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Rituximab for the Treatment of Multiple Sclerosis: A Retrospective Observational Study of 50 Cases from Morocco, and Literature Review

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ABSTRACT

Introduction : Rituximab (RTX) showed to be effective and relatively safe in the treatment of relapsing-remitting and progressive forms of multiple sclerosis (MS), both in the phase II setting and in some observational studies. **Objective:** To investigate the effectiveness and safety of rituximab in MS. **Patients and methods:** We report a retrospective observational study to describe the effectiveness and safety of off-label rituximab in the treatment of a population of Moroccan MS patients including 50 relapsing-remitting (RRMS) and progressive multiple sclerosis (PMS) subjects. **Results:** Our study showed that the RTX treatment was associated with the mean ARR decreasing by 0.72 at one year follow up. EDSS scores improved after 1 year of treatment with RTX by a score of 0.5-1.0 in 31 (62%) patients and remained stable in the second year of therapy. It should be emphasized that the mean reduction in EDSS was more significant in the RRMS subgroup compared to the PMS group (RRMS-25, SPMS-6, PPMS-0). EDSS score remained same in 12 patients (24%), of which 9 had RRMS and 3 SPMS. EDSS worsened after 2 years from RTX in 7 (14%) patients (5 SPMS, 2PPMS). Follow up MRI Brain with contrast at one year, show new T2 lesions in 6 patients (12%), with no enhancing lesions either old or new. Concerning safety issues in our patients, we observed a frequency of infusion associated reactions inferior to the data reported in other studies. Majority of patients (98%) tolerated RTX infusion well. **Conclusion:** RTX could be an effective and safe treatment in RRMS. Some selected PMS patients could also benefit from this treatment.

Keywords: Multiple Sclerosis, Rituximab, CD20.

INTRODUCTION

Multiple sclerosis (MS) is the most common chronic, immune-mediated inflammatory that profoundly alters both cellular and humoral immune systems, causing demyelination and neuronal loss in the central nervous system (CNS), often leading to the accumulation of irreversible clinical disability in young adults worldwide. Along this line, increasing attention is being paid to anti-CD20 monoclonal antibodies (mAb) capable of destroying B cells for the treatment of MS, conventionally treated with

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cellular immunity strategies. Rituximab (RTX) showed to be effective and relatively safe in the treatment of MS both in the phase II setting and in some observational studies [1,2].

Upon differentiation into plasmablasts, B cells begin to downregulate the expression of CD20, which is not expressed by plasma cells (Figure 1). Thus, some plasmablasts and virtually all plasma cells are refractory to anti-CD20 B cell depletion. In contrast, memory B cells retain CD20 and are depleted with anti-CD20. CD20 is a transmembrane, non-glycosylated phosphoprotein of 33-37 kDa expressed in tetramers associated with lipid rafts on the surface of cells of the human B-cell lineage from pre-B cells to naïve and memory B cells. The function of CD20 is not fully elucidated, though the structure predicts major hydrophobic regions and it has been described as having features of a calcium channel with possible roles in B cell activation and differentiation. Thus, the efficacy of B cell depletion in MS has placed a focus on a potential role for effector/memory B cells with the capacity to present antigen to autoreactive T cells. B cells demonstrated also a gradual differentiation into a stable plasma cell population, showing expression of markers involved in B-cell survival and plasmablast differentiation (CD27 and CD38) [1,2].

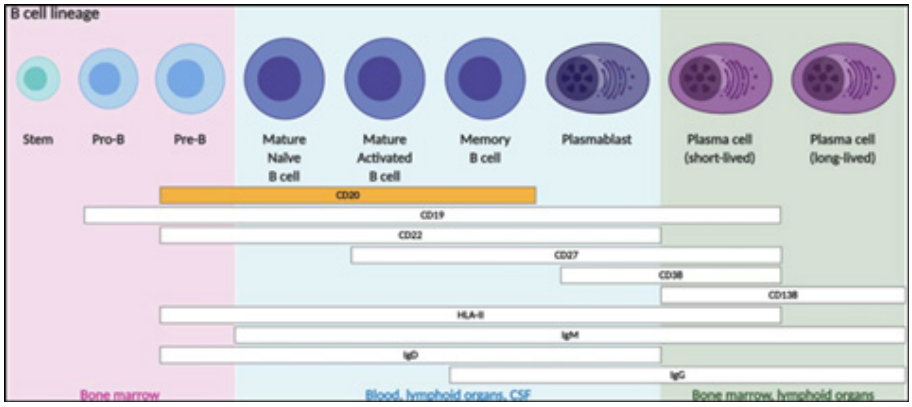

Figure 1. Summary of the B-cell maturation stages, defined according to the expression of specific cell-surface antigens.

B cells first undergo differentiation into plasmablasts, which begin to secrete antibodies. Antibodies are a secreted version of the BCR and are also known as immunoglobulins (Ig). Plasmablasts can further differentiate into long-lived plasma cells that migrate back to the bone marrow and secrete antibodies into the blood for decades. After activation, B cells can also undergo a fate decision to become long-lived memory B cells. The function of memory B cells is to provide fast and efficient antibody responses upon re-exposure to the same antigen [1,2].

Proinflammatory cells, particularly CD27+ memory B cells, induce autoreactive, autoproliferative, proinflammatory T cells (including TH17 cells), which in turn play a crucial role in CNS inflammatory cascades (Figure 2). Within the B cell pool, the memory subset harbours most of the potentially pathogenic MS-associated cells. However, deficiencies in protective (anti-inflammatory or regulatory) B cells in other subsets could be equally important in the pathophysiology of MS [3,4].

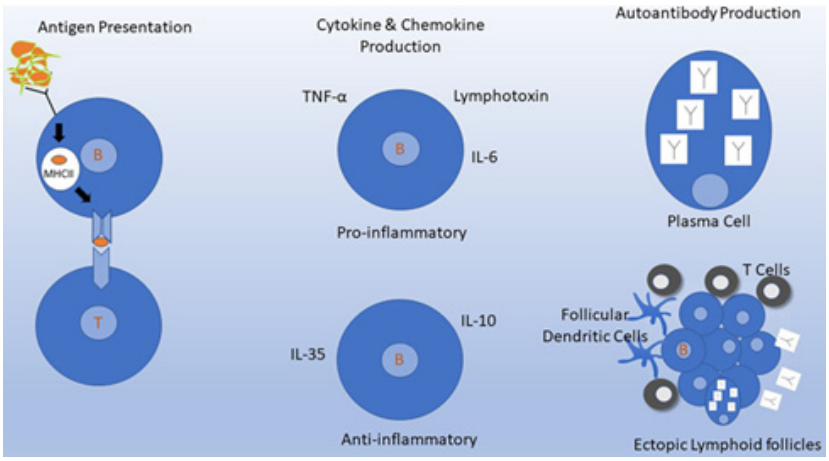

Figure 2. Mechanisms of B cells in MS pathogenesis.

Various antiinflammatory regulatory B cells (B regs) cell subsets have been described that can suppress inflammatory immune responses. An in vitro study has shown that CD19+CD24+CD27+ B cells are more efficient than transitional B cells at suppressing CD4+ T cell proliferation and the expression of IFNγ and IL-17. The mechanism of immunosuppression differs between the subsets and further investigation is needed to fully understand the contributions of other specific B-lymphocyte subgroups present in CSF in determining disease phenotype [3,4].

All of these clones tend to persist within the CNS and can be shared among different CNS compartments and the periphery, suggesting bidirectional trafficking of distinct B cell clones between the CNS and the periphery. Thus, B cells can dynamically traffic into and out of the CNS, and can potentially carry, process, and present CNS antigens in the deep cervical lymph nodes, make their way back into the CNS via the thoracic duct, systemic circulation, and the various brain barriers, infiltrate the brain parenchyma, populate ectopic lymphoid follicles, and trigger another bout of CNS targeted inflammation [5,6].

During the last few years, there has been a dramatic evolution in several key concepts of MS immune pathophysiology. MS has been historically considered as an autoimmune disease of the CNS mediated by CD4+ T cells reactive to myelin antigens. According to this model, the autoimmunity processes directed to the CNS are induced by the imbalance between CNS-reactive effector T cells of the helper-1 (Th1) and Th17 type and regulatory T cells (Treg). Therapies (e.g., interferon beta and glatiramer acetate) developed on the basis of this theory decrease the relapse rate by approximately one third but do not fully prevent the occurrence of exacerbations or accumulation of disabilities, and they are largely ineffective against purely progressive forms of MS. Evidence now suggests the inflammation in MS stems from more complex and bidirectional interactions between T cells and antigen-presenting cells (APCs) such as B cells and myeloid cells (macrophages, dendritic cells and microglia) [1].

The original impetus for targeting humoral activity in MS was based on the long-standing recognition of abnormally increased B cells, plasmablasts and plasma cells in MS lesions, meninges and the cerebrospinal fluid (CSF) of MS patients and their number is positively associated with intrathecal inflammation (e.g., increased immunoglobulin synthesis rates in the CNS, CSF-restricted oligoclonal bands OCBs, the predominance of memory and the lack of naive B cells in the CSF, antibodies bound to myelin fragments within phagocytic cells in the CNS parenchyma, Ig and complement detection in demyelinated lesions), as well as the meningeal-based ectopic B-cell follicles adjacent to areas of focal cortical demyelination, which can contribute to the demyelination, axonal damage and disease progression through several antibody-dependent and -independent mechanisms. These data are indirectly confirmed by the efficacy of plasmapheresis and immunoadsorption in treating steroid-resistant MS relapses. Furthermore, the demonstration of the strong efficacy of selective B-cell-depleting therapies (such as anti-CD20 monoclonal antibodies), pointing out the key role of B cells in triggering MS disease activity [1,3,4,7].

Initially, B-cell depletion both in the periphery and in the CNS was expected to exert its effect by diminishing the production of autoantibodies. However, the rapid onset of the profound effects of CD20 B cell-targeted therapies has prompted a reevaluation of the humoral immune response in MS. The concept holds that clinical benefit preceded humoral change/autoantibody synthesis. B cells play key roles in mediating disease activity and pro-inflammatory pathogenicity such as antigen presentation, cytokine production and antibody production by CNS-infiltrating B cells, and finally to a formation of ectopic lymphoid organs in the CNS [1,3,4,7].

Peripheral mature B cells can cross the blood-brain-barrier (BBB) into the CNS via parenchymal vessels into the perivascular space and via post-capillary venules into the subarachnoid and Virchow-Robin spaces. They can also cross the blood-CSF barrier via the choroid plexus into the CSF, and via the blood-leptomeningeal interphase. In early and active focal demyelinating lesions, CD20+ B cells are mainly located focally in the perivascular space of only one or a few larger veins and have pro-inflammatory functions. Conversely, a more abundant plasma cell and B cell infiltrate can be found in the perivascular space, parenchyma and in the meninges, mainly within deep cortical sulci, creating an intracerebral milieu that sustains chronic CNS-compartmentalized inflammation and also directly mediates or exacerbates cortical pathology, degenerative mechanisms and disease progression, which can be maintained in the absence of ongoing relapse biology and characterizes longstanding disease. These abnormalities are accentuated in a subgroup of patients who have a high level of brain inflammation, extensive and active subpial grey matter demyelination, and a rapidly progressive clinical disease course, suggesting that B cell accumulation causes or contributes to the worse clinical course. In particular, meningeal inflammation has been associated with a gradient of neuronal, astrocyte and oligodendrocyte loss from the surface inwards, accompanied by microglial activation in subpial grey matter lesions that is greatest in the most external cortical layers and lower in the inner layers close to the white matter (Figure 3) [1,4,6].

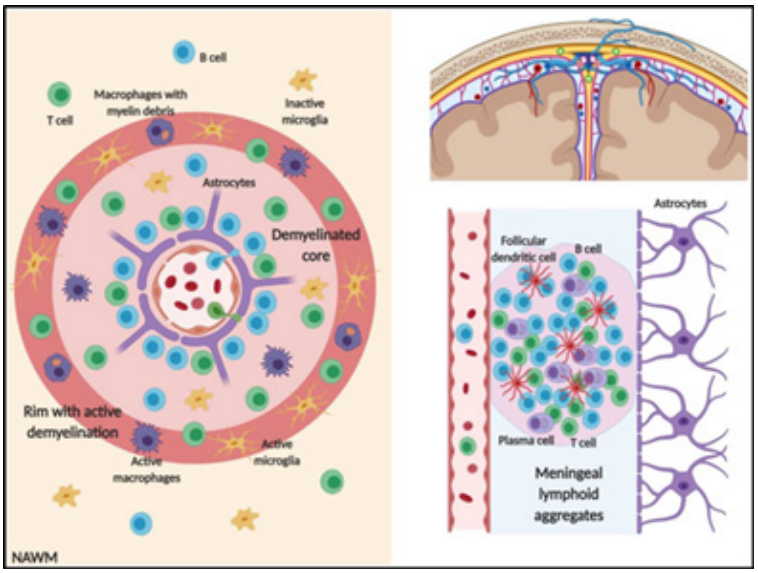

Figure 3. Summary of the involvement of B cells in the immune pathophysiology and pathology of MS.

Additional data lending support to its effectiveness and safety when treating this neurological condition are of clinical relevance. We report thus a retrospective observational study to describe the effectiveness and safety of off-label RTX in the treatment of a population of Moroccan MS patients including 50 relapsing-remitting (RRMS) and progressive (PMS) MS subjects.

Definitions

Relapse was defined by the presence of new or worsening neurological symptoms, lasting more than 24 h, in the absence of fever or significant infectious processes and accompanied by objective changes in the neurological examination.

Confirmed improvement in disease (CID) and confirmed worsening of disability (CWD) were defined by a decrease or increase, respectively, of one point in EDSS (if EDSS was <6) or of 0.5 point (if EDSS was 6 or more) persisting after 6 months.

Clinical activity was defined as the presence of relapses and/or CWD and radiological activity was defined as the presence of new T2 and/or gadolinium enhancing lesions on MRI scan.

No evidence of disease activity (NEDA) was defined as absence of clinical and radiological activity, so evidence of disease activity (EDA) was defined by the presence of any activity, whether clinical or radiological.

MATERIALS AND METHODS

Study Population, Data collection and monitoring

Types of studies

All MS patients who were treated with RTX in two university hospitals in Marrakech (Morocco), from December 2018 to December 2022 were retrospectively evaluated.

Types of participants

Both males and females, adult patients aged 18-65 years old with MS included in this study, presented either RRMS or PMS (secondary progressive SPMS and primary progressive PPMS), fulfilled the McDonald 2017 diagnosis criteria, and received treatment with RTX between December 2018 and December 2022.

Patient baseline demographic and clinical characteristics-Data collection

Data collected at baseline included age, gender, first symptoms, disease duration since onset, treatment duration, detailed history of relapse including date of relapse, previous therapies, discontinuation date and reasons for switching to RTX [relapse and/or GEL(s)], MS phenotype, number of clinical relapses in the previous 2 years, the date of RTX start, dosage of RTX and adverse events (AEs). Relapses and Gd+ lesions were included if occurring at least 3 months after the first DMT dose. Magnetic resonance imaging (MRI) of brain was done at baseline and every year on follow up. MRI scans were performed according to standard follow-up guidelines and MRI protocols, using 1.5-T or 3-T MRI scanners. Laboratory tests including CD 19/20 B cell counts were performed at baseline and every 6 months after the first RTX infusion. Follow-up ended in December 2022 and the data were updated every 6 months during the follow-up visits. Decision regarding next dose was based on CD 19/20 counts and clinical response. A re-treatment was given when the CD19 counts were above 1%. All patients underwent complete blood counts (CBC), urine analysis, chest x-ray posteroanterior (PA) projection (CXR PA view), renal function tests (RFT), liver function test (LFT), Hepatitis B surface antigen test (HBsAg), Human Immunodeficiency

Virus (HIV), AntiHepatitis C antibody (Anti-HCV), IgG Hepatitis B core antibody IgG (Anti-HBc IgG). Treatment duration was defined as the interval between baseline and the last available neurological follow-up.

Primary and secondary outcomes

The disease durations at the initial RTX infusions ranged from 0 year to 8 years. The median time between infusions was 7.1months (range: 2.6-27.3months); patients received a median (range) of 6 (3-12) RTX infusions during the follow-up. The annualized relapse rate (ARR) and the expanded disability status scale (EDSS) before and after RTX, the time to first relapse (TTFR), the time to EDSS progression, and the percentage of patients with NEDA after RTX or potential side effects were counted from RTX initiation, every 6 months thereafter, 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 evaluated in patients that had at least one-year follow-up EDSS assessment (to allow progression confirmation). The radiological outcome measure was MRI activity expressed as presence of new T2 weighted (as compared to baseline) and/or Gadolinium + lesions at brain MRI performed 1 year from baseline according to local clinical practice and change from baseline to week 96 in brain volume (brain parenchymal fraction) on brain MRI scans.

Treatment regimen and protocol for RTX infusion

RTX induction and maintenance regimens were classified according to the protocols applied at participating centers as follows:

1. For the induction regimen, two 500 mg infusions administered : Two 500 mg infusions 15 days apart in RRMS (median intensity regimen) and 1000 mg intravenous twice 2 weeks apart every 24th week in four cycles in PPMS patients.
2. Maintenance regimens were classified as follows:

- Median intensity regimen: re-infusion of a single dose of 500 mg as a slow infusion every 6-9 months, based on reappearance of CD19+ (exceeding 1% of peripheral mononuclear cells) or CD27+ memory cells (exceeding 0.05%).
- High intensity regimen: patients with progressive MS either PPMS or SPMS received a high intensity regimen of 2 gm every 6 months.

Patients were premedicated with dexchlorpheniramine, intravenous Methylprednisolone (100-500 mg, one hour prior to infusion) and oral paracetamol 650 mg, 1 h prior to RTX infusion to prevent allergic reactions. RTX 500 mg was added to 500 ml normal saline for infusion. RTX was given as a very slow infusion to minimize infusion reactions. Infusion rate was started at 4 ml/hr and slowly increased every 15 min (4 ml/hr. for 15 min followed by, 8 ml/hr. for 15 min, 16 ml/hr. for 15 min, 32 ml/hr. for 15 min, 48 ml/hr. for 15 min), as tolerated to a maximum rate of 64 ml/hr, to continue till the end of the infusion. Infusion typically lasts nine to ten hours especially during the first infusion. If there were no reactions infusions times were shortened to six hours during further infusions, but the infusion rates were never reduced below 6 h.

Statistical analysis

Descriptive statistics on patient characteristics for the entire cohort were provided via mean and standard deviation for continuous variables and proportion for categorical variables. Median and range were provided for non-normally distributed continuous variables. Differences in baseline characteristics were tested using the Wilcoxon rank sum test for continuous variables and Fisher exact test for categorical variables. For the outcomes Gd1 and T2 MRI lesions, the number of patients with positive scans per patient with valid scans was calculated, and the differences in these proportions were tested in logistic regression models. For the outcomes; clinical relapses, AEs, and drug survival, person-years and yearly incidence were calculated, and Kaplan-Meier curves and Cox proportional hazards models were used, with time from the first administration of RTX as timescale.

Limits

Our study has several limitations. The most important ones are the retrospective design, the absence of a control group, the relatively small number of patients and the short follow-up time.

RESULTS

Of the 50 patients starting RTX, 34 (68%) were RRMS and 16 (32%) were progressive (2 PPMS and 14 SPMS). In 20 cases (40%), RTX was the initial treatment, while the remaining 30 (60%) patients, switched from other disease-modifying therapies (DMTs): natalizumab (30%) and fingolimod (50%) were the most commonly used drugs, followed by interferons (16%). Reasons for RTX initiation were mainly poor efficacy (77%), lack of tolerance or AE related to previous MS therapy (12%), and John Cunningham virus (JCV)-positive status (11%) in patients treated with natalizumab. Median (range) washout periods for first- and second-line therapies was 1.09 months.

Rituximab effectiveness

Clinical outcomes

We considered three primary efficacy outcomes: (1) changes in ARRs when comparing the periods before and after the RTX therapy, (2) changes in mean EDSS scores when comparing the periods before and after the RTX therapy, and (3) the likelihood of participants experiencing relapses after RTX therapy.

Efficacy outcomes: ARRs

The median follow-up time was 2.9 years (range: 1.8-4.9 years). 32 RRMS patients (94%) had no relapses during follow up. Our study showed that the RTX treatment was associated with the mean ARR decreasing by 0.72 at one year follow up (95% confidence interval, 0.55-1.27). The decrease versus the year before RTX start was significant in the RRMS group and in the SPMS group (p<.0002) and did not reach significance in the PPMS 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.

Efficacy outcomes: relapse likelihoods

Relapses following the RTX therapy occurred in only 12% of patients.

Efficacy outcomes: EDSS score progression

Our patients had an EDSS evaluation at baseline and at 6 and 12 months. The median time to progression for the 50 patients who had at least 1 year EDSS assessment was 2.3 years. EDSS scores improved after 1 year of treatment with RTX by a score of 0.5-1.0 in 31 (62%) patients and remained stable in the second year of therapy. It should be emphasized that the mean reduction in EDSS was more significant in the RRMS subgroup compared to the PMS group (RRMS-25, SPMS-6, PPMS-0). EDSS score remained same in 12 patients (24%), of which 9 had RRMS and 3 SPMS. EDSS worsened after 2 years from RTX in 7 (14%) patients (5 SPMS, 2PPMS). 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 and for SPMS as compared to RRMS patients. In the overall cohort, 86% of patients did not experience CDW. For progressive patients, even if in a reduced sample size, the medium EDSS worsened at 12 months.

The data on RRMS are in line with some other real-life studies, where the EDSS ameliorated for RR patients at 12 months. However, other studies demonstrated a stability of the EDSS score at 12 months for RRMS.

NEDA-3 status

The NEDA-3 status was evaluated at 12 months from the start of RTX therapy, and up to the last follow-up: 28 of the 50 patients (56%) continued to demonstrate NEDA status up to their last follow-up, consistent with the result reported by D'Amico et al.

When analyzing the patients that used RTX as a first-line treatment vs. escalation from other DMT (40% and 60%, respectively) no differences were found in CWD, radiological activity and NEDA. However, fewer patients that used RTX as first line treatment experienced relapses.

Radiological outcomes

MRI activity

All of our patients had brain MRI at baseline and a 12-month MRI after the RTX initiation. 22 patients (44%) (20 RRMS, 2 SPMS) had new T2 and/ or GD+ lesions on the baseline scan. Follow up MRI Brain with contrast at one year, show new T2 lesions in 6 patients (12%), with no enhancing lesions either old or new. In 3 patients (2 RRMS, 1 SPMS) without active lesions at baseline, new T2 lesions were found in the 12-month MRI after the RTX initiation.

Regarding radiological disease activity, the significant 12-month MRI activity reduction showed in our patients corroborates the efficacy of RTX therapy on MRI measures of ongoing disease activity, documented in other studies.

Change from baseline to week 96 in brain volume, measured by MRI

RTX don't stop the progression of brain atrophy in our patients.

Safety outcomes: Rituximab tolerability

The primary safety outcome was the occurrence of adverse events (AEs), including infusion-assocated reactions (IARs), infections, and hematological disorders.

Concerning safety issues in our patients, we observed a frequency of IARs inferior to the data reported in other studies. Majority of patients (98%) tolerated RTX infusion well. Mild infusion reaction was seen in 1 patients and resolved spontaneously. None of the patients had tuberculosis, infection or malignancies, conversely from what has been reported in another studies, where infectious AEs were more represented. No patient developed serious AEs. There were no PML cases in our series, although 6 of our patients were JCV antibody positive. However, the median follow-up time of 2.9 years is very short and continued monitoring for these serious AEs will be essential.

As expected, there was a striking and stable reduction of CD19+ B cell concentration after RTX initiation, while CD4+ and CD8+ levels did not appear considerably influenced by RTX treatment.

Withdrawal of rituximab treatment

RTX was discontinued in 5 patients (10%). The median (range) time to switch was 3.2 years. CWD was the reason for withdrawal in 4 patients (8%), all of whom had PMS (2 SPMS, 2 PPMS). In one patient SPMS (2%), RTX was withdrawn due to inflammatory activity (isolated radiological activity). One of these patients underwent autologous hematopoietic stem cell transplantation and 4 PMS were switched to OCR. No case of RTX discontinuation due to side effects was reported.

Our observational study that included both RRMS and progressive patients, with a mean follow-up time of 34 months, confirms the previously shown good efficacy and safety profile of RTX therapy for MS patients, to those reported in randomized controlled trials of B cell therapies (Table 1).

Table 1. Clinical Trials and Real-World Data and Retrospective Studies obtained from the wide off-label use of rituximab, to assess its efficacy in MS patients [12].

Clinical Trial	Study type	Decrease in ARR after RTX	Decrease in radiological activity after RTX	EDSS change after 1 year of RTX
1-Bar-Or et al (2008)	Open label phase 1 RRM	-ARR decreased from 1.27 to 0.25 at week 24 and to 0.18 at week 72 -GAD-enhancing lesions was also reduced from 1.31 at baseline to 0.73 at week 4 after the first course and further to 0.05 at week 48 and to 0 at week 72. The mean number of new T2 lesions decreased as well, from 0.92 at week 4 to 0 at week 72, with a significant reduction also in the volume of the lesions		
2-Hauser SL et al. (2008)	Double-blind,placebo-controlled, manufacturersponsored, 48 week trial (HERMES) Trial Group	The proportion of patients with relapses was reduced in the rituximab group	Patients who received rituximab had a reduction in total gadolinium enhancing lesions counts and T2 lesions volume	
3-Naismith RT et al. (2010)	Phase I/II trial (RIVITaLISe)	Rituximab induced a significant reduction of GAD-enhancing lesions in comparison to INFb or glatiramer acetate. a reduction in ARR has also been observed		
4-STRIX-MS trial (2016)	Open-label, phase II trial	Superior effect in reducing disease activity in RRMS compared to first-line treatments during the first year after switch (Class IV evidence), whereas neurologic impairment assessed by EDSS did not show any progression or improvement of statistical significance, as well as scores for patient-perceived impact of disease on daily life		
5-Honce JM et al. (2019)	Double-blind, placebo-controlled, randomized	A single cycle of rituximab followed by a moderate efficacy/high safety DMT as glatiramer acetate may provide a superior efficacy than glatiramer acetate alone in RRMS, although this benefit does not seem to be long-lasting.		
6-Cheshmavar M et al.2021)	Phase II/III, openlabel, randomized clinical trial	EDSS increased after 12 months from 3.05 ± 1.01 to 4.14 ± 0.91 in the rituximab group ($p < 0.001$), and from 3.22 ± 1.20 to 4.60 ± 0.67 in the glatiramer acetate group ($p < 0.001$). No statistically significant differences in EDSS scores were observed between the two groups=apparent lack of efficacy of both treatments in controlling EDSS progression		
Retrospective studies	**Study type**	**Decrease in ARR after RTX**	**Decrease in radiological activity after RTX**	**EDSS change after 1 year of RTX** **NEDA**
Salzer et al. (2016)	The largest retrospective observational study	Significantly lower ARR (0.044 for RRMS, to 0.038 for SPMS, and to 0.015 for PPMS)	Reduction of CEL=from 26.2% at baseline to 4.6%	Unchanged in patients with RRMS but increased by 0.5 and 1.0 in patients with SPMS and PPMS 21.
Zecca et al. (2019)	Retrospective study	Decrease in ARR was observed for RRMS (0.09), SPMS (0.06), and PPMS (0.07)	84.2	In the multivariable analysis, the risk of EDSS progression was higher for PPMS (p=0.0005) and SPMS (p=0.013) as compared with RRMS patients.
Alping et al. (2016)	Observational retrospective study	2%	GEL in 1.%	PPMS=
Disanto G et al (2020)		No relapses were reported in the 12 months after de escalation of rituximab dose from 1,000 to 500 mg/6 months	Only three new T2 lesions in brain/spinal cord (all of which without contrast enhancement and clinically asymptomatic) were detected	EDSS scores maintained approximately stable, as well as serum NFL concentration,

Airas et al. (2020)	Retrospective observational study	RTX significantly reduced ARR in both RRMS and SPMS	RTX reduced the mean number of GAD-enhancing lesions in RRMS patients.	EDSS remained substantially stable in all MS group. In particular, among patients with progressive forms, 45% had stable EDSS during the study, whereas 18% of PPMS and 20% of SPMS patients even had an improvement.
Bellinvia A (2020)		RTX (1,000 mg, 6 monthly) significantly reduced the ARR from 0.75 to 0.36 at 12 months ($p<0.001$), with no differences between RR and progressive patients	MRI activity was reduced from 88% to 8.3% at follow-up. NEDA status at 12 months was observed in about 60% of patients.	13 (23.2%; 10 PMS, and 3 RRMS) showed a progression at 6 months compared with baseline, whereas only one progressive patient showed a progression at 12 months.
Yamout BI et al. (2018)	Retrospective cohort study	Reduction of ARR by approximately 89% (relapse-free in 79% in the RRMS and 90% in the PMS group)	92.6% in the RRMS and from 82% in the PMS group were free from any new lesions, and 74% achieved NEDA at 1 year of treatment.	No EDSS score progression in both RRMS and PMS patients. Interestingly, there was a trend of improvement in terms of EDSS in RRMS, whereas in the PMS group, it was substantially unchanged.
Alcalá C . (2018)	Retrospective university hospital-based study	RTX significantly reduced ARR by 88.4%	RTX reduced the number of GAD-enhancing lesions from 2.56 to 0.06. NEDA status was reached in 70% of the total sample (74.2% of RRMS patients, and 67% of the PMS patients).	A decrease of 0.3 EDSS points in the first year and no variation in the second year of therapy were detected. Considering only PMS patients, most of them remained stable after rituximab treatment, without significant changes in the EDSS score.
Hellgren et al. (2020)	Retrospective, observational, registry-based, longitudinal study	highly significant reduction of ARR induced by rituximab with a global reduction by 87%	New inflammatory lesions decreased from 58% at baseline to 26%. GEL dropped from 47% at baseline to 6% at 1 year after RTX	
Mathew T et al. (2020)		No relapses in 97% of treated patients	No lesions either old or new at 1 year	Improved by 0.5 to 2.0 points in 85% of patients. remained stable in 12.5% (9 SPMS and 1 PPMS), and worsened in 2.5% (2 SPMS patients).
Naegelin et al. (2019)	Retrospective cohort study	A significantly lower EDSS score during a mean follow-up of 3.5 years and a ignificantly delayed time to confirmed disability progression or patients treated with rituximab compared with matched patients never treated with rituximab, suggesting a potential therapeutic benefit of Rituximab also in SPMS		
Scotti B et al. (2018)	Retrospective observational study	Efficacy of rituximab in MS treatment, both in RRMS and PMS in terms of number of new relapses, EDSS worsening, new T2 and GAD+ lesions, and proportion of patients without evidence of disease activity during treatment.		
RIFUND-MS study		RTX is superior to dimethyl fumarate in preventing relapses over 24 months in patients with early RRMS		
OLYMPUS trial	Double-blind, placebo controlled, manufacturersponsored trial	RTX marginally reduced the time to CWD status, but the difference did not reach statistical significance except in a preplanned subgroup of young patients (<51 years of age with GEL in the baseline MRI)		

RRMS=relapsing-remitting forms of of multiple sclerosis

PMS=progressive forms of multiple sclerosis

ARR=Annualized relapse rate

GEL=gadolinium-enhanced lesion

EDSS= Expanded Disability Status Scale

CWD= Clinical worsening of disease

CURRENT STATUS OF THE ART

The role of B cells in MS immune pathophysiology

Antigen presentation

B cells recognize antigen via the antigen binding domains of their B cell receptor (BCR) leading to their activation. B cells have been demonstrated to contribute to cascades of cellular immune interaction in the periphery by the expression of class-II major histocompatibility complex (MHC class II), to act as antigen presenting cells (APCs) to T cells, thus promoting T-cell activation and proliferation, to interact with APCs to influence antigen trafficking, and to be directly involved in the production of cytokines and chemokines exerting both anti- and pro-inflammatory actions and contributing to oligodendrocyte and neuronal damage [1,2,7].

Cytokine production

B cells in MS are skewed toward a pro-inflammatory cytokine profile, which can drive T cells and myeloid cells

and enhance pathogenic immune responses. The binding of autoantigen to B cell receptor (BCR) also causent aberrant B cells to produce more than 20 co-stimulatory cytokines that perpetuate the inflammatory milieu. In MS, B cells are also recognized to have not only an abnormal propensity to produce pro-inflammatory cytokines (interleukin 6, tumor necrosis factor alpha), but also a deficient capacity to produce regulatory cytokines (interleukin 10 and 35, transforming growth factor-β, granzyme B) by regulatory B (B-reg) cells. Due to such an abnormal cytokine response profile, B cells can induce aberrant pro-inflammatory Th1, Th17 and myeloid cell responses, contributing to the cellular immune cascades involved in disease activity, an effect that is mitigated by B cell depletion. Other B cell-secreted cytokines upregulated in MS include granulocyte macrophage-colony-stimulating factor (GM-CSF), which increases mobility and activity of myeloid populations. Moreover, B-cell activation factor (BAFF) produced by astrocytes, is an important survival factor balancing pro-inflammatory and regulatory B-cell subtypes, and is upregulated in MS lesions. In addition, B cells in patients with MS produce some as yet unidentified cytotoxic mediators into the CSF, that are toxic to oligodendrocytes and neurons [1,4,6-8].

Antibody production

The presentation of antigen to CD4 T cells by B cells, results in crosstalk between the two cell types and signaling leading to B cell differentiation into antibody secreting cells. It has been shown that MS in OCBs-positive patients shows a more aggressive course than OCBs-negative patients. OCBs of the IgG type are present in most patients with MS, and OCBs of the IgM type are present in 30%-40% of patients. These OCBs are made up by plasma cells and plasmablasts generated from a restricted numbers of B cell clones that persist within the CNS of the same individual and are shared by different CNS compartments and the periphery, but differ among individual patients. Despite this, the antigenic targets of the aberrant immune cell activation in MS remain incompletely defined and the long-term contribution of autoantibodies is largely unknown. The antibodies that make up these OCBs primarily recognize ubiquitous intracellular proteins but not specific antigens that are shared across MS patients, suggesting a humoral response to debris from dead-cells rather than a primary pathogenic response. Oligoclonal Ig bands found in the cerebrospinal fluid (CSF) of most MS patients to the potassium channel KIR4, to myelin antigens such as myelin basic protein (MBP), proteolipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG) or to axoglial proteins around the Ranvier's nodes as neurofascin43 and contactin-2, do not seem to have any pathogenic role in MS [1,4,6-8].

Antibody secreting cells do not express CD20 on their surface, and the highly efficacy of B cell depletion therapies in the rapid decrease of clinical and MRI disease activity has therefore been attributed to antigen-presentation and cytokine secretion and is is unlikely to result from the removal of any pathogenic antibodies, which have relatively long half-life. Therefore, autoantibodies are an unlikely primary pathogenic mechanism in MS. However, anti myelin/oligodendrocyte glycoprotein antibodies have been shown to contribute to demyelination in the experimental allergic encephalomyelitis (EAE) model, and demyelinating MS lesions contain immunoglobulins and activated complement, which may suggest antibody-mediated damage at least in some patients [6,7,9].

Formation of ectopic lymphoid follicles (ELFs) or tertiary lymphoid organs

B cells that have been attracted to the brain of MS patients, with the appropriate help from T cells, can proliferate, aggregate, and generate meningeal inflammation and eventually ectopic immunocompetent germinal center-like structures, called also tertiary lymphoid organs, which are associated with more severe cortical pathology and more aggressive disease course. These B cell-rich ectopic lymphoid structures, which were described in secondary-progressive (SP) MS, RRMS, and active primary-progressive MS (PPMS), can serve as a reservoir of memory-B cells and autoreactive plasmablasts and plasma cells, perpetuating autoimmune disease. In addition, they can secrete soluble factors that were shown to be cytotoxic to both oligodendrocytes and neurons. B cells residing in ELFs appear relatively protected from anti-B cell therapy: this may in part be due to paracrine secretion of BAFF. In MS, this is further compounded by a relative lack of drug access to the CNS [6,7].

The extent of meningeal inflammation and the levels of pro-inflammatory cytokines in the CSF of MS patients have been associated with the severity of subpial cortical demyelination, promoting also a graded pattern of neuronal loss and microglial activation consistent with a 'surface-in' process possibly mediated by one or more toxic substances contained in the CSF [1].

The following table summarizes the pathophysiologic mecanims of B cells in MS **(Table 2)**.

Table 2. Evidence for potential pathophysiologic functions of B lymphocytes in MS [3].

Synthesis of intrathecal oligoclonal bands
Production of antibodies against myelin components in blood and CSF
B-cell accumulation and activated complement deposition in brain lesions
Meningeal B-cell agregates in secondary progressive MS
Increased number of plasmoblasts in blood and CSF
Antigen presentation, cytokine production, stimulation, and regulation of autoreactive proinflammatory T cells
Induction and regulation of the proliferation of autoreactive, proinflammatory T cells (including TH17 cells) homing to the CNS
Induction of neuronal apoptosis and oligodendroglial cytotoxicity

Mechanisms of action of RTX in MS

Mechanisms of action of anti CD20 therapies in MS

Monoclonal antibodies (mAb) belong to the immunoglobulin G (IgG1 kappa) isotype which bind specifically with their fragment antigen-binding (Fab) region to the epitope of the target molecule. The binding of the fragment crystallizable (Fc) region can lyse a target cell through at least four possible different mechanisms: (i) antibody-dependent cellular cytotoxicity (ADCC); (ii) complement-dependent cytotoxicity (CDC); (iii) antibody-dependent cellular phagocytosis (ADCP); and (iv) induction of cell apoptosis. IgG monoclonal antibodies typically a have a half-life of 21 days [2,10].

The first-generation of mAb were entirely murine in structure, sometimes leading to potentially fatal immune responses. Second-generation biologics were engineered as either chimeric (combining human Fc-regions with murine variable regions=two-thirds human) or humanized (the variable region containing relatively more human protein=90% human). Third generation biologics are fully human mAb, yet these still appear to induce production of anti-human mAb. The mAb currently licensed for in MS have proven high efficacy in phase 3 studies and are therefore used in patients with high disease activity. Fully human mAb, such as ofatumumab, are the least immunogenic category of mAb. Ofatumumab should have a lower incidence of production of ADAs than the chimeric antibody RTX or the humanized antibody ocrelizumab (OCR) [2,10].

mAb targeting specific Fab domains of CD20-expressing lymphocytes B represent an important treatment option for patients with MS. Currently available anti-CD20 mAb induce B-cell depletion mainly through ADCC, CDC and ADCP. These anti-CD20 therapies include RTX and ublituximab (chimeric), OCR (humanized), and ofatumumab (fully human) (Figure 4). Three anti-CD20 mAbs are currently available with OCR and ofatumumab labeled for treatment of MS and RTX frequently used off-label anti-CD20. mAb further differ in their structure, immunogenicity (chimeric, humanized, fully human or glycoengineered), the CD20 epitope they recognize, the relative degree of ADCC and CDC they exert, route of administration (intravenous or subcutaneous), pharmacokinetics, and required infusion times. RTX, OCR and ublituximab bind to the large extracellular loop of CD20, while ofatumumab binds to the large and the small extracellular loops (Figure 5). RTX required a 10-fold higher concentration of CD20 on the surface of target cells to induce CDC than was needed by ofatumumab, and binds to CD20 less tightly and has a higher dissociation rate from CD20 than ofatumumab. This indicates that ofatumumab is less dependent on the density of CD20 on the surface of target cells than RTX [1,2,3,6,8,11].

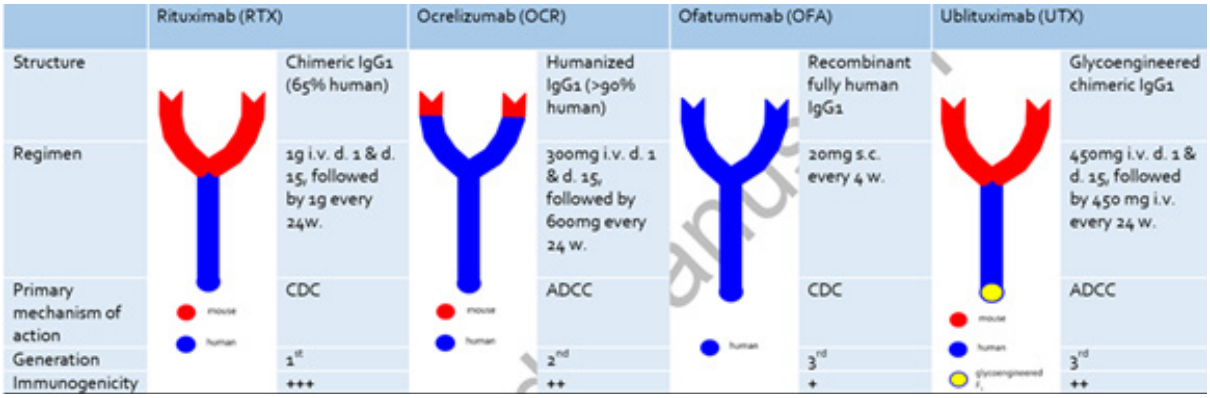

Figure 4. Overview of CD20-monoclonal antibodies currently implemented in Multiple Sclerosis.

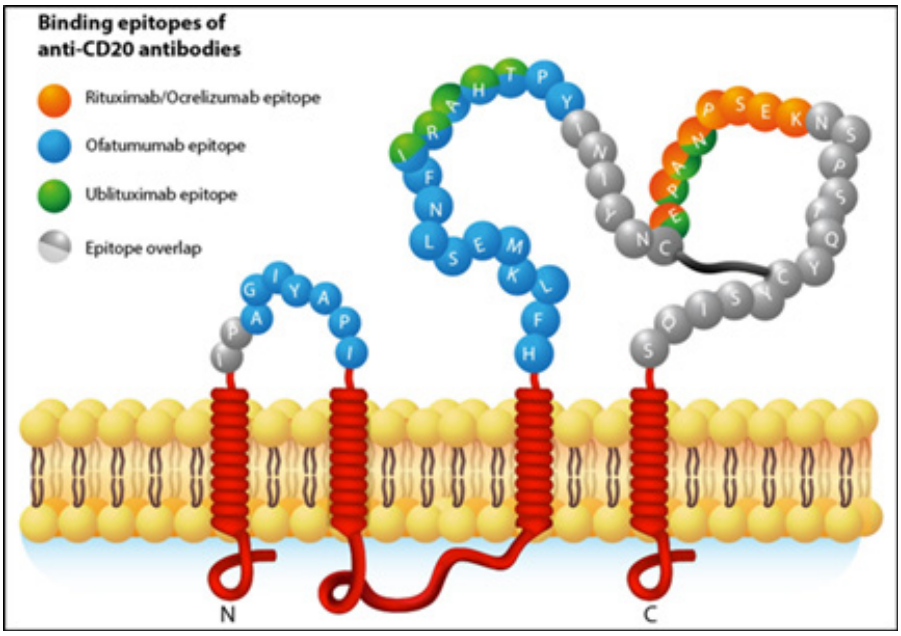

Figure 5. Target epitopes of antiCD20 monoclonal antibodies of interest.

Effect on circulating B and T cells, and B cells in lymphoid organs

B cell changes

Anti-CD20 therapies rapidly and almost completely deplete circulating CD20+ B cells, but limitedly penetrate lymphoid organs. As only 15% of circulating lymphocytes are B-lymphocytes, and most B cells reside in the bone marrow and secondary lymphoid organs, only a small decrease in total lymphocyte counts are usually observed. Furthermore, data from humans indicate that anti-CD20 mAb induces a significant, but often incomplete, depletion of B cells in the bone marrow, spleen, and lymph nodes [6].

Administration of anti-CD20 therapies causes selective loss of B cell lineage cells responsible for antigen presentation and cytokine production which remain low for at least 1-2 years, without affecting B cell reconstitution or preexisting humoral immunity. It is important that both early (precursor pro-B cells) and late maturation stages (long-lived plasma cells) are not depleted because they do not express CD19 or CD20. Following B-cell therapy, repopulating B cells consist of larger numbers of CD20-negative early naïve B cells and fewer antigen-educated memory B cells and plasmablasts, possibly explaining the continuing suppression of MS disease activity noted even after B-cell reconstitution has occurred. The practical implication of differential CD20 expression is that anti-CD20 mAb tend not to substantially reduce IgG antibody levels despite profound depletion of CD20+ B cells, because plasma cells that produce most IgG are not depleted by anti-CD20 mAbs and B regulatory cells producing anti-inflammatory cytokines increase [3,6].

The infusion of anti-CD20 mAb promotes a rapid depletion of CD20+ B cells within hours, mainly occurring in the liver. A negligible peripheral B cell count can be seen as early as 4 days following infusion and depletion reaches the nadir typically after 8 weeks. Radiological benefit is demonstrable at 4 weeks and clinical benefit apparent at an average of 8 weeks. The duration of effect of the specific anti-CD20 mAb is variable but thought to be typically 6 to 9 months depending on dose and features of mAb. The recovery of total B-cell numbers generally occurs after 12 months; in particular, the repopulated B-cell compartment mostly includes naïve cells, while the depletion of memory B cells (MBCs) may persist in peripheral blood even 5 years after treatment [1,7,8,12].

CD19 in contrast to CD20 is expressed also on pro-B cells and plasma cells. B-cell counts are usually determined using CD19, which largely overlaps with CD20 during B-cell differentiation and also is less prone to potential antibody interference in presence of anti-CD20 antibodies. Various studies have indicated almost complete (>98%) depletion of circulating CD19+ B cells within two weeks of infusion of anti-CD20 mAb. Furthermore, CD19-directed mAb have a broader coverage of the B-cell lineage [1,3,6,7,13,14].

T cell changes

Anti-CD20 treatment also alters T cell function and markedly reduces the proliferation and pro-inflammatory cytokine production of CD4+ (Th1 cell and Th17 cell) and CD8+ T cells (IFNγ and IL-17), while increasing regulatory T cells. In a study, a significant reduction in both blood (by 12% from baseline) and CSF (by > 50%) T cell counts was observed, 20-32 weeks after treatment with RTX was completed. A

minor subpopulation of CD3 T lymphocytes, CD8 more than CD4 T cells, also display the CD20 antigen [1,3,4,6,7,14,15].

Within the T cell pool, almost complete depletion of CD20+ T cells was observed at 1-2 weeks post-treatment and only partial repopulation had occurred by weeks 25-52 (3% frequency). Most of the surviving CD20+ T cells were CD4+ T cells, although these cells made up only 36% of CD20+ T cells (vs. 60% CD8+ cells) pre-treatment. However, treatment with anti-CD20 mAbs did not have any significant effect on circulating NK cell counts. These quantitative and qualitative changes in both cellular and humoral arms of the adaptive immune system clearly form the basis for the therapeutic efficacy of anti-CD20 mAb in MS [16].

Innate immune cells

Treatment with anti-CD20 mAbs did not have any significant effect on circulating monocyte counts.

Effect on B cells and immunoglobulins in the CSF

Although anti-CD20 mAb almost do not cross the BBB due to their large size (approximately 150 kDa) and the CSF concentration of RTX has been estimated to reach only 0.1% of that in serum after intravenous administration, they eliminate B cells in the CSF and the CNS perivascular space without a detectable effect on the IgG index or oligoclonal bands, which heralds low pathogenetic impact of autoreactive Igs in MS. Depletion of B cells in the blood is accompanied by significant reductions of these cells in CSF, observed at 20- to 32-weeks post-treatment completion. Moreover, B cells reconstituting after anti-CD20 treatment produce less pro-inflammatory and more regulatory cytokines in the CSF for at least six months. In a phase 2 trial of patients with RRMS receiving RTX as add-on therapy, decreases of both B- and T-lymphocyte counts were observed in CSF [3,6,12,15,17].

Growing evidence supports the hypothesis that the progressive phase of MS might be associated with intrathecal compartmentalization of inflammatory cells and meningeal B-cell follicles that may drive inflammation behind a closed BBB and the lack of efficacy of RTX in PPMS may be attributed to the very low concentrations in the CSF achieved after IV administration, insufficient to affect the compartmentalized CNS inflammation, which arguably drives progressive MS. Thus, several studies investigated the effect of intrathecal administration of of RTX in MS, as intrathecal RTX administration showed a 20-fold increase in CSF bioavailability compared to i.v. infusion (2% vs 0.1% after i.v. administration). The effect of double-blind combination of RTX by IV and intra-thecal (IT) injection vs placebo was tested in the RIVITALISE study. Although IT RTX nearly completely depleted B cells in the CSF, this effect lasted only 3 months, B cells in CNS tissue were inadequately depleted, T cells were not depleted, and neurofilament light chain (a marker for axonal damage) did not change. Interestingly, aftrer administration of low-dose RTX via lumbar puncture as single doses (1-25 mg) or via intraventricular catheter, CSF clearance of RTX is rapid, likely mediated through Fc-receptor-mediated immunoglobulin efflux, in turn leading to a profound depletion of peripheral B cells for up to 12 months. The authors concluded that the intrathecal RTX administration might be effective on intrathecal B cells and it could be adopted to reduce systemic doses, thus reducing risks. However, Intrathecal administration of ultra-low doses of RTX does not seem to efficiently suppress biomarkers of inflammation or neurodegeneration in PMS. Moreover, the B-cell depletion in the periphery was complete for up to 12 months, but incomplete and transient in the CSF and CNS, without any change in the number or appearance of leptomeningeal enhancement. Together with insufficient saturation of CD20, low CSF levels of lytic complement and cytotoxic CD56dim Natural-killer cells, this may have contributed to decreased efficacy of RTX in the CNS and inefficient intrathecal B-cell depletion. These findings provided more evidence for the difficulty of targeting the inflammatory process in the CNS and meninges [1,3,6,8,12,18-20].

There is also a rare, small subsets of CD3+ CD4 and CD8 T cells that express low levels of CD20 and such T cells are also depleted by anti-CD20 mAbs. The proportion of CD20+ T cells in blood are increased in MS patients, they have a proinflammatory phenotype, and accumulate in the CSF where they correlate with disease severity, and may therefore mediate treatment effects of anti CD20 therapy [3,6,12,14,20].

Rituximab

Pharmacology and pharmacodynamics

RTX is a second-generation mouse-human chimeric IgG1 mAb to CD20 and the first mAbs marketed since 1997, in the category of B cell depleting therapies, targeting the CD20 antigen on B cells. Compared to OCR, RTX binds weaker to the low-affinity variant of FcɣRIIIa, which is present in over 80% of MS patients. These data may explain why RTX induces more CDC (and less ADCC) and displays a higher incidence of IARs when compared to OCR. It causes rapid and complete depletion of B cells in in peripheral blood and to a certain extent in peripheral lymph nodes and in bone marrow and, to a lesser degree, also in the CSF. Interestingly, one report also indicates that immunoglobulin M OCBs are a potential marker for more active inflammatory MS and patients manifesting these OCBs might therefore better respond to RTX. Due to its intravenous route of application, its bioavailability is

100% and the terminal elimination half-lives is 22 days. RTX was initially approved for various therapeutic indications, including onco-hematologic and auto-immune diseases; non-Hodgkin's lymphoma (NHL), chronic lymphocytic leukemia (CLL), rheumatoid arthritis (RA), granulomatosis with polyangiitis, and microscopic polyangiitis, pemphigus vulgaris, and was being used off-label in several neurological diseases, including neuromyelitis optica spectrum disorder (NMOSD), myasthenia gravis, chronic inflammatory demyelinating neuropathy paraproteinemic neuropathies and MS. Historically, RTX has been used more often in treatment of NMOSD in the United States (US) but has higher rates of use in European nations for MS [12,17,21].

For commercial reasons, RTX never progressed to phase 3 studies in RRMS. Contemporary use of RTX in MS is off-label with variable uptake worldwide; it has largely been supplanted by OCR, a humanised anti-CD20 agent, for the treatment of both RRMS and PPMS after three phase three clinical trials [7,22].

Dosing regimens and pharmacokinetic aspects of Rituximab

In randomized clinical trials, the induction regimens were grouped as follows [8] :

- Two 375 mg/m2 infusions 15 days apart.
- Two 500-1000 mg infusions 15 days apart in RRMS and 1000 mg intravenous twice 2 weeks apart every 24th week in four cycles in PPMS patients.
- Four 375 mg/m2 infusions every week for 4 weeks.

The magnitude and duration of B-cell depletion is dependent upon dose, treatment intervals

and the duration of treatment. Due to the absence of formal head-to-head trials for therapy regimen comparisons for RTX in MS, there are neither consensus nor treatment guidelines on therapy protocols. The B-cell repopulation has a significant individual variability. At the beginning, basic dosing and interval strategies for RTX in MS have been adopted from RTX usage in oncology and RA. It has been shown that an almost complete B-cell depletion occurs within a fortnight of infusion, usually persisting for 6-12 months and suggests that relapse risk remains low with extended infusion intervals of RTX. Following RTX administration in RA patients of either four weekly infusions of 375 mg/m2 or two 1,000 mg infusions two weeks apart, naïve B cells returned to baseline levels after 12-16 months. In contrast, CD27+ memory B cells were present at 25% of their baseline level at 25 months. In a study by Yamouth et al., induction with RTX 2000 mg was associated with no evidence of disease activity [8,17,23-25].

In other studies, the initial RTX dose required to achieve the clinical effects and B-cell depletion, and the time to B-cell repopulation may considerably vary with a reported prolonged B-cell depletion lasting over 3 years following a single dose of RTX. In a study involving 439 PPMS patients, about 40% of them had recovered peripheral B cells 48 weeks after their last dose (2×1000 mg 2 weeks apart). In another study 26 RRMS patients had a reconstitution to a mean of 35% of baseline counts, 48 weeks after 2×1000 mg, 2 weeks apart, in particular with a greater amount of naïve B cells rather than memory B cells, producing less proinflammatory and more regulatory cytokines. While overall B-cell repopulation rate depends on RTX dosing (250-2000mg), it is of interest that a single RTX cycle (2×1000mg RTX, 2 weeks apart or 3×375mg/m2 every fourth week) leads to long-term suppression of the memory B-cell compartment. Morever, the development of PML in patients treated could be a fearful complication, so it remains unclear whether high doses of RTX are safe or necessary for sustained clinical efficacy in inflammatory diseases [2,8,11,12,26].

Common approaches include the IV administration of two 500-1000 mg at day 0 and 14. This can be followed by single or repeat double doses of 500 mg rituximab intravenously at 6 months intervals. Alternative approaches include the administration of 375 mg/m2 at intervals following B cell repopulation measured by CD19 or CD20 of 1% or more. However, no further study specifically compared the use of a standard (e.g. RTX 2000 mg) versus personalized induction schedule. A Swedish study investigating exclusively RTX patients (n = 822), of which 32.6% received an induction dose of 2000mg, demonstrated discontinuations due to AEs to be 5.2% (mean follow-up time 21.8 months). Reducing the induction dose to 1000 mg or 500 mg may reduce AEs, and maintain effectiveness as previous studies have shown no significant difference in B-cell reconstitution at 6 months after induction doses of 1000 mg and 2000 mg [22].

Interestingly, different maintenance regimens (i.e. fixed vs cytofluorimetric based) were not associated with ARR or time to first relapse, 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. Nowadays, in European countries, and for most of the neuro-immunological diseases including MS, RTX doses of 500 mg are typically administered every 6-9 months. Indeed, The currently used dosing strategy in Sweden consists of one intravenous dose of 500mg RTX every 6 months, since it was demonstrated that this regimen can determine a CD19+ B-cell suppression at 6 months after infusion comparable to the 1000 mg dose one with a better safety profile and a substantial cost-saving (given that the cost of RTX is related to the dose

administered). Anti-CD20 dose interval extension (beyond the regular 6- month interval) could be also considered in patients with RRMS with stable disease especially in case of increased susceptibility to infections, lowered immunoglobulin levels, scheduling of vaccinations, or planning of pregnancy, without incurring risk of return of inflammatory disease activity in the short to medium term. An optimized therapy scheme could potentially improve the efficacy and safety profile. Thus, further studies are needed to find the optimal dose, to identify the administration interval and route of administration, possibly individualized by adjustment to immunological parameters (memory B cell reappearance) and disease activity. It may be interesting to investigate if a reduced dosing schedule adjusted to CD19 cell concentrations or immunoglobulin replacement can reduce the risk of infections, while preserving efficacy and the favorable safety profile [8,17,23-25].

Monitoring and screening

It has been suggested that monitoring circulating memory B cells (CD19+ and CD27+), 5 months after infusion could be a viable strategy to control relapsing MS, in order to schedule a personalized treatment regimen (RTX re-infusion) and to identify "early re-populators" at risk of disease relapse in order to retreat them before disease progression and avoid the overtreatment of patients with sustained B-cell depletion over time. RTX is associated with rapid almost complete depletion of CD19+ B cells from weeks 2 to 24. By week 48, CD19 cells had returned to 31% of baseline. B-cell depletion resulted in markedly diminished proinflammatory Th1 and Th17 responses of CD4 and CD8 T lymphocytes. Re-infusions based on CD19+ cells reappearance was defined when CD19+ B cells reach 1% of lymphocyte counts; however, other criteria are also applied, including 2% of CD19+ B cells, while re-infusions based on CD27+ memory B (CD19+) cells reemergence when this population exceeded 0.05% of peripheral blood mononuclear cells in the first 2 years and 0.1% in the following years [8,14,26].

Apart from routine laboratory tests, baseline immunoglobulin levels and serum levels of IgG every 6 months should be determined to adjust the dose of RTX or even substitute immunoglobulins in the case of low levels, as a reduced baseline level of IgG has been associated with higher risk for severe infections. Recent studies of hypogammaglobulinemia during RTX therapy suggested manifold approaches to the problem: closer monitoring of CD19+ B cells before re-administration of RTX in patients with high EDSS scores, monitoring of CD27+ memory B cells in peripheral blood, research of concomitant leukopenia and hypogammaglobulinemia, assessment of vaccine responses in the setting of recurrent infections. Current guidelines advise providing immunoglobulin replacement therapy in hypogammaglobulinemic patients provided they develop recurrent infections, fail to respond to polysaccharide (T cell-independent) and protein (T cell-dependent) vaccines or exhibit IgG levels of under 1-2 g/l. Currently, there is no evidence to suggest monitoring anti-JCV antibodies in patients on RTX [12].

Clinical efficacy of rituximab in MS

RTX is not approved from Food and Drug Administration (FDA) nor European Medicines Agency (EMA) for use in MS and can only be administered off-label for this indication. However, most publications regarding the off-label use of RTX in MS came from a single country, namely, Sweden, where RTX has become the most commonly used DMT for all MS subtypes nationwide, not only as an alternative when previous DMT was ineffective, but also as a first-line therapy, accounting for almost 40% of all DMT used for MS. By June 2017, over 50% of all treatment naïve subjects with MS in Sweden and 10-15 % of all treated MS patients in Norway received RTX as their first DMT and this growth has recently resulted in the Swedish Ministry of Health and Social Affairs planning a risk-benefit analysis of off-label RTX use [12,26,27].

After successful phase II clinical testing for RRMS and phase II/III trial for PPMS, the manufacturer stopped further RTX development for MS and promoted the humanized, anti-CD20 antibody OCR for this indication, recently added to the MS drugs armamentarium. However, evidence for the efficacy and safety of RTX is rising, and it remains the mainstay of off-label MS treatment in different countries as second-line therapy in RRMS patients with suboptimal response to the first-line DMT (escalation therapy) or first-line therapy in highly active MS patients, especially at the early stages of the disease, when B-cell related phenomena are pronounced. In addition, RTX may be a therapeutic option in some PMS patients, for whom there is currently no any approved effective treatment. Furthermore, in case of the presence of a concurrent autoimmune disease, this drug should be considered as a serious choice. The use of RTX to remove B immune cells with CD20 expression makes it possible to interrupt the inflammatory cycle and immunemediated myelin degeneration and achieve extended periods between relapses [11,13,20,27,28].

Although it does not hold regulatory approval for this indication, this anti-CD20+ antibody, with the same mechanism of action as OCR, should be also considered as a therapeutic option for RRMS and some PMS patients, given its good and well-known efficacy and safety profile with a low discontinuation rate, faster onset of action, long duration of action, convenient administration regimen, favorable

cost-effectiveness profile, emerging from clinical trials and the wide real-world use as monotherapy for RR and some progressive forms. Therefore, RTX has become an interesting option for patients with MS in in several countries, including developing countries [17,29].

Clinical efficacy of Rituximab in RRMS phenotypes

Clinical Trials

A small case series in 2004 first described a favorable experience with RTX in four RPMS patients, which was corroborated in a phase 2, multicenter randomized 48-week control trial (HERMES study) including 104 RRMS patients which demonstrated a drastic reduction in number of total contrast enhancing lesions on MRI when compared to placebo. RTX was also associated with fewer clinical relapses at 48 weeks, with 20.3% of patients in the RTX group experiencing relapses versus 40.0% of patients in the placebo group [5,26,30].

According to Naismith et al., 2010, Hauser et al., 2008, RTX led to 88% and 91% reductions in T1 gadolinium enhancing lesions in RRMS clinical trials. Relapse rates were lower in patients treated with RTX than those who received placebo (14.5% vs. 34.3% at week 24 and 20.3% vs. 40% at week 48) (Hauser et al., 2008). Despite the efficacy of RTX in RRMS, studies were stopped following phase II trials and the focus shifted to OCR and ofatumumab [31].

RIFUND-MS provides evidence that rituximab given as 1000 mg followed by 500 mg every 6 months is superior to dimethyl fumarate in preventing relapses over 24 months in patients with early relapsing-remitting multiple sclerosis [5,26,30].

Another population-based Swedish study explored the efficacy and drug discontinuation rates among 494 patients with newly diagnosed RRMS across all more frequent DMT. The ARR and/or neuroradiologic disease activity were lower for RTX compared to all other DMT [31].

Real-world data and retrospective studies

Besides clinical trials, a large number of studies have used real-world data, obtained from the wide off-label use of RTX, to assess its efficacy and safety in MS patients. Salzer et al. (2016) ; Disanto et al. (2020) ; Zecca et al. (2020) ; Naegelin et al. (2019) ; Hellgren et al (2020) ; Alcalá C (2018) ; Airas et al (2020) ; Bellinvia A (2020) ; Yamout BI et al (2018). Although further exploration of efficacy has not been carried out in phase-III RCTs, several other observational studies have confirmed a significant reduction of disease activity with RTX [1].

In a cohort of MS patients with an aggressive form of the disease, followed for a mean time of 30 months, RTX was safe and useful for controlling the inflammatory activity, and has shown a high efficacy over relapses and the progression of short-term disability in patients with active RRMS. RTX helped to achieve NEDA status in both RRMS and PMS (PPMS-PPMS and SPMS). Based on these benefits, RTX has been largely administrated out of label in RRMS patients who experienced disease activity on the standard therapies, and also in PMS [32].

Meta-analysis

RTX was the first anti-CD20 mAb tested in MS by several groups in RRMS. Different meta-analysis, including sevral studies showed a significant decrease of ARR, gadolinium-enhanced lesions (GEL) on MRI, and EDSS score. Based on these benefits, RTX has been widely administered off-label to RRMS patients who experience disease activity on the standard therapies [18,19,23,33].

Indirect comparisons of Rituximab with other DMT

Currently, no head-to-head RCTs comparing RTX with other DMT have been completed and several ongoing clinical trials are comparing DMT, including RTX. Previous retrospective observational studies suggest superior efficacy of RTX with regard to rate of clinical relapses, radiologic disease activity, compared with interferon (INF), glatiramer acetate (GA), dimethyl fumarate (DMF) in treatment-naive patients with RRMS starting a first DMT and significantly better effectiveness of RTX compared with fingolimod (FGL) in patients switching from natalizumab (NTZ) due to John Cunningham virus (JCV) antibody positivity. In a retrospective study by Alcalá et al., RTX has proven to be an effective and safe therapeutic alternative in a small cohort of RRMS patients after fingolimod withdrawal due to suboptimal response or side effects [34,35].

Compared with natalizumab, ARR and GAD+ lesions were numerically lower but did not reach statistical significance. Both NTZ and RTX demonstrated superiority compared with FGL, in suppressing clinical and neuroradiological disease activity in patients with RRMS switching from INF/GA due to breakthrough disease and naive patients. Furthermore, patients with RRMS that stopped NTZ treatment, the switch to off-label RTX resulted efficacious in preventing disease reactivation or rebound and in maintaining radiological stability. RTX resulted to be a valid post-NTZ treatment option, for cases where NTZ administration cannot be continued for any reasons (positive JCV serology). Depending on the previous therapeutic regimen, we strive a latency of 2-8 weeks (6-8 weeks in the case of NTZ, before induction with RTX. During RTX therapy, we monitor patients clinically at least every 6 months and radiologically at least yearly [12,31,36,37,38].

Another small retrospective study, including patients with RRMS all of which had failed first-line therapy (IFN and glatiramer) and second-line therapy (NTZ/FGL), confirmed RTX as a safe and effective second-or third-line DMT and can be regarded as an off-label salvage therapy for active resistant RRMS, who despite treatment with high efficacy drugs are still experiencing MS relapses. No patients had a clinical relapse, MRI activity was not detected and the EDSS scores improved [13,38,39,40,41].

Real-world studies also demonstrated a lower discontinuation rate compared with INF, GA, DMF, FGL, and NTZ, related to a good benefit/risk profile, and a good compliance. The most common cause of treatment discontinuation was pregnancy for RTX, disease breakthrough and AEs for injectable DMT and DMF, disease breakthrough for FGL, and positive JCV serology for NTZ, giving rise to increased risk for PML with increasing treatment duration [34].

Given the importance of starting treatment in RRMS with active disease as early as possible to reduce disability accumulation, RTX was evaluated as induction therapy (add-on therapy). The study results indicate that induction therapy with RTX followed by GA was superior to placebo induction and GA monotherapy in reaching NEDA in patients with active MS, although the effect appeared to be temporally limited. Thus, a single dose of RTX is, by itself, an inadequate induction agent in MS. It is unknown whether multiple doses of RTX every 6 months would have a more sustained effect, including beyond the expected return of B lymphocytes after cessation of the intervention [8,17].

Case reports

Several case reports convincingly demonstrated that RTX not only mitigated or arrested progression of a fulminant disease course but also led to clinical improvement [3].

Clinical efficacy of Rituximab in SPMS and PPMS phenotypes

A further knowledge gap is represented by the use of RTX for the treatment of PPMS; indeed, even if data showed that younger PPMS patients, particularly those with inflammatory lesions, may benefit from RTX, the effectiveness of RTX in PPMS needs to be further explored also taking into account specific clinical variables, such as age, disease duration, comorbidities and evidence of inflammatory activity defined by clinical relapses, progression rate and MRI data [8].

In patients with an active progressive disease, the ARR significantly improved compared with

the reported pre-administration drug. However, discrepant results have been reported in studies on the effects of RTX on degrees of disability among PMS patients especially in active cases. In some publications, most of them remained stable after RTX treatment, without significant changes in the EDSS score, while in others it increased or dimished. Naegelin et al showed a significantly lower EDSS score during a mean follow-up of 3.5 years and a significantly delayed time to confirmed disability progression for patients treated with RTX compared with matched patients never treated with RTX, suggesting a potential therapeutic benefit of RTX also in PMS. However, a meta-analysis with a massive collection of data from MS patients can thoroughly address the mentioned issue [13,32,39,40].

In 2009, A phase II/III randomized double-blind, placebo-controlled manufacturer sponsored multicenter trial of 439 patients with PPMS (**The OLYMPUS trial**) studied the effect of RTX on adult patients with PPMS. At week 96, treatment with RTX, compared with placebo was associated with a reduction in the proportion of patients with CWD, defined by an increase in the EDSS score sustained for 24 weeks. A statistically significant effect of RTX on CWD rate was demonstrated in patients younger than 51 years with baseline GAD-enhancing lesions. RTX treatment was also associated with significantly lower increase in T2 lesion volume and with lower worsening in the Multiple Sclerosis Functional Composite (MSFC) timed 25-foot walk test (therefore in the ambulation) at week 96, whereas brain volume decrease was similar to placebo. It is thus likely that RTX may have a better effect early in the disease course when the inflammatory component is most prominent. As possible biomarkers, GEL representing inflammatory processes may serve as good response to RTX treatment [7,13,28,34].

Safety and tolerability

Infusion associated reactions (IARs)

Defined as those reported during or within 24 hours of an infusion. There are no studies comparing the safety profile of different anti-CD20 therapies. Evaluating IARs across studies is challenging given different premedication regimens [26].

Globally, RTX has a good safety and tolerability profile, despite a higher rate of IARs compared with FGL (26% vs 7%). Two smaller studies reported 25-26% of patients being affected by IARs. In two randomized clinical trials, IARs are relatively common with use of RTX in MS, appearing in 67.1% (placebo: 23.1%) and 78.3% of patients (placebo: 40.0%) respectively, after the first infusion, likely due to cytokine release accompanying CD20 B cell lysis. IARs levels decreased to those observed in placebo arms with subsequent infusions. The vast majority of these reactions are mild-to-moderate in severity not inducing hospitalization or treatment discontinuation and include fever, rush, and chills. Other frequent IARs include nausea, vomiting,

pruritus, angioedema, throat irritation, bronchospasm, hypotension, rhinitis, urticaria, headache, myalgia, dizziness, and hypertension. The IARs typically arise 30-120 min after initiating the first infusion and usually resolve with slow withdrawal, infusion discontinuation or symptomatic treatment. Allergic anaphylactic reactions are less commonly observed and the incidence of severe hypersensitivity reactions is < 10% in cancer patients treated with RTX and they rarely necessitate treatment discontinuation. At least 30-60min before RTX infusion, premedication with an antihistamine and methylprednisolone (100 mg or an equivalent) is recommended to prevent IARs. In addition, paracetamol can be administered on the day of the infusion to avoid side effects such as headaches and the patients should be monitored after the infusion for 1h. These AEs could be influenced by peripheral B cell level and CD16 expression and it is possible to speculate that previous immunosuppressive treatment could influence B and NK cell activities, reducing the risk of IARs after RTX administration. However, larger studies are needed in the attempt to find a predictive model for these events and identify the patients who may need a more aggressive premedication before RTX infusion [6,8,11,20,27,41,42].

In the phase III studies of OCR, infusion reactions were reported in 34% of the patients treated with OCR, versus 10% treated with IFNB-1a or placebo, in OPERA I and II, and in 40% treated with OCR versus 26% with placebo in the ORATORIO trial. For ofatumumab, a similar level of infusion reactions have been reported (41%-66% vs 15% for placebo). However, preliminary results from an ongoing phase III trial, reported a similar incidence of IARs between patients continuing RTX and those switched to OCR and suggested a correlation between levels of CD19/CD20 B cells and risk of IAR (with a decrease by 74% of the risk when CD19 and/or CD20 were ≤1%), suggesting that switching between them is safe.These results indicate that the use of RTX in patients with RRMS is associated with adverse events that are frequent but not serious and occur less frequently with subsequent infusions [8,12,35].

The low frequency of IARs in our patient could be explained by the use of low doses of RTX for induction and maintenance regimen, asscіated with efficious premedication [8,12,35].

Susceptibility to infections

Long-term safety of RTX is well documented not only in MS but also in other conditions, such as RA, where prolonged exposure for 11 years was well tolerated and not associated with increased safety risks, including serious opportunistic infections and progressive multifocal leukoencephalopathy (PML). However, other studies have reported an increased risk for different types of infections, mainly affecting respiratory and urinary systems in patients treated with RTX, especially after longer treatment periods, compared with NTZ, FGL, IFN beta, and GA. However, RTX had a lower incidence of herpetic infections than FGL or NTZ. In the RCT of RTX in PPMS, serious infections occurred in 4.5% of RTX-treated patients and in <1.0% for placebo, however, with no clear association to the number of infusions, which corroborates findings from large RA trials [2,8,17,26].

Reactivation of tuberculosis, viruses (hepatitis B, herpes zoster and HIV) have been reported in patients treated with anti-CD20 medications. Consequently, all patients should be screened for latent infections before starting treatment. Indeed, especially in endemically affected areas or populations, the risk of tuberculosis reactivation should be considered through specific prescreening and active surveillance with latent tuberculosis testing. Although less frequently reported, other possibly RTX associated infections include cytomegalovirus (CMV) and herpes simplex virus (HSV) [8].

A new retrospective study, comprising RRMS and SPMS patients treated with RTX (n=311) and RRMS patients treated with OCR (n=161), found that OCR, but not RTX, was associated with a decrease in IgG of 0.16 g/L with each infusion (a reduction that may increase susceptibility to infections), whereas IgM decreased to a similar extent with both drugs. Infections and serious advers events were more common in the OCR group. In another recent study, frequency of reported infections (especially oral herpes, urinary tract infections, and nasopharyngitis) was nearly two times higher with OCR [43,44].

The minimal effect on serum immunoglobulin levels is explained by the fact that RTX and other CD20-targeted treatments do not directly deplete plasma cells because these cells do not express CD20 (except a small population of CD20+ plasmablasts). However, long-term treatment with anti-CD20 agents can cause sustained hypogammaglobulinaemia (≥4 months) and the attendant increased risk of severe infections. Hypogammaglobulinaemia, not explicitly defined in the HERMES study but commonly defined as a serum IgG level of less than 6 g/L, was seen more frequently in patient treated with RTX compared to placebo group (7.9% vs 3.0%), and represents a complication in over half of patients treated with mid to long-term B cell depleting therapy (64% in a British cohort of 50 patients, 52% in an Italian cohort of 21 patients treated for NMO or NMOSD). IgM hypogammaglobulinemia, even though more frequent, is less clinically significant than IgG hypogammaglobulinemia, while IgA depletion is even more seldom (IgG 38%, IgM 56%, IgA 18% in an NMOSD cohort treated with RTX). Anti-CD20 mAb-induced hypogammaglobulinemia accumulates

incrementally following successive treatment courses, reaching a nadir typically after several cycles (mean nadir of IgG of 4.5 g/l recorded after a mean of 3.4 years on RTX). Moreover, low gamma-globulin baseline levels may be more relevant than treatment duration/cumulative RTX doses in predicting the development of hypogammaglobulinemia. For all these reasons, the measurement of total serum immunoglobulins before starting RTX and at least yearly during treatment is strongly recommended. Upon ceasing RTX treatment, seldom, persistent hypogammaglobulinemia develops, putatively owing to pre-existing B cell maturation defects or due to long-lasting effects on bone marrow B cells. The underlying mechanism for development of hypogammaglobulinemia is not known, but may result from depletion of CD27+ memory B cells, plasmablasts, bone marrow plasma cells (by impairing maturation of naïve B cells) or because of the increased likelihood of T cells interacting with non-B cell APCs and/or due to diminished B-cell-secreted cytokines such as B-cell activating factor (BAFF) or interleukin 6 [4,7,8,12,26].

Late-onset neutropenia (LON), a severe adverse event, is defined as an absolute neutrophil count of < 1.5 × 10 to the power of 9/L occurring > 4 weeks following the last dose and was described as a rare complication during RTX treatment. RTX-related immunogenicity may have been the pathophysiological mechanism behind a LON and is associated with a higher infection rate during the neutropenic period. Patients treated with rituximab should be screened for hypogammaglobulinemia and neutropenia, as these may present independent risk factors for developing infections [8,11].

While development of progressive multifocal leukoencephalopathy (PML) remains a potential risk in MS patients treated with anti-CD20 B cell depleting therapies, this risk remains rare and its incidence is estimated to be one case per 32,000. Cases of PML have not been reported in patients treated with RTX strictly for MS. PML has occurred in patients treated with RTX for other conditions such as CLL and RA or following other immunosuppressive treatments in the setting of B-cell lymphoma and rarely in rheumatic diseases. However, CLL is itself a risk factor for PML. Recently, in the nationwide register-based cohort study conducted in Sweden, one case of RTX-related PML was described (the patient had switched from NTZ within 6 months before the diagnosis of PML. The mechanisms underlying viral reactivation after RTX treatment could also involve the changes in T-lymphocyte activity after B-lymphocyte depletion due to the alteration of T-lymphocyte cytokine profiles. However, even if there are no specific recommendations to screen patients for JCV prior to administration of RTX, it is important for clinicians to keep in mind that RTX may be associated to PML, and it is crucial to suspend therapy in the event of signs and symptoms suggestive of PML, and urgently carry out a specific workup in order to reduce morbidity and mortality [2,8,34].

COVID 19 infection risk

Accordingly, the Society of Italian Neurologists (SIN), the Association of British Neurologists (ABN) MS and Neuroimmunology Advisory Group practical guidance recommended to delay further infusions of anti-CD20 drugs, as that anti-CD20 therapies may probably increase the risk of COVID-19 infection and that infection severity may be greater in those treated with anti-CD20 for a longer period of time. Complete B-cell depletion and the decrease of immunoglobulin G (IgG) level in both patients and the persistent viremia in blood samples could be correlated with increased morbidity, suggesting that B-cell function might be one important mechanism in resolving SARSCoV-2 infection. Particularly referring to OCR, it has been recommended to consider the initiation of this drug only if a high-efficacy drug is required and the use of NTZ is contraindicated. However, several data have shown encouraging results, suggesting that immunosuppression, or at the least the moderate immunosuppression induced by DMTs, may have a protective effect against the development of severe COVID-19 infection [8].

Malignancies

Sporadic cases of malignancies in RTX-treated MS patients have been reported. In a large Swedish nationwide study, no higher risk of malignancies was found in RTX patients compared to the general population. The most common invasive cancers in RTX treated patients were breast, melanoma, colon and nonmelanoma skin cancer. No imbalance in subtypes of invasive cancers was found [2,8,11,26].

Anti drug antibodies (ADAs)

Due to the chimeric nature of RTX, the frequency of ADAs is higher than that reported with OCR, which decreased after repeated RTX infusions, and was associated with incomplete or unmaintained B-cell depletion, but not with infusion reactions, adverse events, or treatment failure, with a strong suppression of disease activity observed in both antibody-positive and antibody-negative patients. Such a failure could relate to the higher level of immunological activity found in the earlier relapsing stage of the disease. In the OLYMPUS study, 20 out of 286 (7%) patients with PPMS who received RTX tested positive for human anti-chimeric antibodies (HACA) during the treatment or safety follow up. A large cross-sectional study by Dunn and collaborators, including patients receiving off-label RTX for MS (both RRMS and

PMS), reported the development of antirituximab antibodies in 34% of patients (a percentage higher to that observed in clinical trials). Recently, a large cross-sectional real world using a more sensitive technique showed ADA in 38% of the RRMS patients and in 27% of PPMS. Interestingly, a negative relationship was found between the number of infusions and the frequency of ADAs. However, few cases of delayed hypersensitivity reactions, associated with ADAs forming immune complexes and observed in RTX use for other indications, have been reported in MS. Actually, there is no consensus on the matter of whether RTX treatment should be stopped based on the presence of ADA when evidence of disease activity is absent, as previously discussed in an article by Phiel and Hillert. These findings corroborate the idea of the non-inferiority, in terms of tolerability and safety, of RTX to OCR. Thus, in the absence of head-to-head trials, the choice of RTX or OCR should be made carefully on the basis of efficacy and safety issues [2,8,12,17,26,45,46,47].

Other Aes

Studies of RTX in MS and non-MS populations have reported several AEs involving cardiovascular system (i.e., angina pectoris, cardiac arrhythmias, heart failure and/or myocardial infarction), upper and lower airways (i.e., bronchospasm, chest pain, dyspnoea, cough, rhinitis), gastrointestinal system (i.e., vomiting, abdominal pain, dysphagia, stomatitis, constipation, dyspepsia, anorexia, Reflux disease, abdominal pain, diarrhea, gastritis, pharyngolaryngeal pain), musculoskeletal and connective (i.e., myalgias, arthralgias, arthritis ; hypertonia, pain), nervous system (paresthesia, hypoesthesia, agitation, insomnia, vasodilatation, dizziness, anxiety, fatigue, neuropsychiatric disorders), skin (i.e., rash, itching, pruritus, alopecia) and endocrine system [8,12,45].

Vaccinations

Data from the oncology and rheumatology literature have shown that the response to vaccination may be ineffective in patients receiving RTX. It is recommended to wait at least 6 months after RTX for vaccination, while patients should be advised to complete any required vaccinations at least 6 weeks prior to RTX initiation. Particularly, vaccinations for hepatitis B, pneumococcus, tetanus toxoid every 10 years and for influenza annually should be undertaken for patient considered for RTX therapy. EMA and FDA labels allow inactivated vaccines to be given to patients receiving RTX, whereas live-attenuated or live vaccines are not recommended during RTX treatment and until B-cell recovery since, currently, there are no sufficient data on the potential risk of vaccination with this kind of vaccines [8].

Available data indicates significantly reduced humoral immune response to SARS-CoV2 vaccines (15-60% developing antibodies) in patients on RTX compared to healthy controls. However, there is growing evidence that T cell responses may be preserved or even augmented under anti-CD20 mAbs, potentially mitigating the loss of antibody-mediated vaccine efficacy [8].

Pregnancy and breastfeeding

Since the therapeutic effect of anti-CD20 therapies last much longer than their pharmacological half-life, they could be an option for women who wants to become pregnant. RTX is classified as a pregnancy category C drug as there are no adequate and well-controlled studies of RTX in pregnant women. Thus, women are usually advised to attempt conception about 3 to 3.5 months after last infusion of RTX. The European Medicines Agency (EMA) and the FDA recommend that pregnant women should not receive RTX infusion, unless the possible benefit outweighs the potential risk. A study analyzing 90 live birth outcomes of women inadvertently conceiving during or less than 12 months after the treatment of rituximab reported 22 premature births, one neonatal death after 6 weeks, 11 newborns with hematological changes (B-cell deficiency, neutropenia, thrombocytopenia, anemia, and lymphopenia), and two inborn malformations. Anti-CD20 mAbs can be actively transported across placental barrier during second and third semester and subsequently deplete fetal B cells, although low B cell counts have also been reported in newborns when the mother was exposed to RTX even longer prior to conception. Thus, the use of RTX in the pregnant population should be extremely limited but is worth consideration in severe severe cases refractory to first-line agents, after coordination with high-risk obstetrics and pediatrics is advisable [8,12,47-51].

Few studies have examined the effect of RTX administration during lactation in humans. One case study reported levels of RTX in milk 240 times below maternal serum concentrations. In addition, IgG is normally degraded in the gut, also in infants. Thus, due to the lack of largest and studies and definite recommendations, to avoid potential harm to the newborn, women are still advised not to breastfeed during and up to 6 months after discontinuing the treatment [8,12,52-55].

A large observational cohort study, including 586 women with MS onset, showed a relapse rate 1 year post-partum significantly higher in women who suspended natalizumab within 6 months before conception and in women untreated within 1 year before conception compared with women who suspended rituximab in the 6 months before conception. Moreover, in the suspended rituximab women, only one maternal relapse occurred during pregnancy and only one of four patients who relapsed in the first quarter after delivery experienced new GAD+ lesions. These results suggest a prolonged protective effect on MS disease activity of

rituximab, which can encompass pregnancy and postpartum period, without the high risk of disease reactivation or rebound described with natalizumab withdrawal before pregnancy [8,12,56-58].

Pediatric MS patients

As of today, only FGL has been approved for pediatric-onset MS (POMS). In contrast, experiences with RTX in POMS are very limited. A Swedish case series of 14 POMS patients reported favorable outcomes upon RTX treatment, where 500-1000 mg RTX every 6-12 months induced a clinically and neuroradiologically stable disease in 13 out of 14 patients (93%) during a median treatment duration of 23.6months. No serious adverse events were reported and the drug survival was 86% [26,50,59,60].

Cost effectiveness

An American pharmacoeconomic study demonstrated that the off-label use of single dose of 500 or 1000 mg of RTX twice yearly is less expensive than most of the currently available FDA-approved DMTs. The cost of RTX varies greatly between countries. For example, in Sweden, a yearly treatment course with two doses of 500 mg costs 2400 €, while the corresponding cost in the United States is around 7000 €. Introduction of cheaper biosimilars will reduce costs further, but real-world data on their tolerability and effectiveness in MS are currently lacking [8,26,61,62].

RTX is the most cost effective of the three available antiCD20 Ab. The most expensive annual listed price is attached to KESIMPTA (ofatumumab) with an annual listed price of $83,000. In addition, even if the stated price of the recently approved OCR is within the range or less than other current approved DMTs with an annual cost of twice-a-year infusions of $65,000, it remains significantly more expensive than RTX. The prohibitive costs of these newly approved medications will prevent its usage by most MS specialists in the resource limited settings. This makes RTX a very attractive option in developing countries where no other approved B cell therapies are available [2,8,61-63].

Due to its low yearly price which is lower than all injectables, oral therapies, and mAbs, and in view of its good safety and efficacy profile, it has become the DMT of choice for Syrian and Palestinian refugees in Lebanon who have limited financial coverage for all MS therapies. So another advantage of RTX, in addition to efficacy and safety, is that it is a cost-effective therapy [23,64-68].

Biosimilars

Since 2015, FDA and EMA have approved several biosimilars of RTX, such as while other biosimilars are to date in the pipeline. CT-P10 (Truxima®) is the first biosimilar approved for use in all indications reported for the originator RTX. Similar efficacy (CD19+ lymphocyte depletion, relapse rate and evolution of MRI activity), safety, and tolerability were observed in comparaison with its originator RTX. Finally, with a price ranging from 15 to 30% lower than the originator molecule (MabThera®), the development of RTX biosimilars may also significantly contribute to cost savings for healthcare systems [8].

However, although the annual cost of RTX is lower than that of most MS drugs, its access is not universal because its cost remains high for some patients and healthcare services. Biosimilars could represent a relatively cheaper and safe therapeutic alternative and could improve access to a highly efficient therapy for MS in low- or middle income countries (Table 3) [8].

Table 3. Approximate price for the different alternatives (swedish prices), lowest to highest

Rituximab biosimilars	€ 200-400 depending on dose yearly
AHSCT	€ 40 000-80 000 as a one-time cost, averaging approx. € 2000 yearly assuming "saving" 30 years other tx
Ofatumumab	€ 13 000 yearly
Natalizumab	€ 17 000 yearly
Ocrelizumab	€ 17 000 yearly
Ublituximab	Not available presently

Future directions

While anti-CD20 mAbs deplete mainly circulating B cells, it is unclear whether B cells should be depleted also from the CNS or other compartments (eg, bone marrow or lymphatic tissue). The long-term safety of prolonged B cell depletion and the duration of depletion of peripheral B cells are still unknown. Maintenance therapies that would prevent re-emergence of pathogenic B cells after cessation of anti-B cell therapies or divert them toward a regulatory profile should be developed [6].

Using mAbs to CD19, such as inebilizumab (MEDI-551), which targets also pro-B cells, plasmablasts, and plasma cells may provide more complete and prolonged B cell depletion. However, it is still unclear whether depleting broader range of B cells entails greater clinical benefits or more potentially serious adverse events, which result from negatively affecting

B cell reconstitution due to the elimination of earlier stages in the bone marrow or reducing humoral immunity by elimination of antibody-producing cells [6].

Additional approaches with a potential to target B cells that have not yet been explored as MS treatments or have not progressed past phase-II clinical trials include the use of other B cell-targeting mAbs such as epratuzumab (antiCD22, a negative regulator of BCR-derived activation signals), daratumumab (anti-CD38 that depletes plasmablasts and some plasma cells), LTbR-IgG (anti-lymphotoxin beta receptor that would reduce the formation of ectopic germinal centers), NNC114-0005 (anti-IL21, an important cytokine for Ab formation), otilimab (anti-GM-CSF that blocks pro-inflammatory myeloid cell response), belimumab and talabumab (anti-BAFF), VAY736 (anti-BAFF receptor), hBCMA-Fc (human BCMA fused to IgG1 Fc), and mAbs to costimulatory molecules that would prevent B cell activation. In addition, several small molecules that target B cell signaling (through BTK, PI3 kinase, or Janus kinases), proteasome that is involved with plasma cell differentiation, or Epstein-Barr virus, which infects B cells and is believed to be involved in MS etiology, may provide novel mechanisms of targeting B cells and possibly other cells involved in the immune pathogenesis of MS [6].

CONCLUSION

RTX was associated with reduced disease activity, and reduced disability levels in patients with RRMS. RTX was well tolerated and sufficiently safe for treating MS, with minimal and manageable IARs.

Despite few limitations, our study adds to the published literature confirming that RTX was well-tolerated and effective in reducing relapse rate and stabilizing disease in relapsing-remitting and progressive MS patients in our real-world clinical practice setting. However, future multicentric and comparative trials are needed to evaluate the long-term efficacy and tolerability of this low-cost therapy compared with other mAb used for MS.

ETHICS STATMENT

The study involving human participants was reviewed and approved by Ethic Committee of University Hospital of Marrakech, Morocco. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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