

## Rituximab in neuromyelitis optica: A review of literature

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### Abstract

Neuromyelitis optica spectrum disorders, or neuromyelitis optica (NMO), is an autoimmune disease of the central nervous system that must be distinguished from multiple sclerosis. Therapeutic approaches to relapse prevention in NMO include immunosuppressants and monoclonal antibodies. Rituximab, a monoclonal antibody that targets CD20 antigen expressed on the surface of pre-B, mature B-lymphocytes and a small subset of T-lymphocytes, has been widely used for the treatment of NMO. In this review, we aim to summarize global experience with rituximab in NMO. We identified 13 observational studies that involved a total of 209

NMO patients treated with rituximab. Majority of rituximab-treated patients evidenced stabilization or improvements in their disability scores compared to pre-treatment period and 66% of patients remained relapse-free during treatment period. Monitoring rituximab treatment response with CD19+ or CD27+ cell counts appears to improve treatment outcomes. We offer clinical pointers on rituximab use for NMO based on the literature and authors' experience, and pose questions that would need to be addressed in future studies.

**Key words:** Neuromyelitis optica; Rituximab; Longitudinally extensive transverse myelitis; Optic neuritis; CD19+; CD27+

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**Core tip:** Relapsing neuromyelitis optica (NMO) is an autoimmune disorder of the central nervous system that often results in severe disability and death if untreated. Rituximab, an anti-CD20 monoclonal antibody, appears to be a promising treatment option for NMO. In this review, we summarize the results of 13 observational studies that assessed efficacy of Rituximab in neuromyelitis optica. On average, 66% of patients remained relapse-free during treatment period and in the majority of patients disability scores have stabilized or improved. Monitoring response to rituximab with CD19+ and CD 27+ cell counts appears to improve outcomes.

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### INTRODUCTION

Neuromyelitis optica spectrum disorders, hereafter

referred to as “Neuromyelitis optica (NMO)”, is an autoimmune disease of the central nervous system (CNS)<sup>[1]</sup>. Diagnostic criteria for NMO have undergone several revisions in recent years, but core clinical syndromes - longitudinally extensive transverse myelitis and optic neuritis, have been retained. The most recent iteration of the diagnostic criteria for NMO is based on International Panel for NMO Diagnosis consensus paper<sup>[2]</sup>. In Aquaporin-4-IgG (AQP4-IgG) seropositive patients, diagnosis can be made after a single NMO-compatible relapse. AQP4-IgG seronegative NMO criteria include evidence of dissemination in space as well as at least one well-recognized syndrome of NMO, such as ON, LETM or intractable vomiting/hiccups<sup>[2]</sup>.

NMO has been reported worldwide with prevalence ranging from 0.52-4.4/100000<sup>[3]</sup>. In Western counties, NMO is rare relative to multiple sclerosis (MS) - ratio of 1:50-100<sup>[4-6]</sup>, but in the developing counties, NMO may constitute up to 40% of all CNS autoimmune diseases<sup>[7]</sup>. Prognosis and treatment in NMO and MS are different. Five-year mortality of untreated relapsing NMO was 68% - a much higher rate than in MS - and half of the surviving patients had permanent monoplegia or paraplegia<sup>[8]</sup>. Disease modifying therapies for relapsing MS, such as Interferon and the remarkably effective Natalizumab, fail to prevent, and may even precipitate, relapses of NMO<sup>[9,10]</sup>. Current strategies for relapse prevention in NMO include immunosuppressants and monoclonal antibodies<sup>[11,12]</sup>, but efficacy of these approaches has not been tested in randomized clinical trials. One of the most promising agents for NMO is rituximab (RTX), a monoclonal antibody that targets CD20 antigen expressed on the surface of pre-B, mature B-lymphocytes (but not normal plasma cells)<sup>[13]</sup> and a small subset of T-lymphocytes<sup>[14]</sup>. Our review aims to summarize global experience with RTX for the treatment of NMO and offer clinical pointers based on the literature and authors' experience.

## RATIONALE FOR B-CELL DEPLETING THERAPY IN NEUROMYELITIS OPTICA

Landmark pathologic study by Lucchinetti *et al.*<sup>[15]</sup> concluded that “the pronounced Ig reactivity co-localizing with complement activation at sites of vessel damage may be due to a specific antibody targeted to a vascular antigen”<sup>[15]</sup>. This prediction was borne out two years later when Lennon *et al.*<sup>[16]</sup> discovered an exquisitely NMO-specific autoantibody directed against AQP-4, a water channel found in astrocytic end-feet<sup>[16]</sup>. Current conceptualization of NMO pathogenesis is that anti-AQP4 auto-antibody binds to AQP-4<sup>[17]</sup> and initiates complement-mediated astrocyte injury and inflammatory reaction that secondarily affects oligodendrocytes and leads to demyelination and neuronal loss<sup>[18]</sup>. This hypothesis successfully explains many features of NMO, but does not account for the diversity of observed pathologic findings<sup>[19]</sup>, nor for disease pathogenesis in anti-AQP4- Ab seronegative

NMO patients, who comprise approximately 30% of NMO cases in the United States<sup>[5]</sup>.

In view of the central role of humoral autoimmunity to NMO pathogenesis, it is not surprising that B cell lineage depletion would be proposed as a rational therapeutic strategy. Indeed, shortly after discovery of anti-AQP-4 Ab, Cree *et al.*<sup>[20]</sup> reported an open label study of RTX in NMO that demonstrated high efficacy of the drug in all but one of their patients. A number of reports on RTX efficacy in NMO from different parts of the globe have since appeared. This review included all English-language studies that involved 5 or more RTX-treated NMO patients and recorded either relapse rate/number before and after treatment with RTX, or expanded disability status scale (EDSS) scores before and after treatment with RTX, or both outcome measures. We searched PubMed for “Neuromyelitis Optica” and “Rituximab” and cross-checked references. We identified 25 articles and finally 13 articles were included in this study, which met our inclusion criteria. Two articles were excluded for multiple treatments used; four were excluded for other diseases included; four were excluded for having less than 5 patients; and another two articles were excluded for not documenting treatment effect. Two unpublished case series of RTX-treated NMO that were presented at recent international neurologic conferences were also included in this review; additional data was obtained from the authors<sup>[21,22]</sup>.

## EFFICACY OF RTX IN NMO

Thirteen studies met our inclusion criteria. The total number of treated patients was 209, of whom the overwhelming majority were women (approximately 90%). Table 1 summarizes demographic and clinical data from the 13 studies. Four out of the thirteen studies reported median annualized relapse rate (ARR) before and after RTX<sup>[20,23-25]</sup>. Median ARR prior to treatment ranged from 1.7-2.6 and it decreased to 0-0.4 during the treatment period, which was usually 1-2 years. Two studies reported change in mean ARR<sup>[26,27]</sup>, which decreased from 1.2-2.4 pre-treatment to 0-0.3 after treatment was started. The remaining seven studies specified total number of relapses before and after RTX as detailed in Table 1<sup>[21-22,28-32]</sup>. In 11 out of 13 studies, 48%-75% of patients were relapse-free during treatment period. There were two exceptions: in the study by Lindsey *et al.*<sup>[32]</sup> only 3 out of 9 patients (33%) were relapse-free; this study was critiqued for possible under-dosing of RTX<sup>[33]</sup>. In the study of Yang *et al.*<sup>[28]</sup> none of the 5 patients experienced any further relapses while on RTX.

In all but one study, some patients “failed to respond” to treatment. Javed *et al.*<sup>[22]</sup> characterized nearly 33% of their NMO patients as “non-responders” based on the fact that RTX failed to delay further relapses, which occurred within 2.5 mo post treatment<sup>[22]</sup> (Table 1). Phenomenon of disease rebound in the immediate post-

**Table 1 Case series of rituximab in neuromyelitis optica**

Ref.	Country; Type of study	No. of patients (n = 209)	Mean age at RTX; % Female	% Anti-AQP4 Ab seropositive	RTX Protocol /treatment duration	ARR before RTX	ARR after RTX	% Relapse-free	EDSS (median) before --> after RTX
Cree <i>et al</i> <sup>[20]</sup>	United States; Retrospective	8	37 <sup>1</sup> ; 88%	N/A	A- treatment B- retreatment	2.6 (median)	0 (median)	75% (6/8 pts at 12 mo f/u)	7.5--> 5.5
Jacob <i>et al</i> <sup>[23]</sup>	United States/ England; Retrospective	25	43 <sup>1</sup> ; 88%	70%	A or B; median interval between cycles-8 mo 19 mo follow up	1.7 (median)	0 (median)	72% (17/25 at 12 mo estimated)	7--> 5 2 patients deceased
Bomprezzi <i>et al</i> <sup>[31]</sup>	United States; Retrospective	18	46 (+/-12); 83%	67%	B	15 pts-RTX tx and 7 had relapses. 42% (5/12) showed "positive treatment effects", the other 7 continued to relapse despite RTX therapy		53% (8/15)	Severe disability from NMO' - 10 patients
Bedi <i>et al</i> <sup>[24]</sup>	United States; Retrospective	23	46 <sup>1</sup> ; 91%	72%	A or B; 32.5 mo	1.87 (median)	0 (median)	74 % (17/23 pts)	7--> 5.5
Pellkofer <i>et al</i> <sup>[30]</sup>	Germany; Prospective	10	47 <sup>1</sup> ; 90%	100%	B; number of cycles of RTX 1-5	Ever before RTX: 1.3 mo, 12 m before RTX: 2.4 mo, 24 m before RTX: 1.72 mo, With RTX: 0.93 mo		50% (5/10 at 12 mo estimated)	6--> 6.5 <sup>1</sup> 1 patient deceased
Javed <i>et al</i> <sup>[22]</sup>	United States; Retrospective	15	34; N/A	N/A	B; patients were given RTX 1g x1 usually 6-9 mo after the initial dose	2/10 had 2 relapses in 6 mo post RX. 5 non-responders had mean of 1.45 (median 1) relapses in mean 12.2 (median 10) mo		67% (RTX delayed further relapses for 9 mo or more)	N/A
Gredler <i>et al</i> <sup>[26]</sup>	Austria; Retrospective	6	38; 83%	66%	375 mg/m <sup>2</sup> ; no of infusions 3-16 (mean = 6.67), interval between infusions 3.3-11 mo	2.5 (mean) <sup>1</sup>	0.4 (mean) <sup>1</sup>	67% (4/6)	5.25--> 2.25 <sup>1</sup>
Ip <i>et al</i> <sup>[25]</sup>	China; Prospective	7	52; 85%	66%	A or B: Mean # trx courses: 2.85. median 2	Mean ARR = 2.4 median ARR = 2 <sup>1</sup> 5 became relapse free. 2 had 50% reduction over median 24 mo		71 % (5/7)	8--> 7
Lindsey <i>et al</i> <sup>[32]</sup>	United States; Retrospective	9	N/A; 89%	60%	A or B: Mean duration: 74.2 mo	3 pts with early relapses in first month after RTX, 4 pts (including 1 pt with early relapse) with later relapses		33% (3/9)	3.5--> 4.3 <sup>1</sup>
Kim <i>et al</i> <sup>[27]</sup>	South Korea; Retrospective	30	38.4 (± 10.5); 90%	77%	A or B; mean 61 mo (range 49-82 mo), median 60 mo	2.4 (mean)	0.3 (mean)	70% (21/30 at 2 yr f/u)	4--> 3
Yang <i>et al</i> <sup>[28]</sup>	China; Prospective	5	42 <sup>1</sup> ; N/A	80%	100 mg (50-59 mg/m <sup>2</sup> ) RTX IV 1 dose/wk for 3 cons wk; mean duration: 12.2 mo	1.16 <sup>1</sup> (mean)	0 <sup>1</sup> (mean)	100%	4.5--> 4
Mealy <i>et al</i> <sup>[29]</sup>	United States; Retrospective	30	45 <sup>1</sup> ; 83%	50%	B; median of 20 mo (range 5-83 mo)	Total pretreatment ARR- 2.89	Total post-treatment ARR- 0.33 Median ARR was 0.24; mean was 1.02 (SD = 1.36)	67% (20/30)	N/A
Farber <i>et al</i> <sup>[21]</sup>	United States; Retrospective	23	38; 100%	74%	Mean of 22 mo (range 2-96 mo)			48% (11/23)	N/A

<sup>1</sup>Estimated based on results table or manuscript when possible. A or B (in RTX protocol column): There were two treatment protocols used- Protocol A with 4 doses RTX 375 mg/m<sup>2</sup> IV wk for 4 wk; Protocol B with 2 doses of RTX 1000 mg IV 2 wk apart. NMO: Neuromyelitis optica; ARR: Annual relapse rate; EDSS: Expanded disability status scale; RTX: Rituximab; AQP4: Ab - aquaporin 4 antibody; N/A: Not available.

induction period was documented by Perumal *et al*<sup>[34]</sup> in 6 out of their 17 patients; however most patients with

post-induction relapses evidenced disease stabilization with further RTX dosing and so need not be necessarily classified as “true non-responders”. Perumal *et al.*<sup>[34]</sup> hypothesized that cytokine release and increases in BAFF and AQP4 levels that immediately follow RTX infusion<sup>[35]</sup> may precipitate a post-infusion relapse in highly active NMO patients.

Continual disease activity can occur in RTX-treated NMO patients with complete depletion of B cells<sup>[29,30,32,36]</sup>. Risk factors that would predict non-responsiveness to RTX are presently unknown. It was suggested that RTX non-responders may require not only B-lymphocyte elimination with RTX, but an additional, “broad-spectrum immune-suppressant” to achieve disease remission<sup>[31]</sup>. This strategy has been successfully adopted in the treatment of Rheumatoid Arthritis, where RTX is often combined with Methotrexate or Cyclophosphamide<sup>[13]</sup>. We discuss potential mechanisms that may explain lack of response to RTX in “Variability in responses to RTX treatment” section below.

EDSS scores before and after RTX were reported in 9 out of 13 studies. In 7 of the 9 studies, EDSS at last follow-up was lower than prior to RTX initiation. Exceptions were the studies by Lindsey *et al.*<sup>[32]</sup> and Pellkofer *et al.*<sup>[30]</sup>, in which EDSS at last follow-up increased by 0.8 and 0.5, respectively, compared to pre-treatment EDSS<sup>[30,32]</sup>.

## ADVERSE EVENTS

Two studies recorded fatal outcomes in RTX-treated NMO patients. In the study of Jacob *et al.*<sup>[23]</sup>, one patient died from a brainstem NMO relapse and another succumbed to suspected septicemia. Pellkofer *et al.*<sup>[30]</sup> reported one death due to presumed cardiovascular failure that occurred 3 d after a rituximab infusion.

Adverse events were not systematically documented across the studies, so estimates of their prevalence are not possible. A number of infections have been observed - mostly, herpetic rashes and tuberculosis reactivation. RTX treatment carries a small risk of progressive multifocal leukoencephalopathy (PML) - 1 case per 25000 individuals in one large cohort of patients with rheumatoid arthritis<sup>[37]</sup>. No cases of PML in RTX-treated NMO patients have been reported to date, though there was a single case report of PML in NMO patients treated with azathioprine<sup>[38]</sup>. Overall, adverse events profile of RTX in NMO appears to be consistent with known safety profile of the drug<sup>[13]</sup>. Infusion reaction to RTX are very common, but can usually be mitigated by pre-treatment with intravenous steroids anti-histamine and slow titration of RTX.

## DOSING OF RTX AND BIOMARKERS OF TREATMENT RESPONSE

The majority of studies used one of two “induction protocols”: 375 mg/m<sup>2</sup> IV once a week for four consecutive weeks (“protocol A” in Table 1), or 1000 mg

IV infused two weeks apart (“protocol B”). Timing of subsequent doses either followed a fixed schedule - with typical time to the next infusion cycle of 6-9 mo or was based on monitoring parameters. The most commonly used test for monitoring B cell suppression was CD19+ count assessed by flow cytometry. Since RTX interferes with the direct analysis of CD20 cell surface antigen *via* flow cytometry due to its mechanism of action, CD19+ antigen, which is largely co-expressed with CD20, is used as a surrogate marker to assess extent of B cell depletion<sup>[26]</sup>. However, CD19+ count may also overestimate degree of B cell depletion<sup>[39]</sup>. RTX typically depletes CD 19+ counts to undetectable levels (< 10 cells per  $\mu$ L) within 2-4 wk of infusion<sup>[13]</sup>.

Table 2 summarizes the use of biomarkers to monitor treatment response to RTX in NMO. Several studies showed that CD19+ B cell population greater than 1% of lymphocyte total is a risk factor of a relapse. Farber *et al.*<sup>[21]</sup> measured CD19 counts post-relapse and during periods of stability, and noted higher B cell counts in the immediate post-relapse period. Yang *et al.*<sup>[28]</sup> suppressed CD19+ count to less than 1% in all their patients and were able to achieved complete eradication of relapses, despite lower doses of RTX used<sup>[27]</sup>. Pellkofer *et al.*<sup>[30]</sup> used monthly, highly sensitive flow cytometry measurements to demonstrate that complete B cell suppression led to sustained clinical stabilization in most patients. Bomprezzi *et al.*<sup>[31]</sup> showed that B cells become undetectable within 2 wk of the first dose of RTX, but rise to 2%-12% at the time of a relapse<sup>[31]</sup>. The early rise in CD19+ cells correlated with radiologically proven relapses, and 5 out of 7 patients experienced a relapse when CD19+ B cell population exceeded the 1% threshold<sup>[31]</sup>. In summary, preponderance of evidence favors suppressing CD19+ B cell to  $\leq$  1% of the total lymphocyte count in NMO patients for maximal efficacy.

Efficacy of “low dose” RTX on CD19 counts was assessed in two studies. Yang *et al.*<sup>[28]</sup> used RTX 100 mg infusion once a week for 3 consecutive weeks, which was followed by the next RTX 100 mg dose when CD19+ cells were > 1% and the memory CD19+ CD27+ B cells were > 0.05%. In this regimen, CD19+ cells started to increase in 4 of the 5 patients approximately 140 d after the initial RTX infusion, necessitating a re-infusion<sup>[28]</sup>. In the Greenberg *et al.*<sup>[40]</sup> study, RTX 100 mg dose resulted in early re-population of B cells compared to the 1000 mg dose<sup>[40]</sup>. The median number of days for CD19 population to reach threshold of 2% was 133 d in the 100-mg per dose arm vs 259 d in the 1000-mg per dose arm<sup>[40]</sup>.

Kim *et al.*<sup>[27]</sup> proposed that CD27+ memory B cells may be a more relevant biomarker of pathogenic B cells depletion in NMO than CD19+ B cells<sup>[26]</sup>. Memory B cells can elicit larger and faster responses to antigen than naive B cells, and so may be more relevant to disease pathogenesis. Re-emergence of CD27+ memory B cells above the therapeutic target (< 0.05% of PBMCs) may occur even when CD19+ B cells levels were < 0.5% of



**Table 2 Monitoring parameters in Neuromyelitis optica patients treated with rituximab**

Ref.	Monitoring parameter/comments
Cree <i>et al</i> <sup>[20]</sup>	CD19 levels- when detectable, patients were re-treated. CD 19 followed bimonthly. 2 protocols-planned infusions every 6 mo or 12 mo
Jacob <i>et al</i> <sup>[23]</sup>	CD19 not routinely monitored. Some RTX given when B-cell counts detectable either 6 or 12 mo in intervals or when CD19+ became detectable
Bomprezzi <i>et al</i> <sup>[31]</sup>	Flow cytometry used to test circulating B cells. Suggest clinical relapses occurring while on RTX therapy correlate with reconstitution of circulating B cells. Correlated that even early rise in CD20+ cells correlated with radiologically proven relapses. B cells had re-sent between 2% and 12% at time of new attack. Total of 7 patients relapsed after RTX-5 had acute event when B cell counts just returned to greater than 1%, whereas 2 patients continued to relapse despite B cells being undetectable. Detected significant variability in timing of reconstitution of normal values, which implies that scheduling of doses of RTX can be adjusted accordingly
Bedi <i>et al</i> <sup>[24]</sup>	CD19 cell counts planned every 2-3 mo, but not collected systematically for report
Pellkofer <i>et al</i> <sup>[30]</sup>	Measured lymphocyte subsets by flow cytometry; B cell depletion defined as counts below $0.01 \times 10^9$ /L. B cells became undetectable in 9 out of 10 patients within 14 d after 1st dose. Time of B-cell repopulation varied. After 3 patients experienced a relapse shortly after reappearance of B cells, RTX given at fixed interval every 6 to 9 mo, which this led to improved outcomes
Javed <i>et al</i> <sup>[22]</sup>	"Non-responders" were defined as clinical attack < 6 mo post rituximab treatment, when B cell count was still undetectable
Gredler <i>et al</i> <sup>[26]</sup>	Flow cytometry used; B cells quantified using following combinations of monoclonal antibodies: CD3/19/45, 19/27/45, 19/38/45. Two patients out of 6 had relapses while B-cells were absent
Lindsey <i>et al</i> <sup>[32]</sup>	4 patients had relapses after more than 1 mo when peripheral B cell count "very low". Case 1: CD19 increased to 250 cells/ $\mu$ L had sensory relapse, no further symptoms for 18 mo; Case 2: Had relapses with CD19 count of 0; Case 3, 4, 6 no further relapses; Case 5: CD19 1 cells/ $\mu$ L at 10 mo, 12 cells/ $\mu$ L at 13 mo and subsequent relapses; Case 7--continued to have relapses with 1 cell/ $\mu$ L at 7 mo, 4 cells/ $\mu$ L at 12 mo. Case 8: CD19 count 3 cells/ $\mu$ L, with continued relapses; Case 9: continued relapses with CD19 1 cells/ $\mu$ L
Kim <i>et al</i> <sup>[27]</sup>	Blood samples obtained every 6 wk in 1st year, every 8 wk in second year. Therapeutic target for CD 27+ memory B cell depletion was less than 0.05% of PBMCs. Patients received additional infusion of 375 mg/m <sup>2</sup> if frequency of re-emerging memory CD27+ B cells in PBMCs exceeded 0.1% by flow cytometry. CD 19 B cells counts measure- less than $0.01 \times 10^9$ /L or less than 0.5% of PBMCs (considered B cell depletion in prior studies. 60%-65% relapses occurred when CD19 were depleted. Authors argue CD27+ more informative biomarker than CD19
Yang <i>et al</i> <sup>[28]</sup>	Goal of CD19+ B cells to less than or equal to 1%, as well as CD19 CD27 B cells to less than or equal to 0.05% of PBMCs. All with no relapses despite low doses of RTX (100 mg single infusion and follow up infusion at mean of 35 wk)
Mealy <i>et al</i> <sup>[29]</sup>	CD19 cell counts tested monthly, repeated dosing scheduled on detection of CD19 greater than 1% of total lymphocyte population or at regular 6 mo intervals
Farber <i>et al</i> <sup>[21]</sup>	Total of 23 relapses, of which 70% occurred when B cells < 1% of lymphocytes. 7 relapses (30%) occurred when B cells greater or equal to 1% of lymphocytes. CD19 > 1% was associated with higher rate of relapses

RTX: Rituximab.

PBMCs. Perhaps, presence of memory cells in patients with ostensible absence of CD19+ cells can be explained by a recent study in which loss of CD19 surface antigen from healthy donor B cells exposed to rituximab *in vitro* was not necessarily associated with B cell death<sup>[41]</sup>. In the study of Kim *et al*<sup>[27]</sup>, no relapses were observed in 29 out of 30 patients in whom CD27+ memory B cell fraction was below the therapeutic target. This important finding is corroborated by the small series of Yang *et al*<sup>[28]</sup>, cited above. More studies are needed to determine if CD27+ should replace CD19+ as the biomarker of choice in monitoring response to RTX in NMO.

Pellkofer *et al*<sup>[30]</sup> studied utility of AQP4-Ab, total B cell counts and B cell fostering cytokines, such as BAFF (B cell activating factor) or APRIL (a proliferation-inducing ligand) as biomarkers in NMO. They found that disease activity correlated with B cell depletion, but not with AQP4-Ab or APRIL levels<sup>[30]</sup>. Relationship between CD19 counts and Anti-AQP4 titers was analyzed by Jarius *et al*<sup>[17]</sup>, who concluded that administration of RTX was followed by a "prompt and marked decline" in AQP-4 Ab, though the auto-antibody remained detectable in nearly all patients<sup>[17]</sup>.

## VARIABILITY IN RESPONSES TO RTX TREATMENT

The mechanisms responsible for variability in RTX treatment responses are unclear. An early, post-infusion relapse may be related to incomplete elimination of pathologic B cells as well as transient increase in B cell activating factor, anti-AQP4 Ab titers and other cytokines following infusion<sup>[34]</sup>. Greenberg *et al*<sup>[40]</sup> reported an NMO patient who was "resistant" to RTX: after an initial fall of CD19 cell count to 0, patient continued to experience clinical relapses and marked early return of B cells at 91 d after RTX, which could not be suppressed by further doses of RTX. This appears to be an exceptional case, as most "true" non-responders in the Greenberg *et al*<sup>[40]</sup> study - 6 out of 8 - had CD19 count below 2% at the time of relapse. Kim *et al*<sup>[27]</sup> also noted that 13 out of 20 relapses (65%) occurred even when CD19 B cell fraction was less than 0.5% PBMCs<sup>[27]</sup>. Lindsey *et al*<sup>[32]</sup> ask whether in patients with continued relapses despite complete B cell suppression, pathogenic T-cell may play a relatively more prominent role in pathogenesis. It is also possible RTX does not completely eliminate pathogenic clones in the periphery

or that CD19+ count overestimates the degree of peripheral B cells depletion<sup>[41]</sup>. Furthermore, peripherally administered monoclonal antibodies have limited penetration across the blood-brain barrier - typically CSF concentration is < 0.1% of serum antibody concentration<sup>[42]</sup>. Although CSF Rituximab concentration may be considerably higher if blood-brain barrier is perturbed<sup>[39]</sup>, its concentration may still be insufficient for elimination of B cells from CSF.

## CONCLUSIONS AND QUESTIONS FOR FUTURE STUDIES

Thirteen observational studies from across the world (Table 1) have documented stabilization or improvement in disability scores in a majority of NMO patients upon initiation of RTX. Pooling data across the studies shows that 66% of patients were relapse-free throughout the treatment period (typically 1-2 years). It is possible that with a more rigorous monitoring of response with CD19+ or CD27+ biomarkers and improved strategies to avoid relapses in the post-induction period even more impressive results could be achieved. Although the studies in our review have been uncontrolled and mostly retrospective and so subject to various biases (e.g., ascertainment bias, selection bias, publication bias), they are consistent in demonstrating robust treatment response. Considering the natural history of untreated NMO<sup>[8]</sup>, it would seem highly unlikely that the observed reduction of relapses and improved disability scores in RTX-treated patients is accounted for solely by artifacts of data collection or regression to the mean. The accumulated weight of evidence, in authors' opinion, casts doubt on the possibility of genuine clinical equipoise in NMO at the present time.

Important questions remain with regard to place of RTX in the treatment algorithm of NMO. A recent retrospective review concluded that RTX had the lowest failure rate compared to the commonly used oral immunosuppressants<sup>[5]</sup>. Based on this data, and overall efficacy of RTX in the published studies, RTX should be strongly considered in any NMO patient who continues to relapse on oral immunosuppressants<sup>[13]</sup>. The question of whether RTX should replace prior treatment or be combined with it remains unresolved. Combination therapies, in wide use for rheumatologic diseases, have not received sufficient attention in neuro-immunology and will need to be studied more in the future. An acute treatment often used during NMO relapse is plasma exchange (PLEX)<sup>[43]</sup>. Efficacy and safety of PLEX in other refractory systemic autoimmune disease have been shown in several studies<sup>[44,45]</sup> and its role as maintenance therapy of NMO is currently being investigated<sup>[46]</sup>.

Should RTX be the agent of choice for all previously untreated patients with NMO? The authors would consider RTX as a first-line therapy in a patient with aggressive disease course as well as in the older NMO

patients, who tend to have worse outcomes<sup>[47]</sup>. It is less clear whether risk-to-benefit ratio calculation would favor RTX in milder and earlier cases, e.g., in an AQP4-Ab seropositive patient after single relapse.

What would be the optimal timing for initiating RTX? If, as suggested by some studies<sup>[22,32,34]</sup>, RTX could exacerbate NMO in the immediate post-infusion period, it would probably be safer to initiate RTX after a period of stability rather than during an acute exacerbation. This question requires further study as the data is conflicting. When switching a patient to RTX, a prudent recommendation is to avoid discontinuing prior therapy prematurely, as delay in starting RTX could put the patient at risk of relapse<sup>[24]</sup>. In our practice, we routinely continue treatment with an oral immunosuppressant for at least one month after RTX is started. With regard to timing of repeat RTX cycles, the literature supports use of CD19+ and, possibly, CD27+ cell count, to monitor treatment response (Table 2). One goal of treatment should be to keep these counts below threshold levels.

Important questions remain regarding long term safety and efficacy of RTX, and duration of therapy. There is little data with regard to long-term safety of RTX in NMO, but the long-term safety record of RTX in rheumatoid arthritis is reassuring<sup>[48]</sup>. Would it be safe, from NMO standpoint, to discontinue treatment with RTX after a (prolonged) period of stability? A recent study documented that a period of several years of no disease activity after RTX is discontinued is possible in some patients, though 2 out of 4 patients in that series experienced relapses after years of quiescence<sup>[49]</sup>. Considering the potentially devastating consequences of NMO relapse, routine discontinuation of RTX and "watchful waiting" is probably not advisable.

It is hoped that randomized clinical trials, several of which are under way now (e.g.,<sup>[50]</sup>) as well as multi-center collaborative observational studies based on NMO registries, such as online NMOBase registry ([www.msbase.org](http://www.msbase.org)), could provide data on long-term safety and efficacy of RTX in NMO and help resolve the unanswered questions raised in our review. Quality of observational studies in NMO could be improved by adherence to accepted guidelines<sup>[51]</sup>, especially with respect to reporting outcomes (relapse rates and disability scores).

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## REFERENCES

- 1 **Bergamaschi R**, Ghezzi A. Devic's neuromyelitis optica: clinical features and prognostic factors. *Neurol Sci* 2004; **25** Suppl 4: S364-S367 [PMID: 15727235]
- 2 **Weinshenker BG**. NMO diagnostic criteria: an update. Paper presented at: ACTRIMS/ECTRIMS Annual meeting. Boston, MA,

- 2014, September 10-13
- 3 **Marrie RA**, Gryba C. The incidence and prevalence of neuromyelitis optica: a systematic review. *Int J MS Care* 2013; **15**: 113-118 [PMID: 24453773 DOI: 10.7224/1537-2073.2012-048]
  - 4 **Bizzoco E**, Lolli F, Repice AM, Hakiki B, Falcini M, Barilaro A, Taiuti R, Siracusa G, Amato MP, Biagioli T, Lori S, Moretti M, Vinattieri A, Nencini P, Massacesi L, Matà S. Prevalence of neuromyelitis optica spectrum disorder and phenotype distribution. *J Neurol* 2009; **256**: 1891-1898 [PMID: 19479168 DOI: 10.1007/s00415-009-5171-x]
  - 5 **Mealy MA**, Wingerchuk DM, Greenberg BM, Levy M. Epidemiology of neuromyelitis optica in the United States: a multicenter analysis. *Arch Neurol* 2012; **69**: 1176-1180 [PMID: 22733096 DOI: 10.1001/archneurol.2012.314]
  - 6 **Collongues N**, Marignier R, Zéphir H, Papeix C, Blanc F, Ritleng C, Tchikviladze M, Outteryck O, Vukusic S, Fleury M, Fontaine B, Brassat D, Clanet M, Milh M, Pelletier J, Audoin B, Ruet A, Lebrun-Frenay C, Thouvenot E, Camu W, Debouverie M, Créange A, Moreau T, Labauge P, Castelnovo G, Edan G, Le Page E, Defer G, Barroso B, Heinzlef O, Gout O, Rodriguez D, Wiertlewski S, Laplaud D, Borgel F, Tourniaire P, Grimaud J, Brochet B, Vermersch P, Confavreux C, de Seze J. Neuromyelitis optica in France: a multicenter study of 125 patients. *Neurology* 2010; **74**: 736-742 [PMID: 20194912 DOI: 10.1212/WNL.0b013e3181d31e35]
  - 7 **Siritho S**, Nakashima I, Takahashi T, Fujihara K, Prayoonwiwat N. AQP4 antibody-positive Thai cases: clinical features and diagnostic problems. *Neurology* 2011; **77**: 827-834 [PMID: 21813785 DOI: 10.1212/WNL.0b013e31822c61b1]
  - 8 **Wingerchuk DM**, Hogancamp WF, O'Brien PC, Weinshenker BG. The clinical course of neuromyelitis optica (Devic's syndrome). *Neurology* 1999; **53**: 1107-1114 [PMID: 10496275 DOI: 10.1212/WNL.53.5.1107]
  - 9 **Kleiter I**, Hellwig K, Berthele A, Kämpfel T, Linker RA, Hartung HP, Paul F, Aktas O. Failure of natalizumab to prevent relapses in neuromyelitis optica. *Arch Neurol* 2012; **69**: 239-245 [PMID: 22332191 DOI: 10.1001/archneurol.2011.216]
  - 10 **Palace J**, Leite MI, Nairne A, Vincent A. Interferon Beta treatment in neuromyelitis optica: increase in relapses and aquaporin 4 antibody titers. *Arch Neurol* 2010; **67**: 1016-1017 [PMID: 20697055 DOI: 10.1001/archneurol.2010.188]
  - 11 **Papadopoulos MC**, Bennett JL, Verkman AS. Treatment of neuromyelitis optica: state-of-the-art and emerging therapies. *Nat Rev Neurol* 2014; **10**: 493-506 [PMID: 25112508 DOI: 10.1038/nrneurol.2014.141]
  - 12 **Trebst C**, Jarius S, Berthele A, Paul F, Schippling S, Wildemann B, Borisow N, Kleiter I, Aktas O, Kämpfel T. Update on the diagnosis and treatment of neuromyelitis optica: recommendations of the Neuromyelitis Optica Study Group (NEMOS). *J Neurol* 2014; **261**: 1-16 [PMID: 24272588 DOI: 10.1007/s00415-013-7169-7]
  - 13 Rituximab prescribing information. On October, 12, 2014. Available from: URL: [http://www.gene.com/download/pdf/rituxan\\_prescribing.pdf](http://www.gene.com/download/pdf/rituxan_prescribing.pdf)
  - 14 **Palanichamy A**, Jahn S, Nickles D, Derstine M, Abounasr A, Hauser SL, Baranzini SE, Leppert D, von Büdingen HC. Rituximab efficiently depletes increased CD20-expressing T cells in multiple sclerosis patients. *J Immunol* 2014; **193**: 580-586 [PMID: 24928997 DOI: 10.4049/jimmunol.1400118]
  - 15 **Lucchinetti CF**, Mandler RN, McGavern D, Bruck W, Gleich G, Ransohoff RM, Trebst C, Weinshenker B, Wingerchuk D, Parisi JE, Lassmann H. A role for humoral mechanisms in the pathogenesis of Devic's neuromyelitis optica. *Brain* 2002; **125**: 1450-1461 [PMID: 12076996 DOI: 10.1093/brain/awf151]
  - 16 **Lennon VA**, Wingerchuk DM, Kryzer TJ, Pittock SJ, Lucchinetti CF, Fujihara K, Nakashima I, Weinshenker BG. A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. *Lancet* 2004; **364**: 2106-2112 [PMID: 15589308 DOI: 10.1016/S0140-6736(04)17551-X]
  - 17 **Jarius S**, Aboul-Enein F, Waters P, Kuenz B, Hauser A, Berger T, Lang W, Reindl M, Vincent A, Kristoferitsch W. Antibody to aquaporin-4 in the long-term course of neuromyelitis optica. *Brain* 2008; **131**: 3072-3080 [PMID: 18945724 DOI: 10.1093/brain/awn240]
  - 18 **Verkman AS**, Phuan PW, Asavapanumas N, Tradtrantip L. Biology of AQP4 and anti-AQP4 antibody: therapeutic implications for NMO. *Brain Pathol* 2013; **23**: 684-695 [PMID: 24118484 DOI: 10.1111/bpa.12085]
  - 19 **Misu T**, Höftberger R, Fujihara K, Wimmer I, Takai Y, Nishiyama S, Nakashima I, Konno H, Bradl M, Garzuly F, Itoyama Y, Aoki M, Lassmann H. Presence of six different lesion types suggests diverse mechanisms of tissue injury in neuromyelitis optica. *Acta Neuropathol* 2013; **125**: 815-827 [PMID: 23579868 DOI: 10.1007/s00401-013-1116-7]
  - 20 **Cree BA**, Lamb S, Morgan K, Chen A, Waubant E, Genain C. An open label study of the effects of rituximab in neuromyelitis optica. *Neurology* 2005; **64**: 1270-1272 [PMID: 15824362 DOI: 10.1212/01.WNL.0000159399.81861.D5]
  - 21 **Farber R**, Klineova S, Katz-Sand I, Fabian M, Krieger S, Lublin F. Neuromyelitis optica patients on rituximab: predicting response to therapy. *Mult Scler* 2014; **20** (S1): 501-510
  - 22 **Javed A**, Arnason B, Reder AT. Breakthrough relapses after rituximab in NMO patients: incidence and other treatment options. *Mult Scler* 2008; **14**: S49
  - 23 **Jacob A**, Weinshenker BG, Violich I, McLinskey N, Krupp L, Fox RJ, Wingerchuk DM, Boggild M, Constantinescu CS, Miller A, De Angelis T, Matiello M, Cree BA. Treatment of neuromyelitis optica with rituximab: retrospective analysis of 25 patients. *Arch Neurol* 2008; **65**: 1443-1448 [PMID: 18779415 DOI: 10.1001/archneur.65.11.noc80069]
  - 24 **Bedi GS**, Brown AD, Delgado SR, Usmani N, Lam BL, Sheremata WA. Impact of rituximab on relapse rate and disability in neuromyelitis optica. *Mult Scler* 2011; **17**: 1225-1230 [PMID: 21622594 DOI: 10.1177/1352458511404586]
  - 25 **Ip VH**, Lau AY, Au LW, Fan FS, Chan AY, Mok VC, Wong KS. Rituximab reduces attacks in Chinese patients with neuromyelitis optica spectrum disorders. *J Neurol Sci* 2013; **324**: 38-39 [PMID: 23040959 DOI: 10.1016/j.jns.2012.09.024]
  - 26 **Gredler V**, Mader S, Schanda K, Hegen H, Di Pauli F, Kuenz B, Deisenhammer F, Berger T, Reindl M, Lutterotti A. Clinical and immunological follow-up of B cell depleting therapy in CNS demyelinating diseases. *J Neurol Sci* 2013; **328**: 77-82 [PMID: 23522498 DOI: 10.1016/j.jns.2013.02.024]
  - 27 **Kim SH**, Huh SY, Lee SJ, Joung A, Kim HJ. A 5-year follow-up of rituximab treatment in patients with neuromyelitis optica spectrum disorder. *JAMA Neurol* 2013; **70**: 1110-1117 [PMID: 23897062 DOI: 10.1001/jamaneurol.2013.3071]
  - 28 **Yang CS**, Yang L, Li T, Zhang DQ, Jin WN, Li MS, Su N, Zhangning N, Liu Q, Shao ZH, Yu C, Shi FD. Responsiveness to reduced dosage of rituximab in Chinese patients with neuromyelitis optica. *Neurology* 2013; **81**: 710-713 [PMID: 23884041 DOI: 10.1212/WNL.0b013e3182a1aac7]
  - 29 **Mealy MA**, Wingerchuk DM, Palace J, Greenberg BM, Levy M. Comparison of relapse and treatment failure rates among patients with neuromyelitis optica: multicenter study of treatment efficacy. *JAMA Neurol* 2014; **71**: 324-330 [PMID: 24445513 DOI: 10.1001/jamaneurol.2013.5699]
  - 30 **Pellkofer HL**, Krumbholz M, Berthele A, Hemmer B, Gerdes LA, Havla J, Bittner R, Canis M, Meinl E, Hohlfeld R, Kuempfel T. Long-term follow-up of patients with neuromyelitis optica after repeated therapy with rituximab. *Neurology* 2011; **76**: 1310-1315 [PMID: 21482945 DOI: 10.1212/WNL.0b013e3182152881]
  - 31 **Bomprezzi R**, Postevka E, Campagnolo D, Vollmer TL. A review of cases of neuromyelitis optica. *Neurologist* 2011; **17**: 98-104 [PMID: 21364364 DOI: 10.1097/NRL.0b013e31820a9d35]
  - 32 **Lindsey JW**, Meulmester KM, Brod SA, Nelson F, Wolinsky JS. Variable results after rituximab in neuromyelitis optica. *J Neurol Sci* 2012; **317**: 103-105 [PMID: 22405926 DOI: 10.1016/j.jns.2012.02.017]
  - 33 **Kim SH**, Kim HJ. Lack of response to rituximab therapy in patients with neuromyelitis optica: true non-responders or insufficient treatment? *J Neurol Sci* 2012; **319**: 171; author reply 172 [PMID: 22405926 DOI: 10.1016/j.jns.2012.02.017]

- 22633442]
- 34 **Perumal J**, Kister I, Howard J, Herbert J. Disease exacerbation after Rituximab induction in Neuromyelitis Optica. *Neurol Neuroimmunol Neuroinflamm* 2015; **2**: e61 [DOI: 10.1212/NXI.0000000000000061]
  - 35 **Nakashima I**, Takahashi T, Cree BA, Kim HJ, Suzuki C, Genain CP, Vincent T, Fujihara K, Itoyama Y, Bar-Or A. Transient increases in anti-aquaporin-4 antibody titers following rituximab treatment in neuromyelitis optica, in association with elevated serum BAFF levels. *J Clin Neurosci* 2011; **18**: 997-998 [PMID: 21565508 DOI: 10.1016/j.jocn.2010.12.011]
  - 36 **Capobianco M**, Malucchi S, di Sapio A, Gilli F, Sala A, Bottero R, Marnetto F, Doriguzzi Bozzo C, Bertolotto A. Variable responses to rituximab treatment in neuromyelitis optica (Devic's disease). *Neurol Sci* 2007; **28**: 209-211 [PMID: 17690854 DOI: 10.1007/s10072-007-0823-z]
  - 37 **Clifford DB**, Ances B, Costello C, Rosen-Schmidt S, Andersson M, Parks D, Perry A, Yerra R, Schmidt R, Alvarez E, Tyler KL. Rituximab-associated progressive multifocal leukoencephalopathy in rheumatoid arthritis. *Arch Neurol* 2011; **68**: 1156-1164 [PMID: 21555606 DOI: 10.1001/archneurol.2011.103]
  - 38 **Flanagan EP**, Aksamit AJ, Kumar N, Morparia NP, Keegan BM, Weinshenker BG. Simultaneous PML-IRIS and myelitis in a patient with neuromyelitis optica spectrum disorder. *Neurology: Clinical Practice* 3.5, 2013: 448-451
  - 39 **Petereit HF**, Rubbert-Roth A. Rituximab levels in cerebrospinal fluid of patients with neurological autoimmune disorders. *Mult Scler* 2009; **15**: 189-192 [PMID: 18971221 DOI: 10.1177/1352458508098268]
  - 40 **Greenberg BM**, Graves D, Remington G, Hardeman P, Mann M, Karandikar N, Stuve O, Monson N, Frohman E. Rituximab dosing and monitoring strategies in neuromyelitis optica patients: creating strategies for therapeutic success. *Mult Scler* 2012; **18**: 1022-1026 [PMID: 22261118 DOI: 10.1177/1352458511432896]
  - 41 **Jones JD**, Hamilton BJ, Rigby WF. Rituximab mediates loss of CD19 on B cells in the absence of cell death. *Arthritis Rheum* 2012; **64**: 3111-3118 [PMID: 22674374 DOI: 10.1002/art.34560]
  - 42 **Tran JQ**, Rana J, Barkhof F, Melamed I, Gevorkyan H, Wattjes MP, de Jong R, Broszofsky K, Ray S, Xu L, Zhao J, Parr E, Cadavid D. Randomized phase I trials of the safety/tolerability of anti-LINGO-1 monoclonal antibody BIIB033. *Neurol Neuroimmunol Neuroinflamm* 2014; **1**: e18 [PMID: 25340070]
  - 43 **Schwartz J**, Winters JL, Padmanabhan A, Balogun RA, Delaney M, Linenberger ML, Szczepiorkowski ZM, Williams ME, Wu Y, Shaz BH. Guidelines on the use of therapeutic apheresis in clinical practice-evidence-based approach from the Writing Committee of the American Society for Apheresis: the sixth special issue. *J Clin Apher* 2013; **28**: 145-284 [PMID: 23868759 DOI: 10.1002/jca.21276]
  - 44 **Cid J**, Carbassé G, Andreu B, Baltanás A, Garcia-Carulla A, Lozano M. Efficacy and safety of plasma exchange: an 11-year single-center experience of 2730 procedures in 317 patients. *Transfus Apher Sci* 2014; **51**: 209-214 [PMID: 25217991]
  - 45 **Pons-Estel GJ**, Salerni GE, Serrano RM, Gomez-Puerta JA, Plasin MA, Aldasoro E, Lozano M, Cid J, Cervera R, Espinosa G. Therapeutic plasma exchange for the management of refractory systemic autoimmune diseases: report of 31 cases and review of the literature. *Autoimmun Rev* 2011; **10**: 679-684 [PMID: 21569864]
  - 46 **ClinicalTrials.gov**. Maintenance Plasma Exchange for Neuromyelitis Optica (MultiPLEX) [Accessed 2015 January 6]. Available from: URL: <https://clinicaltrials.gov/ct2/show/NCT01500681>
  - 47 **Collongues N**, Marignier R, Jacob A, Leite M, Siva A, Paul F, Zephir H, Akman-Demir G, Elson L, Jarius S, Papeix C, Mutch K, Saip S, Wildemann B, Kitley J, Karabudak R, Aktas O, Kucsu D, Altintas A, Palace J, Confavreux C, De Seze J. Characterization of neuromyelitis optica and neuromyelitis optica spectrum disorder patients with a late onset. *Mult Scler* 2013; **20**: 1086-1094 [PMID: 24323817 DOI: 10.1177/1352458513515085]
  - 48 **van Vollenhoven RF**, Emery P, Bingham CO, Keystone EC, Fleischmann RM, Furst DE, Tyson N, Collinson N, Lehane PB. Long-term safety of rituximab in rheumatoid arthritis: 9.5-year follow-up of the global clinical trial programme with a focus on adverse events of interest in RA patients. *Ann Rheum Dis* 2013; **72**: 1496-1502 [PMID: 23136242]
  - 49 **Weinfurter K**, Graves J, Ness J, Krupp L, Milazzo M, Waubant E. Prolonged Remission in Neuromyelitis Optica Following Cessation of Rituximab Treatment. *J Child Neurol* 2014; pii: 0883073814553974 [PMID: 25387545]
  - 50 **ClinicalTrials.gov**. A Double-masked, Placebo-controlled Study With Open Label Period to Evaluate MEDI-551 in Neuromyelitis Optica and Neuromyelitis Optica Spectrum Disorders. [Accessed 2015 January 6]. Available from: URL: <https://clinicaltrials.gov/ct2/show/NCT02200770>
  - 51 **von Elm E**, Altman DG, Egger M, Pocock SJ, Göttsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Prev Med* 2007; **45**: 247-251 [PMID: 17950122 DOI: 10.1016/j.ypmed.2007.08.012]

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