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Treatment of multiple sclerosis with rituximab: A multicentric Italian–Swiss experience

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Abstract

Background: Rituximab, an anti-CD20 monoclonal antibody leading to B lymphocyte depletion, is increasingly used as an off-label treatment option for multiple sclerosis (MS).

Objective: To investigate the effectiveness and safety of rituximab in relapsing–remitting (RR) and progressive MS.

Methods: This is a multicenter, retrospective study on consecutive MS patients treated off-label with rituximab in 22 Italian and 1 Swiss MS centers. Relapse rate, time to first relapse, Expanded Disability Status Scale (EDSS) progression, incidence of adverse events, and radiological outcomes from 2009 to 2019 were analyzed.

Results: A total of 355/451 enrolled subjects had at least one follow-up visit and were included in the outcome analysis. Annualized relapse rate significantly decreases after rituximab initiation versus the pre-rituximab start year in RRMS (from 0.86 to 0.09, $p < .0001$) and in secondary-progressive (SP) MS (from 0.34 to 0.06, $p < .0001$) and had a slight decrease in primary-progressive (PP) MS patients (from 0.12 to 0.07, $p = 0.45$). After 3 years from rituximab start, the proportion of patients with a confirmed EDSS progression was 14.6% in the RRMS group, 24.7% in the SPMS group, and 41.5% in the PPMS group. No major safety concerns arose.

Conclusion: Consistently with other observational studies, our data show effectiveness of rituximab in reducing disease activity in patients with MS.

Keywords: Rituximab, multiple sclerosis, relapsing–remitting, primary progressive, secondary progressive, real life

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Introduction

Multiple sclerosis (MS) is a chronic immune-mediated inflammatory disease affecting the central nervous system.[1] Several lines of evidence support the relevance of B lymphocytes in the pathogenesis of MS.[2] Rituximab (RTX), the first anti-CD20 monoclonal antibody marketed since 1998, is currently approved for B-cell malignancies, rheumatoid arthritis, and some vasculitis.[3,4] This chimeric antibody achieves circulating B-cell depletion mainly by complement-dependent cytotoxicity and only to a lesser extent by antibody-dependent cell-mediated cytotoxicity.[2] After successful phase II clinical testing for relapsing–remitting MS (RRMS)[5] and phase II/III trial for primary-progressive MS (PPMS),[6] the manufacturer stopped further RTX development for MS and promoted the humanized, anti-CD20

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antibody ocrelizumab for this indication, recently added to the MS drugs armamentarium.

Ocrelizumab efficacy and safety have been indeed assessed in three randomized trials, showing superiority over interferon beta 1-a in the treatment of active RRMS[7,8] and an effect in reducing disability progression in PPMS.[9]

Albeit the ocrelizumab safety profile seems overall reassuring, a signal of increased breast cancer risk during treatment was reported.[7,8] In addition, opportunistic infections were seen in the ocrelizumab rheumatoid arthritis development program.[10] Notably, the costs of a treatment with ocrelizumab are considerable.

In the meantime, RTX has been extensively used as an *off-label* therapy in MS[11–13]and several other neuroimmunological conditions with encouraging results.[3,4] Despite indicating that RTX is generally well tolerated and safe even in the long term,[3] data concerning its use in MS are scarce.

Therefore, we aimed to report on the effectiveness and safety of RTX in the treatment of a large population of Italian and Swiss MS patients.

Materials and methods

Sample population

We designed a multicenter, retrospective study based on prospectively collected data, involving 1 Swiss and 22 Italian MS centers. The ethics committee of the coordinating center (Genova) approved the study. Raw data collection and data exchange were approved by the local ethics committees at all centers. Written permission for the use of anonymized personal clinical data for research purposes and written informed consent were obtained from all study participants. Data were coded before being accessed and centrally analyzed.

Consecutive patients with a diagnosis of MS based on McDonald Criteria[14] who started treatment with RTX from January 2009 until May 2019 were enrolled.

Induction and maintenance treatment regimen

RTX infusion regimens were classified according to the induction and maintenance protocols applied at participating centers.

The induction regimens were grouped as follows:

(a) Two 375 mg/m^2 infusions 15 days apart.
(b) Two 1000 mg infusions 15 days apart.
(c) Four 375 mg/m^2 infusions every week for 4 weeks.

The maintenance regimens were grouped as follows:

(a) Fixed time-point (6 months) re-infusions of 1000 mg 15 days apart.
(b) Cytofluorimetric-based reinfusion regimens (re-infusions based on CD19+ or CD27+ cells reappearance).[15]

Re-infusions based on CD19+ cells reappearance was defined as CD19+ cells exceeding the 1% of peripheral blood mononuclear cells, while re-infusions based on CD27+ memory B (CD19+) cells re-emergence when this population exceeded 0.05% of peripheral blood mononuclear cells in the first 2 years and 0.1% in the following years.[15]

Patients who did not follow one of these induction/maintenance regimens were excluded from the follow-up analysis.

Data collection and monitoring

Demographic and clinical data were collected at baseline (the date of RTX start) and every 6 months thereafter. A common database template with predefined criteria for data categorization was filled in with data derived from source data of local databases and patient's charts. All files were merged and further processed for data cleaning and analysis. Treatment duration was defined as the interval between baseline and the last available neurological follow-up.

Neurological examination with neurological disability assessed by the Expanded Disability Status Scale (EDSS) was performed at baseline and every 6 ± 2 months thereafter according to clinical practice. Evaluations performed outside this timeframe were excluded from the analyses. The treating neurologists at the participating centers are certified for EDSS assessment (https://www-neurostatus-net.eoc.netbib.ch/). Visit reports referring to an EDSS score served as data source.

Brain magnetic resonance imaging (MRI) including T2 weighted as well as Gadolinium (GD+)-enhanced T1-weighted sequences were performed at least once a year under RTX treatment. Scans performed from 3 months after RTX initiation and within 2 months before baseline, and those performed after 12 ± 2 months

were considered for the analysis. Images were reviewed by the treating neurologist and at least by one neuroradiologist for new T2 lesions and GD+ lesions.

Outcome measures

The annualized relapse rate (ARR), the time to first relapse (TTFR), and the time to EDSS progression were the clinical outcome measures. MRI activity expressed as presence of new T2 (as compared to baseline) and/or GD+ lesions at brain MRI performed at 1 year from baseline according to local clinical practice was the radiological outcome measure.

A relapse was defined as a monophasic clinical episode characterized by acute or subacute, newly developing neurological symptoms or reactivation of pre-existing neurological deficits for a minimum of 24 hours, with or without recovery, in the absence of fever or infection, occurring at least 30 days after the preceding episode. The occurrence of fatigue, mental symptoms, and/or vegetative symptoms without any additional signs were not classified as a relapse. Relapses were counted from RTX initiation to 24 months, or to the last available follow-up, or the date of treatment switch/drop out, whichever came first.

EDSS was assessed 6 months before RTX initiation, at the time of RTX start, and repeated every 6 months. Disability progression was defined as an increase from baseline of at least 1.5 EDSS points if baseline EDSS score was 0, 1.0 points if baseline EDSS score was between 0.5 and 5.0, or 0.5 points if baseline EDSS score was $\geqslant$5.5, sustained at 6 months. Disability progression was evaluated in patients that had at least one-year follow-up EDSS assessment (to allow progression confirmation).

Safety

All the infusion-related reactions (IRRs), defined as any adverse events (AEs) occurring during RTX in-hospital infusions and AEs, defined as any untoward medical occurrence during RTX treatment, even without a causal relationship with the treatment, were reported.

According to clinical practice, at participating centers, nurses responsible for patient's monitoring prospectively recorded any infusion reactions related to RTX on a paper form. In case of new emerging symptoms, an experienced neurologist was called to evaluate the patient and to record any adverse event. Paper forms were reviewed to ascertain IRRs.

During the 6-month follow-up visits, treating neurologists actively asked for non-infusion-related AEs that may have emerged since the previous evaluation. Any such reports were recorded in the respective visit report. Visit reports were reviewed to ascertain IRRs.

AEs severity was categorized as mild (grade 1), moderate (grade 2), severe (grade 3), or serious (grades 4 and 5).[16]

Statistical analysis

ARR pre and post RTX start were compared by a mixed-effect negative binomial model accounting for the repeated measures analysis. Negative binomial and Cox regression analyses were used to assess the influence of the demographic, clinical, and treatment regimen characteristics on the clinical outcome measures during follow-up.

The baseline variables collected were age, sex, disease duration since onset, presence of any comorbidities and/or autoimmune comorbidities, EDSS (assessed 6 months prior to the RTX start and at the time of RTX start), number of relapses in the previous 2 years, presence of active lesions on the brain or spinal cord MRI in the year prior to RTX induction, and previous MS treatments.

Only factors significantly associated with the outcomes at univariate analysis (with a p value $\leqslant$0.05) were included in a multivariate model with a stepwise procedure (p for inclusion $<$0.05).

SAS 9.3 (Institute Inc., Cary, NC, USA) and R software (version 3.5.0) were used for the computation.

Results

Patients' demographics and clinical characteristics

Four hundred and fifty-one (95.6%) of 472 included patients completed the induction regimen and 355/451 (78.7%) had at least one follow-up visit after the induction period. Of these, 83 (18.4%) patients from the Center of Lugano were already reported in the publication by Scotti et al.,[13] and of this subgroup, 21 patients were further reported in the case series by Naegelin et al.[17]

The follow-up of these patients has been updated for the present analysis.

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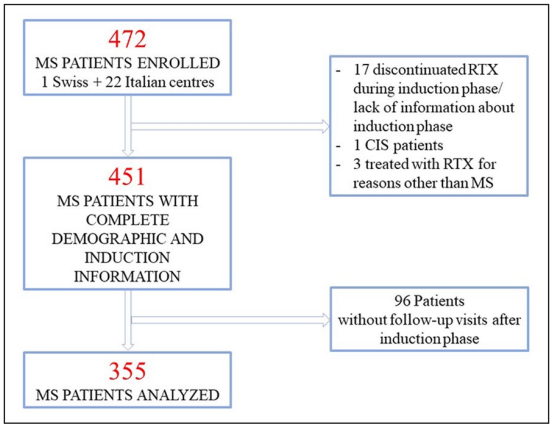

Figure 1. Flow chart of MS patients enrolled in the study and retained in the final analysis.

Three patients discontinued RTX treatment during the induction phase; two were for patients' decision and one was due to an IRR (Figure 1).

Demographic and clinical characteristics as well as induction/maintenance regimens of the cohort with at least one follow-up visit ($N=355$) are reported in Table 1 (the characteristics of the whole cohort ($N=451$) are reported in the Supplementary Appendix Table 1).

Of the 355 patients starting RTX and with at least one follow-up visit, 188 (53.0%) were RRMS and 167 (47.0%) were progressive (43 PPMS and 124 SPMS). Only 37 (10.4%) patients were treatment-naïve, while the remaining 318 (89.6%) switched from other disease-modifying therapies (DMTs): natalizumab (22.6%) and fingolimod (23.3%) were the most commonly used drugs, followed by interferons (13.8%), dimethyl fumarate (9.1%), and other therapies such as mithoxantrone and azathioprine (10.4%). Reasons for RTX initiation were mainly poor efficacy (55.0%), lack of tolerance or AE (16.7%) related to previous MS therapy, and John Cunningham virus (JCV)-positive status (11.3%) in patients treated with natalizumab.

The median follow-up time was 1.9 years (range: 0.5–8.8 years) and the median time between infusions was 7.1 months (range: 2.6–27.3 months); patients received a median (range) of 2 (0–16) RTX infusions during the follow-up.

Forty-four patients (12.4%) switched to another therapy during follow-up. The median (range) time to switch was 1.8 (0.5–8.8) years: 20 patients switched to ocrelizumab, 4 patients switched to dimethyl fumarate, 4 switched to mithoxantrone, 3 patients underwent hematopoietic stem cell transplantation, 2 switched to natalizumab, and 2 to fingolimod while for the remaining 9 patients other treatment options were chosen (including interferon beta-1, glatiramer acetate, and methylprednisolone).

Treatment regimens

Ninety-two percent of patients ($n=417$) followed an induction regimen based on two infusions of 375 mg/m^2 ($n=25$) or 1000 mg 15 days apart ($n=392$), while only 27 patients were infused with 375 mg/m^2 in four weekly infusions.

Out of the 355 patients with follow-up, 221 (62.3%) received a fixed time-point maintenance regimen, while 132 (37.2%) received a cytofluorimetric-based reinfusion regimen. Of the latter group, 34 (25.8%) were re-infused according to the CD19+, and 98 (74.2%) to the CD27+ lymphocytes repopulation. Of note, all the patients following the maintenance regimen based on CD27+ repopulation were enrolled only in two centers (Ospedale Policlinico San Martino and Ospedale Micone, Genoa, Italy).

Seven patients (2.0%) did not follow any of these induction regimens and were infused with a single 1000 mg dose infusion. Two patients (0.6%) did not follow the maintenance regimens previously described and were excluded from the analysis.

Relapse activity

The ARR in the 2 years and in the year before RTX start was 0.67 (95% confidence interval (CI): 0.59–0.76) and 0.86 (95% CI: 0.73–0.99) in the RRMS group, 0.25 (95% CI: 0.20–0.32) and 0.34 (95% CI: 0.25–0.45) in the SPMS group, and 0.12 (95% CI: 0.06–0.20) and 0.12 (95% CI: 0.04–0.25) in the PPMS group. The ARR in the 2 years after the RTX start decreased to 0.09 (95% CI: 0.07–0.13) in the RRMS group, to 0.06 (95% CI: 0.04–0.10) in the SPMS group, and to 0.07 in the PPMS patients (95% CI: 0.03–0.13; Figure 2). The decrease versus the year before RTX start was significant in the RRMS group and in the SPMS group ($p<.0001$) and did not reach significance in the PPMS group ($p=0.45$).

Factors associated with the ARR and TTFR at univariate analysis for the RRMS cohort are reported in Table 2. A higher number of relapses in the year preceding the first RTX induction (hazard ratio (HR) = 1.37 (95% CI: 1.08–1.74), $p=0.011$) was associated with a shorter TTFR over a 2-year period

Table 1. Baseline characteristics and rituximab treatment data for 355 enrolled MS patients with at least one visit of follow-up treated with rituximab at 22 Italian and 1 Swiss centers by MS subtype.

Characteristics	All patients (*N*=355)	Relapsing–remitting (*N*=188)	Primary progressive (*N*=43)	Secondary progressive (*N*=124)
Age at MS onset, years, mean (SD)	29.9 (11.0)	28.3 (10.2)	41.4 (10.4)	28.5 (10.0)
Gender (female), *n* (%)	241 (67.9)	134 (71.3)	27 (62.8)	80 (64.5)
MS duration, years, mean (SD)	12.9 (9.3)	10.7 (8.4)	6.6 (4.7)	18.4 (9.0)
Comorbidity, *n* (%)	146 (41.2)	71 (38.0)	17 (39.5)	58 (46.8)
Autoimmune comorbidity, *n* (%)	47 (13.3)	35 (18.7)	2 (4.7)	10 (8.1)
EDSS 6 months pre-RTX, median (range)	4.5 (0.0–8.5)	2.5 (0.0–8.5)	5.0 (1.5–7.5)	6 (1.5–8.5)
EDSS at RTX start, median (range)	4.5 (0.0–8.5)	3 (0.0–8.5)	5.5 (1.5–7.5)	6 (2.0–8.5)
No. relapse 2-year pre-RTX, median (range)	1 (0–10)	1 (0–10)	0 (0–3)	0 (0–4)
No. relapse 1 year pre-RTX, median (range)	0 (0–7)	1 (0–7)	0 (0–2)	0 (0–5)
Presence of active MRI 1 year pre-RTX, *n* (%)	159/286 (55.6)	100/144 (69.4)	17/34 (50.0)	42/108 (38.9)
Presence of spinal lesions 1 year pre-RTX, *n* (%)	110/256 (43.0)	70/123 (56.9)	11/27 (40.7)	29/106 (27.4)
Naïve patients, *n* (%)	37 (10.4)	19 (10.1)	14 (32.6)	4 (3.2)
Previous treatments, median (range)	2 (0–7)	3 (0–7)	1 (0–4)	3 (0–7)
First-line previous treatment, median (range)	1 (0–6)	2 (0–6)	0 (0–3)	2 (0–5)
Second-line previous treatment, median (range)	1 (0–4)	1 (0–3)	0 (0–2)	1 (0–4)
Last DMT before RTX, *n* (%)	*N*=318	*N*=169	*N*=29	*N*=120
Alemtuzumab	3 (0.9)	1 (0.6)	0 (0.0)	2 (1.7)
Cyclophosphamide	16 (5.0)	6 (3.6)	3 (10.3)	7 (5.8)
Daclizumab	1 (0.3)	1 (0.6)	0 (0.0)	0 (0.0)
Dimethyl fumarate	29 (9.1)	16 (9.5)	3 (10.3)	10 (8.3)
Fingolimod	74 (23.3)	34 (20.1)	8 (27.6)	32 (26.7)
Glatiramer acetate	28 (8.8)	15 (8.9)	1 (3.5)	12 (10.0)
Interferons	44 (13.8)	19 (11.2)	3 (10.3)	22 (18.3)
Natalizumab	72 (22.6)	57 (33.7)	3 (10.3)	12 (10.0)
HSCT	3 (0.9)	2 (1.2)	0 (0.0)	1 (0.8)
Teriflunomide	15 (4.7)	8 (4.7)	1 (3.5)	6 (5.0)
Other	33 (10.4)	10 (5.9)	7 (24.1)	16 (13.3)
Reasons for changing to RTX, *n* (%)	*N*=318	*N*=169	*N*=29	*N*=120
Inefficacy	175 (55.0)	79 (46.8)	18 (62.1)	78 (65.0)
Intolerance/adverse event	53 (16.7)	24 (14.2)	9 (31.0)	20 (16.7)
JCV+	36 (11.3)	27 (16.0)	1 (3.5)	8 (6.7)
Pregnancy	8 (2.5)	7 (4.1)	1 (3.5)	0 (0.0)
Other	46 (14.5)	32 (18.9)	0 (0.0)	14 (11.7)
Induction regimen, *n* (%)				
Two 375 mg/m^2 infusions 15 days apart	19 (5.4)	13 (6.9)	2 (4.7)	4 (3.2)
Two 1000 mg infusions 15 days apart	315 (88.7)	159 (84.6)	39 (90.7)	117 (94.4)
Four 375 mg/m^2 infusions every week for 4 weeks	14 (3.9)	12 (6.4)	0 (0.0)	2 (1.6)
1000 mg infusion every 6 months (no induction)	7 (2.0)	4 (2.1)	2 (4.7)	1 (0.8)
Maintenance regimen, *n* (%)				
Fixed time points (6 months) infusions of 1000 mg	221 (62.2)	127 (67.5)	15 (34.9)	79 (63.7)
Cytofluorimetric-based reinfusion regimens	132 (37.2)	60 (31.9)	28 (65.1)	44 (35.5)
Re-infusion based on CD19+ cells reappearance	34/132 (25.8)	29/60 (48.3)	0/28 (0.0)	5/44 (11.4)

(Continued)

Table 1. (Continued)

Characteristics	All patients (*N*=355)	Relapsing–remitting (*N*=188)	Primary progressive (*N*=43)	Secondary progressive (*N*=124)
Re-infusion based on CD27+ cells reappearance	98/132 (74.2)	31/60 (51.7)	28/28 (100)	39/44 (88.6)
Other	2 (0.6)	1 (0.6)	0 (0.0)	1 (0.8)
Follow-up time since RTX start, years, median (range)	1.9 (0.5–8.8)	2.0 (0.5–8.1)	1.9 (0.5–6.0)	1.6 (0.5–8.8)
No. of RTX infusions excluding the first treatment course, median (range)	2 (0–16)	3 (0–16)	1 (0–8)	1 (0–11)
RTX dose/infusions excluding the first treatment course, median (1st–3rd quartiles)	1000 (750–1000)	1000 (700–1000)	750 (656–875)	1000 (750–1000)
Infusion interval, excluding the first treatment course, days, median (range)	217 (78–832)	207 (78–832)	293 (98–669)	244 (110–525)

MS: multiple sclerosis; RTX: rituximab; SD: standard deviation; EDSS: Expanded Disability Status Scale; DMT: disease-modifying treatment; HSCT: hematopoietic stem cell transplantation; JCV+: Positive for John Cunningham virus; MRI: magnetic resonance imaging.

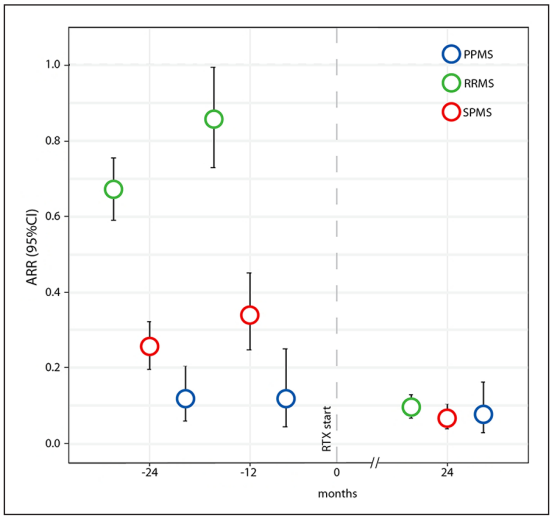

Figure 2. Annualized relapse rate in the 2 years, in the year preceding rituximab treatment, and 2 years post rituximab treatment initiation, according to MS course.

and to a higher ARR under RTX (rate ratio (RR) = 1.45 (95% CI: 1.03–2.14), $p = 0.025$). An induction regimen of 375 mg/m^2 four times every week for 1 month or two times 15 days apart showed a trend for a shorter TTFR with an HR = 2.31 (95% CI: 0.91–5.84, $p = 0.076$).

When assessing the kinetic of relapse rate reduction after RTX start, we found that ARR started decreasing during the first 3 months after RTX initiation (ARR = 0.18 (95% CI: 0.11–0.20)), decreased further at month 6 (ARR = 0.11 (95% CI: 0.06–0.20)), and remained consistently low at subsequent 3-month intervals until 2 years after RTX start (Figure 3).

EDSS progression

The median (range) baseline EDSS score was 3.0 (0.0–8.5) in RRMS, 6.0 (2.0–8.5) in SPMS, and 5.5 (1.5–7.5) in PPMS patients.

The median time to progression for the 291 patients who had at least 1 year EDSS assessment was 1.6 (0.3–8.8) years. The proportion of patients with a 6-month confirmed EDSS progression after 3 years from RTX start was 14.6% (standard error (SE) = 0.07) in the RRMS group, 24.7% (SE = 0.11) in the SPMS group, and 41.5% (SE = 0.17) in the PPMS group.

In the univariate analysis, being a PPMS or an SPMS patient as compared to an RRMS patient (HR = 3.69 (95% CI: 1.97–6.94), $p < 0.0001$ and HR = 2.03 (95% CI: 1.14–3.62), $p = 0.017$, respectively) and being a treatment naïve patient (HR = 2.01 (95% CI: 1.05–3.85), $p = 0.036$) were the only factor associated with an increased risk of EDSS progression during RTX treatment over 3 years (Table 3 and Figure 4). The multivariable analysis retained only the disease type in the final model: the risk of EDSS progression was higher for PPMS as compared to RRMS patients (HR = 3.28 (95% CI: 1.68–6.40), $p = 0.0005$) and for SPMS as compared to RRMS patients (HR = 2.09 (95% CI: 1.17–3.74), $p = 0.013$).

Table 2. Comparison of factors associated with 2-year annualized relapse rate and time to first relapse at 2 years in 188 relapsing–remitting patients.

Factors	2-year ARR		2-year TTFR	
	RR (95% CI)	*p* value	HR (95% CI)	*p* value
Age at MS onset, years	1.0 (0.95–1.05)	0.926	1.0 (0.96–1.04)	0.976
Disease duration, years	0.98 (0.92–1.04)	0.430	0.97 (0.92–1.02)	0.275
Gender (female vs male)	1.03 (0.38–2.81)	0.958	1.01 (0.42–2.44)	0.979
Presence of comorbidity	0.50 (0.18–1.30)	0.162	0.63 (0.26–1.51)	0.296
Presence of autoimmune comorbidities	1.45 (0.47–4.42)	0.516	1.69 (0.67–4.27)	0.268
EDSS 6 months prior RTX	1.04 (0.81–1.32)	0.767	1.03 (0.83–1.27)	0.811
Number of relapse 1 year before RTX	1.45 (1.03–2.14)	0.025	1.37 (1.08–1.74)	0.011
Presence of active MRI 1 year before RTX	1.26 (0.38–4.25)	0.708	2.21 (0.63–7.71)	0.212
Presence of spinal lesion 1 year before RTX	1.63 (0.54–5.15)	0.393	1.34 (0.49–3.70)	0.568
Treatment history (naïve vs previously treated)	1.0 (0.18–4.60)	0.966	0.84 (0.20–3.56)	0.808
Induction regimen (375 mg/m^2 (four infusions a month or 15 days apart) vs two 1000 mg infusions 15 days apart)	1.79 (0.53–6.10)	0.338	2.31 (0.91–5.84)	0.076
Maintenance regimen (fixed vs cytofluorimetric based)	1.45 (0.53–4.17)	0.477	1.11 (0.46–2.68)	0.815

ARR: annualized relapse rate; TTFR: time to first relapse; MRI: magnetic resonance imaging; RTX: rituximab; RR: rate ratio; HR: hazard ratio; CI: confidence interval; EDSS: Expanded Disability Status Scale.

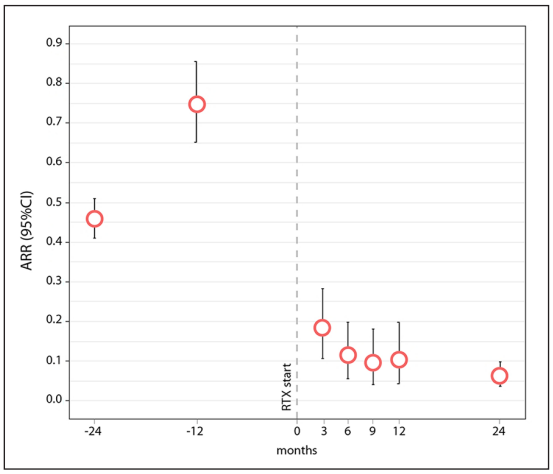

Figure 3. Trend of annualized relapse rate in the 2 years and in the year preceding rituximab treatment and 2 years post rituximab treatment initiation, all MS courses.
EDSS: Expanded Disability Status Scale; HR: hazard ratio; CI: confidence interval; RTX: rituximab; PPMS: primary-progressive patients; SPMS: secondary-progressive patients; MRI: magnetic resonance imaging.

MRI activity

Among the 365/451 (80.9%) patients with available brain MRI at baseline, 213 (58.4%) had new T2 and/or GD+ lesions on the baseline scan.

One hundred and seventy-one patients (46.8% of those with baseline MRI) had a 12-month MRI after the RTX initiation. Of these patients, 27 (15.8%) had new T2 and/or GD+ lesions; 7 patients (4.1%) had GD+ lesions and 23 (13.4%) had new T2 lesions.

In six patients (3 RRMS, 2 SPMS, and 1 PPMS) without active lesions at baseline, new T2 lesions were found in the 12-month MRI after the RTX initiation.

Safety

Out of 414 patients who completed the induction regimen and having this information available, 98 (23.7%) experienced at least one IRR (mainly skin rash, hypotension, dyspnoea, and nausea), 79 during the first infusion, and 19 during subsequent infusions. Three of them (3.1%) experienced a serious IRR (2 hypotension and 1 allergic reaction), remaining IRRs were all mild or moderate in intensity. Among the 317 with at least 6 months follow-up and available AEs data, 146 (46.1%) and 14 (4.4%) patients experienced at least one mild or moderate-intensity AE and one serious AE, respectively. Infections (particularly respiratory and urinary tract infections) were the most common AEs reported in our population (Table 4). Only eight patients had to be withdrawn from RTX due to AEs (mainly infections) and one death occurred due to a mediastinal neoplasm.

Table 3. Comparison of factors associated with 3 year EDSS progression in 291 enrolled patients.

Factors	Time to EDSS progression	
	HR (95% CI)	*p* value
Age at MS onset, years	1.01 (0.99–1.04)	0.159
Disease duration, years	0.99 (0.97–1.02)	0.670
Gender (female vs male)	0.67 (0.40–1.11)	0.120
Presence of comorbidity	0.61 (0.36–1.03)	0.065
Presence of autoimmune comorbidities	0.65 (0.26–1.61)	0.349
EDSS 6 months prior RTX	1.11 (0.97–1.25)	0.120
Relapsing remitting (as reference)		
Primary progressive	3.69 (1.97–6.94)	<0.0001
Secondary progressive	2.03 (1.14–3.62)	0.017
Number of relapse 1 year before RTX	1.00 (0.75–1.33)	0.992
Presence of active MRI 1 year before RTX	0.58 (0.32–1.05)	0.070
Presence of spinal lesion 1 year before RTX	0.59 (0.31–1.13)	0.111
Treatment history (naïve vs previously treated)	2.01 (1.05–3.85)	0.036
Induction regimen (two 1000 mg infusions 15 days apart vs 375 mg/m² (four infusions a month or 15 days apart))	1.03 (0.44–2.40)	0.943
Maintenance regimen (fixed vs cytofluorimetric based)	1.15 (0.68–1.94)	0.596

EDSS: Expanded Disability Status Scale; HR: hazard ratio; CI: confidence interval; RTX: rituximab; MRI: magnetic resonance imaging.

Discussion

RTX showed to be effective and relatively safe in the treatment of MS both in the phase II setting[5,15–18] and in some observational studies.[11–13,19] Additional data lending support to its effectiveness and safety when treating this neurological condition are of clinical relevance.

We describe here a large population of Caucasian MS patients treated with RTX in the clinical practice setting, including over 350 relapsing–remitting and progressive MS subjects.

During a median RTX treatment of 1.9 years, relapse rate was significantly reduced in patients with RRMS. This is in line with the results provided by the phase II study showing a decreased ARR in the RTX compared to the placebo group of active RRMS patients.[5] Several observational studies also demonstrated a favorable effect of RTX on relapses: the number of patients experiencing relapses was 3/82 over a median (interquartile range) follow-up of 1.5 (1.0–2.5) years,[13] 59/822 over a mean (standard deviation (SD)) follow-up of 21.8 (14.3) months,[12] and 15/89 over a mean follow-up of 22.2 (24.8) months.[20] This beneficial effect on relapses was also evident in progressive MS subtypes, corresponding to 47.6%, 32.2%, and 33.7% of the patients included in the above-mentioned studies and to 42.9% of our population. Of note, when analyzed separately, PPMS patients showed unchanged ARR after RTX start as compared to previous years, probably reflecting the lower number of events in this small disease group.

When analyzed according to 3-month treatment periods, ARR progressively decreased during the first and second trimesters of RTX therapy and stabilized thereafter. This is consistent with the results from the phase II study, showing that in RRMS patients ARR was significantly decreased at 24 weeks after RTX start as compared to placebo (0.37 vs 0.84, $p = 0.04$) and remained at similar values afterwards in the RTX arm. Notably, the OPERA studies[8] assessing the efficacy and safety of ocrelizumab showed almost complete suppression of GD+ enhancing lesions 6 months after treatment start. Overall, these results suggest that considering clinical and/or radiological rebaselining at this time point appears a reasonable strategy to fully estimate the anti-CD20 compounds' effectiveness.

The analysis of factors predicting a higher relapse activity under RTX start indicates the pre-treatment relapses as the main predictor. TTFR was delayed in those patients receiving an induction regimen of two 1000 mg infusions 15 days apart as compared to other induction schemes, being this result possibly biased by the relatively low number of patients. In a study by Yamouth et al., induction with RTX 2000 mg was associated with no evidence of disease activity.[20] No further study specifically compared the use of a standard (e.g. RTX 2000 mg) versus

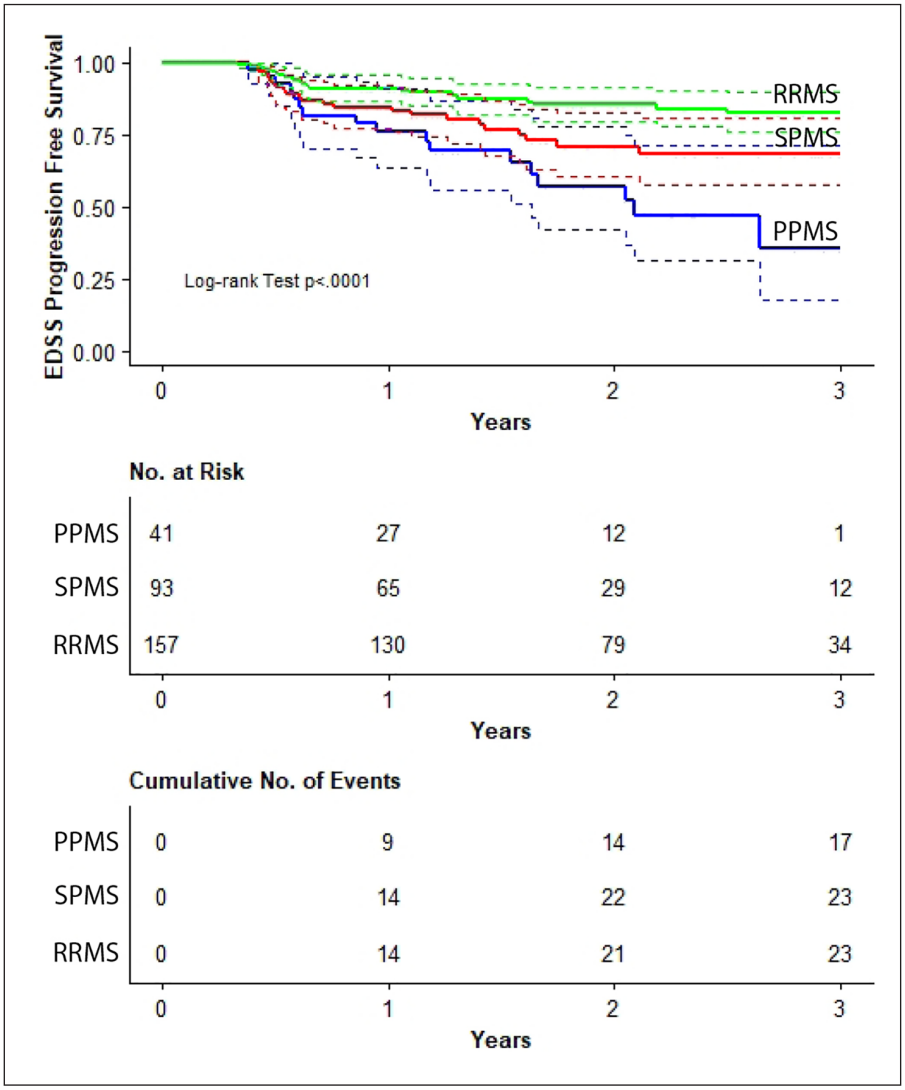

Figure 4. Kaplan–Meier plot for the risk of confirmed EDSS progression during rituximab treatment over 3 years according to the MS course.

Table 4. Adverse events (AE) for 317 multiple sclerosis patients treated with rituximab.

Adverse events	No. of patients (%)
Fever	27 (8.5%)
Infections (all the infections)	103 (34.5%)
Gastrointestinal infections (colitis)	9 (2.8%)
Respiratory tract infections (pneumonia, bronchitis, sinusitis, rhinitis, tonsillitis)	56 (17.7%)
Urinary infections (cystitis, candida)	34 (10.7%)
Herpes	24 (7.6%)
Other infections	8 (2.5%)
Other AE (headache, fatigue, hypertension, skin disorder)	72 (22.7%)

AE: adverse events.

personalized induction schedule. Interestingly, different maintenance regimens (i.e. fixed vs cytofluorimetric based) were not associated with ARR or time to first relapse in our analysis, being this result in line with Salzer et al., showing no difference in clinical relapses and MRI activity between

MS patients receiving <750 vs >750 mg at each maintenance RTX infusion.[12]

While with ours and other observational studies lacking control groups,[12,13,20] it is not possible to estimate whether RTX produces any benefits on disability progression, a recently published study suggests advantage of RTX over no or other therapy options in the prevention of disability progression in SPMS.[17] In addition, compared to placebo, RTX reduced disability progression in specific subgroups of PPMS patients treated within a phase II/III trial, that is, <51-year-old subjects with GD+ lesions at brain MRI.[6] This is also in line with the results from ORATORIO trial showing benefit of ocrelizumab over placebo in preventing disability progression in PPMS.[9] AEs observed under RTX were within the expected range, concerning mostly IRRs and infections. Similarly to other observational cohorts,[12,13,20] both infusion related and unrelated AEs involved approximately one quarter of the patients each and were rarely reported to be serious. These figures were higher during the phase II study,[5] likely reflecting different study design and populations.

Our study has several limitations. The most important ones are the retrospective design and the absence of a control group. However, data were collected prospectively within local databases or registries, partially mitigating possible reporting biases. Moreover, we could collect MRI data only in a limited proportion of patients with heterogeneous timeframes, thus precluding a more solid radiological description of our sample. The limited number of patients receiving a specific induction or maintenance RTX regimen precludes from definitive conclusions about the most appropriate treatment schedule.

Overall, our study adds to the published literature confirming that RTX is effective and relatively safe in the treatment of multiple sclerosis.

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Author Contributions

C.Z. acquired the data, participated in data analysis and interpretation, and contributed to manuscript draft. F.B. managed, analyzed, and interpreted the data and contributed to manuscript draft. G.N. acquired the data, participated in data analysis and interpretation, and contributed to manuscript draft. M.C. acquired the data, participated in data analysis and interpretation, and contributed to manuscript draft. R.L. acquired the data, participated in data analysis and interpretation, and contributed to manuscript draft. J.F. acquired the data, participated in data analysis and interpretation, and contributed to manuscript draft. A.M.R. acquired the data, participated in data analysis and interpretation, and contributed to manuscript draft. B.H. acquired the data, participated in data analysis and interpretation, and contributed to manuscript draft. S.R. acquired the data, participated in data analysis and interpretation, and contributed to manuscript draft. S.B. acquired the data, participated in data analysis and interpretation, and contributed to manuscript draft. E.C. acquired the data, participated in data analysis and interpretation, and contributed to manuscript draft. L.B. acquired the data, participated in data analysis and interpretation, and contributed to manuscript draft. G.M. acquired the data, participated in data analysis and interpretation, and contributed to manuscript draft. P.C. acquired the data, participated in data analysis and interpretation, and contributed to manuscript draft. A.D.S. acquired the data, participated in data analysis and interpretation, and contributed to manuscript draft. E.S. acquired the data, participated in data analysis and interpretation, and contributed to manuscript draft. S.B. acquired the data, participated in data analysis and interpretation, and contributed to manuscript draft. G.T.M. acquired the data, participated in data analysis and interpretation, and contributed to manuscript draft. I.M. participated in data analysis and interpretation and contributed to manuscript draft. I.M. acquired the data, participated in data analysis and interpretation, and contributed to manuscript draft. G.B. acquired the data, participated in data analysis and interpretation, and contributed to manuscript draft. S.M. acquired the data, participated in data analysis and interpretation, and contributed to manuscript draft. A.N. acquired the data, participated in data analysis and interpretation, and contributed to manuscript draft. G.C. acquired the data, participated in data analysis and interpretation, and contributed to manuscript draft. C.M. acquired the data, participated in data

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Supplemental material

Supplemental material for this article is available online.

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