Vollenhoven RF, Emery P, Bingham CO Jr, Keystone EC, Fleischmann RM, Furst DE et al (2013) Long-term safety of rituximab in rheumatoid arthritis: 95-year follow-up of the global clinical trial programme with a focus on adverse events of interest in RA patients. Ann Rheum Dis 72(9):1496–1502
52. Zhang Y, Cofield S, Cutter G, Krieger S, Wolinsky JS, Lublin F (2022) Predictors of disease activity and worsening in relapsing-remitting multiple sclerosis. Neurol Clin Pract 12(4):e58–e65
53. Andorra M, Freire A, Zubizarreta I, de Rosbo NK, Bos SD, Rinas M et al (2024) Predicting disease severity in multiple sclerosis using multimodal data and machine learning. J Neurol 271(3):1133–1149
54. Starvaggi Cucuzza C, Longinetti E, Ruffin N, Evertsson B, Kockum I, Jagodic M et al (2023) Sustained low relapse rate with highly variable B-cell repopulation dynamics with extended rituximab dosing intervals in multiple sclerosis. Neurol Neuroimmunol Neuroinflamm. https://doi.org/10.1212/NXI.0000000000200056
55. Selmaj K, Cree BAC, Barnett M, Thompson A, Hartung HP (2024) Multiple sclerosis: time for early treatment with high-efficacy drugs. J Neurol 271(1):105–115
56. Svenningsson A, Frisell T, Burman J, Salzer J, Fink K, Hallberg S et al (2022) Safety and efficacy of rituximab versus dimethyl fumarate in patients with relapsing-remitting multiple sclerosis or clinically isolated syndrome in Sweden: a rater-blinded, phase 3, randomised controlled trial. Lancet Neurol 21:693–703. https://doi.org/10.1016/S1474-4422(22)00209-5
57. Alping P, Neovius M, Piehl F, Frisell T (2024) Real-world healthcare cost savings and reduced relapse rate with off-label rituximab versus disease-modifying treatments approved for relapsing-remitting multiple sclerosis: a nationwide cost-effectiveness study. Ann Neurol 95(6):1099–1111
58. Roos I, Hughes S, McDonnell G, Malpas CB, Sharmin S, Boz C et al (2023) Rituximab vs ocrelizumab in relapsing-remitting multiple sclerosis. JAMA Neurol 80(8):789–797

Authors and Affiliations

Clara Grazia Chisari[1,2] · Salvatore Lo Fermo[2] · Alessia Di Sapio[3] · Maria Pia Amato[4,5] · Giuseppe Salemi[6] · Ilaria Pesci[7] · Erica Curti[8] · Diana Ferraro[9] · Alessandra Lugaresi[10,11] · Luca Massacesi[12] · Matilde Inglese[13,14] · Paola Gazzola[15] · Sabrina Realmuto[16] · Cristina Fioretti[17] · Umberto Aguglia[18,19] · Sara Montepietra[20] · Massimo Filippi[21,22,23,24,25] · Francesco Patti[1,2] on behalf of the Italian Multiple Sclerosis and Related Disorders Register Group

✉ Francesco Patti
patti@unict.it

[1] Department of Medical and Surgical Sciences and Advanced Technologies "GF Ingrassia", University of Catania, Catania, Italy

[2] UOS Multiple Sclerosis, Neurology Clinic, "G. Rodolico-San Marco" University Hospital, Catania, Italy

[3] Department of Neurology, Regional Referral Multiple Sclerosis Center, University Hospital San Luigi Gonzaga, Orbassano, Turin, Italy

[4] Department NEUROFARBA, Section of Neurosciences, University of Florence, Florence, Italy

[5] IRCCS Fondazione Don Carlo Gnocchi, Florence, Italy

[6] Department of Biomedicine, Neuroscience and Advanced Diagnostics, University of Palermo, Palermo, Italy

[7] Centro Sclerosi Multipla Unità Operativa Neurologia, Azienda Unità Sanitaria Locale, Ospedale Di Vaio, Fidenza, Parma, Italy

[8] Department of Medicine and Surgery, University of Parma, Parma, Italy

[9] Department of Neuroscience, Azienda Ospedaliera Universitaria, Modena, Italy

[10] IRCCS Istituto delle Scienze Neurologiche di Bologna, Bologna, Italy

[11] Dipartimento di Scienze Biomediche e Neuromotorie, Università di Bologna, Bologna, Italy

[12] Department of Neurosciences Drugs and Child Health, University of Florence, Florence, Italy

[13] Dipartimento Di Neuroscienze, Riabilitazione, Oftalmologia, Genetica E Scienze Materno-Infantili (DINOGMI), Universita' Di Genova, Genoa, Liguria, Italy

[14] IRCCS Ospedale Policlinico San Martino, Genoa, Italy

[15] Neurology Unit, P.A. Micone Hospital, ASL3 Genovese, Genoa, Italy

[16] Multiple Sclerosis Centre, Neurology Unit and Stroke Unit, AOOR "Villa Sofia-Cervello", Palermo, Italy

[17] Multiple Sclerosis Center of Toscana, Leghorn, Italy

[18] Department of Medical and Surgical Sciences, "Magna Graecia" University, Catanzaro, Italy

[19] Regional Epilepsy Centre, Great Metropolitan Hospital, Reggio Calabria, Italy

[20] Neurology Unit, Neuromotor and Rehabilitation Department, AUSL-IRCCS of Reggio Emilia, Reggio Emilia, Italy

[21] Neuroimaging Research Unit, Division of Neuroscience, IRCCS San Raffaele Scientific Institute, Milan, Italy

[22] Neurology Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy

[23] Neurorehabilitation Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy

[24] Vita-Salute San Raffaele University, Milan, Italy

[25] Neurophysiology Service, IRCCS San Raffaele Scientific Institute, Milan, Italy