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Advance in B-cell therapies for the treatment of rheumatic and musculoskeletal diseases

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Advance in B-cell therapies for the treatment of rheumatic and musculoskeletal diseases

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Table of contents

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Editorial: Advance in B-cell therapies for the treatment of rheumatic and musculoskeletal diseases

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Editorial on the Research Topic
Advance in B-cell therapies for the treatment of rheumatic and musculoskeletal diseases

In this Research Topic of Frontiers in Medicine, our aim was to highlight advances in B-cell therapies in various rheumatic and musculoskeletal diseases (RMD) to further refine their use in clinical trial and routine practice. The two most evaluated strategies for B-cell blockade for the treatment of rheumatic and musculoskeletal diseases (RMD) over the last three decades are B-cell depletion and inhibition of B-cell survival factors i.e., B-cell-activating factor (BAFF) and/or A proliferation-inducing ligand (APRIL). Rituximab is a chimeric anti-CD20 monoclonal antibody (mAb). The depth of B-cell depletion and clinical responses may vary, implying potential pathogenic or pharmacodynamic differences between subgroups of patients. Recent data have supported the efficacy of reduced rituximab dose and the different retreatment strategies in its licensed indications; rheumatoid arthritis (RA), granulomatosis with polyangiitis/microscopic polyangiitis and pemphigus vulgaris, although long-term data are still needed to establish the optimal approach (1–5). Despite failure of rituximab in meeting its primary endpoints when investigated in systemic lupus erythematosus (SLE) (6, 7) and primary Sjogren's syndrome (pSS) (8, 9), it may still be used in refractory cases based on clinical effectiveness from case series (10–13). Belimumab, a BAFF-inhibitor, is licensed for patients aged ≥5 years with active, autoantibody-positive SLE who are receiving standard therapy and those aged ≥18 with active lupus nephritis who are receiving standard therapy. Its action on both B-cells and non-B-cells may have contributed to the success of belimumab trials. There remains an unmet need for mechanistic and clinical studies

concerning stratification of patients who would respond best to both rituximab and belimumab to aid personalized therapy.

In RA, the optimal retreatment paradigm for rituximab has not been fully determined. Three strategies are commonly used (3). (i) Fixed retreatment e.g., 2 × 1,000 gm infusions administered every 6 months. Nevertheless, regular retreatment may risk overtreatment in some patients and increases the risk of infections associated with secondary hypogammaglobulinaemia (14). (ii) Retreatment can also be employed based on treat-to-target approach, in line with the European League Against Rheumatism (EULAR) recommendations for RA management whereby target of treatment is remission [DAS28 <2.6, Simple Disease Activity Index (SDAI) <3.3 or Clinical Disease Activity Index (CDAI) <2.8 or at least low disease activity (LDA)] (15). (iii) Retreatment-on-clinical relapse or "on demand." Inherent to this is a degree of instability, with potential clinical implications, such as more short-term corticosteroid use that can be potentially detrimental to long-term outcomes. This could be improved by identifying clinical and biomarkers of imminent relapse. Kim et al. used data from the Korean Rheumatology Biologics registry (KOBIO) and patients who were treated at the Ajou University Hospital, Suwon South Korea. Eighty-two patients were enrolled and those who responded were treated on demand. The mean time-to-retreatment was 16 months. In multivariable analysis, factors associated with longer time-to-retreatment were concomitant use of 2 or more csDMARD and concomitant use of corticosteroid (16). The latter should be interpreted with caution since there was no consistent association with time-to-retreatment when concomitant daily oral prednisolone dose was evaluated. At 5 years, the rituximab retention rate was 72% which was a good outcome from therapeutic perspective. Since some patients appear to be refractory to B-cell depleting therapy in RA, another therapeutic option is through the Janus kinase (JAK) inhibition. Moura and Fonseca discussed in a narrative review article that currently available JAK inhibitors (tofacitinib, baricitinib, upadacitinib, peficitinib, filgotinib, and decernotinib) can affect B-cell activation, proliferation and differentiation and could be beneficial in the pre-clinical or early phase of RA (17).

In pSS, Pavlych et al. conducted a retrospective observational cohort study to compare the effectiveness of rituximab originator (MabTherara®) and rituximab biosimilar (Truxima®) in patients with a disease duration of <5 years and a systemic moderate–high activity [as defined by The EULAR Sjögren's syndrome (SS) disease activity index (ESSDAI) ≥5 points]. Nine and eight patients were treated with the originator and the biosmilar, respectively. At 48 weeks, the mean ESSDAI score was significantly reduced compared to pre-rituximab score in all patients and there was no difference in the change in ESSDAI score from baseline between both treatment arms. Disappointingly, there was no improvement observed in the change in a patient-reported outcome, the EULAR Sjogren's Syndrome Patient Reported Index (ESSPRI) in all patients at weeks 24 and 48 from baseline and between the treatment groups (18).

In SLE, Wise and Stohl wrote a narrative review article and discussed the outcome disparities in randomized controlled trials (RCTs) between rituximab and belimumab. Failure of rituximab in meeting its primary endpoint could be attributed to its poor trial design and to a degree its biological effect i.e., plasma cells do not express CD20 and thus, are insensitive to rituximab. In contrast, in addition to a better trial design including the use of a new composite primary endpoint, the SLE Responder Index (SRI-4) and adequate sample size, plasma cells express B-cell maturation antigen (BCMA) and TNF receptor superfamily member 13b (TACI) of which both are inhibited by belimumab, thus abate ongoing pathogenic autoantibody production by plasma cells (19). These factors could influence the success of belimumab in five RCTs. *Post-hoc* analysis of belimumab RCTs and real-world observational studies identified characteristics of patients would most likely to respond to belimumab including those with high disease activity [the SLE Disease Activity Index 2000 (SLEDAI-2K) ≥10], anti-dsDNA positivity, low complement levels, polyarthritis, non-smoking status, and lack of significant end organ damage (20–22). Plüß et al. reported case series of seven patients who were treated with belimumab for non-approved SLE features (renal = 6 and neuropsychiatric = 1). Following therapy with belimumab, proteinuria was markedly improved in all patients and one patient with dysarthria and ataxia improved (23). Belimumab plus standard therapy has since approved for active lupus nephritis following a positive outcome in a phase III RCT (24). Nevertheless, it is important to note that the effect size of belimumab over its comparator in RCTs overall was rather modest (ranging from 9.7 to 14%), as well as an RCT in patients of black African ancestry failed to meet its primary endpoint at 52 weeks (25). Another subgroup of patient who may not respond well to belimumab is those who develop secondary non-depletion non response (2NDNR) to rituximab which is associated with anti-rituximab antibodies (26). Hassan et al. conducted an observational cohort study and compared the effectiveness of switching those with 2NDNR to rituximab to either belimumab ($N = 8$) or an alternative humanized anti-CD20 agent ($N = 6$; ocrelizumab = 3, ofatumumab = 2, obinutuzumab = 1). All patients in the latter group achieved SRI-4 response while only 1/8 patient in the former group met SRI-4 response. Moreover, 2/8 patients in the former developed lupus nephritis including one *de novo* Class II and V nephritis (27). This study suggests that patients who developed 2NDNR to rituximab should be switched within the same biologic class i.e., humanized or type 2 anti-CD20 mAbs.

In a narrative review article, Parodis et al. discussed other promising strategies to improve B-cell blockade in SLE including plasma cell inhibition using proteasome inhibitor such as bortezomib, the next generation anti-CD20 mAbs including

obinutuzumab, targeting B-cell intracellular signaling through inhibition of Bruton's Tyrosine Kinase (BTK) and the use of chimeric auto-antigen receptor (CAAR) T-cells that have been genetically engineered to kill human autoreactive B-cells (28). They also discussed since BAFF level rose following treatment with rituximab, combining therapy of rituximab and belimumab would be a logical approach. This was supported by the results from BEAT-LUPUS RCT where add on add-on belimumab was superior over rituximab alone in prolonging the time-to-severe SLE flare and in reducing anti-dsDNA antibody titres (29). Another alternative is sequential therapy for which Petricca et al. reported a case report of a patient with severe and refractory lupus nephritis and bullous pemphigus who responded to treatment with rituximab, followed by belimumab (30).

Finally from B-cell biomarker perspective, You et al. compared peripheral blood mononuclear cells of 57 SLE patients and 50 healthy controls using flow cytometry. They found that double negative B-cells (DN Bcells; CD19+CD27–lgD–) were associated with lupus nephritis and positively correlated with proteinuria level. The proportion of lupus nephritis patients who achieved remission following a therapy was higher in SLE patients with low DN Bcells than in patients with high pre-treatment DN Bcells rates; 83 and 25% respectively, thus could be a promising prognostic biomarker in lupus nephritis (31).

We thank the contributing authors for shedding light on the advance in B-cell therapies in our Research Topic, with the ultimate goal of improving the care of patients with RMD. Future research agenda will build on this progress and should focus on better biomarkers that may allow prediction of active disease, prognosis and/or response to therapy through the application of new technologies and clinical efficacy of novel B-cell-targeted therapies with stratification of therapy to disease manifestation and their long-term safety particularly in terms of the risk of severe infection and major cardiovascular events.

Author contributions

MM wrote the first draft of the Editorial, which was then reviewed by SA and KC who revised it critically for important intellectual content and final approval of the version published. All authors have agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Conflict of interest

MM has received consultancy fees from Aurinia Pharmaceuticals and UCB. KC received consultancy fees from Eli Lilly, AbbVie, and Pfizer.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Double Negative B Cell Is Associated With Renal Impairment in Systemic Lupus Erythematosus and Acts as a Marker for Nephritis Remission

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Objective: Recent studies on double negative B cells (DN B cells) suggested that they have potential pathogenic roles in systemic lupus erythematosus (SLE). This study aimed to determine the circulating DN B cells in SLE patients and analyzed the clinical significance of this cell subset.

Methods: Fifty-seven SLE patients and fifty healthy controls (HCs) were recruited in this study. Among the 57 SLE patients, 25 had lupus nephritis (LN). All patients were followed up for 24 weeks. Peripheral B cell subsets were analyzed by flow cytometry.

Results: DN B cells were significantly elevated in the SLE patients, especially in the patients with LN ($p < 0.01$). DN B showed a positive correlation with 24-h urine protein excretion (24 h-UPE) levels ($r = 0.444$, $p = 0.034$) in LN patients, and inversely correlated with evaluated glomerular filtration rate (eGFR) ($r = -0.351$, $p = 0.011$). DN B cells had a positive correlation with plasma cells ($r = 0.484$, $p < 0.001$) and memory B cells ($r = 0.703$, $p < 0.001$). After treatment, decreased DN B cells were associated with LN alleviation ($p = 0.002$). In the follow-up, the remission rate of LN patients with decreased DN B cells was significantly higher than LN patients with increased DN B cells (83.33 vs. 25.00%, $p = 0.030$) at week 24.

Conclusions: This study suggests that the peripheral DN B cells are positively correlated with the severity of renal damage in LN patients and may potentially be used as a prognostic marker in LN.

Keywords: double negative B cells, systemic lupus erythematosus, lupus nephritis, 24-h urine protein excretion, remission

INTRODUCTION

Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease with heterogeneous clinical manifestations that causes organ damage, and is triggered by a loss of self-tolerance, and autoreactive immune responses (1–3). Lupus nephritis (LN) is a frequent complication of SLE which can lead to significant illness and even death, despite great advances in treatment

over the recent decades (4–7). The development of LN involves multiple pathogenic pathways, including inflammatory cell infiltration, autoantibody production, aberrant apoptosis, immune complex deposition, and complement activation (4, 8, 9). B cells are prominently involved in the pathogenesis of SLE and connect innate immunity with adaptive immunity, since they can both act as effector cells responding to antigens in humoral immunity and sense the environment and present autoantigens to T cells as antigen-presenting cells (10–12). Hyperactive polyclonal B cells could produce excessive pathogenic autoantibodies, cytokines, and chemokines (10, 13, 14).

The B cell compartment is severely unbalanced in patients with active SLE (15–18). In a previous study, Wei et al. described the expansion of B cells as characterized by lacking both IgD and CD27 (double negative; DN) in SLE and postulated that they represent a novel subset of memory cells (19). Double negative B cells (DN B cells) both in healthy subjects and SLE patients express switched and mutated antibodies. It has been proposed that DN B cells are involved in the pathogenesis of SLE. DN B cells were reported to be correlated with anti-dsDNA and anti-RNP/Sm autoantibodies (19–21). However, the detailed clinical relevance of DN B cells in SLE remains unclear.

In this study, we determined the circulating DN B cells in SLE patients and analyzed the clinical significance of this cell subset. Our study shows that DN B cells are positively correlated with 24-h urine protein excretion (24 h-UPE) in patients with lupus nephritis regardless of disease activity and decreased DN B cells are associated with renal alleviation. Thus, our finding suggests that DN B cells may be potential therapeutic targets for the treatment of lupus nephritis.

MATERIALS AND METHODS

Patients and Healthy Controls

Patients with SLE satisfying the Systemic Lupus International Collaborating Clinics classification criteria were recruited from the Department of Rheumatology and Immunology in Peking University People's Hospital between February 2016 and January 2017. Fifty age-matched healthy controls (HCs) were enrolled. All participants were older than 16 years of age. All patients received standard-of-care therapy. The medications received by the patients were prednisone and other immunosuppressants, which were shown in **Table S1**. This study was approved by the ethics committee of Peking University People's Hospital. All patients provided informed consent to donate their blood samples and clinical information for research, and written consent was given by each individual.

Flow Cytometry Analysis

Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Hypaque density gradient centrifugation. For surface staining, PBMCs were stained for 30 min in the dark at 4°C with the monoclonal antibodies Alexa Fluor 700 mouse anti-human CD3 (Biolegend), PE-CF594 mouse anti-human CD19 (BD Biosciences), PE mouse anti-human CD24 (eBioscience), APC mouse anti human CD27 (Biolegend), BV421 mouse anti-human CD38 (BD Biosciences), FITC mouse anti-human IgD (Biolegend), and PerCp-Cy5.5 mouse anti-human CD20 (Biolegend). Based different expression of surface markers, B cell subsets were identified: DN B cells (CD19+CD27–lgD–), memory B cells (CD19+CD27+), and plasma B cells (CD19+CD20–CD27+CD38+). Samples were examined on a BD FACS Aria II flow cytometer and data were analyzed by FlowJo version X.

TABLE 1 | Demographic characteristics in patients with systemic lupus erythematosus.

Clinical characteristics	Values
Age, years	30.96 ± 9.84
Disease duration (months)	62.43 ± 63.13
SLEDAI-2K	11.74 ± 3.93
Gender (female: male)	53:4
Rash (%)	29/57 (50.88)
Arthritis (%)	27/57 (47.37)
Alopecia (%)	21/57 (36.84)
Lupus nephritis (%)	25/57 (43.86)
Pericarditis (%)	1/57 (1.75)
Thrombocytopenia (%)	12/57 (21.05)
Vasculitis (%)	6/57 (10.53)
Photosensitivity (%)	1/57 (1.75)
Ulceration (%)	5/57 (8.77)
Leukopenia (%)	3/57 (5.26)
Myositis (%)	3/57 (5.26)
Fever (%)	7/57 (12.28)
Headache (%)	2/57 (3.51)
Anemia (%)	19/55 (33.93)
Decreased C3 (%)	36/57 (63.16)
Decreased C4 (%)	35/57 (61.40)
Increased CRP (%)	12/53 (22.64)
Increased ESR (%)	24/56 (42.86)
ANA (+) (%)	42/45 (93.33)
Anti-dsDNA Ab (+) (%)	39/56 (69.64)
Anti-Sm (+) (%)	11/45 (24.44)
Anti-RNP (+) (%)	18/45 (40.00)
Anti-SSA (+) (%)	24/45 (53.33)
Anti-SSB (+) (%)	5/45 (11.11)
Proteinuria (+) (%)	37/55 (67.27)
Urine sediment erythrocytes (+) (%)	19/55 (34.55)
Urine sediment leukocytes (+) (%)	31/55 (56.36)

SLEDAI-2K, systemic lupus erythematosus disease activity index 2000; LN, lupus nephritis; C3, complement 3; C4, complement 4; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; ANA, antinuclear antibodies; Anti-dsDNA Ab, anti-double-stranded DNA antibody; anti-Sm, anti-Sm antibody; Anti-RNP, anti ribonucleoprotein; Anti-SSA, anti-Ro/SSA antibody; anti-SSB, anti-La/SSB antibody. Numerical data presented as mean ± SD were analyzed using Student's t-test, Mann-Whitney U-test or Pearson's Chi-squared test. $p < 0.05$ was taken as significant.

Clinical and Laboratory Evaluation

Clinical and laboratory data were collected at baseline, week 6, 12, and 24. Patient follow-ups were as scheduled by the treating physician. The following features of SLE were included in this study: rash, alopecia, photosensitivity, ulceration, myositis, fever, arthritis, leukopenia, thrombocytopenia, lupus nephritis, pericarditis, vasculitis, and NPSLE. Disease activity according to the Systemic Lupus Erythematosus Disease Activity Index-2000 (SLEDAI)-2K was recorded. A diagnosis of LN was made if patients fulfilled the American College of Rheumatology renal criteria (persistent proteinuria >0.5 g per day or >3+ by dipstick and/or cellular casts, including red cells, hemoglobin, granular, tubular, or mixed) at any time during the study. White cell and platelet counts $<3.50 \times 10^9$/L and 125×10^9/L were regarded as leukocytopenia and thrombocytopenia, respectively. All patients underwent extensive serological examinations, including tests of antinuclear antibody (ANA), anti-dsDNA antibody (anti-dsDNA), anti-Sm antibody (anti-Sm), anti-Ro/SSA antibody (anti-SSA), anti-La/SSB antibody (anti-SSB), anti-RNP antibody (anti-RNP), complement component C3, complement component C4, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), immunoglobulin A (IgA), immunoglobulin G (IgG), and immunoglobulin M (IgM). C3, C4, IgA, IgG, and IgM were tested by ELISA with normal ranges of 0.79–1.52 g/L, 0.16–0.38 g/L, 0.82–4.53 g/L, 7.2–16.8 g/L, and 0.46–3.04 g/L. Anti-SSA and anti-SSB were measured by ELISA, and ANA was measured by indirect immunofluorescence. CRP was examined by immunonephelometry assays and values equal to or more than 8 mg/L were considered positive. Serum IFN-alpha2, IL-6, IL-2, IL-21, IL-7, IFN-gamma, IL-12p70, IL-15, IL-17A, BCA-1, IL-10, and TGF-beta were measured by ELISA. Complete renal remission (CR) in this study was defined as 24 h-UPE was <0.5 g/day and absence of hemoglobinuria or pyuria after therapy. Partly remissive (PR) LN was defined as a decrease of 24 h-UPE >50% after therapy.

Statistical Analysis

SPSS 22.0 for windows and GraphPad Prism 7 were used to analyze the data. Data were presented as mean ± standard deviation and statistical significance between the two groups was assessed with the non-parametric Mann-Whitney test, paired *t*-test, and X^2 test. Spearman's rank correlation coefficient was applied to calculate the correlations. Bonferroni correction was performed to adjust multiple comparisons in our correlation analysis. The Kaplan–Meier method was applied to compare the renal remission of the two groups during the follow-up. A value of $p < 0.05$ was considered to be significant.

RESULTS

Characteristics of SLE Patients

In this study, 57 SLE patients and 50 healthy controls with matched demographic features (age, 30.96 ± 9.84 vs. 30.52 ± 5.66, $p = 0.399$; male/female, 4/53 versus 7/43, in SLE and HC, respectively, $p = 0.386$) were recruited. Detailed characteristics of SLE patients are shown in **Table 1**. The SLE patients had a mean disease duration of 62.43 months, ranging from 0 to 240 months and the mean SLEDAI of the patients was 11.74, ranging from 6 to 27 (**Table 1**).

Circulating DN B Cells Increased in SLE Patients

As shown in **Figures 1A,B**, we evaluated the levels of CD19+CD27-IgD- DN B cells in PBMCs of HCs and SLE patients by flow cytometry. Compared with HCs, the SLE patients showed a significant increase of DN B cells (13.70 ± 8.28 vs. 5.95 ± 4.09%, $p < 0.01$; **Figure 1C**).

Increased DN B Cells Were Correlated With Renal Involvement in SLE Patients

The percentages of DN B cells were compared between patient groups with or without autoimmune clinical and laboratory features. Among the 57 SLE patients in our study (**Table 1**), 25 were diagnosed as LN. Compared with peripheral blood DN B cell levels of 11.86 ± 7.48 % in non-LN patients, increased peripheral blood DN B cell levels of 16.06 ± 8.79 % in LN patients were observed ($p = 0.030$; **Table 2**, **Figure 2**). We also found the subset from patients with urinary protein

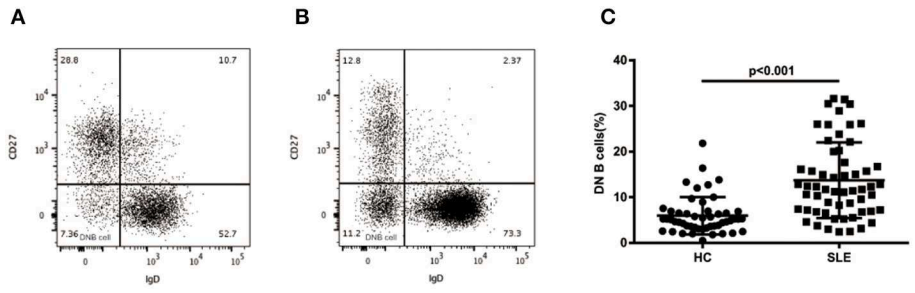

FIGURE 1 | Surface phenotype of peripheral blood DN B cells. Dot plots represent staining of peripheral B cells in healthy subjects **(A)** and SLE **(B)** patients with increased frequency of DN B cells. **(C)** Comparative analysis of blood DN B cells from healthy controls and different groups of SLE patients. Higher levels of DN B cells were detected in SLE patients ($n = 57$) than in healthy subjects ($n = 50$). Differences compared to the HC were assessed by Mann-Whitney test.

was increased significantly ($p = 0.018$; **Table 2**, **Figure 2**). These findings indicated an increase of DN B cell frequency in LN patients. We further analyzed the correlation between DN B cells and laboratory features of SLE. We found that DN B cells were positively correlated with 24 h-UPE levels ($r = 0.444$, $p = 0.034$) in LN patients (**Table 3**, **Figure 3**). The DN B cells had an inverse correlation with eGFR ($r = -0.351$, $p = 0.011$) in SLE patients. These results suggested that high levels of DN B cells were associated with impaired renal function.

TABLE 2 | DN B cells in the presence or absence of clinical or laboratory characteristics.

Characteristics	DN B cells		*p*-value
	Presence (*n*)	Absence (*n*)	
Rash	14.31 ± 9.26 (29)	13.07 ± 7.24 (28)	0.873
Arthritis	12.81 ± 7.33 (27)	14.51 ± 9.10 (30)	0.528
Alopecia	11.13 ± 7.04 (21)	15.20 ± 8.66 (36)	0.078
Lupus nephritis	16.06 ± 8.79 (25)	11.86 ± 7.48 (32)	**0.030**
Pericarditis	10.60 (1)	13.76 ± 8.34 (56)	0.761
Thrombocytopenia	14.86 ± 10.22 (12)	13.39 ± 7.79 (45)	0.845
Vasculitis	15.19 ± 6.82 (6)	13.53 ± 8.47 (51)	0.370
Photosensitivity	26.00 (1)	13.48 ± 8.18 (56)	0.181
Ulceration	13.64 ± 7.46 (5)	13.71 ± 8.42 (52)	0.855
Leukopenia	18.87 ± 10.24 (3)	13.41 ± 8.18 (54)	0.268
Myositis	8.94 ± 5.94 (3)	13.97 ± 8.35 (54)	0.292
Fever	12.68 ± 9.95 (7)	13.84 ± 8.12 (50)	0.451
Headache	10.82 ± 5.77 (2)	13.81 ± 8.37 (55)	0.696
Anemia	13.89 ± 8.77 (19)	13.74 ± 8.21 (37)	0.897
Decreased lymphocyte	15.96 ± 9.34 (28)	11.62 ± 6.64 (28)	0.103
Decreased C3	12.93 ± 7.28 (36)	15.02 ± 9.81 (21)	0.503
Decreased C4	12.67 ± 7.14 (35)	15.34 ± 9.77 (22)	0.342
Increased CRP	14.72 ± 9.53 (12)	13.15 ± 8.12 (41)	0.663
Increased ESR	15.03 ± 8.89 (24)	12.93 ± 7.84 (32)	0.362
ANA	12.98 ± 7.76 (42)	12.87 ± 2.47 (3)	0.716
Anti-dsDNA Ab	12.99 ± 8.04 (39)	15.82 ± 8.72 (17)	0.193
Anti-Sm	13.66 ± 6.39 (11)	13.66 ± 6.39 (34)	0.561
Anti-RNP	13.88 ± 9.10 (18)	12.36 ± 6.34 (27)	0.835
Anti-SSA	12.07 ± 6.39 (24)	13.99 ± 8.65 (21)	0.716
Anti-SSB	8.65 ± 4.65 (5)	13.51 ± 7.66 (40)	0.193
Proteinuria	15.41 ± 8.68 (37)	10.85 ± 6.83 (18)	**0.049**
Urine sediment erythrocytes	15.52 ± 8.63 (19)	13.08 ± 8.18 (36)	0.362
Urine sediment leukocytes	14.88 ± 8.91 (24)	13.18 ± 7.95 (31)	0.508

C3, complement 3; C4, complement 4; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; ANA, antinuclear antibodies; Anti-dsDNA Ab, anti-double-stranded DNA antibody; anti-Sm, anti-Sm antibody; Anti-RNP, anti ribonucleoprotein; Anti-SSA, anti-Ro/SSA antibody; anti-SSB, anti-La/SSB antibody. P-value was determined by Mann-Whitney U-test. $p < 0.05$ was taken as significant. The values in bold represent results with statistical significance.

Since the difference in DN B cells between patients with LN and patients without LN might be associated with variances in the therapies, we summarized the treatments the patients received in **Table S1**. There was no significant difference in most of the treatments between SLE patients with LN and without LN. Compared with non-LN patients, a higher proportion of LN patients received azathioprine treatment (20 vs. 0%, $p = 0.013$; **Table S1**). To exclude the therapeutic influence, the percentages of DN B cells were further compared between LN patients treated with or without azathioprine, and no significant difference was observed (**Figure S1**, $p = 0.362$). These results suggested that the significant difference in DN B cell levels between LN and non-LN patients might not be induced by concurrent treatments.

Correlation Between Plasma Cells or Memory B Cells and DN B Cell Subset in SLE Patients

We further analyzed the correlation between DN B cells and a variety of immune cell subsets in SLE. We found that DN B cells had a positive correlation with plasma cells ($r = 0.484$, $p < 0.001$) and memory B cells ($r = 0.703$, $p < 0.001$) (**Figures 4A,B**). No association was observed in naïve B cells, non-switched memory B cells, Treg, Tfh, Th1, Th2, Th17, and DN B cells. We also measured the concentration of serum cytokines in SLE patients. There was an inverse correlation between serum IL-21 and DN B cells (**Table 4**, **Figure 4**). DN B cells were also inversely correlated with lymphocyte ($r = -0.312$, $p = 0.019$), IgG ($r = -0.326$, $p = 0.020$), and IgM ($r = -0.412$, $p = 0.003$; **Table 3**, **Figure 3**). No association was observed between age or disease duration and DN B cells.

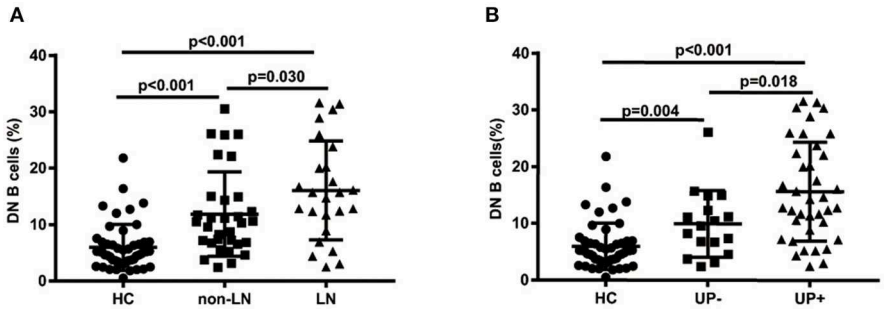

FIGURE 2 | Comparative analysis of blood DN B cells from healthy controls and different groups of SLE patients. **(A)** Frequency of DN B cells in LN patients, non-LN patients, and healthy controls. Higher frequency of DN B cells was detected in LN patients ($n = 25$) than in non-LN patients ($n = 32$). **(B)** Comparison among healthy controls and SLE patients with proteinuria negative ($n = 37$) or positive ($n = 18$). Differences between groups are indicated. Differences were assessed by Mann-Whitney test.

TABLE 3 | Correlations of DN B cells with the laboratory parameters from SLE patients.

Laboratory parameters	DN B cells	
	r	*p*-value
WBC	−0.114	0.402
Hb	−0.076	0.580
PLT	−0.020	0.886
Lymphocyte	−0.312	**0.019***
eGFR	−0.351	**0.011***
ALB	−0.132	0.358
ESR	0.054	0.692
CRP	0.132	0.347
IgA	0.053	0.712
IgG	−0.326	**0.020***
IgM	−0.412	**0.003***
C3	0.087	0.521
C4, g/L	0.232	0.082
Anti-dsDNA Ab	−0.061	0.654
SLEDAI-2K	0.088	0.516
24 h-UPE	0.444	**0.034***
Urine sediment erythrocytes	0.227	0.096
Urine sediment leukocytes	−0.076	0.580

*WBC, white blood cell; Hb, hemoglobin; PLT, platelet; eGFR, evaluated glomerular filtration rate; ALB, albumin; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; C3, complement 3; C4, complement 4; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; Anti-dsDNA Ab, anti-double-stranded DNA antibody; SLEDAI-2K, systemic lupus erythematosus disease activity index 2000; 24 h-UPE, 24-h urine protein excretion. Correlation was examined by Spearman's rank correlation test. $p < 0.05$ was taken as significant. The adjusted p-value is calculated by Bonferroni correction. The values with *represent not significant after correction for multiple comparisons. The values in bold represent results with statistical significance.*

DN B Cell Decreases After Treatment Was Associated With LN Alleviation and Was a Prognostic Marker for Future LN Remission

In the follow-up, we further assessed the effect of DN B cells on LN. Among the 25 LN patients in our study, 19 LN patients had complete follow-up data. Patients with decreased 24 h-UPE were considered as responsive to therapy (responders, $n = 12$), and the others were regarded as failing to respond to therapy (non-responders, $n = 7$). According to the change trends of these subsets at week 6 after treatment, we divided LN patients into two groups: patients with increased DN B cells (Δ DN B cells > 0, $n = 8$) and patients with decreased DN B cells (Δ DN B cells < 0, $n = 11$).

As the frequency of DN B cells was associated with LN involvement and 24 h-UPE level in LN patients, we speculate that effective therapy for LN might reduce the levels of DN B cells. Among LN patients under treatment, the percentages of DN B cells were indeed significantly decreased from 18.82 ± 8.727 to 15.26 ± 7.034% at week 6 in responsive LN patients ($n = 12$, $p = 0.030$; **Figure 5A**), whereas an obvious increase of DN B cells from 13.6 ± 10.51% at week 0 to 16.11 ± 11.61% at week 6 was observed in non-responsive patients ($n = 7$, $p = 0.080$).

The 24 h-UPE reduced from 2.44 ± 2.36 g/day at week 0 to 1.44 ± 1.11 g/day at week 6 in LN patients with decreased DN B cells ($p = 0.002$; **Figure 4B**); however an increase of 24 h-UPE was found (2.4 ± 1.75 g/day at week 0 vs. 3.22 ± 1.78 g/day at week 6) in LN patients with increased DN B cells ($p = 0.469$; **Figure 5B**).

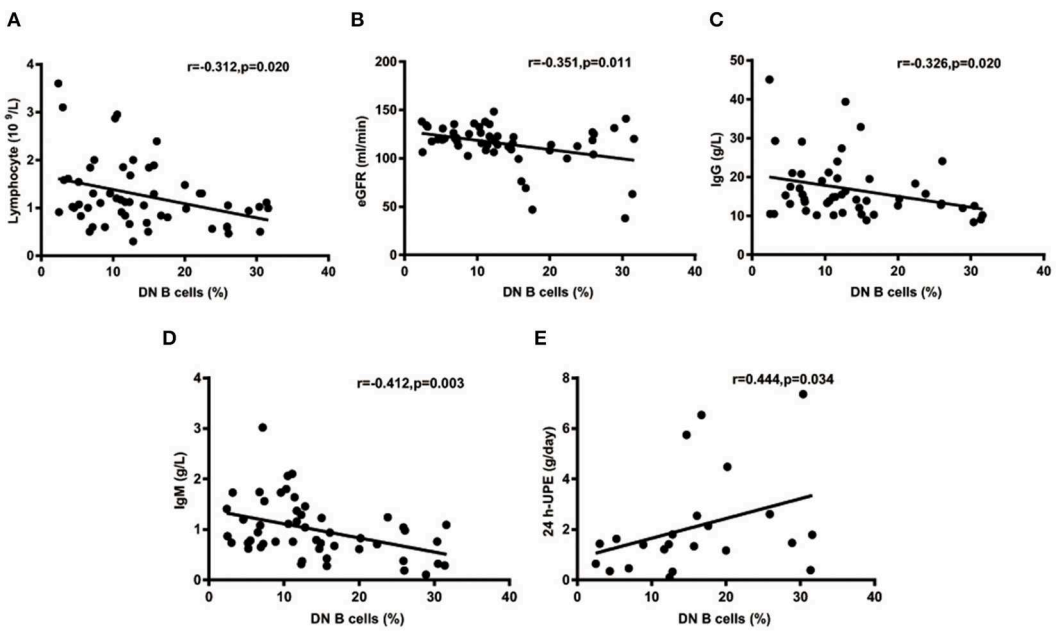

FIGURE 3 | Correlations of circulating DN B cells in SLE with laboratory values. **(A–D)** DN B cells had an inverse correlation with Lymphocyte, eGFR, IgG, and IgM. **(E)** DN B cells had a positive correlation with 24 h-UPE. High DN B cells were associated with high 24 h-UPE in LN patients. *P* was calculated according to Spearman's correlation test.

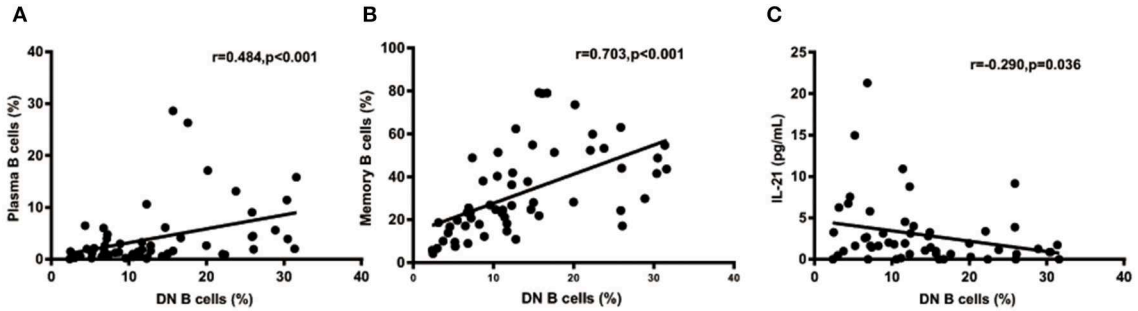

FIGURE 4 | DN B cells in SLE patients are correlated with other subsets and cytokines. DN B cells had a positive correlation with plasma B cells **(A)** and memory B cells **(B)**. **(C)** An inverse correlation was observed between DN B cells and IL-21. *P* was calculated according to Spearman's correlation test.

TABLE 4 | Correlation analysis for serum cytokines and DN B cells.

Cytokines	DN B cells	
	r	*p*-value
IFN-alpha2	−0.079	0.571
IL-6	−0.001	0.992
IL-2	−0.112	0.414
IL-21	−0.290	**0.036***
IL-7	−0.257	0.058
IFN-gamma	−0.157	0.254
IL-12p70	0.046	0.740
IL-15	−0.258	0.060
IL-17A	−0.232	0.092
BCA-1	0.022	0.874
IL-10	−0.164	0.232
TGF-beta	0.141	0.313

*Correlation was examined by Spearman's rank correlation test. $p < 0.05$ was taken as significant. The adjusted p-value is calculated by Bonferroni correction. The value with *represents not significant after correction for multiple comparisons. The values in bold represent results with statistical significance.*

As mentioned above, LN patients were divided into two groups according to the changes of DN B cells at week 6. During the follow-up period of 24 weeks, the remission rate in the group of patients with decreased DN B cells was 81.82%, which was significantly higher than in the other group (25.00%, $p = 0.030$; **Figure 6**).

DISCUSSION

Conventional memory B cells can be distinguished from naïve B cells by the presence of somatic hypermutation in their Ig variable gene sequences (22). CD27 is generally used as a marker to identify memory B cells because its expression correlates with the presence of somatic mutations in Ig genes (23, 24). However, further research elucidated that between 10 and 20% of IgG+ class-switched memory B cells were CD27- (25). Absence of IgD expression on DN B cells indicates that they have undergone class switching, though they do not gain the expression of CD27. DN B cells could represent a novel population of memory cells lacking CD27 expression (19). Early studies demonstrated that DN B cells can be detected in healthy persons, but are expanded in elderly people (26), patients with systemic lupus erythematosus, rheumatoid arthritis (27), Alzheimer's disease (28), non-small cell lung cancer (29), rotavirus (30), and HIV (31). Previous work showed that DN B cells are expanded in SLE patients (19). However, its clinical relevance in SLE is not yet clearly understood.

Our study demonstrated that LN patients had significantly higher DN B cells than non-LN patients, suggesting that DN B cells are involved in the renal damage associated with SLE. This result is consistent with previous studies that showed DN B cells expanded in patients with LN (16, 21). In addition, the levels of DN B cells were positively correlated to 24 h-UPE, suggesting that DN B cells may be involved in the severity of renal damage in LN patients. Consistent with this notion, the kidney damage of LN patients was effectively alleviated by drug treatment, accompanied by the downregulation of DN B cells. This result raised the possibility that DN B cells might be used as a prognostic marker in LN. Previous studies indicate that DN B cells are a heterogeneous subpopulation in SLE patients and DN B cells in the peripheral did not correlate significantly with disease activity (32). Our results were in agreement with this study and no correlation between DN B cells and SLEDAI was observed. The relationship between DN B cells and SLE is likely to be complex. Our data presents new evidence that DN B cells may play pathogenic roles in LN specifically. In previous studies, transcriptomic analysis on DN B cell subsets showed altered expression of multiple chemokine receptors including CCR9, CCR7, and CXCR5. In SLE patients, the alteration of the chemokine signaling network might lead to the chemotaxis of DN B cells into inflammatory tissues, like kidneys, to aggravate the local inflammation. These studies also showed that DN B cells were prone to differentiate into plasma cells, which preferentially produced pathogenic autoreactive antibodies in SLE. Altogether, these reports indicate that DN B cells might migrate to the relevant renal tissues and play a pathogenic role either *in situ* in the kidney or by their active production of autoantibodies.

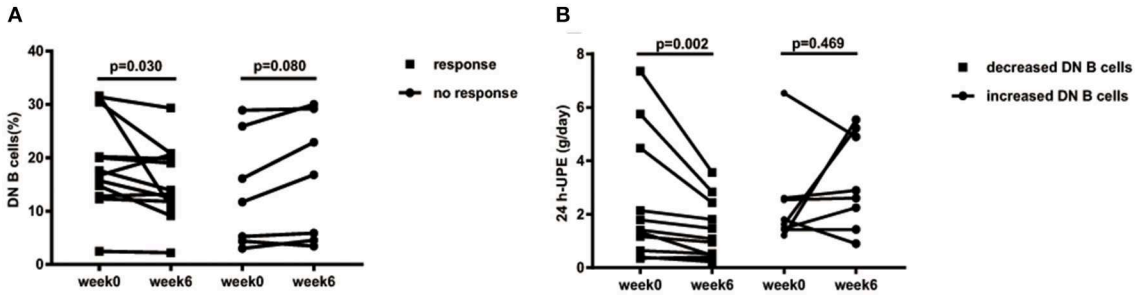

FIGURE 5 | (A) Decreased 24 h-UPE in LN was accompanied by the decrease of DN B cells. Patients who had improvement at week 6 ($n = 12$) presented significantly decreased DN B cells. Those patients with a poor response ($n = 7$) showed a trend of increased DN B cells. **(B)** DN B cells correlated with 24 h-UPE in LN patients. 24 h-UPE decreased in LN patients with decreased DN B cells ($n = 11$). Differences were assessed by Wilcoxon test.

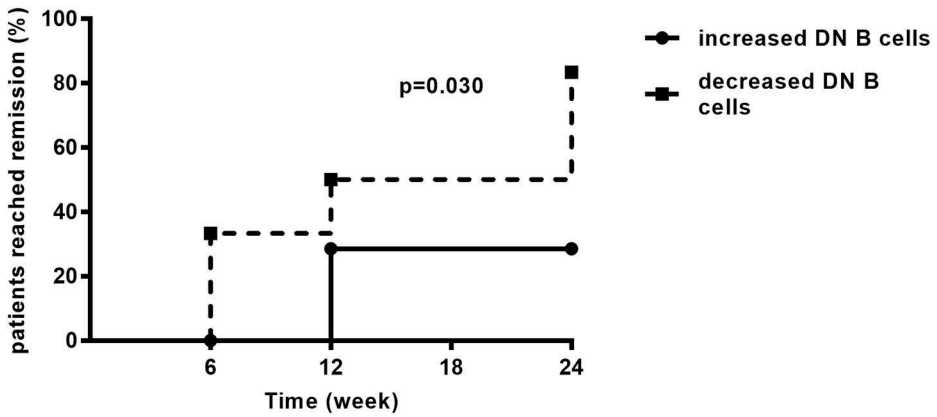

FIGURE 6 | During the follow-up, LN patients achieved remission. The remission rate was higher in LN patients with decreased DN B cells ($n = 11$) than those with increased DN B cells ($n = 8$). Differences were assessed by Kaplan–Meier method.

We found that DN B cells correlated with decreased lymphocytes or eGFRs, which are associated with SLE. As we know, lymphopenia is a typical feature of SLE (33–35). Glomerular filtration rate (GFR) is one of the conventional clinical parameters for detecting ongoing disease activity in lupus-affected kidneys and early relapse of nephritis (36, 37). A low GFR is a dangerous sign of existing kidney disease. Our data presented more evidence that DN B cells were specifically associated with LN development. Previous studies (20, 38) showed that DN B cells were correlated with anti-dsDNA or anti-RNP/Sm autoantibodies, but our study didn't observe such an association. It is possible that the limited cohort size in our study led to the lack of statistical significance. Differences in autoantibody testing technology or genetic background of recruited patients between previous studies and this study might also be possible reasons for this discrepancy.

IL-21 plays a critical role in B-cell differentiation and antibody production (39). Previous studies showed that IL-21 increased the number of transitional B cells, post-switched memory B cells, and plasma cells and promoted serum IgG and IgM production (40, 41). In our study, an inverse correlation was found between serum IL-21 and DN B cells. This may be explained by the possibility that DN B cells consumed IL-21 to differentiate into plasma cells. Our results also showed that increased levels of DN B cells were associated with elevated levels of plasma cells, which also supported the above hypothesis. These results suggest that DN B cells might play a role in the enhanced humoral immune response in SLE. However, the main limitation on our hypothesis is the lack of experimental evidence in this study. Previous studies have shown that the IL-21 receptor (IL-21R) was expressed at high levels in DN B cells, and after binding IL-21 with IL-21R, DN B subsets efficiently differentiated into plasma cells. These studies could provide some supportive experimental clues to our hypothesis.

During the 24-weeks follow-up, the remission rates of LN patients with decreased DN B cells and increased DN B cells at week 6 were 83.33 and 25.00% ($p = 0.030$), respectively. These results demonstrate that patients with decreased DN B cells under treatment are likely to achieve renal remission, and this is the first study showing that DN B cells might be a prognostic marker in LN.

One limitation of this study is that the SLE patients received different treatments during the study. Since therapy could affect the immune phenotypes, it is possible that the difference in DN B cells between patients with LN and patients without LN is associated with the possible variances in the treatments they received. Our analysis showed that there was no significant difference in most of the treatments between LN patients and non-LN patients. Although 20% of LN patients received azathioprine treatment while non-LN patients did not, no difference in the percentages of DN B cells were observed between LN patients treated with or without azathioprine. These results suggest that different treatments might not lead to different DN B cell levels in LN patients and non-LN patients in the current study. However, it is still possible that differences in DN B cells might be induced by different therapies, and future studies with a larger cohort size should be performed to elucidate the possible effects of different treatments on DN B cells.

Another limitation of this study is shown by the adjusted *p*-value of correlation analysis with Bonferroni correction (**Tables 3**, **4**). After the correction, no statistical significance was observed in the adjusted *p*-values. It is possible that the limited cohort size in our study led to the lack of statistical power when correction for multiple comparison was performed.

This study suggests that DN B cells correlate with the severity of renal damage in LN patients. Also, DN B cells may be involved in the pathogenesis of LN. Furthermore, decreased DN B cells are associated with renal alleviation during the follow-up. Specifically, our findings indicate that DN B cells may be used as a prognostic marker in LN.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

This study was approved by the ethics committee of Peking University People's Hospital. All patients provided informed consent to donate their blood samples and clinical information for research, and written consent was given by each individual.

AUTHOR CONTRIBUTIONS

XY performed the experiment and statistical analysis and wrote the draft of the manuscript. ZL and XS designed the study, analyzed the data, and wrote the manuscript. RZ and MS participated in acquiring clinical data and performed some experiments. JH, JC, JL, XZ, XL, and RJ participated in the analyses. All authors contributed to manuscript revision and have read and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2020.00085/full#supplementary-material

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BRIEF RESEARCH REPORT
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Clinical Efficacy of Routinely Administered Belimumab on Proteinuria and Neuropsychiatric Lupus

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Background and Objectives: Belimumab (BEL) is a monoclonal antibody approved for the treatment of active systemic lupus erythematosus (SLE) but not for lupus nephritis (LN) and neuropsychiatric systemic lupus erythematosus (NPSLE). We aimed to assess BEL's effects on these severe, potentially life-threatening manifestations.

Methods: Retrospective observational cohort study using routine clinical data in a case series of patients with SLE receiving BEL.

Results: Sixteen patients received BEL therapy for active SLE. Nine were excluded because they had no LN or NPSLE. Six suffered from LN, and one patient had NPSLE. All LN patients received BEL in addition to standard therapy including glucocorticoids, hydroxychloroquine, and mycophenolate mofetil in five cases, and tacrolimus in one case. Three patients with proteinuria >1,000 mg/g creatinine responded well (one complete, two partial renal responses); all other patients had decreasing proteinuria and a reduction in anti-dsDNA levels. The patient with NPSLE who had failed previous therapies had persistent clinical improvement of cutaneous and neuropsychiatric manifestations. There was one mild allergic reaction and one lower respiratory tract infection, but no other adverse events. One patient discontinued therapy due to a lack of improvement in clinical symptoms, another because of clinical remission.

Conclusions: In our series, BEL led to a decrease of proteinuria in patients with proteinuria of more than 1,000 mg/g creatinine despite standard of care treatment, and led to a marked clinical improvement in one patient with NPSLE. No adverse events were observed. Routinely administered BEL shows clinical efficacy on non-approved manifestations, but careful patient selection is warranted.

Keywords: systemic lupus erythematosus, lupus nephritis, neuropsychiatric lupus erythematosus, belimumab, monoclonal antibodies

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INTRODUCTION

Systemic lupus erythematosus (SLE) is a rare autoimmune disease that can potentially affect every organ system (1). Many organ manifestations can be managed effectively with the available immunosuppressive therapies. Lupus nephritis (LN) and neuropsychiatric lupus erythematosus (NPSLE), however, are two of the organ manifestations that may lead to a worse overall prognosis

(2, 3). LN occurs in up to 38–60% of SLE patients throughout the disease course (4, 5). The prevalence of NPSLE is more challenging to assess due to its clinical heterogeneity. A recent meta-analysis reported neuropsychiatric syndromes to be present in about 50% of all patients (6).

Belimumab (BEL) is a monoclonal antibody targeting B-lymphocyte stimulator (BLyS), and is FDA-approved as an additional treatment for SLE patients with persisting disease activity despite standard of care (SoC). It was investigated in the BLISS-52 and−76 trials (7, 8) and subsequently approved internationally; however, as LN and NPSLE were exclusion criteria in these trials, affected patients were only assessed indirectly (9). It is, therefore, as yet unclear whether or not BEL is of benefit for SLE patients with LN or NPSLE. Here, we aim to assess BEL's efficacy on LN and NPSLE patients who received BEL as in label-treatment, and report our experience using BEL in these off-label manifestations.

METHODS

Population and Setting

This is a retrospective observational single-center cohort study of a case series of SLE patients treated at our center who received BEL in addition to SoC. BEL was initiated for continued SLE disease activity (clinical and/or serological) despite SoC medications. Continued disease activity was defined as frequent flares and requirement for repetitive increases of prednisolone doses, or the inability to taper down prednisone to a dose below 7.5 mg daily. Patients who had flares mostly suffered from relapsing arthritis or persistent skin disease with lower prednisone doses. We collected routine clinical and laboratory data and report on the relevant outcomes for the off-label manifestations, LN, and NPSLE, respectively. Of note, BEL was not specifically initiated for LN or NPSLE, and all patients with LN underwent SoC medication including cyclophosphamide (CYC) or mycophenolate mofetil (MMF). All patients were followed consecutively at regular intervals.

Data Selection and Recording

The data used for analysis consisted of routinely collected clinical and laboratory data. Patients who regularly attend our outpatient clinic were screened for eligibility. Routine clinical and laboratory assessment included the parameters described in the SLE disease activity index 2000 (10); in addition, complete blood count, serum creatinine, blood urea nitrogen, erythrocyte sedimentation rate, c-reactive protein, urinalysis for proteinuria [reported as mg/g creatinine from spot urine as an estimation of proteinuria over 24 h (mg/d)] as well as anti-double-stranded (ds) DNA antibodies and complement factors C3 and C4. Renal responses were defined as complete (<650 mg/d after 6 months), partial (reduction, but >650 mg/d after 6 months), and no response (11).

Statistical Methods

Descriptive statistics were used to characterize the study population. Before-and-after comparisons were used for the assessment of therapeutic effects. Comparisons between groups were assessed using Friedman's test or Kruskal-Wallis Test. All statistical tests were performed using GraphPad Prism (Version 8.2.1 for mac OS, GraphPad Software, La Jolla California USA, www.graphpad.com).

Ethics Statement

While no formal approval is required for the use of routine clinical data, the study protocol was acknowledged by the local Ethics committee (No. 4/8/19), and all patients consented to the use of their routinely collected data as part of their regular medical care.

RESULTS

Of a total number of 16 patients with SLE treated with BEL at our center, nine were excluded due to absent LN or NPSLE. Of the remaining seven patients, six had biopsy-proven LN, and one had a clinically definite diagnosis of NPSLE.

Effect on Lupus Nephritis

The six patients with LN receiving BEL were all female and between 27 and 52 years of age. All had biopsy-proven LN class III, IV, or V, and a positive antibody status for anti-nuclear antibodies (ANA) and anti-ds DNA antibodies (**Table 1**). All patients received BEL intravenously and were followed up at regular intervals. Data collected three and six months after the initiation of BEL therapy are presented in **Table 1**.

Case #1

This 49-year-old patient of Asian ancestry had severe proteinuria of more than 4,000 mg/g creatinine and received BEL in addition to treatment with glucocorticoids (GC), hydroxychloroquine (HCQ), and mycophenolate mofetil (MMF) starting 1 month after renal biopsy. She developed allergic symptoms (fever, erythematous rash), and BEL was discontinued; upon re-exposition, however, no further allergic symptoms have developed, and the patient continues BEL therapy. Proteinuria was markedly reduced during BEL treatment but remained >650 mg/g creatinine after 6 months. She had a partial renal response.

Case #2

This 52-year-old Caucasian patient had undergone a renal transplant for LN almost 13 years before the initiation of BEL, and developed transplant kidney LN. Her background therapy consisted of GC, HCQ, leflunomide (LEF), and tacrolimus (TAC), the latter as part of her post-transplant immunosuppression. With BEL, the patient had mild improvement of proteinuria and clinically stable disease, but no additional benefit. Therefore, BEL and LEF were discontinued after five months as per the patient's wishes. It has to be noted, however, that the patient did not have significant proteinuria at the initiation of BEL.

TABLE 1 | Clinical and laboratory characteristics of the study population.

Patient	Age, sex, ethnicity	Disease duration (years)	Antibodies, complement levels	Renal biopsy (time before BEL initiation)	LN class	Previous therapies	Proteinuria (mg/g creatinine) (Baseline, after 6 months [% reduction])	Current therapy	GC (mg/d) (Baseline, after 6 months [% reduction])
1	49, f, Asian	15	ANA+, dsDNA+, SSA+, SSB+, Histone +, C3↓, C4↓	1 month	IV–G	GC, CYC, HCQ, MMF	4,074, 1202 (−70.5%)	GC, MMF	50, 2.5 (−95%)
2	52, f, Caucasian	19	ANA+, dsDNA+	12.5 years	V–IV	GC, HCQ, TAC	117, 76 (−35.04%)	GC, HCQ, TAC	5, 5 (0%)
3	30, f, Caucasian	4	ANA+, dsDNA+, SSA+, SSB+, Histone +, C3↓, C4↓	4 months	IV–G (A)	GC, HCQ, MMF	346, 162 (−53.18%)	GC, HCQ, MMF	60, 4 (−93.3%)
4	27, f, Caucasian	8	ANA+, dsDNA+, C3↓	13 months	III (A, C)	GC, HCQ, MMF	489, 115 (−76.48%)	GC, HCQ, MMF	6.5, 5 (−15.3%)
5	35, f, Caucasian	4	ANA+, dsDNA+, Histone +, C3↓, C4↓	4 months	III A	GC, MMF	159, 74 (−53.46%)	GC, AZA, HCQ	15, 5 (−66.3%)
6	40, f, Caucasian	17	ANA+, dsDNA+, APLA+	2 months	IV	GC, CYC, AZA, MMF	4,420, 121 (−97.26%)	GC, MMF	15, 5(−66.6%)
7	75, m, Caucasian	19	ANA+, dsDNA+, U1snRNP+, Histone+, C3↓, C4↓	17 years	None	GC, CYC, AZA, MTX, RTX	1,783, 655 (−63.26%)	GC, AZA	20, 2.5 (−87.5%)

ANA, antinuclear antibody; APLA, anti-phospholipid antibodies; AZA, azathioprine; C3/4, complement factor 3/4; CYC, cyclophosphamide; dsDNA; double-stranded DNA; f, female; GC, glucocorticoids; HCQ, hydroxychloroquine; LN, lupus nephritis; m, male; MMF, mycophenolate mofetil; MTX, methotrexate; RTX, rituximab; TAC, tacrolimus.

Case #3

This further Caucasian patient, 30 years of age, received BEL in addition to GC/MMF/HCQ for relapsing arthritis and malar rash four months after her renal biopsy. A further reduction by about 50% after six months of treatment could be demonstrated (**Table 1**). She was also able to taper down her steroid dosage and experienced overall improvement of her quality of life.

Case #4

This 27-year-old patient received BEL in addition to GC, HCQ and MMF starting 13 months after renal biopsy and was shown to have a significant albeit less pronounced reduction in proteinuria (−35%, **Table 1**). She experienced a clinical remission of SLE and wished to discontinue the additional treatment later on.

Case #5

This 35-year-old Caucasian patient received BEL in addition to GC/MMF for persisting skin disease and arthritis starting four months after renal biopsy. She exhibited another significant reduction in proteinuria of 53% after six months. She was also able to reduce her GC doses and reported an overall improvement of quality of life.

Case #6

This patient had undergone multiple treatment regimens, including azathioprine (AZA) and cyclophosphamide (CYC) for severe LN class IV with high-grade proteinuria and stage 4 chronic kidney disease. She had experienced multiple infectious complications requiring antibiotic therapy. Her background regimen consisted of GC and MMF when BEL was initiated two months after her most recent renal biopsy. She had a significant reduction in proteinuria from >4,000 mg/g creatinine to just over 100 mg/g creatinine. In light of further respiratory and urinary tract infections, the dose was reduced, and the administration of BEL was switched from intravenous to subcutaneous application. The patient continued to benefit from BEL therapy and has remained on the treatment for over two years.

Case #7—Effect on Neuropsychiatric Lupus

The patient with NPSLE is a Caucasian gentleman with a diagnosis of SLE at the age of 56 and presented with multi-organ involvement including skin erythema and ulcerations, arthralgia,

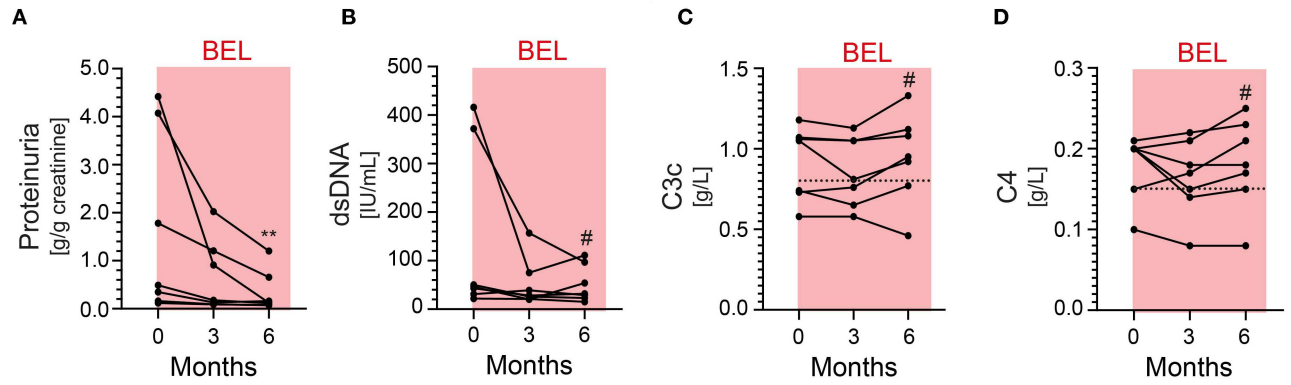

FIGURE 1 | Proteinuria, anti-double-stranded (ds) DNA antibodies and complement levels C3 and C4 at baseline and up to 6 months in patients with LN receiving BEL in addition to standard therapy. **(A)** There is a statistically significant reduction of proteinuria after 6 months (**$p = 0.0015$). The net effect is most pronounced in patients with the highest degree of proteinuria. **(B)** anti-dsDNA antibodies over time. A non-statistically significant decline is observed in patients with the highest baseline levels. **(C,D)** Complement factors C3 and C4 over time. There is no statistically significant difference after 6 months. Dotted lines represent the lower limit of normal (normal range of C3 0.82–1.93 g/L, C4 0.15–0.57 g/L). BEL, belimumab; LN, lupus nephritis. #not significant.

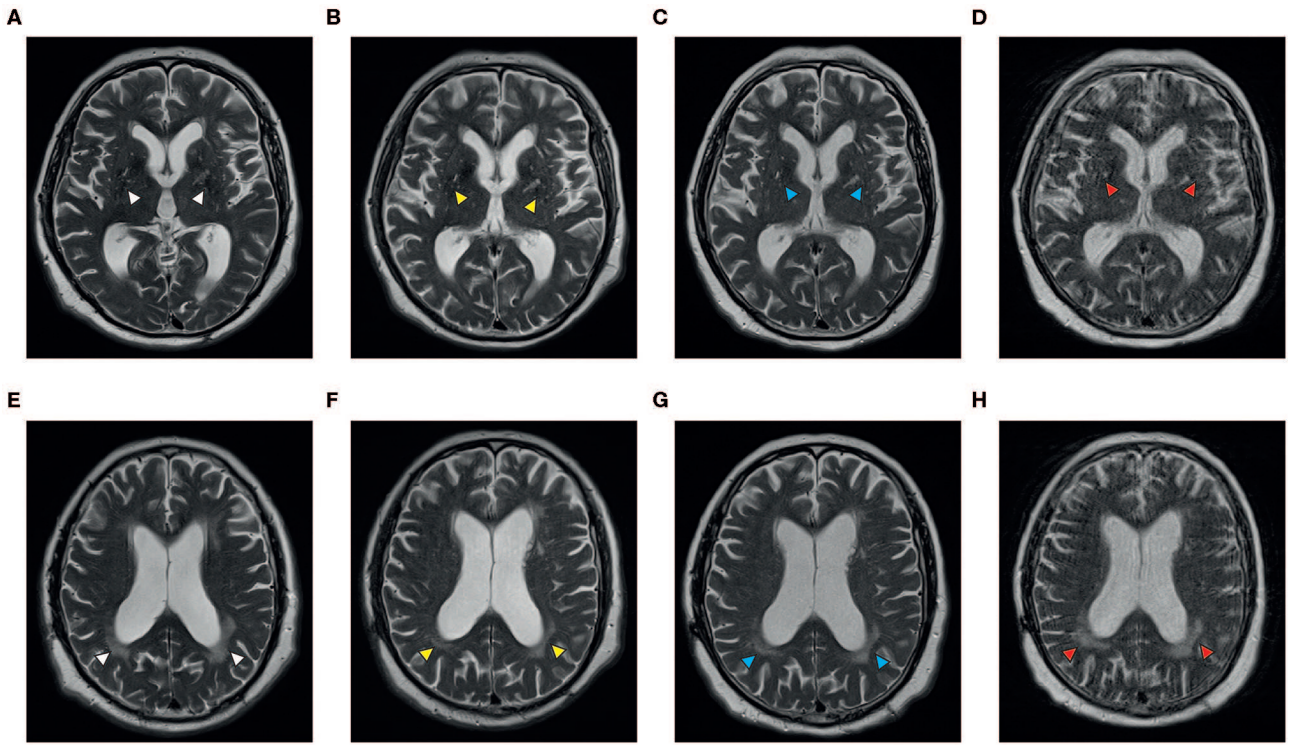

FIGURE 2 | Axial T2-weighted MR images of a patient with NPSLE before **(A,B,E,F)** and during treatment with belimumab **(C,D,G,H)**. Vascular white matter lesions can be demonstrated in the region of the basal ganglia **(A)** and the posterior periventricular matter **(E)** at the onset of neuropsychological symptoms (white arrowheads); there is a progress of the central lesions and formation of anterior white matter involvement within 15 months while the posterior lesions appeared less intense (**B,F,** yellow arrowheads). The panels on the right demonstrate stable disease after four (**C,G,** blue arrowheads) and 22 months (**D,H,** red arrowheads) of treatment with belimumab, respectively. Images courtesy of Prof. Dr. C. Riedel, Department of Neuroradiology, University Medical Center Göttingen.

myalgia, bicytopenia, and pulmonary fibrosis with subsequent pulmonary arterial hypertension. During a flare in 2016, he had developed cerebral manifestations with dysarthria, severe immobilizing ataxia, and concentration deficits. The patient failed or could not tolerate multiple therapies, including HCQ, CYC, MMF, LEF, and AZA, as well as rituximab (RTX) so that the cutaneous and neuropsychiatric disease manifestations remained insufficiently controlled. Therefore, we decided to

initiate treatment with BEL, which was given in conjunction with three doses of methylprednisone (250 mg) and rapidly tapered down. After three intravenous infusions with BEL the cutaneous and, most importantly, neuropsychiatric symptoms (dysarthria and ataxia) improved markedly, enabling the patient to continue a largely independent lifestyle with minimal assistance. Also, findings on cerebral magnetic resonance imaging (MRI) stabilized over time (**Figure 2**). Additionally, this patient also had proteinuria, but he did not have biopsy-proven LN. His first renal biopsy was performed 17 years before BEL initiation. Later on, we performed another renal biopsy in this patient due to increasing proteinuria, which showed focal segmental glomeruosclerosis unrelated to SLE.

DISCUSSION

Of the seven patients analyzed in this study, all showed significantly reduced levels of proteinuria during treatment with BEL, ranging from −35 to −97%. The levels of anti-dsDNA antibodies remained stable or diminished further with SoC plus BEL. Complement levels showed a tendency to normalize. Perhaps most importantly, all patients were able to significantly reduce the glucocorticoid doses and reported a symptomatic improvement of quality of life, although we did not formally assess this with questionnaires. In a recent analysis of the MAINTAIN nephritis trial, cut-off values below 650 mg/d after 6 months and 700 mg/d after 12 months were associated with a more favorable renal prognosis (11). Therefore, it should be highlighted that four out of the seven patients analyzed had a proteinuria of <500 mg/g creatinine, which would be considered as complete renal remission of LN, and, therefore, not prompt an escalation of immunosuppressive therapy. We did, however, include these patients in our analysis as they had biopsy-proven LN and were given BEL for continued disease activity, which might affect their renal outcome in the long run. Another limitation of the study might be that we routinely measure proteinuria in spot urine samples in an outpatient setting. This has been shown to be a reliable alternative (12). In a meta-analysis comparing spot urine to 24-h urine collection, it was argued that both tests correlated, at best, moderately (13). These findings are limited by the fact that only three of the 13 analyzed studies used Bland-Altman analysis as appropriate test for agreement between these two different methods.

The patient with NPSLE experienced a remarkable clinical improvement that allowed him to continue a mostly independent lifestyle. It has to be noted that imaging findings do not always correlate with clinical findings in NPSLE, as MRI lesions may be found in asymptomatic patients (14).

Adjunct therapy with BEL in patients with LN or NPSLE remains off-label: According to the EULAR 2019 standard of care recommendations for LN (15), BEL should currently be considered in patients with *extrarenal* lupus manifestations with poor or no response to first-line treatment.

While the results of further prospective clinical trials are eagerly awaited, a *post-hoc* analysis of the available BLISS trial data of patients with renal manifestations at baseline concluded in favor of BEL (16). Broadening this retrospective data pool to include patients with LN beyond the BLISS trials led to the conclusion that 55.1% of LN patients showed an improvement in renal parameters with BEL, including a reduction in proteinuria of 38% (9).

Interestingly, multiple case reports have been published suggesting a benefit of BEL in patients with LN, both as an adjunct treatment when first- and/or second-line therapies have failed (17–19), and as maintenance therapy after RTX (20–22). A case in which LN was refractory to RTX and then successfully treated with BEL has also been reported (23).

On the other hand, caution has been raised by some authors who witnessed patients developing LN while undergoing therapy with BEL (24, 25). However, a recent extensive review (26) of the treatment of refractory LN concludes that BEL—in combination with RTX, and possibly as monotherapy—will play a role. In the meantime, Glaxo Smith Kline, the manufacturer of Belimumab, has released a news outline that the phase 3 study of BEL in LN reached its primary endpoint (27). The final results have not been published as of yet but are awaited eagerly.

As far as NPSLE itself is concerned, data and case reports are not available. BLyS has, however, been shown to be elevated in the cerebrospinal fluid of NPSLE patients, making it a feasible target (28). A concise review of the available evidence (29) concluded that while, again, the BLISS trials were neither designed nor powered for NPSLE research, BEL appears to have been beneficial for neuropsychiatric symptoms.

In conclusion, the data of our retrospective series of seven cases presented in this study show a favorable effect of BEL on proteinuria in patients with biopsy-proven lupus nephritis as well as on neuropsychiatric manifestations in one patient with severe NPSLE. While further randomized controlled trials and subsequent recommendations concerning these indications are awaited, this data may help other clinicians to find appropriate and safe treatment for their patients.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics committee of the University Medical Center Göttingen. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

MP treated patients, collected and analyzed data, created figures, and co-wrote the manuscript. BT analyzed data, created figures, and reviewed the manuscript. NN collected data and reviewed the manuscript critically. MZ analyzed data and reviewed the manuscript. GM treated patients, analyzed data, and reviewed the manuscript. PK treated patients, conceived the study, collected and analyzed data, created figures, and co-wrote the manuscript.

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REVIEW
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Belimumab and Rituximab in Systemic Lupus Erythematosus: A Tale of Two B Cell-Targeting Agents

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Given the centrality of B cells to systemic lupus erythematosus (SLE), it stands to reason that a candidate therapeutic agent that targets B cells could be efficacious. Both rituximab, a monoclonal antibody (mAb) that binds to CD20 on the surface of B cells, and belimumab, a mAb that binds and neutralizes the B cell survival factor BAFF, have been extensively studied for the treatment of SLE. Despite the greater ability of rituximab to deplete B cells than that of belimumab, randomized controlled trials of rituximab in SLE failed to reach their primary clinical endpoints, whereas the primary clinical endpoints were reached in four independent phase-III clinical trials of belimumab in SLE. Accordingly, belimumab has been approved for treatment of SLE, whereas use of rituximab in SLE remains off-label. Nevertheless, several case series of rituximab have pointed to some utility for rituximab in treating SLE. In this review, we provide a concise summary of the factors that led to belimumab's success in SLE as well an analysis of the elements that may have contributed to the lack of success seen in the rituximab randomized controlled trials in SLE.

Keywords: B cells, B cell activating factor (BAFF), anti-CD20, B cell depletion, B cell depletion therapy

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INTRODUCTION

Systemic lupus erythematous (SLE) is a multi-organ systemic autoimmune disease that is characterized by autoantibody formation, deposition of antibodies into tissues, and complement activation, ultimately culminating in end-organ damage and dysfunction. Manifestations may range from the bothersome, such as alopecia and photosensitive rashes, to the life-threatening, such as myocarditis, cerebritis, and nephritis. While SLE can affect both sexes over a wide age range, it has a clear female predominance and classically affects women in their child-bearing years. The highly protean clinical presentation of SLE tends to be more aggressive in minorities, even when adjusting for socioeconomic factors that are independent of biologic or genetic factors (1).

While the future for patients with SLE is less foreboding today than it was 50 years ago, thanks in large measure to advances in immunosuppression regimens, including glucocorticoids, cyclophosphamide (CYC), and/or mycophenolate (MMF), SLE remains a challenge to treat due to a variety of factors. Its complex pathophysiology hints at processes that will be difficult to control with a single agent, and its heterogenous manifestations remind the provider that one size will likely not fit all. To further complicate the picture, the quintessential SLE patient is often a young woman in her childbearing years, and effective medications, such as CYC, may have dire short-term and long-term consequences regarding fertility, teratogenicity, and carcinogenicity. Renal involvement, which portends a poorer prognosis and is at the forefront of morbidity and mortality

for SLE patients, will eventually develop in up to 50% of SLE patients (2). Minority groups bear a poorer outcome regarding both SLE and lupus nephritis relative to their European or Caucasian counterparts, but regardless of ethnic background, there is a clear consensus that new avenues of research and treatment are desperately needed (1, 3–5).

To that end, B cells are a logical target for new SLE-directed therapies, given the overwhelming evidence implicating B cells as central players in the pathogenesis of SLE. In mice, genetic depletion of B cells from SLE-prone MRL. *lpr* or NZM 2328 mice completely blocks development of disease (6, 7). This protection goes beyond just the elimination of autoantibody production, since re-introduction into B cell-deficient MRL. *lpr* mice of B cells incapable of secreting Ig partially restores susceptibility to disease despite the absence of circulating autoantibodies (8). In human SLE, B cells have been implicated in pathogenic autoantibody production, cytokine production, and antigen presentation. Evidence exists that a loss of self-tolerance in B cell development contributes to the development of autoimmunity, thus prompting antibody production against self-antigens (9–12). Further, B cells also play a key role in T cell activation by serving as antigen-presenting cells (APCs), and B cells importantly contribute to the production of both pro- and anti-inflammatory cytokines (13, 14). Thus, via a variety of mechanisms, aberrant B cell function is linked both directly and indirectly to autoimmunity.

Not surprisingly, B cell-targeting therapy in SLE has attracted major interest over the past several years (**Table 1**). Rituximab (RTX), an anti-CD20 mAb, has been explored in SLE, given its B cell specificity and its efficacy in many other rheumatologic diseases. Belimumab (BEL), a mAb with specificity for B cell activating factor (BAFF), a vital B cell survival and differentiation factor, has also been explored in SLE. While there have been many promising uncontrolled and retrospective reports of RTX in SLE, it has failed to demonstrate efficacy in two independent SLE randomized clinical trials (RCTs). BEL, on the other hand, demonstrated efficacy in each of four independent SLE phase III trials (**Table 2**). The reasons behind these strikingly disparate outcomes are not self-evident, and in this paper, we describe the relevant landmark clinical trials and discuss some of the possible reasons for the difference in outcomes.

Finally, it is very clear that SLE is a complex disease that depends on a variety of pathogenic cellular functions which ultimately stem from a loss of tolerance to self. Many proposed mechanisms for this loss of tolerance are not directly dependent on B cells, and as such, B cell-directed therapy may have little to no clinical impact on discrete subsets of patients. For example, dendritic cells that transition from tolerogenic to immunogenic are unlikely to be affected by B cell-directed therapy, and the delicate interplay between dendritic cells and T regulatory cells to maintain homeostasis is also unlikely to be substantially affected by B cell-directed therapy (21). Accordingly, neither BEL nor RTX (nor any other B cell-targeting agent) will be the "cure-all" for SLE; B cell-targeting agents will comprise *part* of the solution, but they will never comprise the *entire* solution.

RITUXIMAB

RTX is a chimeric mAb that is specific for CD20, a transmembrane protein present on all B-lineage cells other than pro-B cells and plasma cells (22–24). Its engagement of CD20 promotes both cell-mediated and antibody-mediated cytotoxicity, resulting in depletion of $CD20^+$ B cells. First developed and FDA-approved for the treatment of non-Hodgkin's lymphoma, RTX has made a successful foray into rheumatology, with it being indisputably beneficial in the management of rheumatoid arthritis and ANCA-associated vasculitides (25, 26). RTX may also have a beneficial role in IgG4-related disease, inflammatory myopathies, cryoglobulinemia, and sarcoidosis (15, 16, 25, 27–29).

RTX was first explored for SLE in 2002, when five of six SLE patients with refractory disease clinically responded to a combination of RTX, CYC, and high dose corticosteroids (30). Looney et al. (31) then evaluated RTX in a phase I/II dose-escalating trial ($n = 18$) and found improvement in disease activity in 11 of these patients. Another study evaluated open-label RTX in 24 patients, many of whom who had failed conventional therapy, and found benefit regarding many disease parameters, including nephritis (32). An additional retrospective study of 45 SLE patients also found RTX to be beneficial; 89% of these patients achieved either full or partial remission after administration of RTX despite having a history of poor responsiveness or non-responsiveness to conventional therapy (33).

Given the many case series and anecdotes of RTX's success in SLE, the Exploratory Phase II/III SLE Evaluation of Rituximab (EXPLORER) RCT set out to critically assess RTX in non-renal SLE with moderate-to-severe disease (34). Patients ($n = 257$) on one immunosuppressant drug at a stable dose were treated with standard-of-care (SOC) therapy plus either RTX

TABLE 1 | Drug targets.

Drug	Structure	Target	Cells affected	Mean terminal elimination half life
Rituximab	Chimeric IgG1 mAb	Surface CD20	All B-lineage cells, excluding plasma cells and pro-B cells	∼3 weeks
Belimumab	Human IgG1 mAb	Soluble BAFF (BLyS)	All cells that express one or more BAFF receptors (BR3, BCMA, TACI); predominantly B cells and to a much lesser extent, T cells	∼9–14 days

mAb, monoclonal antibody; BAFF, B cell activating factor; BLyS, B lymphocyte stimulator; BR3, BAFF receptor 3; BCMA, B cell maturation antigen; TACI, transmembrane activator and CAML (calcium-modulator and cyclophilin ligand) interactor.

TABLE 2 | Landmark trials.

Drug	Trial name (n = subjects enrolled)	Trial design	Primary end point	Trial outcome
Rituximab*	EXPLORER ($n = 257$) (15)	Two arms; SOC + RTX vs. SOC + PBO	Achieving a major or partial clinical response at week 52 via the BILAG	No difference between RTX and PBO
	LUNAR ($n = 144$) (16)	Two arms; MMF + CS + RTX vs. MMF + RTX + PBO	Composite rate of complete and partial renal response at week 52	No difference between RTX and PBO
Belimumab**	BLISS-52 ($n = 867$) (17)	Three arms; SOC + BEL 1 mg/kg vs. SOC + BEL 10 mg/kg vs. SOC + PBO	SRI-4 response at week 52	Higher rates of response in both BEL 1 mg/kg (51%; $p = 0.0189$) and BEL 10 mg/kg (58%; $p = 0.0024$) compared to placebo (44%)
	BLISS-76 ($n = 819$) (18)	Three arms; SOC + BEL 1 mg/kg vs. SOC + BEL 10 mg/kg vs. SOC + PBO	SRI-4 response at week 52	Higher rate of response in BEL 10 mg/kg arm (43.2%) compared to placebo (33.5%) ($p = 0.017$)
	BLISS-SC ($n = 836$) (19)	Two arms; SOC + BEL 200 mg SC weekly vs. SOC + PBO	SRI-4 response at week 52	Higher rate of response in BEL arm (61.4%) compared to placebo (48.4%) ($p = 0.0006$)
	BEL113750 ($n = 677$) (20)	Two arms; SOC + BEL 10 mg/kg vs. SOC + PBO	SRI-4 response at week 52	Higher rate of response in BEL arm (53.8%) compared to placebo (40.1%) ($p = 0.0001$)

Rituximab 1,000 mg administered intravenously at weeks 0, 2, 24, and 26.

**Belimumab administered intravenously at weeks 0, 2, 4, and every 4 weeks thereafter, unless otherwise indicated.*

EXPLORER: exploratory phase II/III SLE evaluation of rituximab. SOC, standard of care; RTX, rituximab; PBO, placebo; BILAG, British Isles Lupus Assessment Group; LUNAR, Lupus nephritis assessment with rituximab; MMF, mycophenolate mofetil; CS, corticosteroids; BEL, belimumab; SRI-4, systemic lupus erythematosus responder index.

(two intravenous [IV] 1,000 mg doses 14 days apart at the start of the trial and at 6 months) or placebo. Major exclusion criteria included severe central nervous system involvement, organ-threatening SLE, recent (within 12 weeks of screening) use of CYC or a calcineurin inhibitor, and pregnancy or planning for pregnancy. During the trial, SOC therapy, which may have included methotrexate (MTX), azathioprine (AZA), mycophenolate mofetil (MMF), and/or corticosteroids, was continued at the discretion of the treating physician.

The primary end point was achieving and maintaining a major clinical response or a partial clinical response at week 52 via the BILAG. Secondary endpoints included average BILAG over 52 weeks, the proportion of patients with a partial clinical response at week 52, the time to first moderate or severe disease flare, and improvement in quality life, among others. RTX's steroid-sparing ability was also assessed.

To the dismay of many, no differences were detected between the RTX and placebo cohorts in achieving the primary or secondary endpoints. This did not change even when patients who did not attain complete B cell depletion were excluded. Nevertheless, among African-American/Hispanic patients, those treated with RTX compared to those treated with placebo had higher rates of major (13.8 vs. 9.4%, respectively) and partial (20.0 vs. 6.3%, respectively) clinical responses ($p = 0.04$). Within this subgroup of patients, RTX treatment led to reduction in anti-dsDNA titers ($p = 0.006$) and normalization of complement levels ($p = 0.0188$) relative to placebo.

Whereas the EXPLORER trial evaluated RTX in non-renal lupus, the Lupus Nephritis Assessment with Rituximab (LUNAR) trial evaluated RTX in lupus nephritis (35). This double-blind, placebo-controlled RCT, using a RTX-dosing regimen similar to that used in the EXPLORER trial, evaluated 144 patients with biopsy-proven class III or class IV nephritis. All patients received corticosteroids [1,000 mg on day 1 and again within three days, followed by weight-based prednisone (maximum 60 mg/daily), which was tapered to $\leq$10mg/daily by week 16] and MMF from day 1, with a goal dose of 3 g daily as tolerated.

The primary endpoint was the composite rate of complete and partial renal response at week 52. Complete renal response included a normal serum creatinine if it was abnormal at baseline or a serum creatinine of <115% of baseline if it was normal at baseline; an inactive urinary sediment (<5 RBCs/hpf and no RBC casts), and a urine protein:creatinine ratio <0.5. Partial renal response included a serum creatinine of <115% of baseline, RBCs/hpf <50% above baseline without RBC casts, and at least a 50% decrease in the urine protein:creatinine ratio to <1.0 or to $\leq$3.0, if the baseline ratio was >3.0. Secondary end points were similar to the EXPLORER trial and included sustainment of complete renal remission from week 24 to 52 as well as time to complete renal response.

Once again, no differences were detected between the RTX and placebo cohorts in achieving the primary or secondary endpoints. Complete renal response rates were 30% in the placebo group vs. 26% in the RTX group, whereas partial response rates favored RTX (31%) compared to placebo (15%). Along the same lines, among partial responders, 32% of RTX-treated patients had complete resolution of proteinuria compared to just 9% of placebo-treated patients. Similar to the LUNAR study, the African-American population trended toward more clinical improvement with RTX, although this trend did not achieve statistical significance. Additionally, eight patients in the placebo arm required CYC rescue therapy at week 52, whereas no patients in the RTX arm required such intervention.

Despite these disappointing findings, rheumatologists continue to use RTX in the clinical setting, often with excellent and encouraging results. Garcia-Carrasco described 52 patients with refractory disease who were treated with RTX (36). Not only did RTX control disease activity in several patients from a musculoskeletal and hematologic standpoint, but it also led to complete or partial renal remission in 10 of the 13 lupus nephritis patients. Terrier et al. (37) described 136 SLE patients, 42 of whom with nephritis, and also found a wide range of benefits, including control of lupus nephritis. This has been corroborated through several other case series and retrospective studies in both renal and non-renal SLE (38–41).

BELIMUMAB

BEL is a human IgG1λ mAb directed at BAFF (also known as B lymphocyte stimulator [BLyS]). BAFF is a vital B cell survival and differentiation factor that is produced by myeloid-lineage cells (42–44). Deletion of the *Baff* gene prevents development of disease in SLE-prone mice (45), and pharmacologic neutralization of BAFF in such mice ameliorates disease (46–48). In humans, BAFF levels are greater in SLE patients than in healthy control subjects, and BAFF levels correlate with disease activity (17–19, 49, 50).

BEL binds to soluble BAFF, thereby preventing BAFF from binding to its three B cell receptors: TACI, BCMA, and BR3 (20, 51, 52). BEL was approved for adult and pediatric SLE in 2011 and 2019, respectively, and was the first FDA-approved drug for SLE in over 50 years. To date, it remains the only biologic approved for SLE. Unlike RTX, which was developed outside the realm of rheumatology, BEL was developed with SLE in mind.

BEL was studied in two large double-blind phase III RCTs, BLISS-52 (n = 865) and BLISS-76 (n = 819) (53, 54). Each trial enrolled SLE patients with active disease (excluding those with active CNS involvement or nephritis) who, in addition to background SOC, received IV BEL 1, 10 mg/kg, or placebo at weeks 0, 2, 4, and then every 4 weeks. All patients were required to be on stable doses of corticosteroids, non-steroidal anti-inflammatories, anti-malarials, and other immunosuppressants for the 30 days prior to the start of the trial. The three arms in both trials were similar in average daily prednisone use, percentage of patients taking >7.5 mg prednisone daily, and use of background medications such as hydroxychloroquine, MTX, AZA, and MMF.

The primary endpoint in both trials was the Systemic Lupus Erythematosus Responder Index (SRI)-4 at week 52, defined as ≥4 point reduction in the Safety of Estrogens in Lupus Erythematosus National Assessment-Systemic Lupus Erythematosus Disease Activity Index (SELENA-SLEDAI) score, no new British Isles Lupus Activity Group (BILAG) A organ domain score and ≤1 new BILAG B score, and no worsening in Physicians Global Assessment (PGA) score. Secondary endpoints included SRI-4 response rate at week 76 (for BLISS-76 only), change in PGA score at week 24, and percentage of patients with a mean prednisone dose reduction of ≥25% from baseline and ≤7.5 mg/day during weeks 40–52.

At week 52 in BLISS-52, a greater percentage of patients in the BEL 1 mg/kg arm (51%; $p = 0.0189$) and in the 10 mg/kg arm (58%; $p = 0.0024$) achieved an SRI-4 response than in the placebo arm (44%). Further, there were also significant improvements in median time to first flare, as well as a steroid sparing effect in the BEL 10 mg/kg arm ($p = 0.0036$ and 0.0032, respectively). In BLISS-76, the SRI response at week 52 was greater in the BEL 10 mg/kg arm than in the placebo arm (43.2 vs. 33.5%; $p = 0.017$). While there were some trends toward reduced glucocorticoid use in each BEL arm, not all achieved statistical significance.

Given the success of these two phase III RCTs with IV BEL, subcutaneous (SC) BEL was evaluated in another double-blind phase III RCT, BLISS-SC (55). Patients (n = 836) received SOC and either BEL 200 mg SC every week or placebo, and the primary outcome was SRI-4 response at week 52. Secondary endpoints were time to first flare and reduction in corticosteroid use. Once again, a greater response rate was achieved by patients in the BEL group (61.4%) than in the placebo group (48.4%) ($p = 0.0006$).

Subsequently, IV BEL (10 mg/kg plus SOC) was again assessed vs. placebo (plus SOC) in the Asia-based phase III RCT, BEL113750 (n = 677) (56). Primary outcome was SRI-4 response at week 52, and for the fourth time in phase III RCTs, response was greater in the BEL arm than in the placebo arm (53.8 vs. 40.1%; $p = 0.0001$). Secondary end points, including rates of severe flares and reduction in cumulative steroid exposure, were also greater in the BEL arm than in the placebo arm.

Importantly, BEL has achieved success in "real world" settings. In the OBSErve studies, an ongoing international set of observational studies of BEL use in routine clinical practice in over 700 patients, efficacy and a steroid-sparing effect for BEL have been documented (57–60). Additional observational studies of BEL from Italy (n = 67), Greece (n = 91), Sweden (n = 58), and Spain (n = 23) have confirmed the efficacy and steroid-sparing effect of BEL (61–64). Moreover, at time of writing, positive results have been reported (albeit not yet published) for BLISS-LN, a RCT to evaluate BEL's efficacy in lupus nephritis (65). Both *post-hoc* analysis of phase III trials and examination of "real-life" belimumab-treated patients suggest that patients who have high disease activity (SLEDAI-2K >10), anti-dsDNA positivity, polyarthritis, non-smoking status, and lack of significant end organ damage have the highest probability of responding to BEL treatment (62, 66, 67).

While BEL has demonstrated efficacy both in clinical trials and in real-world settings and has a safe long-term side-effect profile, it is not a panacea for all SLE patients. In clinical trials, at least 40% of SLE patients did not demonstrate a clinically meaningful response to BEL, suggesting that disease activity depended on other pathways. Targeting dendritic cells, type I interferon, or Janus kinase-signal transduction may offer additional control over SLE disease activity, and the future will tell if combination therapy with these (or others) and BEL should be pursued. Additionally, given its cost, BEL is rarely available as a first-line treatment to SLE patients. Finally, the EMBRACE trial, which focused on the efficacy of BEL in patients of self-identified black race, did not meet its primary endpoint (SRI response rate with a modification for proteinuria at week 52), although some trends in favor of BEL were noted (68). As a general approach, it may

TABLE 3 | Rituximab/belimumab paradox.

Paradigm	Hypothesis
Trial design	Liberal use of CS in EXPLORER and concurrent MMF use in LUNAR may have blunted the differences between placebo and RTX, while BEL trials had stricter requirements for background SOC therapy.
	Rigorous composite response in LUNAR may have been too conservative to detect significance, while BEL's primary outcome (SRI-4) was able to detect subtle changes in disease activity.
	Large numbers of patients in BEL trials resulted in adequate powering, while RTX trials may have not enrolled enough patients for adequate powering.
	The SRI-4 used in BEL trials was based on analysis and assessment of prior phase II trials; a similar approach was not taken for RTX trials.
SLE phenotype	SLE phenotypes with aggressive and refractory manifestations may be highly B cell-driven and respond dramatically to RTX, whereas those with more mild phenotypes may respond less well.
	BEL's more widespread effects on the immune system (including on T cells) may allow for better control of mild-moderate disease phenotypes.
B regulatory cells (Bregs)	Bregs are involved in regulatory functions of the immune system. Depletion by RTX may aggravate autoimmune response, whereas they may be spared by BEL.
Plasma cells	RTX spares plasma cells, thereby allowing continued pathogenic autoantibody production. Receptors for BAFF are present on plasma cells, so plasma cell function may be inhibited by BEL.
B cell depletion	B cells may require "priming" by certain factors prior to become sensitive to RTX. No such "priming" may be needed for sensitivity to BEL.
Effect on non-B cells	BEL may modulate non-B-cell elements of the immune system that contribute to SLE activity, whereas RTX is B-cell specific.

CS, corticosteroids; EXPLORER, exploratory phase II/III SLE evaluation of rituximab; MMF, mycophenolate mofetil; LUNAR, Lupus nephritis assessment with rituximab; RTX, rituximab; BEL, belimumab; BEL, belimumab; SRI-4, systemic lupus erythematosus responder index.

prudent for the treating provider to consider treatment with less expensive traditional non-biologic agents such as AZA or MMF prior to pursuing BEL.

DISPARATE OUTCOMES

Although RTX and BEL each target B cells, the two RTX RCTs failed to meet primary endpoints, whereas BEL met its primary endpoint in each of the four published phase III BEL RCTs. This begs the question, "why," and we offer several possibilities to explain the apparent "RTX/BEL paradox" (**Table 3**).

A failed trial does not *a priori* mean that the tested drug failed—the trial design, rather than the trial drug, may have failed. The EXPLORER trial allowed for very liberal use of corticosteroids, which may have led to spuriously inflated responses in the placebo arm, thereby blunting a real difference between RTX-treated and placebo-treated patients. On the flip side, the primary outcomes in the LUNAR trial may have been too restrictive. By utilizing a composite response (the sum of those with either full or partial response), rather by focusing separately on full responses and partial responses, the LUNAR trial may have compromised its ability to detect differences in partial response rates between RTX-treated and placebo-treated patients. Indeed, the trial was powered to detect a 20% increase in complete renal response and a 5% increase in partial renal response, but this powering scheme would have missed a difference between the RTX and placebo arms composed primarily of partial renal responses. Moreover, the RTX and placebo arms each received MMF, a medication known to induce remission of lupus nephritis. Whereas addition of MMF was ethically imperative, it likely blunted the difference in response rates between the two groups. Had a greater number of subjects been enrolled into the trial, a statistically significant difference may have emerged. Indeed, the ethical mandate to include an effective SOC drug (MMF) in the control arm of the LUNAR trial highlights a logistic constraint in SLE clinical trials in general, in that the ethically unavoidable use of effective SOC drugs likely blunts differences between control groups and treatment groups. Consequently, positive signals from the experimental treatment may be "buried" and not appreciated.

Whereas trial design may have doomed the RTX trials, trial design likely contributed to the success of the BEL trials. These phase III trials enrolled large numbers of SLE patients without organ-threatening disease, and the trials were powered at 90% to detect a 14% absolute improvement in the SRI response rate with BEL 10 mg/kg compared to placebo. Further, the SRI used as the primary endpoint was created after rigorous post hoc analysis of the SLE phase II BEL trial (69). That is, the BEL trials were larger than were the RTX trials, and the primary endpoint in the SLE phase III BEL trials was chosen following extensive empiric experience and analyses, an approach not taken in the SLE RTX RCTs.

Beyond the concerns surrounding the design of the RTX trials, the clinical reports of RTX's efficacy in "real world" settings are in a very particular subset of SLE—patients with refractory disease that have inadequately responded to SOC therapy such as MMF or CYC for lupus nephritis. It may be that ongoing disease activity in such patients is highly rooted in aberrant B cell function, so the effectiveness of RTX is enhanced. Indeed, African–American and Hispanic patients trended toward improvement in both the EXPLORER and LUNAR trials, consistent with their harboring more aggressive disease than patients of European descent. It may be that severe, aggressive phenotypes are greatly based in aberrant B cell function, whereas the more mild disease phenotypes are less B cell-driven.

Further, one must recognize that not all B cells are equal or are created equal. Whereas some B cells unquestionably are main culprits in autoimmune diseases such as SLE, other B cells, such as B regulatory cells (Bregs) likely have a role in down-regulating the immune response, rather than stoking the autoimmune fire. In murine SLE models, complete depletion of B cells (including Bregs) in young mice leads to accelerated disease, while adoptive transfer of Bregs into B cell-depleted mice improves survival (70, 71). Indeed, Bregs increase in humans in response to high levels of inflammation and autoimmunity (72), likely reflecting a homeostatic attempt to downregulate a dysregulated immune response and mediated, at least in part, through inhibition of $CD4^+$ T cell proliferation and expansion

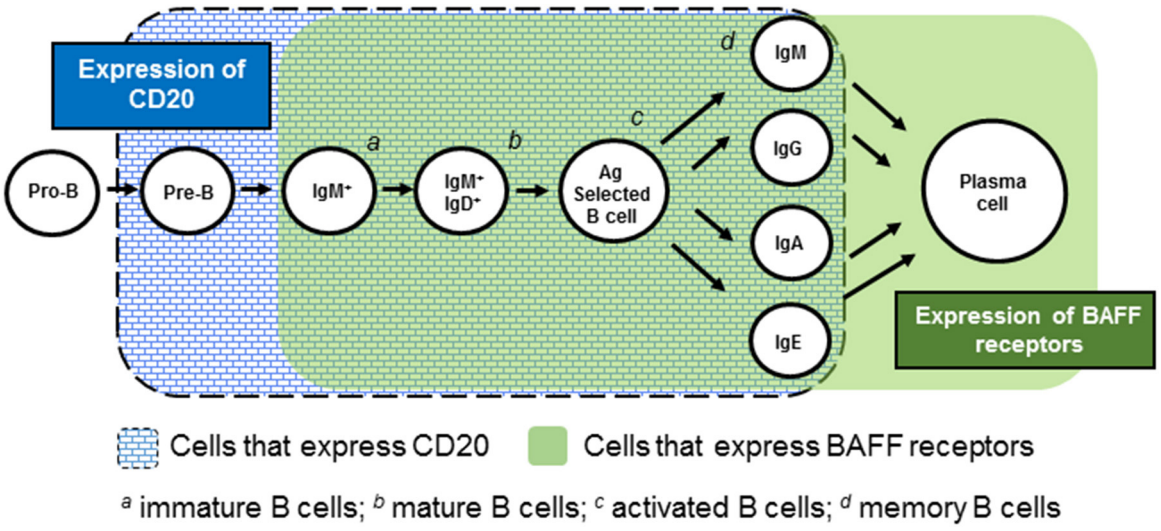

FIGURE 1 | Expression of CD20 and BAFF receptors during B cell ontogeny.

of regulatory T cell populations (73). With this in mind, perhaps the profound B cell depletion induced by RTX eliminates a key player (Bregs) in the regulation of the immune response. Given murine studies that demonstrate that autoreactive B cells are preferentially dependent on BAFF for their survival (74, 75), it may be that BEL preferentially downregulates autoreactive (pathogenic) B cells while (relatively) sparing Bregs, thereby favoring resolution of the ongoing autoimmune response.

Another possible explanation for the "RTX/BEL paradox" has to do with the differential effects of RTX and BEL on plasma cells (**Figure 1**). Whereas these cells express BAFF receptors and hence, may be sensitive to the BAFF-neutralizing effect of BEL, plasma cells do not express CD20 and thus, are insensitive to RTX. Accordingly, RTX will not abate ongoing pathogenic autoantibody production by plasma cells, whereas BEL may have some effect. Indeed, bortezomib (which profoundly depletes plasma cells while sparing mature B cells), has a notable beneficial effect on disease activity (including nephritis) and survival in SLE-prone mice, a finding replicated in small human case series (76–78). BEL's ability to target plasma cells may well have contributed to its success in clinical trials, whereas RTX's inability to target plasma cells may have contributed to its failures in clinical trials.

Not only may the kinds of cells targeted (or not targeted) by RTX have contributed to its failure in clinical trials, but the ability of RTX to deplete B cells may not have been as profound as presumed in the RCTs. In murine models, the anatomic location, microenvironment, and state of B cell differentiation play large roles in determining the susceptibility of a particular $CD20^+$ B cell to RTX (79). Despite abundant CD20 expression by some B cell populations, they are not fully depleted by RTX, perhaps due to undefined survival signals or other protective factors. Indeed B cells need to be, in a sense, primed and ready for CD20-targeted B cell depletion to occur (80). This may help explain why patients with aggressive and recalcitrant disease have high response rates to RTX in the "real-world" setting. Patients with high levels of diseases may harbor B cells in a "primed" state that are "ripe" for RTX-mediated B cell depletion, whereas patients with mild disease (who typically are not treated with RTX in the "real world" but were included in RCTs) may harbor B cells less primed for RTX-mediated B cell depletion. In contrast, as discussed above, autoreactive B cells may be more dependent on BAFF (and hence, more sensitive to BAFF neutralization) than are their non-autoreactive counterparts (75, 81). Accordingly, even though BEL may not promote extensive B depletion, the B cells that are depleted by BEL may preferentially be those that are autoreactive and pathogenic.

Finally, while RTX and BEL each target B cells, differences in their effects on non-B cells may contribute to the "RTX/BEL paradox." Whereas CD20 is highly restricted to B cells, BAFF receptors are expressed on other cells as well (82, 83). For example, TACI is expressed on monocytes, and BAFF appears to be directly involved in monocyte differentiation and activation (83). TACI is also expressed on certain T cell subsets, and BEL's interference with BAFF binding to T cell TACI may modulate T cell function (84). Indeed, BAFF has effects on T cell proliferation, cytokine production, and differentiation (85, 86), so although T cell depletion does not occur following BEL administration (87), interference with T cell function and differentiation may have enough of an effect to control SLE disease activity.

FUTURE PROSPECTS

Whereas BEL significantly promoted clinical responses in RCTs, its effect overall was rather modest. RTX, on the other hand, failed to significantly promote clinical responses in RCTs but may have great potential in the treatment of aggressive SLE. Accordingly, these two agents, when given in combination, may complement each other and lead to a synergistic therapeutic effect. Small case series have indeed reported excellent disease control in patients

treated with RTX followed by BEL (88, 89), and randomized trials formally testing such sequential therapy are being performed (NCT03312907, NCT02260934). Given that BAFF levels increase following RTX infusions (90, 91), BEL administration following RTX may blunt the rise in BAFF levels and delay reconstitution of pathogenic autoreactive B cells, thereby resulting in higher rates of clinical response. This is supported by Ramsköld et al.'s (92) findings that compared to those with higher baseline B cells, patients with lower baseline B cells levels experienced improved disease activity after 24 months of BEL.

On the flip side, administration of BEL prior to RTX may mobilize memory B cells from the tissue to the circulation and facilitate greater RTX-mediated depletion of pathogenic B cells. To that end, BLISS-BELIEVE (NCT03312907) is a randomized placebo-controlled clinical trial that is evaluating 200 patients randomized to one of three arms: BEL SC 200 mg weekly for 52 weeks plus placebo at weeks 4 and 6; BEL SC 200 mg weekly for 52 weeks plus RTX 1,000 mg at weeks 4 and 6; or, BEL SC 200 mg weekly plus standard of care for 104 weeks (93). Per the trial design, patients in the first two arms will receive BEL for only 52 weeks, followed by 52 weeks of observation in order to better characterize remission and the effect of the regimen on maintenance of remission. At time of writing, preliminary results are not yet available, and time will show if this combination therapy will have a place in the treatment of SLE. Additionally, it is imperative that the side effect profile of this combination regimen must also be explored in depth, and if there are increased rates of any adverse events, this must be weighed against any potential benefits.

CONCLUSION

While BEL has expanded the rheumatologist's armamentarium for SLE, RTX's performance in clinical trials has been disappointing. Yet, a myriad of published case series and "real-world" clinical practice point to RTX having a role in treating active SLE. The reasons behind RTX's failure vs. BEL's success in clinical trials are likely multifaceted, stemming both from differences in design of the trials and from differences in the biologic effects of the two agents. In any case, neither RTX alone nor BEL alone is a panacea for SLE, and just as two heads are often better than one, so too these two B cell-targeting agents (RTX and BEL) may be better than either one alone. Rather than being stuck on a "RTX/BEL paradox," perhaps we will ultimately be able to embrace a "RTX/BEL synergy."

AUTHOR CONTRIBUTIONS

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REVIEW
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B Cell Therapy in Systemic Lupus Erythematosus: From Rationale to Clinical Practice

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B cell hyperactivity and breach of tolerance constitute hallmarks of systemic lupus erythematosus (SLE). The heterogeneity of disease manifestations and relatively rare prevalence of SLE have posed difficulties in trial design and contributed to a slow pace for drug development. The anti-BAFF monoclonal antibody belimumab is still the sole targeted therapy licensed for SLE, lending credence to the widely accepted notion that B cells play central roles in lupus pathogenesis. However, more therapeutic agents directed toward B cells or B cell-related pathways are used off-label or have been trialed in SLE. The anti-CD20 monoclonal antibody rituximab has been used to treat refractory SLE during the last two decades, and the anti-type I IFN receptor anifrolumab is currently awaiting approval after one phase III clinical trial which met its primary endpoint and one phase III trial which met key secondary endpoints. While the latter does not directly affect the maturation and antibody production activity of B cells, it is expected to affect the contribution of B cells in proinflammatory cytokine excretion. The proteasome inhibitor bortezomib, primarily directed toward the plasma cells, has been used in few severe cases as an escape regimen. Collectively, current clinical experience and primary results of ongoing clinical trials prophesy that B cell therapies of selective targets will have an established place in the future personalized therapeutic management of lupus patients.

Keywords: B cells, systemic lupus erythematosus, therapy, biologics, plasma cells, plasmablasts, lupus nephritis

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INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease that can affect multiple organ systems (1). The treatment of SLE has traditionally been non-specific, with antimalarial agents as the therapeutic cornerstone due to the wide variety of beneficial effects associated with their use (2, 3), and broad immunosuppression being used to hamper the inflammatory state and protect against end-organ damage accrual (4–6). Several of the medications used to treat patients with SLE still have not received approval by regulatory drug agencies. Following the timeline of drug development in the field of rheumatology at large, the development of new therapies for SLE has been hampered due to several reasons.

First, the pronounced heterogeneity of clinical phenotypes poses challenges in developing outcome measures which unanimously and reliably capture response to treatment regarding activity in the afflicted organs, and which also reflect the global SLE disease activity. As a result,

the lack of reliable measures for treatment evaluation makes it challenging to design clinical trials to assess drug efficacy. Recruitment of participants has been slow and inadequate in organ-specific trials, whereas the applicability of currently available outcome measures has been questioned in clinically heterogeneous study populations. Borrowed from e.g., rheumatoid arthritis (RA), the treat-to-target concept has also gained attention in SLE (7), and composite measures have been developed to serve as tools for assessing clinical improvement. The SLE Responder Index (SRI) (8) was initially designed to serve as an outcome measure in clinical trials of belimumab (9–11), and the British Isles Lupus Assessment Group (BILAG)-based combined lupus assessment (BICLA) was first used in a phase IIb clinical trial of epratuzumab (12). They were both developed to reflect improvement in SLE disease activity. Other composite tools have been developed to reflect low disease activity, e.g., the Lupus Low Disease Activity State (LLDAS) (13), or remission, e.g., the Definitions of Remission in SLE (DORIS) (14). Both LLDAS and DORIS were designed to be applicable on specific evaluation occasions, and are independent of preceding degree of activity.

Using such tools, the first successful trials (10, 11) resulted in the approval of the first biological agent for the treatment of SLE about one decade ago (15). This agent was belimumab, a monoclonal antibody against the B cell activating cytokine BAFF, further discussed later, and the target was no other than B cells of early maturation stages, lending credence to the historical notion that they have a central role in lupus pathogenesis (16). Indeed, even before the official approval of belimumab as a treatment option, several therapies targeting B cells at different developmental stages have been used off-label (17). This review summarizes the rationale and clinical application of the B cell therapy panorama in SLE.

B CELLS IN SLE

The complex SLE disease is characterized by loss of self-tolerance, which leads to immune responses toward endogenous nuclear and cytoplasmic material. In response to these autoantigens, clones of plasma cells produce autoantibodies, which are considered a hallmark of the disease. Autoantibodies may induce inflammation through the formation of immune complexes and through activation of Fc-γ receptors. Arguing for a pathogenic role, autoantibodies such as anti-Smith (Sm) and anti-double stranded DNA (anti-dsDNA) are associated with the clinical presentation of the disease (18), and the level of anti-dsDNA frequently correlates with SLE disease activity (19).

Apart from the production of autoantibodies, B cells play additional roles in the pathogenesis of SLE. In lupus prone mice, B cells that do not secrete autoantibodies are still important to disease progression (20). This indicates that other B cell functions, such as antigen presentation to T cells may be of importance. Furthermore, B cells display hyperactivity in SLE (21), as well as increased expression of several toll-like receptors (TLRs) compared with healthy individuals (22), which may contribute to the inflammatory state. Thus, B cells are important players in several aspects of the SLE pathogenesis, and reducing the stimulation and numbers of B cells has been an important part of drug research.

B cells initially develop in the fetal liver and adult bone marrow and can be characterized by the use of surface markers such as CD19, CD20 and CD22, expressed at different stages of maturation. The development and survival of B cells depend upon stimulation by the B cell activating factor belonging to the tumor necrosis factor (TNF) family (BAFF), also known as B lymphocyte stimulator (BLyS). BAFF is a member of the TNF ligand superfamily of proteins, and is mainly produced by myeloid and stromal cells (23). Stimulation with BAFF improves B cell survival, proliferation, and antibody production through binding to three known receptors expressed in B cells at different stages of maturation, i.e., the BAFF-Receptor (BAFF-R; also known as BLyS receptor 3, BR3), transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI), and B cell maturation antigen (BCMA). BAFF transgenic mice develop symptoms characteristic of SLE (24), and BAFF levels are increased in patients with SLE compared with healthy controls and correlate with disease activity (25–28). In addition to BAFF, B cells are stimulated by cytokines such as a proliferation-inducing ligand (APRIL), which mainly serves as a plasma cell survival factor, interleukin (IL)-6, IL-21 and type I interferons (IFNs).

To inhibit B cell responses in SLE, two main pathways are currently used, i.e., (i) BAFF inhibition, and (ii) B cell depletion targeting the cell surface receptor CD20. The BAFF inhibitor belimumab was the first biological medication approved in 2011 by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for use in SLE. Belimumab is a recombinant human IgG1-λ monoclonal antibody that inhibits the soluble form of BAFF, preventing its interaction with BAFF receptors, thus inhibiting B cell survival and maturation. In contrast, rituximab is a chimeric anti-CD20 IgG1 monoclonal antibody that targets the CD20 molecule on the surface of B cells. This leads to B cell depletion through apoptosis, antibody dependent cell mediated cytotoxicity (ADCC), or antibody-dependent phagocytosis (ADP). Pharmaceuticals directly and indirectly targeting B cells that are used or have been trialed in SLE are illustrated in **Figure 1**, and summarized in **Table 1**.

B CELL DEPLETING THERAPIES

The Rationale for Rituximab

The chimeric anti-CD20 monoclonal antibody rituximab was approved by the FDA in 2006 for use in RA, and has been used off-label in the treatment of refractory SLE (29). The initial uncontrolled studies of rituximab in SLE showed encouraging results with improvements in both the clinical and laboratory compartment of the disease. However, two phase III randomized controlled trials have been performed, the EXPLORER trial in non-renal SLE (30) and the LUNAR trial in renal disease (31), none of which met their primary endpoints of significant reduction of disease activity compared with placebo (32).

The Clinical Trial Failures

Based on experience from rheumatoid arthritis (RA), the most commonly used regimen for rituximab in clinical practice consists of two intravenous infusions of 1,000 mg each, given

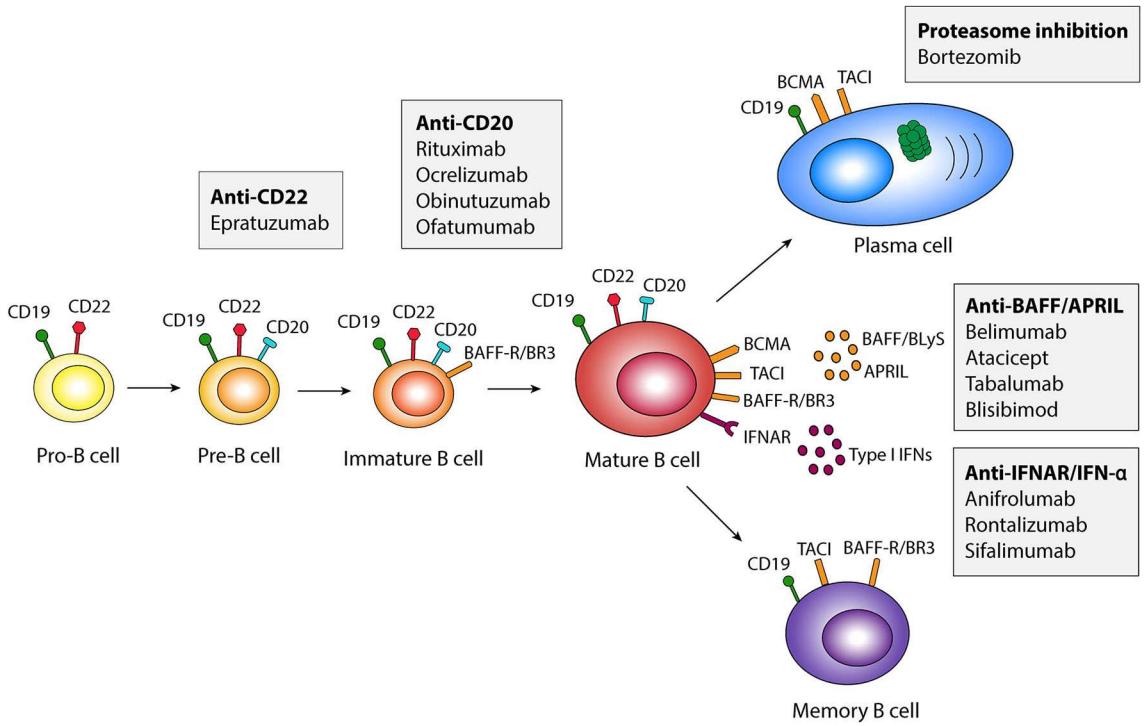

FIGURE 1 | Schematic illustration of pharmaceuticals targeting B cells in different developmental stages. APRIL, a proliferation-inducing ligand; BAFF, B cell activating factor belonging to the tumor necrosis factor family; BAFF-R, BAFF Receptor; BCMA, B cell maturation antigen; BLyS, B lymphocyte activator; BR3, BLyS receptor 3; IFN, interferon; IFNAR, type I IFN receptor; TACI, transmembrane activator and calcium modulator and cyclophilin ligand interactor.

14–21 days apart. In the EXPLORER study, 257 patients with moderate to severe non-renal SLE were randomized to receive rituximab or placebo. Rituximab in EXPLORER was administered at a dose of 1,000 mg at week 0, 2, 24, and 26 on a background of azathioprine, methotrexate, or mycophenolic acid therapy. At week 52, there was no difference between the active treatment and placebo groups in the primary endpoints (30), which comprised achievement and maintenance of a major, partial or no clinical response assessed using the eight British Isles Lupus Assessment Group (BILAG) index organ system scores (33). Nonetheless, in a subgroup analysis, rituximab showed benefit over placebo regarding major clinical response in African-American and Hispanic patients (30). In the LUNAR trial, 144 patients with class III or IV lupus nephritis on mycophenolic acid were randomized to receive placebo or rituximab, again at a dose of 1,000 mg at weeks 0, 2, 24 and 26. Also in this study, rituximab failed to achieve the primary endpoint, and there was no significant difference between the placebo and treatment arms regarding the proportion of patients who achieved complete or partial renal response (31). Afterwards, concerns have been raised regarding the concomitant use of high doses of glucocorticoids and immunosuppressive therapy in the EXPLORER and LUNAR trials, potentially clouding the effect exerted by rituximab. Several other factors may have played roles in the disappointing results of these trials, including inappropriate endpoints, the size of study populations and patient heterogeneity (32).

The Promising Reports From Real-Life Use

Despite the negative clinical trials, the European League Against Rheumatism (EULAR) recommendations for the management of SLE prompt consideration of rituximab for organ-threatening SLE that has been refractory or shown intolerance to standard of care immunosuppressants (4). Moreover, the joint EULAR/European Renal Association—European Dialysis and Transplant Association (ERA-EDTA) recommendations for the management of lupus nephritis (34) and the American College of Rheumatology (ACR) guidelines for the management of renal SLE (35) recommend the use of rituximab as a rescue treatment in active renal SLE that has been non-responsive to standard therapy.

Indeed, targeting CD20 with rituximab has been endorsed in several centers where it is used as an off-label therapeutic option in SLE, mostly for refractory renal disease, either alone or as an add-on treatment to cyclophosphamide or mycophenolic acid (36–43), but also for other organ manifestations when conventional treatment has failed, e.g., severe lupus polyarthritis, hematological aberrancies and neuropsychiatric lupus (43–48). However, the use of rituximab has also raised some concerns regarding untoward effects, such as infusion-related reactions (49–51) and an increased frequency of post-rituximab late-onset neutropenia in SLE compared with other diseases, which calls for an attentive surveillance of rituximab-treated patients (52).

TABLE 1 | Pharmaceuticals with direct or indirect impact on B cells currently used or trialed for systemic lupus erythematosus.

Drug name	Mechanism of action	Phase	Main results	References
B cell depleting agents				
Epratuzumab	Humanized anti-CD22	III	Primary endpoint not met	Clowse et al. (55)
Obinutuzumab	Humanized anti-CD20	II	Primary and secondary endpoints met	Furie et al. (56)
Ocrelizumab	Humanized anti-CD20	III	Primary endpoint not met	Mysler et al. (53)
Ofatumumab	Fully human anti-CD20	R-L	Well-tolerated; reduced proteinuria	Haarhaus et al. (58)
		R-L	Well-tolerated; safe; efficacy implied	Masoud et al. (59)
Rituximab	Chimeric anti-CD20	II/III	Primary and secondary endpoints not met	Merrill et al. (30)
		III	Primary endpoint not met	Rovin et al. (31)
B cell survival factor inhibitors				
Atacicept	Blocks BAFF and APRIL	II/III	Serious infections; terminated	Ginzler et al. (89)
Belimumab	Fully human anti-BAFF	III	Superiority over placebo	Navarra et al. (10)
		III	Superiority over placebo	Furie et al. (11)
		III	Superiority over placebo	Stohl et al. (73)
		III	Superiority over placebo	Zhang et al. (72)
		III/IV	Primary endpoint not met	D'Cruz et al. (77)
Blisibimod	Inhibits soluble and membrane-bound BAFF	IIb	200 mg weekly superior over placebo	Furie et al. (90)
		III	Primary endpoint not met	Merrill et al. (91)
Tabalumab	Human monoclonal antibody binding soluble and membrane-bound BAFF	III	Primary endpoint not met	Isenberg et al. (92)
		III	120 mg every 2 weeks superior over placebo	Merrill et al. (93)
Terminal stage B cell immunomodulators				
Bortezomib	Proteasome inhibitor	II	Frequent adverse reactions	Ishii et al. (109)
		R-L	Efficacy implied	Alexander et al. (107)
		R-L	Efficacy implied	Sjöwall et al. (108)
B cell depletion and survival factor inhibition combined				
Rituximab and belimumab	Chimeric anti-CD20 and fully human anti-BAFF	II	Recruitment completed	Jones et al. (115)
		III	Recruitment completed	Teng et al. (116)
		II	No benefit of add-on belimumab to rituximab and cyclophosphamide; LN	Aranow et al. (117)
		IIa	NET formation reduced; LN	Kraaij et al. (118)
		II	Recruiting; LN	NCT03747159
Agents with indirect impact on B cells				
Anifrolumab	Fully human anti-IFNAR	III	Primary endpoint not met	Furie et al. (126)
		III	Superiority over placebo	Morand et al. (127)
Rontalizumab	Humanized anti-IFN-α	II	Primary endpoint not met	Kalunian et al. (124)
Sifalimumab	Fully human anti-IFN-α	IIb	Superiority over placebo	Khamashta et al. (123)

This table summarizes key clinical trials and observational studies of pharmaceuticals used or trialed for systemic lupus erythematosus, which directly or indirectly impact on B cells. Observational real-life studies are provided when clinical trial data are not available or scarce.

APRIL, a proliferation-inducing ligand; BAFF, B cell activating factor belonging to the tumor necrosis factor family; IFN, interferon; IFNAR, type I IFN receptor; LN, lupus nephritis; NET, neutrophil extracellular trap; R-L, real-life.

B Cell Depleting Therapies Other Than Rituximab

Besides rituximab, some additional biological therapies targeting B cells have been trialed in SLE. The anti-CD20 humanized monoclonal antibody ocrelizumab was evaluated in a phase III trial which included 381 cases with severe lupus nephritis. However, the trial was terminated early due to an imbalance in serious infections in the treatment arm, and ocrelizumab has not been studied further (53). Epratuzumab is a humanized monoclonal antibody directed against CD22, which was well-tolerated and yielded encouraging results in a phase IIb study, with an evident superiority of epratuzumab 2,400 mg monthly in inducing BICLA response compared with placebo (12, 54). Unfortunately, none of the two subsequent phase III trials of epratuzumab in lupus were able to show improvements in response frequencies when compared with placebo (55).

Obinutuzumab is another humanized anti-CD20 monoclonal antibody with superior B cell cytotoxic effects over rituximab implicated for patients with RA and SLE. This drug has been studied in a phase II clinical trial of lupus nephritis (NOBILITY;

NCT02550652), designed to evaluate the safety and efficacy of the type II anti-CD20 monoclonal antibody obinutuzumab in patients with proliferative kidney disease. The first results were reported in the form of a conference abstract, where greater frequencies of complete and partial renal response were observed among patients who received obinutuzumab vs. placebo, both as an add-on to mycophenolate mofetil and glucorticoids (56). Finally, the fully human monoclonal antibody ofatumumab, approved for the treatment of chronic lymphocytic leukemia, has shown encouraging results in smaller groups of patients with lupus manifestations such as autoimmune hemolytic anemia, immune-mediated thrombocytopenia and lupus nephritis (57, 58). These last two agents could be of particular interest for patients in whom rituximab has shown efficacy but infusion reactions have prompted discontinuation (59), or patients who did not achieve complete B cell depletion following treatment with rituximab (50).

INHIBITION OF B CELL SURVIVAL FACTORS

Rationale

Due to its important role in B cell homeostasis, BAFF has been of central interest as a target molecule in B cell pharmacotherapy in SLE. Belimumab, formerly known as Lympho-Stat B, was the first drug to be licensed for SLE in more than 60 years, and is still the sole biological agent approved for use in adult SLE since 2011 and pediatric and adolescent SLE since 2019. The efficacy of belimumab in reducing lupus activity was first shown in two phase III randomized, placebo-controlled clinical trials (10, 11), and patients with serological activity, high BAFF levels, low baseline B cell counts, limited or no organ damage and no exposure to tobacco were later demonstrated to be more benefited (60–67). Belimumab is a recombinant human IgG1-λ monoclonal antibody that specifically binds to the soluble form of BAFF. Normally, the binding of BAFF to B cells prolongs their survival and promotes their maturation and differentiation toward immunoglobulin and autoantibody production (68). BAFF signaling also leads to increases in anti-apoptotic proteins (69). As defective clearance of apoptotic cells is implicated in the pathogenesis of SLE and stimulation of autoantibody production, reductions in anti-apoptotic proteins upon BAFF inhibition may be expected to hamper this B cell-driven component of lupus pathogenesis.

Clinical Trials and Observational Studies of Belimumab

Early trials of belimumab in SLE were inconclusive. A phase II trial that comprised 449 patients failed to meet its primary endpoints (9). However, a significant proportion of study participants (30%) had no elevated titres of antinuclear antibodies (ANA) at baseline, and the validity of their diagnosis was later questioned. To this point, it is important to mention that ANA have been shown to be less common than generally assumed in established cases of SLE (70, 71), which still is a matter of debate.

The first successful randomized controlled trial of belimumab in SLE was the BLISS-52 trial. BLISS-52 comprised 865 patients with a moderate to severe SLE and positivity for immunological markers. Modest but consistent improvements through week 52 were displayed in patients who received belimumab across various clinical outcomes, and the trial met its primary endpoint, i.e., a significantly greater proportion of patients who received belimumab 10 mg/kg at week 0, 2, 4 and thereafter every fourth week met the SRI-4 criteria for response compared with placebo (10). A second phase III clinical trial of similar design, the BLISS-76 trial, comprised 819 patients. The main difference compared with BLISS-52 was that the observation period in BLISS-76 was prolonged to a total of 76 weeks. The primary efficacy endpoint was the same as that in BLISS-52, and was set to the evaluation visit of week 52. Although this endpoint was reached at week 52 with belimumab 10 mg/kg resulting in a greater proportion of SRI-4 responders than placebo, the results of the subsequent study period until week 76 were rather inconclusive (11). Since then, three more phase III trials have been performed. One assessed belimumab efficacy in a North East Asian SLE population (72), and another one assessed the efficacy of subcutaneous administration (73, 74); both reached their primary endpoint, i.e., SRI-4 response frequency at week 52. Another phase III/IV trial assessed the efficacy of belimumab in SLE patients of black race (EMBRACE) using the same primary endpoint, however with a modification in the SLE Disease Activity Index (SLEDAI) assessment for the proteinuria item to meet the SLEDAI-2K standard (75), as compared with scoring according to Safety of Estrogens in Lupus Erythematosus National Assessment (SELENA)-SLEDAI (76) in the original SRI (8). While the primary endpoint of EMBRACE was not achieved, patients with high disease activity were benefited (77). Finally, reports from several real-life clinical settings have confirmed clinical efficacy and steroid-sparing effects (61, 78–84).

The BLISS trials of belimumab excluded patients with severe active lupus nephritis, but a large proportion of study participants had a history of renal involvement and low to moderate proteinuria at the time of inclusion (10, 11). A *post-hoc* analysis demonstrated that these patients benefited from belimumab with regard to several organ-specific aspects, including rates of renal flares (85). A phase III randomized controlled trial has been designed to specifically assess the effect of belimumab as an add-on to standard of care therapy in patients with active renal SLE, i.e., the BLISS-LN trial (NCT01639339), and publication of the first results is awaited. In a recent press release, the pharmaceutical company announced that BLISS-LN met its primary and key secondary endpoints (86), which paves the way for increasing use of B cell-targeted immunomodulation in this severe lupus manifestation (87).

B Cell Survival Factor Inhibitors Other Than Belimumab

Atacicept is another BAFF-blocking biological agent that has been studied as a candidate pharmaceutical for SLE. Being a receptor construct that combines TACI with the Fc portion of human IgG, atacicept blocks the effects of both BAFF and its

homologous B cell cytokine APRIL (88). Unfortunately, a clinical trial of atacicept in lupus nephritis was prematurely terminated due to adverse events in the form of hypogammaglobulinemia and infections (89), but attempts with adjusted dosing have not been totally abandoned.

Blisibimod is a fusion protein consisting of four high-affinity BAFF-binding domains and the Fc domain of human IgG1, and targets both soluble and membrane-bound BAFF. A dose-ranging phase IIb clinical trial (90) determined a safe and effective dose of blisibimob to be further studied in a subsequent phase III clinical trial, which however failed to meet its primary endpoint (91).

Only one of the two phase III clinical trials of tabalumab, a fully human monoclonal antibody that targets soluble and membrane-bound BAFF, met its primary endpoint, i.e., proportion of patients achieving SRI-5 at week 52 (92, 93), and no further development of this drug was therefore planned for SLE. However, it is worth noting that no dose-ranging phase II studies had preceded the phase III trials. Several key outcomes in both trials still justify the rationale of targeting both the cleaved and membrane-bound BAFF counterparts (94, 95).

MODULATING THE TERMINAL MATURATION STAGE OF B CELLS

The Rationale for Proteasome Inhibition

The majority of the immunosuppressants used in SLE exert their therapeutic effects on B cells, plasmablasts and short-lived plasma cells (96). However, to achieve effects beyond this, i.e., on the long-lived plasma cells, the only available alternatives are autologous stem cell transplantation, atacicept (blocking both BAFF and APRIL) and proteasome inhibition (97–99). This was the rationale for using bortezomib in SLE cases resistant to conventional therapy.

Bortezomib is a specific, reversible, and cell permeable dipeptide boronic acid inhibitor of the chymotryptic activity of the 20S subunit of the proteasome, approved for the treatment of multiple myeloma and mantle cell lymphoma (100). Proteasome inhibition causes accumulation of defective immunoglobulin chains, resulting in endoplasmic reticulum stress, misfolded protein response, and subsequent apoptosis of plasma cells (101, 102). In addition, the long-lived plasma cells are vigorous antibody producers, and are thus highly sensitive to proteasome inhibition (99). On the other hand, proteasome inhibitors also effectively function as inhibitors of the production of pro-inflammatory cytokines through the regulation of NF-κB activation (103). Promising results in experimental lupus models and reports on use of bortezomib for allograft rejection in kidney transplantation (104, 105) have given rise to the concept of using bortezomib for patients with refractory lupus (106).

Evidence From Clinical Trials and Observational Studies

Several cases with refractory and life-threating manifestations of SLE in Germany and Sweden were treated with bortezomib and encouraging results were reported (107, 108). In a recent Japanese multicentre double-blind randomized controlled phase II trial, which enrolled 14 patients with persistently raised disease activity, patients were randomized to receive either bortezomib as an add-on therapy to their concomitant immunosuppressants or placebo (109). Unfortunately, albeit obvious clinical efficacy was seen in several patients, some of the patients who received bortezomib experienced adverse reactions, i.e., fever, severe hypersensitivity, or other infusion reactions. The authors recommended to carefully select patients for bortezomib therapy, and use protocols to prevent side-effects.

COMBINING B CELL THERAPIES

Rationale

Since rituximab induces B cell depletion, but also results in elevation of BAFF levels, studies have examined whether the increased BAFF levels may promote re-expansion of autoreactive B cells and by extension an earlier relapse. The effects of rituximab are dependent on the degree of B cell depletion, and incomplete depletion has been shown to be associated with lower frequencies of clinical response (27). In patients with refractory SLE with high levels of anti-dsDNA antibodies, relapse occurred at lower B cell numbers, and plasmablasts represented a larger percentage of the B cell population (110). Following rituximab administration, levels of BAFF rise (111), and BAFF levels are higher at relapse after rituximab treatment compared with disease flare before rituximab treatment (112). Further, quantifiable BAFF in serum has been associated with shorter clinical response to rituximab in patients with refractory SLE (113). Thus, a contributing factor to the lack of efficacy of rituximab in randomized clinical trials may be the increased BAFF levels following rituximab administration. Theoretically, combining rituximab with belimumab could give a more thorough and sustained inhibition of B cell responses, as speculated in early investigations (28, 111, 112, 114). This is currently evaluated in several clinical trials, e.g., BEAT Lupus (115) and BLISS-BELIEVE (116).

It is of particular importance that the merit of combining B cell therapies has also been conceptualized in the context of lupus nephritis. The Rituximab and Belimumab for Lupus Nephritis (CALIBRATE; NCT02260934) (117) and the investigator-initiated Synergetic B cell Immunomodulation in SLE (SynBioSe) trials (SynBioSe 1: NCT02284984; SynBioSe 2: NCT03747159) were designed to assess the efficacy of rituximab and belimumab combined in active lupus nephritis. The proof-of-concept open label SynBioSe 1 is completed, and a first report demonstrated reductions in antinuclear antibodies and neutrophil extracellular trap (NET) formation (118). SynBioSe 2 is currently recruiting, and results may be anticipated by the end of 2023.

PERSPECTIVE: FUTURE WAYS OF TARGETING B CELLS

Autoreactive B cells are indubitably key cells in the pathogenesis of SLE, but the theoretical merit has hitherto seldom culminated in the anticipated outcomes in drug development. The lack of

success in clinical trials has not been for lack of trying. Apart from pharmaceuticals which predominantly exert effects on B cells, numerous other therapeutic modalities have been trialed for SLE, several of them expected to indirectly impact on B cells and B cell functions. For example, in lupus prone mice, targeting other B cell stimulating cytokines, such as IL-6, decreased disease progression, but this strategy did not succeed in subsequent clinical trials (119). Targeting the co-stimulatory molecule CD40 led to modest clinical improvement, but also unacceptable side-effects in the form of thromboembolic events (120).

Activation of the type I IFN pathway is prominent in the pathogenesis of SLE, and type I IFNs stimulate BAFF production. In patients with SLE, the type I IFN pathway is overexpressed, and the IFN-α protein in particular has shown associations with both disease activity (121) and risk of relapse (122). IFNs are pleiotropic cytokines with numerous functions in the immune response equilibrium, including an impact on B cells. Thus, albeit not exclusive, the effects of IFN inhibition are attractive also in the B cell context.

The first reports to support the efficacy of direct IFN-α inhibition in SLE originated from a phase IIb clinical trial of sifalimumab (123). The results were modest, but in favor of sifalimumab. Unfortunately, a phase II trial of the anti-INF-α rontalizumab demonstrated that rontalizumab was superior over placebo in SLE patients with low IFN-regulated gene expression, but not in patients with high IFN gene signature (124), contrary to what expected considering its biologic mechanism.

Following promising results in a phase II clinical trial (125), the type I IFN receptor (IFNAR) inhibitor anifrolumab was evaluated in two phase III trials, i.e., TULIP-1 and TULIP-2. In TULIP-1, the primary outcome, i.e., SRI-4 response, was not met (126). By contrast, a greater proportion of patients receiving anifrolumab vs. placebo in TULIP-2 met the primary outcome, i.e., BICLA (127). Possible reasons for the discrepancy between the TULIP trials may include the choice of outcomes and the study populations. The primary endpoint in TULIP-2 was initially planned to be SRI-4. However, this was changed at a later stage, upon a subanalysis of TULIP-1 where proportions of BICLA unlike SRI-4 responders favored anifrolumab. Notably, in TULIP-2 both SRI-4 and BICLA showed ability to separate treatment arms.

An interesting trend is targeting B cell intracellular signaling, such as through inhibition of Bruton's Tyrosine Kinase (BTK), which is a strategy approved for the treatment of B cell malignancies. Inhibition of BTK has shown efficacy in lupus prone mice, which resulted in reduced kidney damage and increased survival (128). Another development originating in the area of cancer therapy was the chimeric auto-antigen receptor (CAAR) T cells. CAAR-T cells have been genetically engineered to kill human autoreactive B cells specific toward desmoglein-3 in pemphigus vulgaris (129), and in two lupus mice models, use of CAAR-T cells targeting the CD19 surface molecule resulted in reduced kidney damage and increased survival (130). Although long-term data are not available, evidence suggests that the CAAR-T cells acquire a long-term memory phenotype and persist in peripheral tissue of patients.

Epilog

To summarize, B cell hyperactivity and breach of tolerance constitute hallmarks of SLE, and it is widely accepted that B cells play central roles in the pathogenesis. However, the contribution of B cells to disease initiation and perpetuation is less well understood. B cells in SLE constitute the main autoantibody producers and probably facilitate the priming of autoreactive T cells and function as antigen-presenting cells, as well as constitute a source of the cytokines involved in immune dysregulation (131). As a result, many of the therapeutic agents that have been trialed in SLE target B cell-related pathways.

Even though drug development in the field of SLE has been slow, B cell-targeting therapies have been increasingly used during the last two decades and contributed to improved management and improved prognosis. The amount and primary results of ongoing clinical trials prophesy that B cell therapies of selective targets will have an established place in the future personalized therapeutic management of lupus patients.

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All authors contributed to the manuscript draft, critically reviewed all parts of the manuscript, accepted its final version prior to submission, and account for its content.

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frontiers in Medicine

BRIEF RESEARCH REPORT
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Biologic Sequencing in Systemic Lupus Erythematosus: After Secondary Non-response to Rituximab, Switching to Humanised Anti-CD20 Agent Is More Effective Than Belimumab

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Background: Rituximab is commonly used for systemic lupus erythematosus (SLE) but secondary non-depletion and non-response (2NDNR) associated with anti-drug antibodies is a notable problem with repeat rituximab cycles. Other B cell-targeted therapies include other anti-CD20 monoclonal antibodies or belimumab.

Objective: To compare efficacy of switching to alternative anti-CD20 agents vs. belimumab in SLE patients with 2NDNR to rituximab.

Methods: One hundred and twenty five patients received rituximab and had evaluable data. 77/125 received repeat rituximab cycles. Of these, 14/77 (18%) had 2NDNR. 8/14 patients were switched to belimumab (CD20-to-belimumab group) and 6/14 patients were switched to an alternative humanised anti-CD20 agent (CD20-to-CD20 group, ocrelizumab $n = 3$, ofatumumab $n = 2$, obinutuzumab $n = 1$). Efficacy was assessed using the BILAG-2004, SLEDAI-2K, SRI-4, and daily prednisolone requirement at baseline and 6 months.

Results: In the CD20-to-belimumab group, only one patient achieved an SRI-4 and 2/8 patients had new/worsening BILAG-2004 grade A for lupus nephritis. There was no improvement in SLEDAI-2K; median (IQR) was 11.0 (9.5–14.8) at baseline and 10 (9.5–15.5) at 6 months. Median (IQR) prednisolone dose increased from 7.5 mg (4.4–12.5) to 10 mg (6.3–10). In the CD20-to-CD20 group, all 6 patients achieved an SRI-4. Median (IQR) SLEDAI-2K improved from 16.0 (10.3–24.0) at baseline to 5.0 (2.5–6.0) at 6 months. Median (IQR) prednisolone dose decreased from 15 mg (15–15) to 10.5 mg (5.3–15.0).

Conclusion: This is the first assessment of belimumab's efficacy in a post-rituximab population. Our data suggests that patients with 2NDNR to rituximab, which constituted 11% of all patients initiated on this drug, should be switched within the same biologic class to another anti-CD20 agent.

Keywords: B cells, belimumab, immunogenecity, rituximab, systemic lupus erythematosus

INTRODUCTION

Rituximab, a chimeric monoclonal antibody (mAb) is commonly used off-label for the treatment of antibody positive systemic lupus erythematosus (SLE) in patients with severely active disease (including renal manifestations) despite conventional immunosuppressants such as mycophenolate mofetil and cyclophosphamide (1). Although two large phase III randomised controlled trials EXPLORER (non-renal) and LUNAR (renal) failed to meet their primary end points, rituximab appears to be effective in a large number of single-centre open label series (2–4), multi-centre registries (1, 5) and a systematic review of off-label use (6). We previously reported that clinical response to rituximab was better if complete B cell depletion, as measured using highly sensitive flow cytometry [HSFC (7)], was achieved (8). However, in patients with good initial response to a first cycle of rituximab, we found a substantial rate of secondary non-depletion and non-response (2NDNR). We previously defined this as a phenomenon whereby SLE patients who initially responded well to rituximab with B-cell depletion, subsequently experienced; a severe infusion reaction $>$ 24 h during the second infusion of a cycle; failed to completely deplete B-cells; and did not clinically respond during repeat cycles. 2NDNR is associated with anti-rituximab antibodies. Since these patients often had severe disease resistant to other therapies, determining the best follow-on therapy in this situation is crucial.

It is logical to continue to target B cells in these patients given their prior good response to rituximab. There are two potential strategies. First, switching to an alternative anti-CD20 agent, particularly humanised (type I mAb: ocrelizumab, ofatumumab or type II mAb: obinutuzumab), has been reported with good clinical outcomes for the type I anti-CD20 mAbs (8–10) as well as *in vitro* for obinutuzumab (11). None of these anti-CD20 mAbs are currently licensed for use in SLE. Second, switching to belimumab as currently the only biologic agent licensed for treating SLE. Belimumab targets B cells indirectly via B cell activating factor (BAFF) inhibition. BAFF is not only a potent B cell activator, it also plays an important role in B cell proliferation and differentiation (12). Although it is licenced for treating antibody positive SLE with a high degree of disease activity (excluding active renal and neuro-psychiatric complications), its evidence for efficacy is mainly in biologic-naïve patients (13, 14). Neither option has previously been assessed in the context of 2NDNR to rituximab.

BAFF levels are known to significantly increase after B cell depletion, and this may assist in the survival of new B cells emigrating from bone marrow. BAFF levels have also been associated with relapse after rituximab (15). Based on these findings, several trials are in progress using a combination of rituximab and belimumab (16, 17). However, this treatment regimen and trial population are clearly distinct from the rituximab 2NDNR problem.

The objective of this study was to report the comparative efficacy of switching to either (i) belimumab, or (ii) alternative, humanised anti-CD20 agents in SLE patients with prior 2NDNR to rituximab. We hypothesised that both of these B cell targeted agents would have higher response rates in 2NDNR patients than for SLE patients without previous 2NDNR. However, our results showed a marked difference in their efficacy in this population.

METHODS

Patients and Design

A prospective observational study was conducted of all patients with moderate to severe SLE [with at least 1 $\times$ British Isles Lupus Assessment Group (BILAG)-2004 grade A or 2 x BILAG-2004 grade Bs] who were treated with rituximab in Leeds between January 2004 and October 2019. Inclusion criteria were (1) age $\geq$ 18 years old; (2) fulfilling the revised 1997 American College of Rheumatology classification for SLE (18) and (3) at least 6 months follow-up post-rituximab and post-rituximab switch following a 2NDNR (defined below). Total follow up time on each therapy was calculated from the date of therapy initiation until the date of therapy discontinuation / death / last update of data in January 2020.

Rituximab Therapy and 2NDNR

Rituximab (MabThera) was administered to patients if they had moderate to severe SLE despite prior therapy with either mycophenolate mofetil or cyclophosphamide, or with toxicity to these agents, in line with the NHS England criteria (19). Rituximab was administered as 2 $\times$ 1000 mg at weeks 0 and 2, each preceded by 100 mg methylprednisolone. Patients received repeat cycles of the same dose of rituximab if they had a clinical relapse, defined by at least 1 x new BILAG-2004 B, following an initial response at 6 months. In this cohort, we previously reported that 14% of patients with SLE who had previously depleted and responded well to rituximab, subsequently experienced (1) a severe infusion reaction $>$ 24 h during the second infusion of a cycle, (2) failure to deplete CD20+ B cells (naïve and memory) and (3) clinical non-response during repeat cycles. We called this secondary non-depletion and non-response (2NDNR) (8). This phenomenon has also been reported by other groups (20).

Rituximab to Belimumab Switch (CD20-to-Belimumab Group)

Treatment for 8 patients with 2NDNR to rituximab was switched to belimumab. Belimumab was administered using its licensed dose of 10 mg/kg at weeks 0, 2, 4 then every 4 weeks. It was discontinued in patients with non-response or if their condition worsened, requiring other therapies.

Rituximab to Alternative Anti-CD20 mAb Switch (CD20-to-CD20 Group)

For 6 patients with 2NDNR, therapy was switched to a humanised anti-CD20 agent. This was chosen based on availability (compassionate supply from Roche, or individual funding from the NHS England). Three patients were treated with ocrelizumab 2 $\times$ 1000 mg at weeks 0 and 2, each preceded by 100 mg methylprednisolone; for 2 patients we treated with ofatumumab 2 $\times$ 700 mg at weeks 0 and 2, each preceded by 100 mg methylprednisolone; and 1 patient was treated with

obinutuzumab 2 × 1000 mg at weeks 0 and 2, each preceded by 100 mg methylprednisolone.

Treatment choice was determined by availability and funding rather than clinical status. Patients were treated with alternative anti-CD20 agents before the NICE approval and when these agents were available. This depended on compassionate supply from manufacturers (ocrelizumab), individual funding applications until no longer available (ofatumumab), and funding by the hospital trust (obinutuzumab). From the date of the NICE approval for belimumab, patients received belimumab if they met NICE criteria.

Clinical Outcomes

Data were collected as part of the Leeds Connective Tissue Disease and Vasculitis (CONVAS) observational study. Baseline characteristics including demographics, disease activity, previous and concomitant immunosuppressant and daily prednisolone use were collected. Treatment efficacy was assessed using the BILAG-2004 (21), SLE Disease Activity Index version 2000 [SLEDAI-2K (22)], and daily prednisolone requirement at baseline and 6 months after the follow-on therapy.

BILAG-2004 responses at 6 months were determined as follows: (1) major clinical response (MCR) = improvement of all domains rated A/B to grade C/better and no A/B flare between baseline and 6 months; (2) partial clinical response (PCR) = maximum of 1 domain with a persistent grade B with improvement in all other domains and no A or B flare; or (3) no clinical response (NCR) = those not meeting the criteria for major or partial clinical response. Global BILAG-2004 score was calculated as follows: grade A = 12, grade B = 8, grade C = 1, and grades D and E = 0 (23).

SLE Responder Index (SRI-4) was defined by a 4-point improvement in the SLEDAI-2K with no worsening in the BILAG-2004 or in the physicians' global assessment (24).

Laboratory Assessments

Peripheral blood B-cell subsets (naïve, memory B-cells and plasmablasts) were measured using HSFC as previously described (7) at baseline and 6 weeks after treatment with rituximab or alternative anti-CD20 agents without knowledge of clinical status other than time since therapy. Complete B-cell depletion was defined as counts $< 0.0001 \times 10^9$/L and repopulation as $\geq 0.0001 \times 10^9$/L. Anti-dsDNA titres and ENA profile (anti-Ro, -La, -Sm, -Scl-70, -Jo-1, -RNP, -Sm/RNP, -Ribosomal P, -Chromatin) were measured using ImmunoCAP™ chemiluminescent immunoassay by Thermo Fischer Scientific prior to July 2012 and Bioplex 2200 Immunoassay (after July 2012). Complement levels (C3 and C4) (normal range for C3: 0.75–1.65 g/L and for C4: 0.14–0.54 g/L) and total serum immunoglobulin titres were measured by nephelometry. All immunological tests above were analysed at an accredited NHS laboratory.

Ethics Approval

This observational study was approved by the Leeds (East) Research Ethics Committee (REC), 10/H1306/88 and conducted in compliance with the Declaration of Helsinki. All patients gave written informed consent. The off-label use of rituximab, ofatumumab, ocrelizumab and obinutuzumab were all approved by the Leeds Teaching Hospitals NHS Trust Drug and Therapeutic Committee.

Statistical Analyses

Descriptive statistics were summarised using median with interquartile range (IQR) for continuous variables and proportion for categorical variables. Continuous variables were compared using either Student's *t*-tests, Mann-Whitney's test or Kruskall-Wallis test depending on data type and distribution. All statistical analysis was performed using IBM SPSS Statistics v21.0 (IBM Corp, Armonk, New York, USA) and Graph Pad Prism V.6.01 for Windows.

RESULTS

Demographics of Patients With 2NDNR to Rituximab

One hundred and twenty five patients with SLE received rituximab in Leeds over the 15 years follow-up and had evaluable data at 6 months. Of these, 100/125 (80%) had an initial BILAG-2004 response (MCR and PCR). 77/125 (62%) patients suffered a relapse and required repeat cycles of rituximab. Of these, 61/77 (79%) patients maintained BILAG-2004 response, 2/77 (3%) had secondary inefficacy, and 14/77 (18%) developed 2NDNR either in the second cycle (n = 10/77; 13%) or the third cycle (n = 3/40; 8%). Baseline characteristics of the 14 patients with 2NDNR to rituximab are summarised in **Table 1**. Patients who were switched to alternative anti-CD20 agents (CD20-to-CD20 group) were younger at the time of drug initiation, had shorter disease duration, lower number of previous oral immunosuppressants when compared to those who were switched to belimumab (CD20-to-belimumab group). However, the dose of concomitant oral prednisolone, median SLEDAI-2K scores, and the proportion of patients on concomitant anti-malarial and immunosuppressants (IS) were comparatively higher in the CD20-to-CD20 group.

Clinical Outcomes of the CD20-to-Belimumab Group

Eight patients received belimumab after rituximab. All were female with a median (IQR) age at the time of drug initiation of 44.0 years (31.5–56.8). Reasons for failure of rituximab in this subgroup were: (i) primary non-response (never responded) = 1/8; and (ii) 2NDNR = 7/8. At belimumab baseline, 6/8 patients had positive anti-dsDNA and low complement levels in line with data predicting better response to belimumab and the current UK National Institute for Health and Care Excellence (NICE) guidance (25, 26). The other two patients were treated prior to the publication of this guidance.

At 6 months post-belimumab, only one patient achieved an SRI-4. However, belimumab was discontinued for this patient at the 6-month time point due to recurrent chest and urinary tract infections. Another 2/8 patients had a 4-point reduction in SLEDAI-2K (22→18 and 14→10) but failed to achieve SRI-4 due to one having new BILAG-2004 activity in cardiorespiratory

TABLE 1 | Baseline characteristics.

Characteristic	CD20-to-Belimumab Group ($n = 8$)	CD20-to-CD20 Group ($n = 6$)
Age (years), Median (IQR)	44.0 (31.5–56.8)	28.0 (23.3–35.0)
Female:Male	8:0	6:0
Ethnicity, n (%) Caucasian	5/8 (63)	1/6 (17)
Afro Caribbean	3/8 (37)	5/6 (83)
Disease duration at drug initiation (years), Median (IQR)	18.0 (12.8–20.0)	6.5 (6.0–8.5)
Previous Cyclophosphamide, n (%)	3/8 (37)	1/6 (17)
Number of previous oral immunosuppressants*, Median (IQR)	3.0 (2.5–5.0)	1.5 (1.0–2.8)
Prednisolone dose (mg), Median (IQR)	7.5 (4.4–12.5)	15.0 (15.0–15.0)
Concomitant antimalarial, n (%)	4/8 (50)	4/6 (67)
Concomitant IS*, n (%)	4/8 (50)	5/6 (83)
SLEDAI-2K, Median (IQR)	11.0 (9.5–14.8)	16.0 (10.3–24.0)
BILAG-2004 A/B**, n (%) General	1/8 (13)	3/6 (50)
Mucocutaneous	6/8 (75)	3/6 (50)
Neuropsychiatric	1/8 (13)	2/6 (33)
Musculoskeletal	6/8 (75)	3/6 (50)
Cardiorespiratory	1/8 (13)	0/6 (0)
Renal	1/8 (13)	4/6 (67)
Haematological	1/8 (13)	1/6 (17)

**concomitant immunosuppressant (IS) = Azathioprine, Mepacrine, Methotrexate, Mycophenolate Mofetil or Tacrolimus.*

***no patient had activity in gastroenterological or ophthalmic domains of BILAG-2004 so these data not shown.*

domain (Grade E→B) and worsening in general domain (Grade C→B), whilst the other had worsening of both mucocutaneous and renal domains (Grade B→A). A complete breakdown of BILAG-2004 domain scores at baseline and 6 months are shown in **Table 2**.

There was no significant improvement in the SLEDAI-2K post-belimumab; median (IQR) at baseline and 6 months were 11.0 (9.5–14.8) and 10 (9.5–15.5), respectively; $p = 0.629$ (**Figure 1A**). There was no improvement in the Global BILAG-2004 score post-belimumab; median (IQR) at baseline and 6 months were 21.5 (20.0–22.8) and 19.0 (15.3–22.8), respectively; $p = 0.366$ (**Figure 1B**). Median (IQR) prednisolone dose had increased from 7.5 mg (4.4–12.5) at baseline to 10 mg (6.3–10) at 6 months; $p = 0.654$ (**Figure 1C**).

Notably, there were two new episodes of lupus nephritis during belimumab therapy (1 = relapse with Class III nephritis and 1 = *de novo* Class II and V nephritis). Treatment for both patients was switched to intravenous cyclophosphamide therapy.

Total follow up time on therapy in this group was 9.9 patient-years. 4/8 patients continued therapy for longer than 6 months and they all received increased doses of immunosuppressant and prednisolone. Of these, 1/4 patient had initial BILAG-2004 PCR but developed flare of lupus nephritis and stopped at 11 months; 1/4 patient did not meet either an SRI-4 or BILAG-2004 response at 6 months but had BILAG-2004 PCR at 9 months and discontinued therapy at 24 months due to secondary inefficacy; 1/4 patient had two lengthy interruptions to therapy due to unrelated surgical procedures leading to cessation of belimumab at 24 months; and 1/4 patient remains on belimumab at 3 years but still not in clinical remission despite requiring escalation of concomitant oral immunosuppressants and prednisolone.

Immunological Outcomes of the CD20-to-Belimumab Group

Of 6/8 patients with increased anti-dsDNA titre at belimumab baseline, none achieved normalisation of anti-dsDNA titre at 6 months. Furthermore, anti-dsDNA titre did not significantly improve post-belimumab; median (IQR) at baseline and at 6 months were 56 IU/mL (21–132) and 27.5 IU/mL (14.8–104.3), respectively; $p = 0.356$ (**Figure 2A**).

For complement C3 level, median (IQR) at baseline and at 6 months were 1.07 g/L (0.84–1.17) and 1.03 g/L (0.88–1.21), respectively; $p = 0.948$ (**Figure 2B**). 1/8 patient with low level at baseline did not improve post-belimumab. For complement C4 level, median (IQR) at baseline and at 6 months were 0.11 g/L (0.08–0.12) and 0.12 g/L (0.09–0.17), respectively; $p = 0.231$ (**Figure 2C**). 6/8 patients had low level at baseline. Of these, only 1/6 had normalisation of C4 level at 6 months post-belimumab. C3 and/or C4 levels which were normal at baseline ($n = 2$), remained within normal range at 6 months post-belimumab.

Clinical Outcomes of the CD20-to-CD20 Group

Following 2NDNR to rituximab, treatment for 6 patients was switched to humanised anti-CD20 mAbs (3 = ocrelizumab, 2 = ofatumumab, and 1 = obinutuzumab). All 6 patients were female with a median (IQR) age at the time of drug initiation of 28.0 years (23.3–35.0).

Total follow up time on therapy in this group was 31.8 patient-years. Six weeks after treatment with the alternative anti-CD20 agent, complete B cell depletion was achieved in 5/6 patients, while the remaining one had substantially reduced total B cell counts (0.0016×10^9/L).

At 6 months post-switch to alternative anti-CD20 agents, all patients achieved an SRI-4. The median (IQR) SLEDAI-2K score had improved from 16.0 (10.3–24.0) at baseline to 5.0 (2.5–6.0) at 6 months; $p = 0.019$ (**Figure 1A**). The Global BILAG-2004 score significantly improved from 22.5 (18.0–36.8) at baseline to 2.5 (2.0–3.0) at 6 months; $p = 0.009$ (**Figure 1B**). This is also reflected in the BILAG-2004 response, which was MCR for 5/6 patients and PCR for the remainder 1/6 patient, vs. no MCR in the CD20-to-Belimumab group. In fact, 3/8 patients in the CD20-to-Belimumab group had a worsening of BILAG-2004 response. A complete breakdown of BILAG-2004 domains at baseline and 6 months post-alternative anti-CD20 mAbs switch are shown in **Table 2**. Furthermore, median (IQR) prednisolone dose had decreased from 15 mg (15–15) at baseline to 10.5 mg (5.3–15.0) at 6 months; $p = 0.033$ (**Figure 1C**).

TABLE 2 | BILAG-2004 scores.

		Baseline								6 months								
Patient	Biologic	Gen	Muco	Neuro	MSK	Cardio	Renal	Haem	Total score	Gen	Muco	Neuro	MSK	Cardio	Renal	Haem	Total score	BILAG-2004 Response
CD20-to-Belimumab Group																		
1	Bel	E	A	E	B	E	E	C	21	E	B	E	B	E	E	C	17	PCR
2	Bel	E	B	E	E	E	E	C	9	E	B	E	E	E	E	C	9	NCR
3	Bel	A	C	E	C	D	E	B	22	D	C	E	C	E	A	B	22	NCR
4	Bel	D	B	E	B	D	B	C	25	D	A	E	D	D	A	C	25	NCR
5	Bel	D	B	E	B	D	E	C	17	D	B	E	B	D	E	C	17	NCR
6	Bel	C	B	D	A	A	E	D	33	C	C	D	B	D	E	D	10	PCR
7	Bel	E	E	B	A	D	E	C	21	E	E	B	A	D	E	C	21	NCR
8	Bel	C	B	E	A	E	E	C	22	B	B	E	C	B	E	C	26	NCR
CD20-to-CD20 Group																		
9	Ocr	B	A	E	B	D	A	C	41	D	C	E	D	D	D	C	2	MCR
10	Ocr	B	E	E	D	E	A	C	21	D	E	E	D	E	D	C	1	MCR
11	Ocr	E	E	B	B	E	E	C	17	E	E	D	C	E	E	C	2	MCR
12	Ofa	E	E	E	E	E	A	A	24	E	E	E	E	E	B	C	9	PCR
13	Ofa	B	A	B	B	E	A	C	49	D	C	D	D	E	C	C	3	MCR
14	Obi	D	A	D	C	E	D	C	14	D	C	D	C	E	D	C	3	MCR

Ocr, Ocrelizumab; Ofa, Ofatumumab; Obi, Obinutuzumab; MCR, major clinical response; PCR, partial clinical response; NCR, no clinical response.

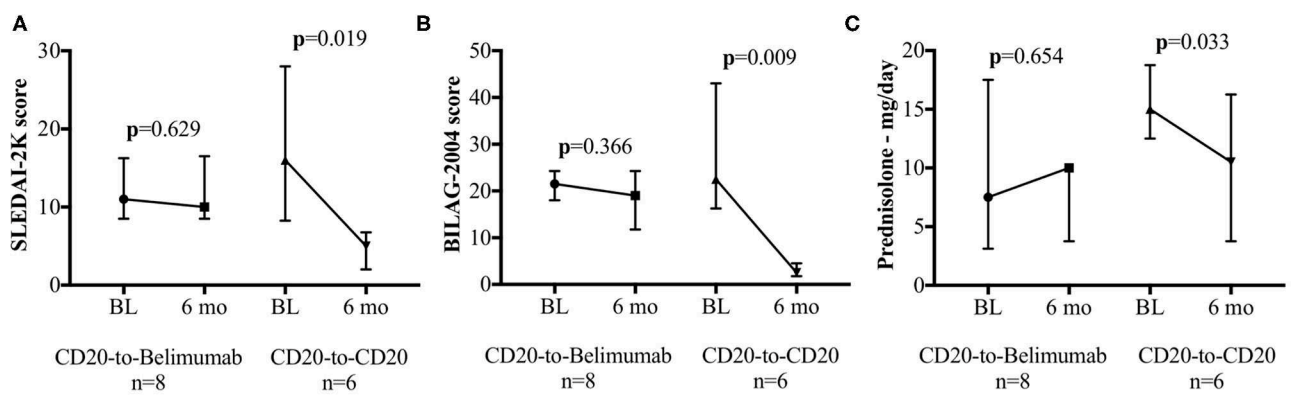

FIGURE 1 | SLEDAI-2K, Global BILAG-2004, and Daily prednisolone dose. Clinical efficacy assessments for the CD20-to-belimumab and the CD20-to-CD20 groups. Each figure shows the pre- and post-treatment results for the **(A)** SLEDAI-2K, **(B)** total BILAG score, and **(C)** daily oral prednisolone requirements in the CD20-to-CD20 group compared to the CD20-to-belimumab group. Points represent median and error bars denote interquartile range. BL, Baseline; 6 mo, 6 months.

Immunological Outcomes of the CD20-to-CD20 Group

In all 6 patients, the anti-dsDNA titres had reduced at 6 months, however none normalised; median (IQR) at baseline and at 6 months were 301.0 IU/ml (137.5–342.3) and 121.5 IU/ml (74.8–259.0), respectively; $p = 0.107$ (**Figure 2A**). For complement C3 and C4 levels, 4/6 had low levels for both at baseline. Of these, 3/4 had normalisation of the C3 and C4 levels at 6 months (**Figures 2B,C**). Those C3 or C4 levels which were normal at baseline ($n = 2$), remained within normal range at 6 months post-alternative anti-CD20 mAbs switch. Median (IQR) for complement C3 level at baseline and at 6 months were 0.53 g/L (0.45–0.89) and 1.18 g/L (0.91–1.21), respectively; $p = 0.087$. For complement C4 level at baseline and at 6 months, median (IQR) were 0.06 g/L (0.05–0.13) and 0.17 g/L (0.15–0.18), respectively; $p = 0.004$.

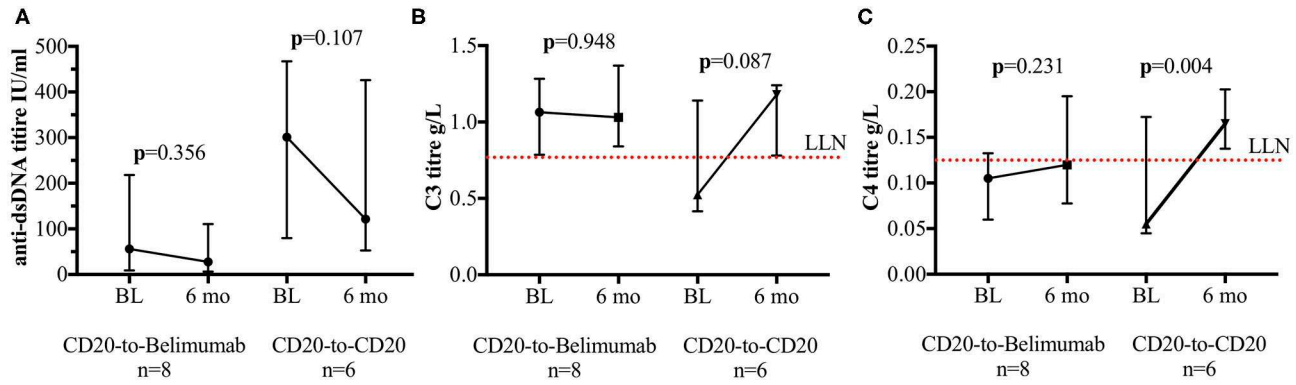

FIGURE 2 | Anti-dsDNA titres and complement levels. Immunological tests for the CD20-to-belimumab and the CD20-to-CD20 groups. Each figure shows the pre- and post-treatment for the **(A)** anti-dsDNA titres; **(B)** complement C3 level and **(C)** complement C4 level. Points represent median and error bars denote interquartile range. The dotted red lines represent lower limit of the normal values of the tests. BL, Baseline; 6 mo, 6 months.

DISCUSSION

In this study, we report the first evidence on biologic switching in SLE, suggesting an important difference in response after rituximab. These data highlight the importance of appropriate biologic sequencing in this growing resistant subgroup.

Many potential therapeutic targets have been explored for SLE. The most prominent target the B cell pathway directly. Most pharmacological agents targeting B cells either deplete them (rituximab, ocrelizumab, ofatumumab, and obinutuzumab); or inhibit BAFF (belimumab, tabalumab, atacicept). Non-B cell targets are diverse and include type I interferon (IFN-I) (27), interleukin (IL) 12/23, CTLA4-CD28 co-stimulation and the Janus kinase/signal transducers and activators of transcription (JAK-STAT) pathway. These agents may still impact on B cells, but only indirectly.

The diversity of these potential targets raises the question of the most appropriate follow-on therapy in patients who either fail to respond to their first biologic or, lose an initial good response (2NDNR). The answer to this question depends on our understanding of the mechanism for inadequate response.

For most manifestations of SLE, we showed that a key determinant of rituximab response is the degree of B cell depletion achieved with therapy (8). This implies that these clinical manifestations are B cell-dependent. Some manifestations of SLE appear to be non-B cell mediated. We showed that most discoid lupus erythematosus and some subacute cutaneous lupus erythematosus lesions either did not respond, worsened or initiated during rituximab therapy despite complete peripheral B cell depletion (28).

Belimumab may also be more effective in a subgroup of SLE patients in whom B cells have a more dominant role. In randomised clinical trials, the difference in response between belimumab and placebo is twice as large in patients with B cell biomarkers (i.e., raised anti-dsDNA titres and low complement levels) (29).

Thus, for patients initially responding well to rituximab (i.e., have proven B cell-dependent manifestations) but subsequently develop pharmacodynamic resistance (2NDNR), switching to any other B cell targeted therapy would appear to be an appropriate strategy. We therefore expected a *higher* SRI-4 response rate than the ∼55% of unselected SLE patients who responded in the pivotal trials of belimumab (12, 13, 30) given the resultant raised BAFF levels following successful depletion of B-cells. We also expected a high response rate to an alternative B cell depleting therapy, provided that depletion could be adequately restored. Surprisingly, we found that the SRI-4 response rate to belimumab was markedly worse in our study than in its trials, especially noting the new episodes of lupus nephritis.

These results may reveal potential complexities of therapeutic BAFF inhibition. After rituximab, there is a marked elevation of serum BAFF; up to 10-fold normal levels (31). Simultaneously, there is a marked shift in B cell dynamics with sustained reduction of naïve B cells that bear the BAFF-R receptor. Thus, there are relatively greater proportions of memory B cells and plasmablasts that express tumour necrosis factor receptor superfamily member 13b (TACI) and B cell maturation antigen (BCMA) receptors instead. These memory B cells and plasmablasts also bind APRIL (3). In particular, TACI signalling may have more complex effects on B cells than the BAFF-R signalling that predominates in rituximab-naïve SLE patients (32). Given these changes, it may therefore be expected that the effects of BAFF blockade in this situation might differ from the general SLE population in which the drug was evaluated in phase III trials.

In contrast, the effectiveness of alternative anti-CD20 agents in patients with 2NDNR to rituximab is entirely consistent with our hypothesis and the correlations we have reported between the degree of B cell depletion and clinical response. Anti-drug antibodies are more likely to occur against chimaeric mAbs. Regular cycles of rituximab may theoretically prevent their development, but the long treatment intervals required for some SLE patients, as well as the underlying propensity for B cell activity and antibody formation in SLE may account for their higher frequency in SLE compared to other diseases in which rituximab is used. The alternative anti-CD20 agents used in this

study were humanised or fully human. There may be other differences between anti-CD20 agents that affect the efficacy of depletion in SLE, which may be particularly important for obinutuzumab (33).

These results may not affect the use of belimumab in SLE patients in general, nor the rationale for the various rituximab-belimumab combination strategies in clinical trials (34). Neither do they provide a comparison of the efficacy of these agents in their more typical patient populations. However, this study emphasises the importance of gathering data on belimumab in real world settings and deeper understanding of the BAFF pathway in SLE.

This study has some limitations. First, the sample size was small to definitively confirm the efficacy of the two strategies used in patients with 2NDNR to rituximab. These findings need to be validated in larger patient cohorts. However, the size of the difference seems large since only one patient in the CD20-to-belimumab group achieved the SRI-4 compared to all of the patients treated with alternative anti-CD20 agents. Second, this study was non-randomised. There was an imbalance in some of the baseline clinical characteristics, particularly patients in the CD20-to-CD20 group who had worse SLE (i.e., higher SLEDAI-2K and higher concomitant daily prednisolone). However, this group still showed better response to therapy compared with the CD20-to-belimumab group. There were some patients with renal involvement at baseline in the CD20-to-CD20 group and not the belimumab group. However, since we observed new episodes of nephritis in the CD20-to-belimumab group, it does not seem promising to further investigate this line of therapy in more severe patients. Our patients were all recruited from the same population and assessed in the same way in a single centre with marked differences in response rates. Lastly, concomitant therapy with immunosuppressant were used in 83% of the patients, thus the overall efficacy could not be attributed to the alternative anti-CD20 agents alone. Nevertheless, our long-term follow-up of a large cohort of rituximab-treated patients is one of the best sources of data available for these more complex questions. It is unlikely that randomised trials will ever be completed for this question. In the absence of randomised trials, and with a clinically important problem, it is appropriate to use the best case series evidence available.

In conclusion, 2NDNR is an increasingly common problem in SLE patients treated with rituximab, often with severe disease. For these patients, our data suggest biologic therapy should be switched within the same class; i.e., to another anti-CD20 agent. This study demonstrates the importance of stratification of therapy in SLE, based on an understanding of the determinants of response.

DATA AVAILABILITY STATEMENT

All datasets presented in this study are included in the article/supplementary material.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Leeds (East) Research Ethics Committee (REC), 10/H1306/88. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SH, MM, EV, PE, and SD: substantial contributions to the conception or design of the work, or the acquisition, analysis or interpretation of data, drafting the work or revising it critically for important intellectual content, and final approval of the version published. SH, MM, and EV: agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors contributed to the article and approved the submitted version.

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Comparison of Rituximab Originator With CT-P10 Biosimilar in Patients With Primary Sjögren's Syndrome: A Retrospective Analysis in a Real-Life Setting

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Introduction: Over the last two decades, rituximab (RTX) has been widely used, albeit off-label, in primary Sjögren's syndrome (pSS). Several studies reported that B lymphocyte depletion with RTX is effective to treat some aspects within the disease spectrum, by reducing disease activity and affecting the inflammation and lymphoid organization that occur in target tissues. Notwithstanding, randomized controlled trials failed to confirm such evidence. With the recent release of several RTX biosimilars on the market, their efficacy and safety compared to the originator must be ascertained across different indications. This study aimed at comparing efficacy and safety of RTX originator and CT-P10 RTX biosimilar in pSS patients in a real-life setting.

Methods: Clinical and laboratory records of pSS patients referring to a tertiary rheumatology clinic were retrospectively evaluated. Patients having received at least two courses of either RTX originator or CT-P10 with complete data at baseline and after 12, 24, 36, and 48 weeks of treatment were enrolled. Disease activity was assessed with the EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI) and its clinical version without the biological domain (clinESSDAI). Patient-reported symptoms were assessed with the EULAR Sjögren's Syndrome Patient-Reported Index (ESSPRI). Adverse events (AEs) occurring during the study period were also recorded.

Results: Nine patients who received RTX originator and eight patients who received CT-P10 were enrolled. Baseline clinical and serological features, including ESSDAI and ESSPRI, were similar in the two treatment groups. An efficient depletion of circulating $CD19^{+}$ B lymphocytes was achieved in both treatment arms. Both RTX originator and CT-P10 significantly reduced ESSDAI and clinESSDAI by week 24, and no difference between the groups was observed at any timepoint. Conversely, changes of ESSPRI overtime did not differ between the two treatment arms and were not statistically significant compared to corresponding baseline values. With regard to safety, at 48 weeks of follow-up, only four mild AEs (two in the RTX originator and two in the CT-P10 group) were observed.

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Conclusion: Our study provides the first evidence that, at 48 weeks of follow-up, RTX originator and CT-P10 display similar efficacy and safety profiles in pSS.

Keywords: primary Sjögren's syndrome, rituximab, ESSDAI, ESSPRI, biosimilar

INTRODUCTION

Primary Sjögren's syndrome (pSS) is a systemic autoimmune disease characterized by mucosal dryness in the majority of patients. General symptoms such as fatigue, weight loss, and fever, as well as extraglandular manifestations involving musculoskeletal system, skin, peripheral and central nervous system, kidneys, and lungs, occur in at least one-third of patients, increasing health care costs and affecting the quality of life (1–3). The evolution into B-cell lymphoma represents one of the main causes of decreased survival in pSS and occurs in about 5% of patients (4).

B cells play a major role in the pathogenesis of pSS via antibody-dependent and -independent mechanisms, and their hyperactivity, along with salivary gland infiltration and development of B-cell follicles containing germinal center–like structures, represents the hallmarks of the disease (4–7).

Therapeutic management of pSS is based on symptomatic treatment of sicca symptoms and broad-spectrum immunosuppression for systemic disease, but data concerning efficacy and safety of the therapeutic options available are often insufficient (8). Although the emergence of biological therapies has increased the therapeutic armamentarium available to treat pSS, their use in clinical practice is limited by the lack of licensing (9). Given the central role of B cells in pSS pathogenesis, a B-cell targeting therapy represents an unarguable and intriguing therapeutic approach in this disease. Rituximab (RTX) is a chimeric murine/human IgG1 monoclonal antibody (with human kappa and IgG1 constant regions and murine light- and heavy-chain variable regions) targeting the CD20 molecule (human B lymphocyte–restricted differentiation antigen, Bp35) found on the surface of most B cells, including pre-B, mature B lymphocytes, and malignant B cells, but not on stem cells, pro–B cells, normal plasma cells, or other normal tissues. There are at least four postulated mechanisms of action for RTX: complement-mediated cytotoxicity, antibody-dependent cell-mediated cytotoxicity, induction of apoptosis, and saturation of the Fc receptors of effector cells, and all of them may contribute to the therapeutic effect in pSS (10, 11).

In pSS, RTX has been tested in four randomized controlled trials (RCTs) (12–15), three prospective cohort studies (16–18), and one case–control study (19). It is evident from most studies that RTX has a positive impact on B-cell numbers and activity, both in the peripheral blood and in salivary glands, but the clinical efficacy of B-cell depletion therapy with RTX in pSS remains controversial (20). In particular, although the majority of studies showed efficacy in at least one of the systemic outcomes analyzed, such as global response, organ-specific response, EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI) reduction, and prednisone reduction, the evidence reported by RCTs is weak. Moreover, an analysis of data from The Trial of Anti–B cell Therapy in Patients With Primary Sjögren's Syndrome shows that RTX is not cost-effective (15). Therefore, the recent EULAR recommendations for the management of Sjögren's syndrome pointed out that the use of RTX should be reserved to selected patients with severe, refractory systemic disease (8).

RTX was the first monoclonal antibody to be approved for the treatment of some type of blood cancer and inflammatory conditions as rheumatoid arthritis, granulomatosis with polyangiitis, microscopic polyangiitis, and pemphigus vulgaris [MabThera® [Roche, Welwyn Garden City, United Kingdom] in Europe and Rituxan® [Genentech, San Francisco, CA] in the USA] (21, 22). As this anti-CD20 monoclonal antibody has now reached patent expiry date, biosimilar versions are in development. In particular, CT-P10 [Truxima® (Celltrion Incheon, Republic of Korea)] has recently been approved in Europe for all indications held by MabThera® (23).

Biosimilars have the potential to broaden patient access to biologics and reduce the economic burden of health care systems. During the development of a biosimilar, data that directly compare the proposed biosimilar with the reference product are required. These comparative data are generated in a stepwise hierarchical process from extensive laboratory-based structural analyses and functional assays, to clinical safety and efficacy (24). Demonstration of similarity in these trials, along with extensive evidence of similarity from other tests, allows the extrapolation of data with the reference compound to the biosimilar in other non-tested indications (25).

Several clinical trials assessing CT-P10 in follicular lymphoma and rheumatoid arthritis (26–33) and a few data concerning the real-world experience (34, 35) have been published so far. However, data concerning this compound in pSS are not available.

To our knowledge, no study has compared efficacy and safety of any approved RTX biosimilar to RTX originator in pSS patients. Therefore, the aim of this retrospective, observational, single-center study was to compare the efficacy and safety of CT-P10 (Truxima®) with RTX originator (MabThera®) in a real-life cohort of patients with pSS.

MATERIALS AND METHODS

Study Population

This was a retrospective, observational, single-center study of pSS patients receiving RTX off-label in a tertiary Rheumatology Unit (ASL1 Avezzano-Sulmona-L'Aquila and University of L'Aquila, Italy). We included 17 consecutive patients with pSS diagnosis, with a disease duration of < 5 years and a systemic moderate–high activity (ESSDAI ≥ 5) (36) who received the first RTX infusion between December 2013 and January 2019.

Patients receiving the first infusion between December 2013 and November 2017 received RTX originator (MabThera®) (nine patients), and those initiating RTX after November 2017 received CT-P10 (Truxima®) (eight patients), because of local health policy regulations. Patients with secondary Sjögren's syndrome; severe cardiac, pulmonary, renal or hematologic failure; a history of cancer in the last 5 years; hepatitis B or hepatitis C infection, human immunodeficiency virus infection, tuberculosis, severe diabetes, and any other chronic disease; or evidence of infection; and if they were unable to understand and to adhere to the treatment were not eligible to start RTX and therefore excluded from this study. Treatment with conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) and corticosteroids was allowed during the study period because of the moderate–high disease activity. However, any change in the dose or schedule was noted.

RTX Administration

Patients received infusion of 1,000 mg of either RTX originator or CT-P10 at day 1 and at day 15 to complete a course of therapy. This course was repeated after 24 weeks. During the study period, all patients received two courses of therapy with RTX (baseline and week 24). To minimize adverse effects, all patients were pretreated with methylprednisolone (40 mg intravenously), paracetamol (1,000 mg orally), and chlorpheniramine (10 mg intravenously).

Clinical and Laboratory Evaluation

Clinical and laboratory evaluations were performed at baseline (W0), week 12 (W12), week 24 (W24), week 36 (W36), and week 48 (W48) of treatment. The disease activity was assessed using the ESSDAI and clinical ESSDAI (clinESSDAI), whereas patient-reported outcomes (PROs) included the EULAR Sjögren's Syndrome Patient-Reported Index (ESSPRI) and the patient's assessment of general health (GH) score [on a 0–100-mm visual analog scale (VAS) with "very poor" and "very well" as anchors]. We also considered the reduction in the daily dose of prednisone. Total lymphocyte count, $CD19^+$ B lymphocytes, serum γ-globulins, immunoglobulin classes (IgG, IgA, and IgM) concentration, and serum rheumatoid factor (RF) were regularly measured. Patients were asked to report the occurrence of systemic or local adverse events (AEs) related to treatment at each visit. AEs were judged as serious if they resulted in death, were life-threatening according to the investigator's own judgment, caused hospital admission, resulted in birth defect (from unplanned pregnancies) or disability, or were important medical events that could have jeopardized the patient or needed intervention to prevent another serious AE, or both.

Study Endpoints

The primary efficacy endpoint of this study was the delta (Δ) ESSDAI, clinESSDAI, and ESSPRI achieved by CT-P10 compared with RTX originator. The primary safety outcome was the number of AEs during the study period. Secondary exploratory endpoints included the percentage of patients achieving a minimal clinically important improvement (MCII) with ESSDAI (drop of at least three points), MCII with ESSPRI (drop of at least one point or 15% of baseline value), and patient acceptable symptom state (PASS) (ESSPRI <5) at W48, laboratory measures, and GH.

TABLE 1 | Demographic and clinical characteristics of patients at baseline.

	CT-P10 n (%)	RTX originator n (%)	*p*-value
Number	8	9	—
Age, mean (SD), years	60.1 (9.1)	51.8 (12.8)	ns
Female gender, n (%)	7 (87)	8 (89)	ns
Disease duration, mean (SD), years	1.29 (1.5)	1.75 (1.6)	ns
Focus score ≥ 1, n (%)	8 (100)	9 (100)	ns
Xerostomia, n (%)	8 (100)	8 (89)	ns
Xerophthalmia, n (%)	8 (100)	9 (100)	ns
Salivary gland enlargement, n (%)	1 (12)	3 (33)	ns
ESSDAI, mean (SD)	12.0 (7.3)	12.6 (6.6)	ns
ClinESSDAI, mean (SD)	11.4 (7.1)	11.4 (6.4)	ns
ESSDAI domains, n (%)			
Constitutional	1 (12)	6 (67)	ns
Lymphoadenopathy	3 (37)	3 (33)	ns
Glandular	1 (12)	3 (33)	ns
Articular	8 (100)	8 (89)	ns
Cutaneous	1 (12)	1 (11)	ns
Pulmonary	1 (12)	1 (11)	ns
Renal	0 (0)	0 (0)	ns
Muscular	0 (0)	0 (0)	ns
PNS	3 (37)	3 (33)	ns
CNS	1 (12)	0 (0)	ns
Hematological	0 (0)	2 (22)	ns
Biological	4 (50)	7 (78)	ns
Hypergammaglobulinemia, n (%)	3 (37)	5 (55)	ns
Reduced complement fractions, n (%)	2 (25)	3 (33)	ns
Autoantibodies, n (%)			
Neither anti-Ro nor anti-La	3 (37)	5 (55)	ns
Anti-Ro only	4 (50)	2 (22)	ns
Anti-Ro and anti-La	1 (12)	2 (22)	ns
RF	5 (62)	4 (44)	ns
ESSPRI, mean (SD)	6.8 (2.1)	7.8 (2.0)	ns
GH, mean (SD)	30 (16.0)	33.3 (22.9)	ns
csDMARDs, n (%)	4 (50)	4 (44)	ns
Prednisone, n (%)	5 (62)	8 (89)	ns

RTX, rituximab; SD, standard deviation; ns, not statistically significant; ESSDAI, EULAR Sjögren's syndrome disease activity index; PNS, peripheral nervous system; CNS, central nervous system; RF, rheumatoid factor; ESSPRI, EULAR Sjögren's Syndrome Patient-Reported Index; GH, global health; csDMARDs, conventional synthetic disease-modifying antirheumatic drugs.

Statistical Analysis

IBM SPSS Statistics 23.0 software was used for statistical analysis. One-way analysis of variance and multiple-comparisons *post hoc* tests were employed to calculate differences between baseline and following timepoints. Differences between the

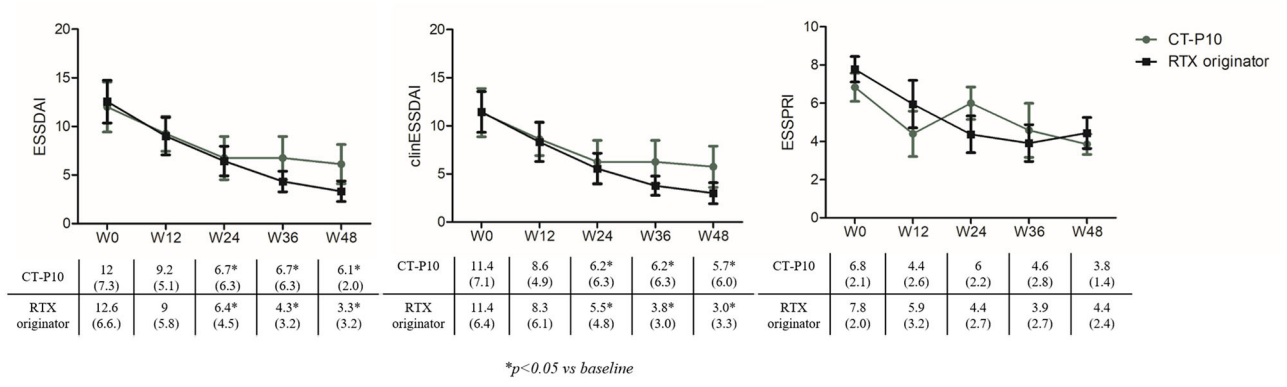

FIGURE 1 | EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI), clinical (clin) ESSDAI, and EULAR Sjögren's Syndrome Patient-Reported Index (ESSPRI) during the study period in the two treatment arms. RTX, rituximab; W, week.

TABLE 2 | Change of disease activity and patient-reported symptoms at the different timepoints compared to baseline.

	W12		W24		W36		W48	
	CT-P10	**RTX originator**	**CT-P10**	**RTX originator**	**CT-P10**	**RTX originator**	**CT-P10**	**RTX originator**
ΔESSDAI, mean (SD)	−2.75 (2.37)	−3.00 (2.18)	−5.12 (2.10)	−5.89 (4.99)	−5.12 (2.10)	−7.67 (5.59)	−5.62 (2.26)	−8.44 (5.03)
ΔclinESSDAI mean (SD)	−2.75 (2.37)	−3.11 (2.26)	−5.12 (2.10)	−5.89 (4.99)	−5.12 (2.10)	−7.67 (5.59)	−5.62 (2.26)	−8.44 (5.03)
ΔESSPRI mean (SD)	−4.1 (4.4)	−3.15 (3.1)	−1.6 (2.3)	−3.3 (2.9)	−4.5 (2.9)	−4.3 (2.6)	−3.46 (2.9)	−3.33 (0.91)

RTX, rituximab; ESSDAI, EULAR Sjögren's Syndrome Disease Activity Index; clin, clinical; ESSPRI, EULAR Sjögren's Syndrome Patient-Reported Index.

TABLE 3 | ESSDAI domains in the study cohort at W48.

	CT-P10 n (%)	**RTX originator n (%)**	***p*-value**
Number	8	9	—
ESSDAI domains			
Constitutional	0 (0)	0 (0)	ns
Lymphoadenopathy	1 (12)	0 (0)	ns
Glandular	0 (0)	1 (11)	ns
Articular	6 (75)	5 (55)	ns
Cutaneous	0 (0)	0 (0)	ns
Pulmonary	1 (12)	1 (11)	ns
Renal	0 (0)	0 (0)	ns
Muscular	0 (0)	0 (0)	ns
PNS	3 (37)	2 (22)	ns
CNS	1 (12)	0 (0)	ns
Hematological	0 (0)	0 (0)	ns
Biological	2 (25)	2 (22)	ns

RTX, rituximab; ESSDAI, EULAR Sjögren's Syndrome Disease Activity Index; ns, not statistically significant; PNS, peripheral nervous system; CNS, central nervous system.

demographic and clinical characteristics of the two treatment groups at baseline and between the two treatment arms at each timepoint were tested with non-parametric Mann–Whitney *U*-test. When required, χ^2 test was also employed. $P < 0.05$ were considered significant.

RESULTS

Baseline Demographic and Clinical Characteristics

Demographic and clinical characteristics of the two treatment groups at baseline are shown in **Table 1**. We included 17 patients: nine in the RTX originator group and eight in the CT-P10 group. We did not observe any significant differences in age, gender, and disease duration, or in clinical and laboratory parameters. None of the included patients displayed B-cell lymhoproliferative disease. The proportion in the use of the different csDMARDs and corticosteroids did not differ in both groups. In detail, three patients were in treatment with methotrexate, one with leflunomide, and six with hydroxychloroquine. Eight patients in the RTX originator group and five patients in the CT-P10 group were also in treatment with prednisone (mean dosage at baseline 6.3 and 4.2 mg, respectively). All 17 patients completed the 12 months' follow-up and are still in treatment.

Primary Endpoints

Figure 1 shows ESSDAI, clinESSDAI, and ESSPRI across the study period. In both treatment groups, ESSDAI and clinESSDAI started to decrease by W12, albeit significantly only from W24. The significant difference with baseline was consistent up to W48; at none of the timepoints the ΔESSDAI and ΔclinESSDAI were significantly different between the two treatment arms (**Table 2**). At W48, MCII with ESSDAI were achieved by all patients regardless of the treatment arm. **Table 3** shows the improvement within each ESSDAI domain in the two

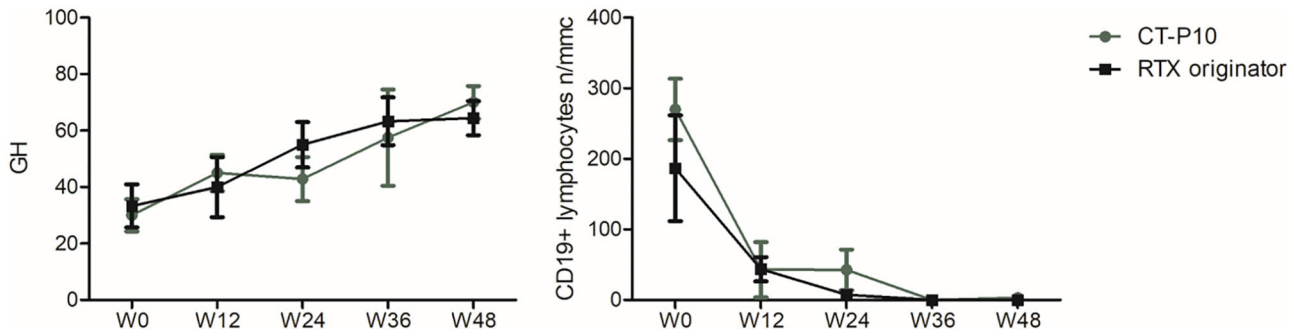

FIGURE 2 | Global health (GH) and CD19$^+$ lymphocyte count during the study period in the two treatment arms. W, week.

treatment groups at W48. Improvement was predominantly seen in constitutional, lymphoadenopathy, glandular, articular, hematological, and biological domains, and still the two cohorts did not display any difference in this regard. In addition, all the five patients (three in the originator group and two in the CT-P10 group) with reduced complement fractions at baseline still displayed it at W48. With regard to ESSPRI, neither CT-P10 nor RTX originator was able to significantly reduce it in line with previous studies (**Figure 1**), and no difference could be observed with regard to the ΔESSPRI between the two treatment groups at any timepoint (**Table 2**). However, MCII with ESSPRI (reduction of at least one point or reduction of 15% of baseline value) was achieved at W48 by six patients (75%) in the CT-P10 group and all nine patients (100%) in the RTX originator group (not statistically significant). PASS was achieved by a similar proportion of patients in the two treatment groups (CT-P10 group: $n = 4$; 50%; RTX originator: $n = 4$; 44%; not statistically significant) at W48.

As far as the safety is concerned, a total of four AEs were recorded in four patients across the 12 months' follow-up: one mild cutaneous reaction (RTX originator group, T6) and three upper airways infections (CT-P10 group, one at T3 and one at T9; RTX originator group, T6), which required antibiotic therapy.

Secondary Clinical and Laboratory Outcomes

GH showed a trend toward improvement starting from W12 in both groups and becoming significant at W48 compared to baseline, with no significant differences in the two groups (RTX originator group W0: 33.3 ± 22.9, W48: 70 ± 17.89; $p = 0.01$; CT-P10 group W0: 30 ± 16, W48: 76.7 ± 11.5; $p = 0.02$) (**Figure 2**). Both RTX originator and CT-P10 effectively depleted circulating B lymphocytes as demonstrated by the serial measurement of CD19$^+$ cells (**Figure 2**). The total lymphocyte count, however, remained unaffected (data not shown). Serum γ-globulins, immunoglobulin classes (IgG, IgA, and IgM) and RF were regularly monitored and did not show significant changes overtime (data not shown).

The majority of patients taking corticosteroids at baseline tapered the dose until withdrawal with only two out of five patients in the CT-P10 group and five out of eight patients in the RTX originator group still on treatment (mean dose 3 mg in both groups).

DISCUSSION

This is the first study demonstrating that CT-P10 and RTX originator show similar efficacy and safety profiles in pSS patients with moderate–high disease activity and disease duration of < 5 years at 48 weeks of follow-up. Concerning the clinical efficacy, in both treatment groups, ESSDAI and clinESSDAI started to slowly decrease by W12, with a significant difference with baseline starting from W24 and remaining sustained up to W48. Furthermore, we did not observe any significant differences in the ΔESSDAI, ΔclinESSDAI, and MCII with ESSDAI between the two treatment arms. Interestingly, this improvement mirrored the dose reduction of prednisone, with its withdrawal in three patients in both groups at W48. In addition, in our cohort, the ESSDAI domains were mainly represented by articular, lymphoadenopathy, and peripheral nervous system involvement in both groups and constitutional in originator. Therefore, our study confirms existing evidence that RTX may be considered a valuable therapeutic option in patients with this specific phenotype. However, despite the generally acknowledged beneficial effects of RTX treatment on biological parameters, clinical outcomes vary between studies (20). Available data on the systemic efficacy of RTX in pSS come from large studies that included more than 400 patients, and the predominant regimen of administration was two doses of 1,000 mg each administered 15 days apart (8, 9). The great majority of these studies showed efficacy in at least one of the systemic outcomes analyzed, considering global response, organ-specific response, ESSDAI reduction, or prednisone reduction, thereby providing a reasonable rationale for the use of this therapy in specific clinical settings. These concepts have been incorporated in the recent EULAR recommendations for the management of Sjögren's syndrome with topical and systemic therapies that reserve the use of RTX to patients with severe refractory disease (8, 9). PROs with VAS have been used in most studies, including two recent large RCTs (14, 15) to assess subjective symptoms. Devauchelle-Pensec et al. (14) found no significant results in the primary outcome (≥30-mm

improvement at week 24 on at least two out of four VAS scores—dryness, fatigue, pain, global, 23 vs. 22%; $p = 0.91$), whereas Bowman et al. (15) found no significant results in the primary outcome (reduction $\geq$ 30% at week 48 in either fatigue or oral dryness VAS, RTX 39.3 vs. placebo 36.8%; $p = 0.76$). This is in line with our findings revealing a failure in achieving a significant reduction of ESSPRI up to W48. Of interest, despite a considerable proportion of patients reached MCII with ESSPRI at W48, namely, a reduction of at least one point or 15% reduction of baseline value, approximately only half of them in each treatment group achieved a PASS, namely, an ESSPRI of < 5. In our study, both treatments also induced a similar improvement of GH, which based on the ESSDAI and ESSPRI values overtime seemed to be more related to systemic disease activity rather than sicca symptoms, fatigue, or pain.

The number of patients underreporting or overreporting their symptoms may influence the results of studies and underscores the uniqueness of individual perception of pSS-related symptoms regardless of disease activity and therefore showing different response patterns to RTX treatment. In this regard, the discrepancy between PROs, objective measurement of glandular function, and systemic disease activity indexes in pSS underpins that they represent complementary perspectives to obtain a holistic view of the individual, and therefore, all of them should be explored and implemented in clinical practice (37). With regard to the effect on B cells, we confirmed that treatment with RTX leads to a nearly complete depletion in the peripheral blood, without any significant differences between the two treatment groups (12, 16). Although we did not observe any change in serum RF, γ-globulins, and immunoglobulin classes, it has been postulated that by interfering with B-cell activation likely one aspect contributing to the amelioration of systemic disease activity in pSS patients may be the lower levels of autoantibodies and proinflammatory cytokines (38). CT-P10 was the first RTX biosimilar (39) and could represent a cost-effective and safe therapeutic alternative to RTX originator, possibly facilitating access to therapy for pSS patients with severe, refractory systemic disease.

Budget impact analysis models estimated the expected changes in expenditure that would occur as a result of the adoption of a new therapeutic intervention. On this basis, it was demonstrated that introduction of CT-P10 could be associated with significant budget savings in European Union countries, for both in-label and off-label indications (40). With the expiry date of patent protection approaching for several originator biologic DMARDs, we have witnessed the development of less expensive competitor products of sufficient similarity, called biosimilars. Regulatory approval is based on the totality of evidence for biosimilarity derived from a comprehensive comparability exercise with the reference medicine (41). This comparability exercise includes extensive physicochemical and structural evaluations, as well as data from preclinical and clinical pharmacokinetic, pharmacodynamic, and immunogenicity assessments. The final step in the development process is confirmatory phase III clinical trial in patients with the specific disease. However, in line with regulatory requirements, approval of CT-P10 in some indications of RTX was based in part on the extrapolation of clinical data collected in other indications, plus a scientific justification based on the consistency of RTX mechanisms of action across indications (42–44). Although there are several discrepancies in assessing and reporting immunogenicity data of biosimilars for CD20 inhibitors, data collected in trials confirmed that immunogenicity parameters of CT-P10 were similar to those of its reference product (26, 27, 30). Furthermore, it was reassuring to observe that the safety profile of CT-P10 was similar to RTX originator also in pSS, with upper airway infections being the most frequent AEs in both groups.

We acknowledge that this study displays some limitations including the retrospective nature, the heterogeneity of clinical spectrum and disease duration, and the small number of patients. In addition, treatment choice between RTX originator and CT-P10 depended on the time of the first infusion and not the physician's decision, because of local health policy issues. Notwithstanding, we believe that given the lack of studies on CT-P10 in pSS, it provides important insight to clinicians who will be required to use this compound in pSS patients.

CONCLUSION

RTX originator is a cornerstone in therapeutic strategies for rheumatic diseases, including pSS; however, economic issues may be a major barrier to access the best available care, particularly in some countries. Therefore, the use of biosimilars can only be expected to increase. Whether the use of biosimilars will be of greater benefit from a societal perspective will depend on their cost-effectiveness and safety. In this perspective, the collection of efficacy and safety data for all in-label and off-label indications is of paramount importance to identify and tackle potential differences among compounds and ultimately does not affect the quality of care for patients with pSS.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary material.

ETHICS STATEMENT

Ethical review and approval was obtained in accordance with local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

All authors drafted and approved the final version of the manuscript.

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CASE REPORT
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Rituximab Followed by Belimumab Controls Severe Lupus Nephritis and Bullous Pemphigoid in Systemic Lupus Erythematosus Refractory to Several Combination Therapies

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Systemic lupus erythematosus (SLE) and bullous pemphigoid (BP) are chronic autoimmune diseases in which B cells play an important pathogenic role in the different stages of the disease. B cell-targeted therapies have been suggested as a new rational approach for treating SLE. Rituximab (RTX), an anti-CD20 chimeric monoclonal antibody, failed to achieve primary endpoints in two clinical trials (EXPLORER and LUNAR) despite multiple observational and retrospective studies showing its beneficial effect on SLE. Moreover, RTX is recommended in cases of BP that is unresponsive to conventional treatments. Belimumab (BLM), a human immunoglobulin G1 λ monoclonal antibody that inhibits soluble B-lymphocyte stimulator (BlyS)/B-cell activating factor (BAFF), is the only biological treatment approved for standard therapy of refractory autoantibody-positive active SLE. Animal models and a few case reports have supported the efficacy of the combined use of RTX followed by BLM as maintenance therapy in severe lupus nephritis (LN), suggesting that their combined use may be more effective than their single use, without compromising safety. In this study, we describe the clinical case of a SLE patient with predominant renal involvement in overlap with BP, refractory to conventional therapy including RTX alone, achieving significant steroid sparing and clinical remission under sequential treatment of RTX-BLM. Moreover, we describe the first case of BP successfully treated with BLM. This case report may encourage further clinical research studies in B lymphocyte targeted combination therapy in patients affected by SLE with major organ involvement or with refractory disease, suggesting that RTX and BLM sequential therapy may be a valid option for the treatment of SLE manifestations, including conventional therapy and RTX-resistant LN.

Keywords: lupus nephritis, bullous pemphigoid, belimumab, rituximab, sequential therapy

INTRODUCTION

Systemic lupus erythematosus (SLE) and bullous pemphigoid (BP) are chronic autoimmune diseases in which B lymphocytes play a primary pathogenic role as they are implicated in the induction and progression of these diseases (1, 2). Only a few cases of patients affected by SLE in overlap with BP have been described in the literature (3–5). B cells exert their pathogenic action not only by producing autoantibodies but also by presenting autoantigens to T lymphocytes and secreting of a wide variety of proinflammatory cytokines, thus perpetuating the activation of the immune system (6). Rituximab (RTX), a chimeric monoclonal antibody that targets CD20 antigen on B cells, is successfully used to treat various autoimmune diseases by depleting B lymphocytes. Although some observational and retrospective studies have shown beneficial effects of RTX in SLE patients (7, 8), it failed to achieve the primary endpoints in the EXPLORER and LUNAR trials (9, 10), probably due to a wrong trial design. Moreover, RTX has been shown to be effective in BP patients who were unresponsive or with unacceptable side effects to conventional immunosuppressive drugs (11–15). However, the position of RTX within the therapeutic flowchart of SLE and BP diseases is still unknown. Belimumab (BLM) is a human immunoglobulin G1 λ monoclonal antibody that inhibits soluble B-lymphocyte stimulator (BlyS)/B-cell activating factor (BAFF) (16), and in 2011, BLM was approved for the treatment of standard therapy-refractory autoantibody-positive active SLE (17, 18). Moreover, BLM has been proven to be effective to treat moderate SLE with skin, articular, and hematologic abnormalities (19), although it is not licensed to treat severe lupus nephritis (LN) (20–22). To date, sequential therapeutic schemes of RTX followed by BLM have not been well-studied. Animal models (23) and few case reports support the efficacy of the combined use of RTX followed by BLM as maintenance therapy in severe LN (24–27), suggesting that their combined use may be more effective than their single use, without compromising safety. In this study, we reported the clinical case of a SLE patient with predominant renal involvement in overlap with BP, refractory to conventional therapy including RTX alone, achieving significant steroid sparing and clinical remission under sequential treatment of RTX-BLM. Moreover, we describe here the first case of BP successfully treated with BLM.

CASE PRESENTATION

We describe the clinical case of a 51-year-old Italian man who was diagnosed as having Undifferentiated Connective Tissue Disease in 2010 because of the presence of Raynaud's phenomenon, arthralgias, positivity for antinuclear antibody (ANA, 1:160 fine speckled), antiphospholipid antibodies (aPL) [(anticardiolipin antibodies (ACLA) IgM, 42 U/ml (normal range <20 U/ml), and anti-β2 Glycoprotein 1 (antiB2GP1) IgM, 38 U/ml (normal range <20 U/ml)], and a mild hypocomplementemia, C3 81 mg/dl (normal range 90–180 mg/dl) and C4 8 mg/dl (normal range 8–32 mg/dl). The patient did not report a family history of rheumatic disorders or a personal history of comorbidities and/or previous major surgery. A treatment with hydroxychloroquine (HQC) 400 mg daily and acetylsalicylic acid 100 mg daily was started. In 2011, the patient developed diffuse bullous skin lesions and a skin biopsy of a trunk lesion showed a typical histological picture for BP. Therefore, topical and oral steroid (0.25 mg/kg daily) therapy was started. Subsequently, the patient developed periorbital and lower limb edema, with proteinuria (6.2 g/daily), and a renal biopsy was performed showing histological findings of diffuse membranous glomerulonephritis associated with moderate mesangial hypercellularity (Class V according to ISN/RPS classification, 2003) (28). Therefore, a diagnosis of SLE was made [due to the presence of nephritis, arthritis, ANA/aPL

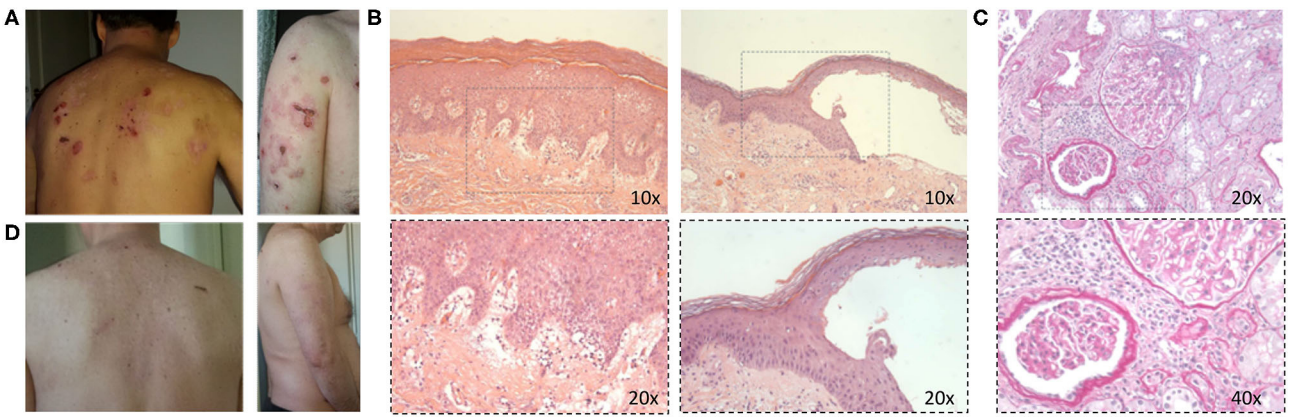

FIGURE 1 | (A) Active bullous pemphigoid skin lesions. **(B)** Histological images in hematoxylin and eosin staining of skin lesion biopsy showing subepidermal blister with a mixed inflammatory infiltrate rich in eosinophils mostly localized in a superficial dermis (10× and 20× magnification, respectively). **(C)** Periodic acid–Schiff staining images of kidney biopsy showing mesangial hypercellularity; the vessels show a slight moderate reduction of the lumen for sclerosis and myointimal hyperplasia, and vasculitic aspects are not observed (20× and 40× magnification, respectively). **(D)** Improvement of skin manifestations after therapy.

positivity (ACLA IgM 46 U/ml and antiB2GP1 IgM 38 U/ml), and complement level reduction (C3 78 mg/dl and C4 8 mg/dl)] with a Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) of 10; intravenous therapy with steroid (methyl-prednisolone pulses 250 mg for three consecutive days) was started followed by oral prednisone 1 mg/kg daily, subsequently tapered, and combined with mycophenolate mofetil (MMF) 2,000 mg daily and angiotensin-converting enzyme inhibitors, obtaining renal and cutaneous remission. In 2013, after steroid discontinuation, the patient experienced a proteinuric flare (2.5 g/daily) and a BP exacerbation. A second renal biopsy was performed confirming the previous histological nephritis class, and the repetition of skin biopsy documented a histological picture of leukocytoclastic vasculitis in overlap with BP. Oral prednisone was restarted at a dose of 0.5 mg/kg daily, with a slow tapering, and MMF dose was increased at 3,000 mg daily, reaching a resolution of the skin manifestations. In 2014, because of a further proteinuric flare (3 g/daily) and BP skin lesions worsening, B cells depletive treatment with RTX (two infusions of 1 g 14 days apart) was administered in association with MMF (3,000 mg daily) with a partial and temporary clinical remission. Therefore, therapy with Tacrolimus (5 mg/daily) in association with MMF was started, but quickly stopped because of a facial cutaneous rash, and followed by a combination treatment of MMF and Cyclosporine (200 mg/daily), discontinued for the same adverse event. In December 2015 and July 2016, two further retreatments with RTX were performed, with partial clinical response. In December 2016, because of the persistence of hypocomplementemia (C3 81 mg/dl, C4 9 mg/dl), proteinuria (3 g/daily) (SLEDAI 6), and BP lesions, the patient repeated skin and kidney biopsy, showing persistence of skin and kidney active inflammation (**Figure 1**). Therefore, RTX was discontinued and intravenous BLM (SLE therapeutic scheme, 10 mg/kg monthly) in association with MMF 2,000 mg daily and low-dose prednisone (10 mg daily) was started, leading to a progressive improvement of both renal and skin manifestations. At 24 weeks of follow up, the patient showed a complete cutaneous remission and a significant reduction until normalization of proteinuria, maintaining a complete clinical remission (SLEDAI 0) up to 52 weeks of follow up, allowing a significant reduction of prednisone dosage to 2.5 mg/daily. At the last clinical assessment, proteinuria was absent with normal complement levels (C3 91 mg/dl, C4 18 mg/dl) (**Figure 2**). Nowadays, the patient is continuing therapy with BLM and MMF and with low dose (2.5 mg/daily) of prednisone without further SLE and BP flares.

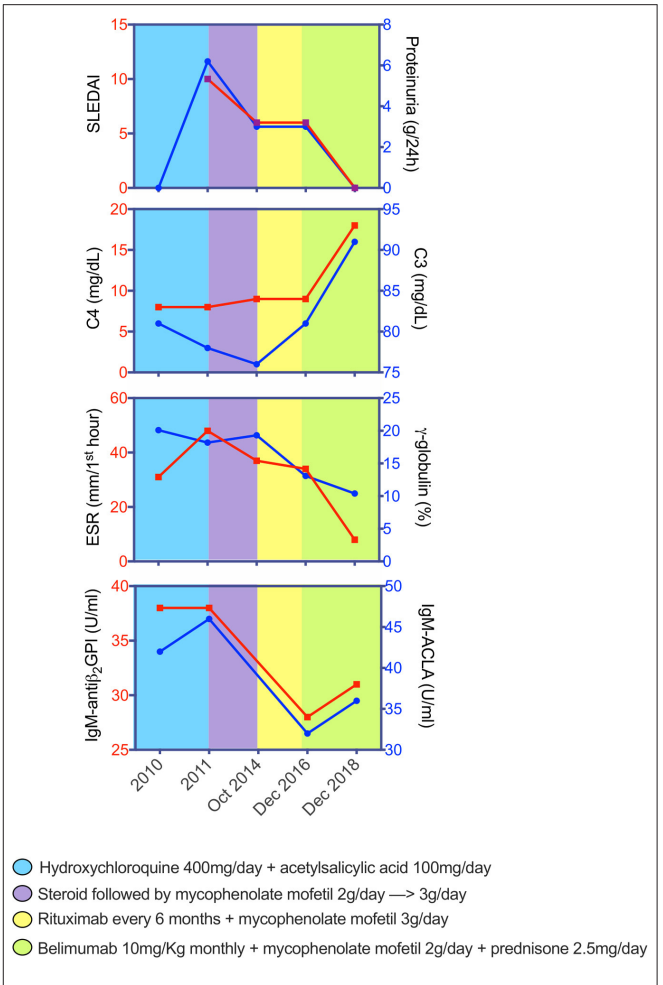

FIGURE 2 | Changes of SLEDAI, proteinuria, and blood parameters across disease course of patient with systemic lupus erythematosus and bullous pemphigoid. Graphs depict the dynamics of each parameter while receiving different therapeutic schemes. SLEDAI, Systemic Lupus Erythematosus Disease Activity Index; C3, fraction 3 of the complement; C4, fraction 4 of the complement; ESR, erythrocyte sedimentation rate; ACLA, anti-cardiolipin antibodies; antiβ2-GPI, anti-β2 Glycoprotein 1 antibodies.

DISCUSSION

The patient described in this clinical case had long-standing relapsing LN and BP, with skin and kidney biopsies showing persistent tissue inflammation. He had been treated with many different schemes, all of them with unsuccessful outcomes. Renal and skin flares were repeatedly treated with RTX, a drug frequently effective in this clinical setting (29). However, in the patient we describe, RTX only helped to achieve partial remission with early relapses. Thus, BLM was used as an additional option. To date, BLM has been used in patients with LN, mainly to maintain remission (25, 30); however, some clinical cases have been described reporting the use of BLM in the treatment of multiple therapy refractory patients with LN (31). Awaiting the results of ongoing clinical trials, we believe that BLM could be added to the list of potential treatments for patients with refractory LN. It is established that B cell hyperactivity is a landmark in SLE (32), and autoantibody positivity significantly characterizes patients with LN. The randomized trials BLISS-52 and BLISS-76 have documented the efficacy of BLM in autoantibody-positive SLE without previous RTX exposure and without major organ involvement (17, 18), and there is a randomized clinical trial demonstrating that BLM plus Standard Therapy (ST) significantly improves LN renal responses compared with ST alone (BLISS-LN trials; Clinicaltrials.gov identifier: NCT01639339; GSK study 114054)

(33). RTX acts by causing a marked B cell depletion (BCD) compared to the more modest effects on BCD induced by BLM. Therefore, the rationale for their consecutive use comes from the observation that significantly higher serum BLyS levels were found during B cell repopulation after RTX treatment, thus leading to disease flares (25, 31, 32). In this context, BCD may lead to ever-increasing levels of BAFF, with an increase in anti-dsDNA antibody levels and disease flare even at low B cell rate (23, 31). Moreover, elevated BLyS plasma levels after RTX would be associated with a paradoxical proliferation of pathogenic B lymphocytes, possibly explaining the therapeutic failure of RTX in clinical trials (34). On the other hand, the increase in BLyS levels could be due to the reduction of its specific receptors that are expressed on B lymphocytes (BAFF-R) (35). Therefore, further studies are needed to address this specific issue.

Currently, two ongoing open-label clinical trials are evaluating the efficacy of sequential therapy of RTX and BLM: the CALIBRATE (Rituximab and Belimumab for Lupus Nephritis, https://clinicaltrials.gov/ct2/show/NCT02260934, 2015) and the SYNBIoSe study (Synergetic B-Cell Immunomodulation in SLE, https://clinicaltrials.gov/ct2/show/NCT02284984, 2015), respectively. Therefore, the rationale for the combination therapy of BLM with RTX could be to operate through complementary and synergistic mechanisms, as demonstrated in preclinical studies in a lupus-prone mouse model (34, 36). BLM induces the mobilization of memory B lymphocytes from tissues despite an overall reduction in peripheral B cells (37). This phenomenon may lead tissue-resident B cells to be more susceptible to depletion by RTX. Moreover, inhibition of high serum BLyS levels could have favorable quantitative and qualitative effects on the reconstitution of B cells after BCD (32). Recently, a randomized, double-blind, placebo-controlled, 104-weeks superiority study (BLISS-BELIEVE) was started, whose objective is to evaluate the efficacy, safety, and tolerability of a combination therapy with subcutaneous BLM and a single cycle of RTX (1,000 mg at weeks 4 and 6 from the beginning of BLM) compared with BLM alone in adult SLE patients (38). In a phase 2, open-label, single-arm proof-of-concept study conducted by Kraaij et al. (39), SLE patients with severe and refractory disease were treated with a combination of RTX (1000 mg at weeks 0 and 2) and BLM (10 mg/kg BLM at weeks 4, 6, 8, and then every 4 weeks) where an increase of BlyS levels upon RTX-mediated BCD was observed, repealed by subsequent BLM treatment, leading to ANA reduction and to regression of excessive NET formation (neutrophil extracellular traps, web-like structures composed of chromatin backbones and granular molecules, released by activated neutrophils through a process called "NETosis"), with a reduction of proteinuria, SLEDAI, and steroid doses.

In conclusion, our clinical case suggests that the RTX and BLM combination therapeutic scheme appears to be safe and successful in achieving a clinically significant response, thus representing a valid option for the treatment of severe SLE manifestations, including LN resistant to conventional therapy and RTX. Moreover, to our knowledge, this is the first case of BP described in the literature successfully treated with BLM.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

Written informed consent was obtained from the patient for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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REVIEW
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JAK Inhibitors and Modulation of B Cell Immune Responses in Rheumatoid Arthritis

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Rheumatoid arthritis (RA) is a chronic, systemic immune-mediated inflammatory disease that can lead to joint destruction, functional disability and substantial comorbidity due to the involvement of multiple organs and systems. B cells have several important roles in RA pathogenesis, namely through autoantibody production, antigen presentation, T cell activation, cytokine release and ectopic lymphoid neogenesis. The success of B cell depletion therapy with rituximab, a monoclonal antibody directed against CD20 expressed by B cells, has further supported B cell intervention in RA development. Despite the efficacy of synthetic and biologic disease modifying anti-rheumatic drugs (DMARDs) in the treatment of RA, few patients reach sustained remission and refractory disease is a concern that needs critical evaluation and close monitoring. Janus kinase (JAK) inhibitors or JAKi are a new class of oral medications recently approved for the treatment of RA. JAK inhibitors suppress the activity of one or more of the JAK family of tyrosine kinases, thus interfering with the JAK-Signal Transducer and Activator of Transcription (STAT) signaling pathway. To date, there are five JAK inhibitors (tofacitinib, baricitinib, upadacitinib, peficitinib and filgotinib) approved in the USA, Europe and/or Japan for RA treatment. Evidence from the literature indicates that JAK inhibitors interfere with B cell functions. In this review, the main results obtained in clinical trials, pharmacokinetic, *in vitro* and *in vivo* studies concerning the effects of JAK inhibitors on B cell immune responses in RA are summarized.

Keywords: JAK-STAT pathway, JAK inhibitors, B cells, cytokines, rheumatoid arthritis

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INTRODUCTION

The success of B cell depletion therapy with rituximab in autoimmune diseases such as rheumatoid arthritis (RA) has reinforced the important role that B cells have in the development of these conditions (1, 2). Indeed, B cells can be responsible for autoantibody production, antigen presentation and T cell activation and/ or cytokine and chemokine release that contribute to disease pathogenesis (3). RA is a chronic, systemic immune-mediated disease that mainly affects the small joints of hands and wrists and, though often ameliorated by treatment, can lead to bone and cartilage destruction (4, 5). Treatment options in RA include non-steroid anti-inflammatory drugs (NSAIDs), corticosteroids, synthetic and/or biologic disease modifying anti-rheumatic drugs (DMARDs). Nevertheless, despite the progresses achieved in the last decades in RA pharmacotherapy, few patients reach sustained remission and refractory disease remains

a significant challenge (6–8). Janus kinase (JAK) inhibitors or JAKi are recently approved oral medications with therapeutic application in myeloproliferative disorders and inflammatory diseases such as RA. JAKi function by inhibiting the activity of one or more of the JAK family of enzymes [JAK1, JAK2, JAK3, and tyrosine kinase 2 (TYK2)], thus interfering with the JAK-Signal Transducer and Activator of Transcription (STAT) signaling pathway (9, 10). There are currently five JAK inhibitors (tofacitinib, baricitinib, upadacitinib, peficitinib, and filgotinib) approved in the USA, Europe and/ or Japan for RA treatment. Furthermore, an additional JAKi (decernotinib) is under investigation for RA treatment in clinical trials (11, 12). Although the number of studies exploring the effect of JAK inhibitors on B cells in the context of RA is limited, evidence from the literature indicates that JAKi also interfere with B cell functions. In this review, we summarize the main results obtained so far in clinical trials, pharmacokinetic, *in vitro* and *in vivo* studies concerning the effects of JAK inhibitors on B cell immune responses in RA.

B CELLS AND RHEUMATOID ARTHRITIS

B cells play several important roles in the development of RA (13). B cells produce autoantibodies, such as rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA), which form immune complexes that deposit in the joints and contribute to the inflammatory process through complement and cellular activation. Furthermore, B cells act as efficient antigen presenting cells (APC) that activate T cells through the expression of costimulatory molecules. B cells also secrete cytokines and/ or chemokines that promote leukocyte infiltration in the joints and the development of ectopic lymphoid structures, thus aggravating angiogenesis, pannus formation and synovial hyperplasia. In addition, the therapeutic efficacy of rituximab, an anti-CD20 monoclonal antibody that specifically depletes B cells, in RA patients has unequivocally supported B cell targeted therapies in RA pathogenesis (1, 2, 14). Of note, previous studies by our group have demonstrated that untreated very early RA patients (with <6 weeks of disease duration) have alterations in circulating memory B cell subpopulations (15); a cytokine profile that supports an early B cell activation (16, 17); and changes in B cell gene expression levels relevant for B cell maturation and differentiation (18). These data reinforce an active role of B cells in RA pathogenesis from early disease onset. Moreover, we have recently shown that in RA, treatment with tumor necrosis factor (TNF)-inhibitors and the interleukin (IL)-6 receptor (IL-6R) antagonist tocilizumab affect B cell phenotype and IgD-CD27- memory B cells in peripheral blood (19). Importantly, clinical relapse observed in B cell depleted RA patients has been associated with B cell repopulation (20–22). In fact, the results observed in RA patients following B cell depletion therapy with rituximab suggest that alterations in the expression of B cell activating factor (BAFF)-binding receptors and an increase in class-switch recombination process, particularly in memory B cell subsets, might be associated with the re-establishment of active disease (23). Interestingly, it has also been recently demonstrated for the first time that the autoantibodies commonly found in RA patients, RF and ACPA, express the inherently autoreactive 9G4 idiotope, thus supporting an activation of autoreactive 9G4+ B cells in RA (24). Additionally, it has been recently suggested that the pattern of B cell distribution in synovial tissue from untreated early RA patients can be associated to a specific pathotype classification with cellular and molecular synovial signatures that might help to predict disease severity, radiographic progression and therapeutic response (25, 26).

CYTOKINES AS KEY PLAYERS IN RHEUMATOID ARTHRITIS PATHOGENESIS

Cytokines are a large family of secreted proteins that play important roles in the immune system, namely in cell differentiation, maturation and signaling. Cytokines can be produced by several types of immune cells, including macrophages, B cells, T cells and mast cells, as well as endothelial cells, fibroblasts and various stromal cells. Of note, cytokines can be major drivers of autoimmunity and inflammation. In RA, several cellular interactions and complex cytokine networks occur that contribute to disease pathogenesis (13). In fact, it has been demonstrated that cytokines including IL-1 beta (IL-1β), IL-2, IL-3, IL-6, IL-7, IL-8, IL-12, IL-15, IL-17, IL-18, IL-19, IL-20, IL-21, IL-23, IL-32, IL-33, IL-35, TNF, interferon-alpha/gamma (IFN-α/γ) and granulocyte-macrophage colony-stimulating factor (GM-CSF) have important roles in RA physiopathology as they contribute to the induction and maintenance of inflammation (13, 27–30). The inflammatory process that develops in RA leads to a cellular infiltration of the synovial membrane, angiogenesis, pannus formation, swelling, and pain. The interactions between B and T cells result in the activation and differentiation of plasma cells, which are responsible for the production of autoantibodies (RF, ACPA). These autoantibodies form immune complexes that can activate complement and stimulate cells such as monocytes by binding to their Fc-gamma receptors (FcγR), triggering cytokine and/ or chemokine release that cause inflammation. Indeed, activated monocytes, neutrophils, and fibroblasts can release high levels of cytokines such as IL-1, IL-6, and TNF, that further activate not only B and T cells, but also chondrocytes and osteoclasts, thus contributing to cartilage and bone destruction (13). Furthermore, cytokines directly related with B cell activation and survival such as A proliferation-inducing ligand (APRIL) and BAFF (31–35), which can be produced by activated monocytes and neutrophils, have been shown to contribute to RA development from an early phase in disease onset (17). Moreover, increased serum levels of BAFF have been suggested to have an important role in B cell triggering during clinical relapse after B cell depletion therapy (23). Previous studies developed by our group have demonstrated that untreated very early RA (VERA) patients (with <6 weeks of disease duration) have a cytokine pattern in circulation that supports an early activation of not only B cells, but also neutrophils and Th17 cells (16, 17) (**Figure 1**). Indeed, we have found that VERA patients have higher serum

levels of APRIL and BAFF when compared to other very early arthritis (non-RA) patients, established RA and healthy controls (17). We also observed that established RA patients have significantly increased synovial fluid levels of APRIL, BAFF and IL-21, a cytokine important for plasma cell differentiation (17) (**Figure 1A**). Additionally, we found that VERA patients have increased serum levels of cytokines that promote neutrophil recruitment and activation (IL-8), Th17 cells polarization (IL-1β and IL-6) and Th17 cells-derived cytokines (IL-17A and IL-22) (16) (**Figure 1B**). Also, the elevated IL-1β, IL-6, IL-8, and IL-17A levels observed in the synovial fluid of established RA patients support a local role for these cytokines in synovial inflammation and bone erosion (16) (**Figures 1B,C**). In fact, IL-17 has been shown to induce osteoclastogenesis, thus contributing for bone resorption (36, 37). Moreover, IL-6 can support the activation and recruitment of autoreactive B cells toward RA synovium (38, 39), leading to an exacerbation of inflammation through autoantibody production and immune complex deposition (40, 41) (**Figure 1C**). Of note, treatment of VERA patients with corticosteroids and methotrexate (MTX), although effective in clinical improvement had no impact on the cytokine pattern in circulation (16, 17). Importantly, the success of biological therapies that directly target key cytokines such as TNF inhibitors (adalimumab, infliximab, etanercept, golimumab and certolizumab); tocilizumab (an IL-6R antagonist) and anakinra (an IL-1R antagonist) in RA further reinforce the relevance of these small proteins in disease development (42–46).

JAK-STAT SIGNALING PATHWAY IN HEALTH AND DISEASE

Cytokines act by binding to cell surface receptors and subsequently activate intracellular signaling cascades, such as the JAK-STAT signaling pathway. JAK-STAT signaling pathway is an evolutionarily conserved pathway that regulates many cellular processes including innate and adaptive immune responses, cell proliferation, differentiation and apoptosis. Activation of this pathway is initiated by binding of a ligand (such as interleukins, interferons, hormones and growth factors) to specific transmembrane receptors (cytokine receptors, G protein-coupled receptors, receptor tyrosine kinases and homodimeric hormone receptors) and culminates in the transcription of target genes (9, 10, 47–49) (**Figure 2**). JAKs, STATs and cell-surface receptors are the main key players of this signal-transduction pathway. JAKs are a family of four members of tyrosine kinases (JAK1, JAK2, JAK3, and TYK2) that selectively associate with the intracellular domains of cell receptors (50, 51) (**Figure 3**). JAK1, JAK2, and TYK2 are ubiquitously expressed, whereas JAK3 expression is mainly restricted to hematopoietic cells (52). Binding of a ligand to a cell surface receptor triggers the receptor dimerization and induces the autophosphorylation and activation of the receptor-associated JAKs. Activated JAKs then phosphorylate critical tyrosine residues on the receptor, which leads to recruitment of specific STATs (49, 51, 53) (**Figure 2**). STATs are a family of proteins named for their dual roles of transducing signals and promoting transcription of specific genes. There are seven members of the STAT family in mammals: STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, and STAT6 (49, 54–57). After binding to the phosphorylated tyrosine residues on the receptor, STATs are phosphorylated by JAKs, which leads to their dissociation from the receptor. STATs form homo- or heterodimers and translocate into the cell nucleus via importins, where they bind to specific DNA regions and activate the transcription of target genes (**Figure 2**). STATs can be dephosphorylated by nuclear protein tyrosine phosphatases (N-PTPs), which leads to the inactivation of STATs. The unphosphorylated STATs associate with exportins to exit the nucleus and return to the cytoplasm where they can be reactivated for further rounds of gene transcription (10, 47, 49, 56). Overall, signaling via the JAK–STAT signaling pathway is a dynamic process that involves the rapid transmission of signal from the cell membrane to the nucleus followed by a highly organized response and subsequent controlled downregulation and attenuation of the initial signal (47–49, 54). Thus, negative regulators of the JAK-STAT signaling pathway also play an essential role. These include protein tyrosine phosphatases (PTPs), which remove phosphate groups from receptors, JAKs and STATs (58); protein inhibitor of activated STAT (PIAS), that prevent the DNA-binding activity of STATs (59, 60); and suppressor of cytokine signaling proteins (SOCS), which form a classical negative feedback loop that switches off the activity of JAKs (61, 62) (**Figure 2**). Disturbances in JAK-STAT signaling pathway, mostly associated with mutations (gain or loss of function) and polymorphisms in JAK and/ or STAT genes (9, 63), have been implicated in the pathogenesis of several diseases including inflammatory skin conditions (psoriasis, atopic dermatitis, alopecia areata, vitiligo) (64–71); cancers (myeloproliferative neoplasms, leukemia) (72, 73); immunodeficiencies (severe combined immune deficiency) (74); and autoimmune disorders such as RA (75–79); psoriatic arthritis (80, 81); systemic lupus erythematosus (82, 83); ankylosing spondylitis (84, 85); systemic sclerosis (86, 87); giant cell arteritis (88); sarcoidosis (89–91) and inflammatory bowel diseases (ulcerative colitis, Crohn's disease) (92, 93). Therefore, targeting JAKs and/ or STATs can be a safe and efficacious strategy for treating these diseases (94).

JAK INHIBITORS AS NEW TREATMENT OPTIONS IN RHEUMATOID ARTHRITIS

JAK-STAT signaling pathway has a critical role in the signal transduction of many pivotal cytokines involved in RA pathogenesis (12, 95, 96) as well as other inflammatory disorders (97). Due to their central role in the immune responses and their association with several cytokine receptors (**Figure 3**), the inhibition of JAKs appeared to be a promising therapeutic strategy in autoimmune diseases (94). JAK inhibitors (JAKi) represent a new class of oral drugs developed in the last decade that directly suppress the enzymatic activity of JAK family members, blocking JAK-STAT signaling pathway (12, 96). Despite the efficacy of biological DMARD treatments that target individual cytokines, biologics are large proteins that may

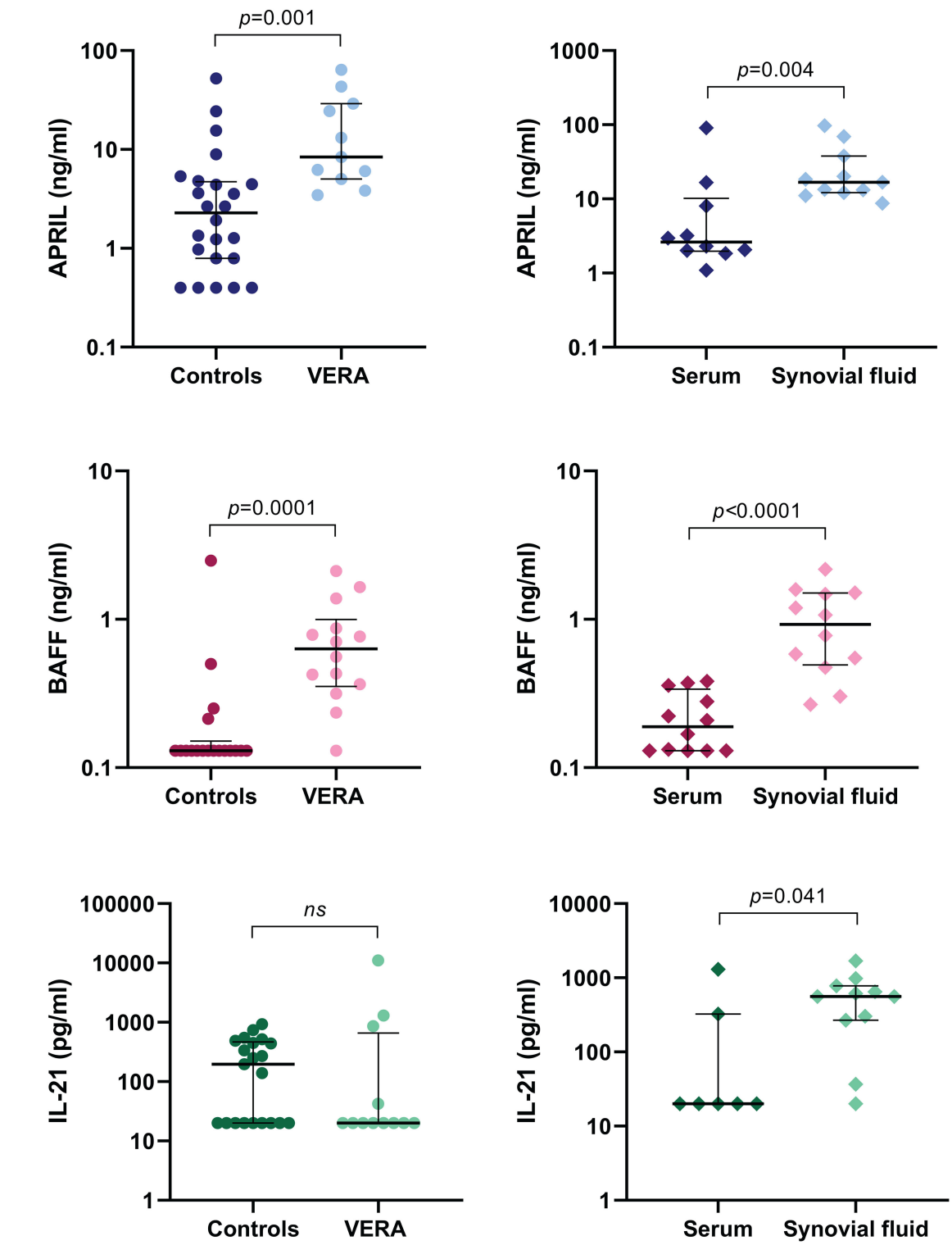
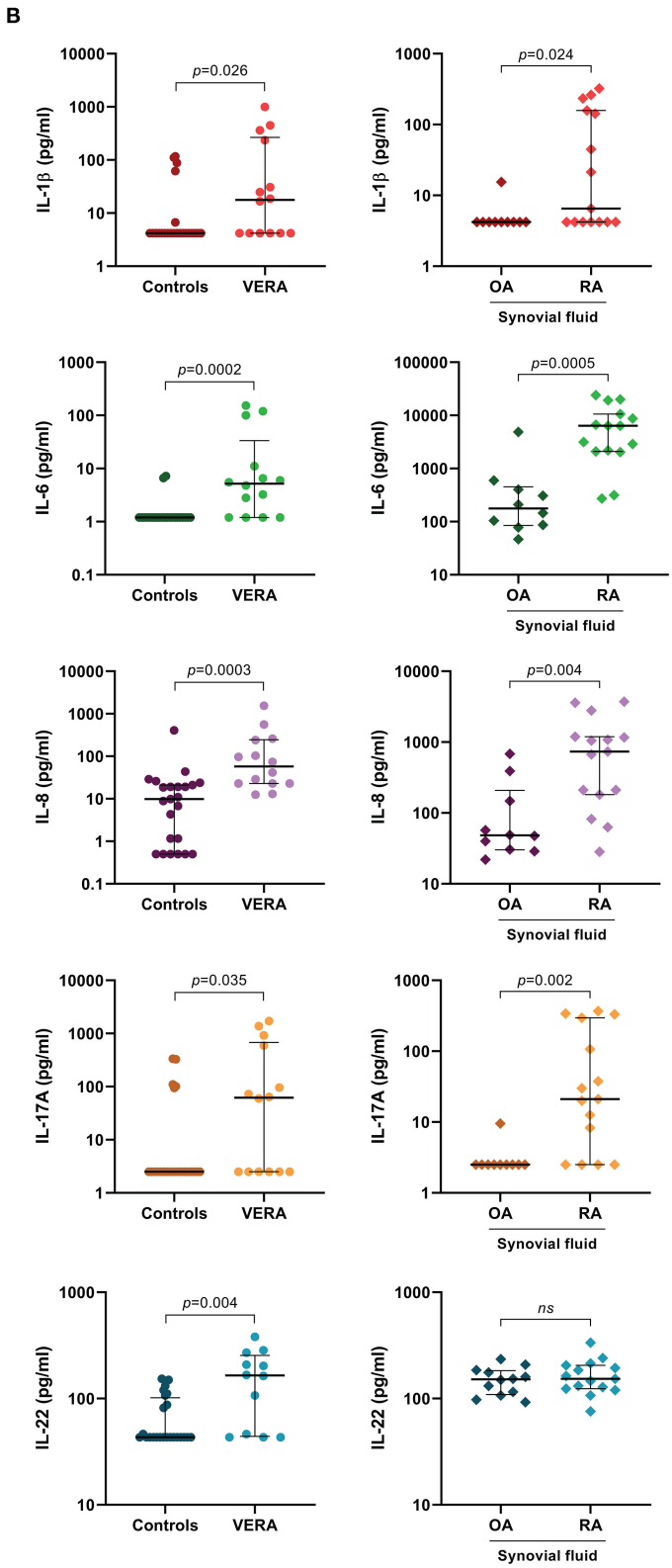


FIGURE 1 | *(Continued)*



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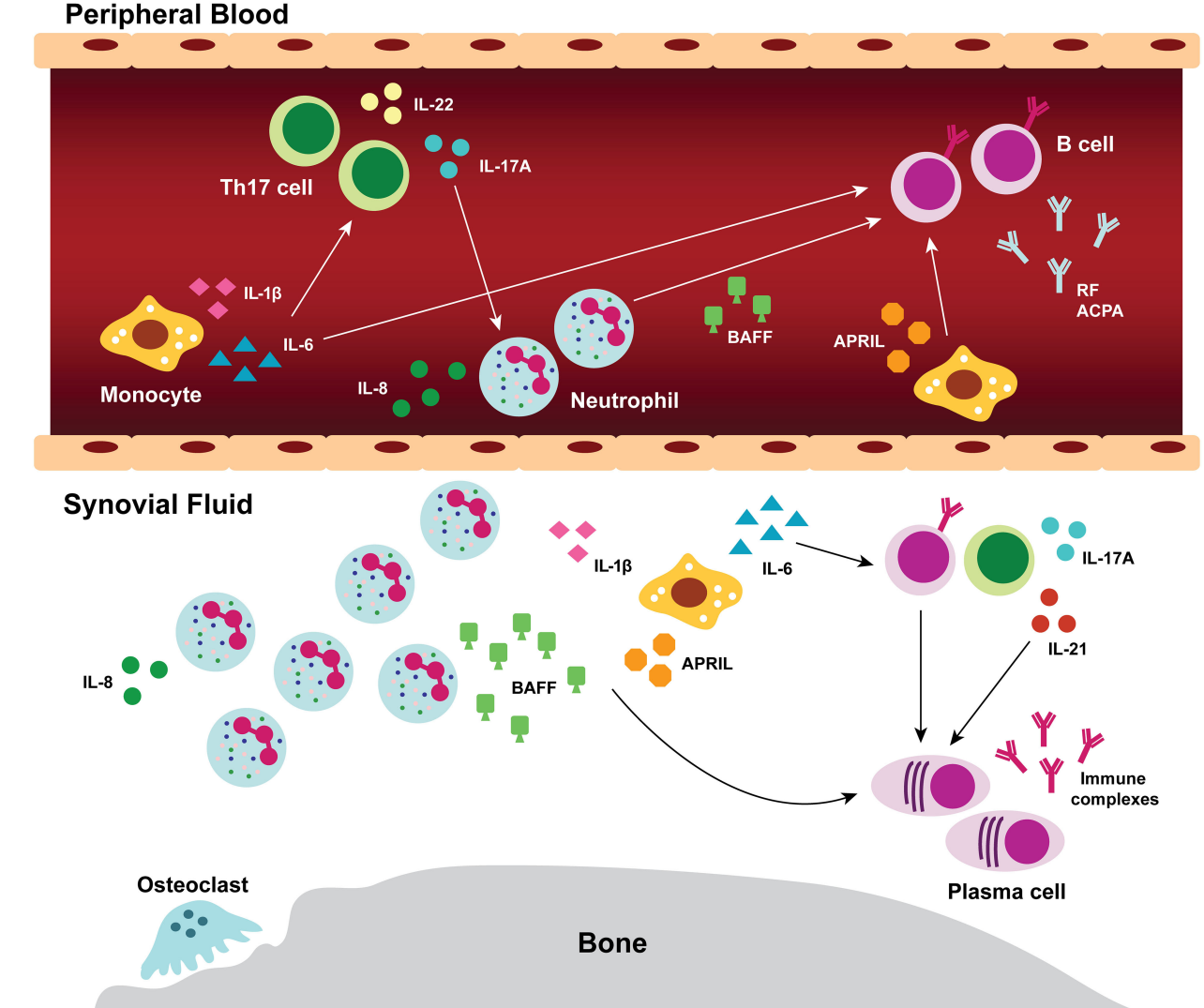

FIGURE 1 | Cytokine profile present in peripheral blood from very early rheumatoid arthritis (VERA) patients and synovial fluid from established RA. A group of cytokines directly related with B cell activation, differentiation and survival was quantified in serum samples from untreated very early rheumatoid arthritis (VERA) patients with <6 weeks of disease duration when compared to healthy controls **(A)**. In addition, serum and synovial fluid samples from established treated RA patients were also analyzed for comparison **(A)**. Cytokines related with neutrophil and Th17 cells activation were also quantified in serum samples from VERA patients and healthy individuals **(B)**. Furthermore, synovial fluid from established treated RA and osteoarthritis (OA) patients was analyzed for comparison **(B)**. Statistical analysis of data was performed with GraphPad Prism (GraphPad Software, San Diego, CA, USA). Lines in graphs represent median values with interquartile range. Non-parametric Mann-Whitney test was used for comparisons between two independent groups. Differences were considered statistically significant for $p < 0.05$. Data represented in Figures 1A,B were adapted from previous published studies by our group (16–18), according to the terms of the Creative Commons license (http://creativecommons.org/licenses/by/4.0/). Figure 1C is an illustration representative of the cytokine profile present in peripheral blood from VERA patients and synovial fluid from established RA supported by previous published studies by our group (16–18). To sum up, RA patients have a cytokine profile in peripheral blood that favors B cells, neutrophils and Th17 cells activation since the first weeks of disease development. In a chronic phase of the disease, the cytokine pattern present locally in the joints supports the intervention of activated monocytes, neutrophils, T and B cells and plasma cell differentiation **(C)**. ACPA, anti-citrullinated protein antibodies; APRIL, a proliferation-inducing ligand; BAFF, B cell activating factor; IL, interleukin; *ns,* non-significant; OA, osteoarthritis; RA, rheumatoid arthritis; RF, rheumatoid factor; Th17, T helper 17; VERA, very early rheumatoid arthritis.

cause immunogenicity and require either intravenous infusion or subcutaneous injection for dosing (98). In contrast, JAK inhibitors are small molecules, orally administered, that can simultaneously suppress the action of multiple cytokines. To date, five JAK inhibitors (tofacitinib, baricitinib, upadacitinib, peficitinib, and filgotinib) have been approved for the treatment of RA.

Tofacitinib

Tofacitinib is an oral JAK inhibitor with selectivity for JAK1 and JAK3 and, to a lesser extent, JAK2 and TYK2. Tofacitinib was the first JAK inhibitor approved by the United States (US) Food and Drug Administration (FDA) (November 2012) and European Medicines Agency (EMA) (March 2017) for the treatment of moderate to severe active RA patients who

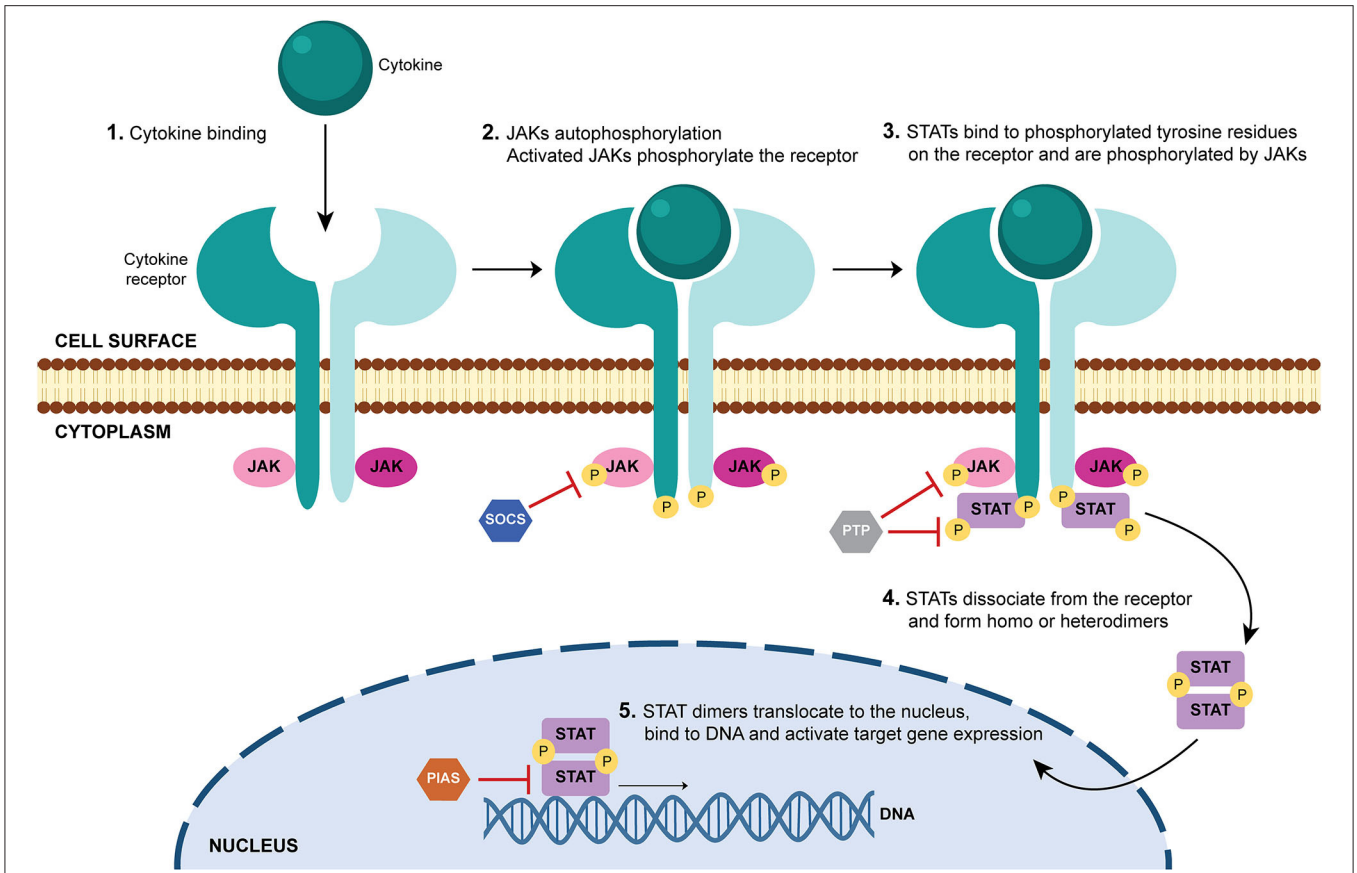

FIGURE 2 | JAK-STAT signaling pathway. When a ligand (usually a cytokine) binds to its receptor in a cell, it triggers the autophosphorylation of the receptor-associated Janus kinases (JAKs). Activated JAKs phosphorylate the intracellular tail of the receptor on critical tyrosine residues, which leads to the recruitment and binding of signal transducer and activator of transcription (STAT) proteins. STATs are phosphorylated by JAKs, which induces their dissociation from the receptor. STATs form homo- or heterodimers and translocate into the cell nucleus, where they bind to specific DNA regions and activate target gene expression. Negative regulators of the JAK-STAT signaling pathway include protein tyrosine phosphatases (PTPs), which remove phosphate groups from receptors, JAKs and STATs; protein inhibitor of activated STAT (PIAS), that prevent the DNA-binding activity of STATs; and suppressor of cytokine signaling proteins (SOCS), which inhibit the activity of JAKs. DNA, deoxyribonucleic acid; JAK, Janus kinase; P, phosphate; PIAS, protein inhibitor of activated STAT; PTP, protein tyrosine phosphatase; SOCS, suppressor of cytokine signaling proteins; STAT, signal transducer and activator of transcription.

had had an inadequate response or intolerance to MTX (76, 78, 99–112). Data from human clinical trial studies have demonstrated the effectiveness of the use of tofacitinib in RA patients not only as a monotherapy (at a dosage of 5 mg twice daily), but also in combination with MTX and the clinical responses have proven to be at least similar to TNF antagonists (78, 103, 105, 107, 109, 112–114). Indeed, tofacitinib has demonstrated efficacy in active RA patients by significantly improving disease activity, physical functioning, health-related quality of life as well as preventing bone erosions and structural joint damage (99, 103, 114–117). Furthermore, safety reports indicate that tofacitinib is generally well-tolerated, has a consistent safety profile (as monotherapy or combination therapy) and sustained efficacy in RA patients. However, adverse events have been described in RA patients after tofacitinib treatment with mild to moderate severity that included nausea, anemia, lymphopenia, neutropenia, lipid profile changes, increase in liver enzymes, cardiovascular events, lower respiratory tract infections, herpes zoster virus (HZV) reactivation, venous thromboembolism, and development of malignancies (76, 78, 109, 112, 114, 118–125). Nevertheless, the overall risk of infection (including serious infection) and mortality rates in RA patients treated with tofacitinib is similar to those observed in RA patients treated with biologic agents (12, 120).

Baricitinib

Baricitinib was the second JAK inhibitor approved for clinical use in RA (in February 2017 by the EMA and in June 2018 by the FDA). Baricitinib is an oral JAK1/JAK2 inhibitor, with moderate activity against TYK2 and significantly less activity against JAK3. Approved dosages (2 and 4 mg once daily) are administered to moderate to severe active RA in adult patients who are intolerant or unresponsive to one or more DMARDs (75, 126–132). Treatment of RA patients with baricitinib monotherapy, or when baricitinib was combined

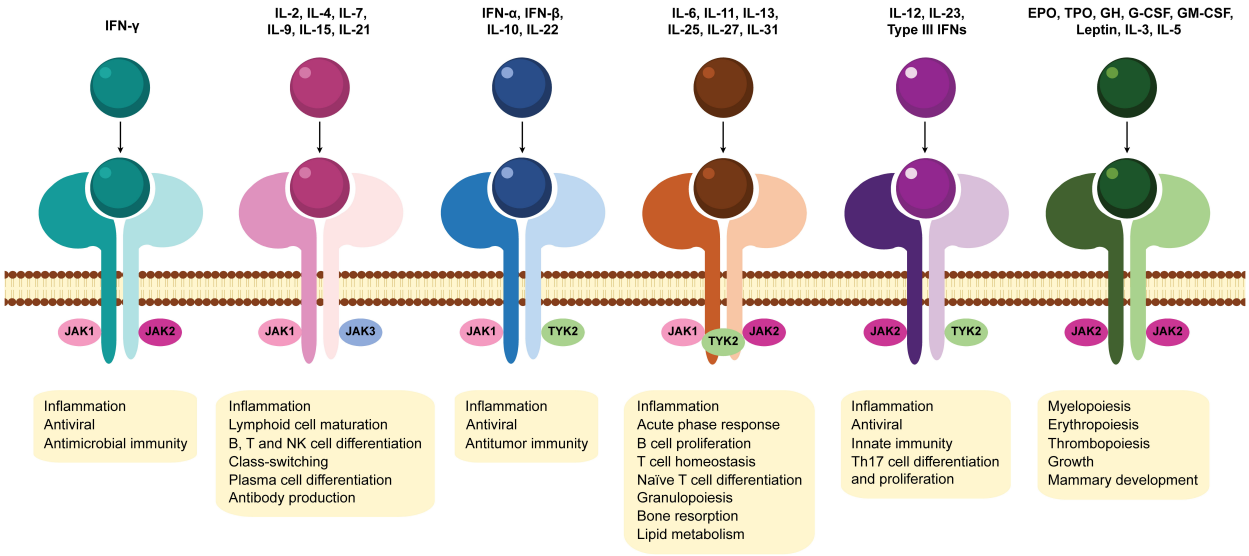

FIGURE 3 | Association of Janus kinases with cytokine receptors and downstream effects of JAK-STAT signaling pathway activation. Janus kinase (JAK) family members include JAK1, JAK2, JAK3, and tyrosine kinase 2 (TYK2). Different JAK combinations with their subsequent downstream effects, each mediated by a specific subset of cytokines are represented. EPO, erythropoietin; GH, growth hormone; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; JAK, Janus kinase; NK, natural killer; Th17, T helper 17; TPO, thrombopoietin; TYK2, tyrosine kinase 2.

with conventional synthetic DMARDs (csDMARDs) such as MTX showed efficacy and had an acceptable safety profile in early active naïve csDMARD-treated RA patients who had exhibited an inadequate response to conventional synthetic or biologic DMARDs (126, 129, 131, 132). Moreover, it has been demonstrated that baricitinib had a similar or improved efficacy when compared to TNF antagonists such as adalimumab (129, 131–134). Of note, treatment of RA patients with baricitinib was associated not only with clinical improvement, but also with inhibition of radiographic joint damage (135, 136). Overall, baricitinib is considered a safe and effective treatment in RA, although some adverse events have been described similarly to what has been observed in tofacitinib treated RA patients (132, 137–139).

Upadacitinib

Upadacitinib is a JAK1-selective inhibitor approved by the FDA (in August 2019) and EMA (in December 2019) for the treatment of RA. Upadacitinib is indicated for the treatment of adults with moderately to severely active RA who fail to adequately respond to, or are intolerant to one or more DMARDs (77, 140–146). Upadacitinib may be used as monotherapy (15 mg or 30 mg once daily) or in combination with MTX as an effective treatment for active RA patients with an inadequate response to conventional or biological DMARDs, with an acceptable safety profile (77, 143–147). Furthermore, it has been demonstrated that upadacitinib was more effective than adalimumab treatment in ameliorating disease activity in RA patients who were concomitantly receiving MTX and significantly prevented radiographic progression (148). In addition, despite being a selective JAK1 inhibitor, upadacitinib has a similar safety profile to less-selective JAKi (139, 143, 146, 147, 149). Nevertheless, longer-term safety data are necessary.

Peficitinib

Peficitinib is a pan-JAK inhibitor with a moderate selectivity for JAK3. It was approved for the treatment of RA in Japan in 2019 and Korea in 2020; and is currently being evaluated by the US FDA to treat adult patients with moderately to severely active RA who show inadequate response to or are intolerant of MTX (150–158). Peficitinib has been tested in RA either as monotherapy (150) or in combination with MTX (151) or csDMARDs (152) and it has been shown to significantly improve disease severity in RA patients who have an inadequate response to conventional therapies. Of note, it has been demonstrated that Peficitinib 50, 100, and 150 mg dosages administered once daily were effective in treating active RA patients, without a significant risk for adverse events (159). Overall, peficitinib has an acceptable safety and tolerability profile with similarly described adverse events as the ones reported with other JAK inhibitors (139, 153–155, 158, 160–162).

Filgotinib

Filgotinib is a JAK1-selective inhibitor recently approved by EMA and in Japan (in September 2020) for the treatment of RA (163–170). Filgotinib is indicated for the treatment of moderate to severe active RA in adults who have responded inadequately to, or who are intolerant to one or more DMARDs. Filgotinib may be used as monotherapy (100 mg or 200 mg once daily) or in combination with MTX (168–170). Of note, similarly to

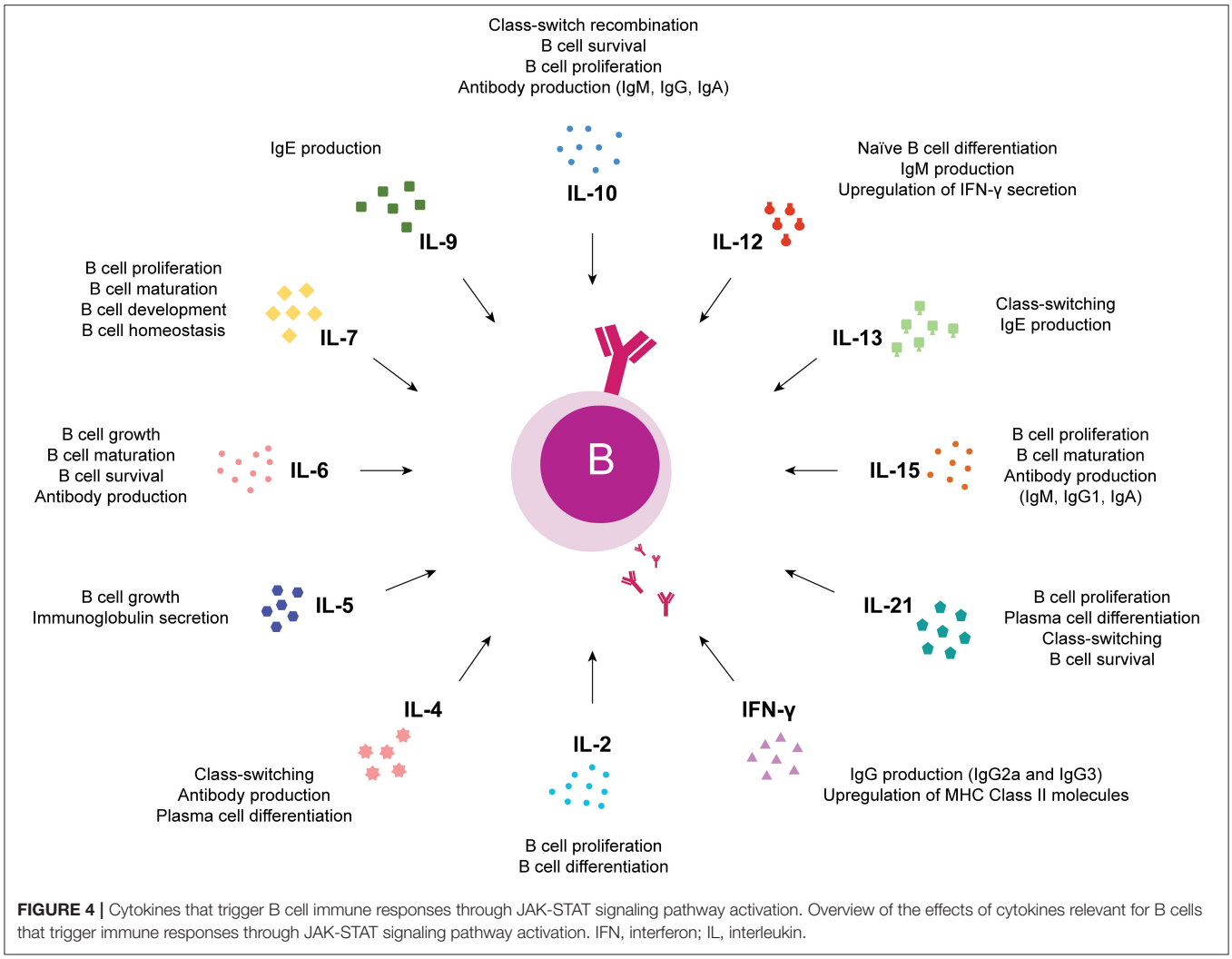

FIGURE 4 | Cytokines that trigger B cell immune responses through JAK-STAT signaling pathway activation. Overview of the effects of cytokines relevant for B cells that trigger immune responses through JAK-STAT signaling pathway activation. IFN, interferon; IL, interleukin.

upadacitinib, another selective JAK1 inhibitor, it has been demonstrated that the risks of serious adverse events did not differ between filgotinib and less-selective JAKi such as tofacitinib (168–171).

In addition to these compounds, another JAK inhibitor, decernotinib, an oral JAK3-inhibitor in Phase IIb studies (172–175), is currently under investigation for the treatment of RA. Overall, results from clinical trials with JAK inhibitors in RA are encouraging (12, 125). JAKi have shown a rapid onset of action and, in case of an adverse event, their short half-life supports a rapid reversal of immunosuppressive effects (176–178). Of note, JAK inhibitors proved efficacious when administered as monotherapy and have demonstrated a comparable or superior efficacy and safety profile to those of biologic agents (179, 180). Importantly, due to the evidence of superiority or non-inferiority of JAK inhibitors when compared to adalimumab emerging from randomized clinical trials (114, 134, 181), the 2020 updated EULAR therapeutic guidelines have recommended the use of JAK inhibitors as an alternative to biologics in RA patients refractory to cDMARDs and having poor prognostic factors, as well as in those failing a previous synthetic or biologic DMARD (182).

EFFECT OF JAK INHIBITORS ON B CELLS: EVIDENCE FROM THE LITERATURE

Studies of the effects of JAK inhibitors on circulating immune cells that play important roles in the pathogenesis of autoimmune diseases may provide insights into immunologic mechanisms associated with clinical outcomes. Due to differences in JAK targeting, JAK inhibitors may also exert distinct immunologic effects. While JAK1, JAK2, and TYK2 are ubiquitously expressed, JAK3 expression is predominantly restricted to hematopoietic cells (50, 183–186), having important roles in immune function and lymphocyte development as described in both humans (74, 187) and mice (188, 189) with JAK3 deficiencies. JAK3 mediates signaling through cytokine receptors that contain the common gamma chain (γc) or IL-2R subunit gamma (IL-2RG) including IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 receptors (51). Also, it has

TABLE 1 | Overview of the impact of JAK inhibitors on B cell immune responses based on pharmacokinetic, *in vitro* and *in vivo* studies.

Description	JAK inhibitor	References
Increase in B cell numbers in peripheral blood	Tofacitinib, Baricitinib, Filgotinib	(163, 164, 204, 205, 214, 226)
Suppression of B cell activation, differentiation and class-switching	Tofacitinib, Baricitinib, Filgotinib	(202, 207, 215, 216, 227)
Impairment of plasmablast development and immunoglobulin secretion	Tofacitinib, Baricitinib	(208, 215, 216)
Inhibition of antibody production	Tofacitinib, Filgotinib	(200, 208–210, 227)
Inhibition of cytokine production relevant for B cell activation and survival	Tofacitinib, Baricitinib, Upadacitinib, Filgotinib	(215, 216, 218, 220, 227)
Downregulation of the antigen presenting cell function of B cells	Baricitinib	(217)
Reduction of T helper cell responses	Baricitinib	(215, 217)
Inhibition of STAT phosphorylation on B cells	Tofacitinib, Baricitinib, Upadacitinib, Filgotinib	(219)
Downregulation of B-cell chemoattractant, activation, survival and differentiation biomarkers	Filgotinib	(226)
Decrease in B cell lymphoid infiltrates in tissues	Filgotinib	(227)

been shown that JAK3 is constitutively associated with CD40, an important B cell co-receptor whose signaling has a wide range of effects on B cells, including cell growth, survival, differentiation, isotype switching, rescue from apoptosis and up-regulation of expression of B7 (CD80), Fas, ICAM-1, CD23 and lymphotoxin (LT)-α (190, 191). In fact, JAK3 activating mutations are found in human hematological malignancies including B-cell lymphomas (192–194). Furthermore, observations in JAK3 knockout mice confirmed JAK3 essential role in B cell division, immunoglobulin gene rearrangement, differentiation and survival (195). Taken together, these data support that the regulation of JAK3 expression and activity is important in B cell development and function (196). Therefore, the use of JAK3 inhibitors such as tofacitinib in autoimmune diseases such as RA might have important consequences in B cell activation and function. Previous studies have shown that the primary targets of tofacitinib during pathological processes in RA are dendritic cells, CD4+ T cells such as Th1 and Th17 and activated B cells, leading to multi-cytokine targeting, decreased synovial inflammation and structural joint damage (117, 197–202). Changes in lymphocyte subsets have been documented with tofacitinib treatment (116, 176, 200, 203, 204). Indeed, phase II and phase III clinical trials involving patients with RA treated with tofacitinib showed a transient increase in total lymphocytes early in treatment, with a gradual decrease over time (204–206). In phase II RA clinical trials, variable changes in T cells were observed with short-term tofacitinib treatment, while B cells and natural killer (NK) cells increased and decreased from baseline, respectively (204, 205). Importantly, no strong association between CD4+ T cell, CD8+ T cell, B cell, or NK cell counts and serious infection incidence rates was observed (204). Although the number of studies exploring the effect of tofacitinib on B cells in the context of RA is limited, results so far indicate that tofacitinib interferes with B cell functions. In fact, it has been suggested that tofacitinib suppresses B cell activation, differentiation and class-switching, but maintains B cell regulatory function (202, 207). Moreover, tofacitinib reduces IgG and RF circulating levels in RA patients, which correlates with disease activity amelioration (200). Additionally, it was shown that tofacitinib severely impaired *in vitro* plasmablast development, immunoglobulin secretion and induction of B-cell fate determining transcription factors from naïve B cells isolated from umbilical cord blood (208). Similar, but less pronounced results were obtained with peripheral blood B cells isolated from healthy blood donors. Indeed, *in vitro* treatment of total peripheral blood B cells with tofacitinib resulted in reduced but not abolished plasmablast development, as well as reduced antibody secretion (208). Furthermore, recent studies developed in murine models of lupus have demonstrated that although tofacitinib treatment did not change B cell numbers, a significant reduction in anti-double stranded DNA (anti-dsDNA) and antinuclear antibodies (ANA) was observed in serum (209, 210). These observations pointed to the potential inability of tofacitinib-treated patients to respond to novel antigens, suggesting that vaccination against new antigens prior to tofacitinib treatment should be considered (208, 211–213). Moreover, *in vitro* activation of B cells isolated from tofacitinib treated polyarthritis patients has revealed that, in the absence of tofacitinib, B cells can be activated again and display a normal or enhanced differentiation (208). This indicates that the inhibitory effect of tofacitinib is terminated as soon as the drug is removed (176, 201, 208). Besides tofacitinib, other JAK inhibitors have been approved or are currently being tested in clinical trials as new potential treatment options for RA and/ or other autoimmune diseases and chronic inflammatory conditions. Thus, new studies concerning the effects of JAK inhibitors on innate and adaptive immune system responses are still emerging. In fact, the diversity of cytokines that trigger B cell immune responses through JAK-STAT signaling pathway activation (**Figure 4**) suggests that other JAK inhibitors, besides JAK3 inhibitors, might have important roles in B cell immunity (**Figure 3**). Changes in lymphocyte numbers (B, T, and NK cells) and subpopulations have been recently demonstrated in active RA patients after treatment with baricitinib (214). An integrated data analysis has been performed based on results from three completed phase III trials comparing placebo with baricitinib treatment (RA-BEAM, RA-BUILD, and RA-BEACON) and one ongoing long-term extension study (RA-BEYOND) in

patients with active RA. Overall, a transient increase in total lymphocyte count was observed in RA patients after 4 weeks of treatment with baricitinib, returning to baseline values by week 12. Moreover, transient changes in T cells and subsets (CD3+, CD4+, CD8+, Th1, Th17, and regulatory T cells) were observed with baricitinib treatment, with cell counts remaining largely within normal reference ranges (214). Additionally, it was shown that CD19+ B cells and B cell subpopulations (including switched memory, non-switched memory, mature naïve, and immature transitional B cells) increased after 4 weeks of baricitinib treatment and remained above baseline or stabilized over time (214). Importantly, baricitinib treatment did not result in increased autoantibody (RF and ACPA) titers, suggesting that the increase in total B cell counts is unlikely to reflect a major expansion of RA antigen-specific B cells (214). Nevertheless, it is possible that some of the class-switched memory B cells, increased by baricitinib in a dose-dependent manner, are regulatory B cells, which inhibit disease progression (214). Of note, the detected changes in lymphocyte subsets were largely consistent across the baricitinib phase III RA clinical trials, which included patients with different responsiveness to prior DMARD therapies and were not associated with increased risk of serious infections (214). Recently, the *in vitro* effects of baricitinib were evaluated on human peripheral blood cells and it was shown that baricitinib modulates both innate and adaptive immune responses similarly to tofacitinib (88, 197, 215). Baricitinib suppressed the expression of costimulatory molecules (CD80/CD86) on monocyte-derived dendritic cells and inhibited T cell proliferation and differentiation of Th1 and Th17 cells. Furthermore, baricitinib suppressed the differentiation of human B cells into plasmablasts by B cell receptor and type-I interferon (IFN) stimuli and inhibited the production of IL-6 from B cells (215). Also, it was recently shown that baricitinib decreased BAFF expression in RA synovial fibroblasts similarly to tofacitinib, thus inhibiting B cell activation locally in the joints (216). The impact of baricitinib on B cells is further supported by studies developed in a mouse model of graft-vs.-host disease (GVHD) in which it was demonstrated that baricitinib inhibited the activation of allogeneic antigen presenting cells (APCs) and prevented GVHD progression (217). It was shown that baricitinib suppressed the expression of major histocompatibility complex (MHC)-II, costimulatory molecules CD80/86 and PD-L1 on B220+ and CD11c+ APCs. Moreover, baricitinib expanded regulatory T cells and downregulated Th1 and Th2 cell responses (217). Studies developed in RA patients and animal models of arthritis treated with upadacitinib have reported decreased circulating numbers of lymphocytes, neutrophils and NK cells (141, 142, 218). Nonetheless, no significant changes were detected in RF and ACPA levels in RA patients after upadacitinib treatment (144). Furthermore, it has been recently shown that upadacitinib has a generally similar profile of *in vitro* cytokine receptor inhibition observed in human leukocyte subpopulations when compared to other JAK inhibitors (219). Particularly, it was observed that upadacitinib inhibited STAT6 phosphorylation on CD19+ B cells triggered by IL-13 stimuli similarly to tofacitinib, baricitinib and filgotinib (219). However, a recent *in vitro* pharmacology study comparing tofacitinib, baricitinib and upadacitinib has revealed that different JAK inhibitors modulate distinct cytokine pathways to varying degrees (220). Notably, it was shown that upadacitinib and tofacitinib were the most potent inhibitors of the JAK1/3-dependent cytokines tested, including IL-4, IL-6 and IL-21, relevant for B cell activation, plasma cell differentiation and humoral immune responses (218, 220). In addition, studies with peficitinib have demonstrated an inhibitory effect of this JAK inhibitor on T cell activation using either a rat adjuvant-induced arthritis model (221) or human peripheral blood mononuclear cells (86, 222). Moreover, it was shown that peficitinib suppressed *in vitro* monocyte chemotactic activity and the proliferation of fibroblast-like synoviocytes from RA patients (79, 223, 224). Interestingly, decreases in neutrophil and total lymphocyte counts were observed after peficitinib treatment, but no significant changes were detected on T cell subpopulations (152–155, 158, 222, 225). Nevertheless, studies on the potential effects of peficitinib treatment on human B cells are currently lacking. Filgotinib was recently approved by EMA for the treatment of RA and clinical trials with this JAK1-selective inhibitor are currently under investigation in other autoimmune diseases. Changes in leukocyte numbers, particularly increases in B cell frequencies, have been reported in RA patients after filgotinib treatment (163, 164, 226). Furthermore, studies exploring the action of this JAKi on B cells have demonstrated that filgotinib directly inhibits human B cell differentiation and IgG production (227). Recent reports in RA patients following treatment with filgotinib have shown significant reductions in markers important for B cell chemotaxis [chemokine (C-X-C motif) ligand 13, CXCL13]; activation and survival (BAFF); regulatory function (IL-10) and germinal center and plasma cell differentiation (IL-2, IL-5, IL-7, and IL-21) (226). Moreover, filgotinib has also been shown to suppress the production of BAFF in human primary salivary gland (SG) epithelial cells and SG organoids (227). Additionally, studies developed in a mouse model of Sjögren syndrome have shown a marked reduction in lymphocytic infiltration of salivary glands after filgotinib treatment, which contributed to disease amelioration (227). Decernotinib is another JAK inhibitor currently under evaluation for the treatment of RA (173–175, 228, 229). Although lymphopenia and neutropenia have been described in decernotinib trials (174, 175), the exact mechanisms of action and effects of this JAKi on B cell immune responses still need to be further clarified. **Table 1** summarizes the impact of currently approved JAK inhibitors on B cell immune responses described in the literature. Overall, additional pharmacological studies of JAKi exploring the effect of different cytokine pathways and/ or JAK targeting in distinct human leukocyte populations remain of clinical importance.

CONCLUSIONS

JAK inhibitors are a new class of oral immunosuppressive drugs with proved efficacy in the treatment of chronic inflammatory conditions and autoimmune diseases such as RA. B cells play several important roles in RA pathogenesis since the first weeks of disease development. Pharmacokinetic, *in vitro* and *in*

vivo studies developed so far with animal models of arthritis or other autoimmune conditions and/ or with human cells from RA patients or other chronic inflammatory disorders have demonstrated that JAK inhibitors (tofacitinib, baricitinib, upadacitinib, peficitinib, filgotinib and decernotinib) can affect B cell activation, proliferation and differentiation. Taking into consideration these B cell effects of JAKi and the relevant role of B cells since early RA onset it is likely that JAKi can have a major impact on the early phase of RA. Nevertheless, further research studies are necessary to clarify the exact mechanisms of action of JAKi on B cells and other immune cell targets not only in currently approved JAK inhibitors, but also in new JAKi under investigation.

AUTHOR CONTRIBUTIONS

RM and JF conceptualized the manuscript. RM reviewed the literature and wrote the manuscript. JF revised the manuscript and contributed with important intellectual input. All authors read and approved the final manuscript.

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Factors Determining Retreatment Time Interval of Rituximab in Korean Patients With Rheumatoid Arthritis

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Unlike other biologic agents for rheumatoid arthritis (RA) that are administered at regular intervals even without flare, rituximab can be administered according to the timing of retreatment determined by the physician. Recently, there has been a tendency to prefer on-demand administration for disease flares rather than regular retreatment. We aimed to investigate the retreatment patterns of rituximab in patients with RA and to identify factors associated with extension of the time interval between retreatment courses. This study included RA patients on rituximab treatment who were enrolled in the Korean Rheumatology Biologics registry (KOBIO) or treated at Ajou University Hospital. Previous or current concomitant conventional synthetic disease-modifying anti-rheumatic drugs (csDMARDs), corticosteroids, number of previous biologic agents, withdrawal, and time intervals of rituximab retreatment were collected. In case of treatment failure, the reasons such as lack of efficacy, adverse events, and others, were also identified. A total of 82 patients were enrolled. The mean follow-up period from the first cycle of rituximab was 46.1 months, and the mean interval between the retreatment courses was 16.3 months. The persistent rates of rituximab after 5 years was 72.4%. Concomitant use of at least two csDMARDs ($\beta = 4.672$; 95% CI: 0.089–9.255, $p = 0.046$) and concomitant use of corticosteroids ($\beta = 7.602$; 95% CI: 0.924–14.28, $p = 0.026$) were independent factors for extending the time interval between the retreatment courses. In conclusion, RA patients treated with rituximab in Korea show high persistence rates. Concomitant use of two or more csDMARDs and concomitant use of corticosteroids with rituximab are associating factors of extending the retreatment time interval. These findings should be considered when selecting rituximab as a treatment for patients with RA.

Keywords: rituximab, rheumatoid arthritis, treatment response, adverse event, safety

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disorder that primarily affects the synovial joints. It is characterised by joint pain and functional disability that lead to reduced quality of life and a high socioeconomic burden (1). As an autoimmune disease, complex interactions among B cells, T cells, macrophages, neutrophils, dendritic cells, fibroblasts, and osteoclasts play crucial roles in initiating and maintaining inflammation of the joints (2). Among these, B cells appear to contribute significantly to the development of RA by producing pathogenic autoantibodies, presenting self-antigens to T cells, and secreting inflammatory cytokines (3).

Therefore, B cells have emerged as therapeutic targets in treatment approaches involving direct depletion through monoclonal antibodies (mAb), inhibition of pro-inflammatory soluble factors or co-stimulatory molecules, and interruption of B cell activation or engagement of inhibitory checkpoint receptors (4). Despite the development of B cell-targeted treatment, the first therapeutic anti-CD20 mAb remains a crucial modality, with a long history of successful clinical use. In RA, the only biological agent approved for specific B cell-targeted therapy is rituximab, a chimeric monoclonal antibody against the CD20 antigen of B cells (5).

Rituximab was first used as a treatment for RA in 2006 and was approved for use with methotrexate (MTX) in patients with inappropriate responses to more than one anti-tumour necrosis factor agent (6). Clinical studies of rituximab have established its efficacy and safety as a protocol administered every 6 months after baseline. As such, the authorised dose regimen is intravenous infusion of 1,000 mg on days 1 and 15 every 24 weeks (7). However, although administration at intervals of 6 months may be the most appropriate, clinical responses may vary depending on seropositivity, biomarkers, and genetic markers given that reconstitution of the peripheral B cells usually occurs 6–9 months after rituximab administration (8, 9). Thus, determining the optimal timing for retreatment is challenging with respect to the duration of the effect. Further, safety is also of primary concern as repeated administration of rituximab may cause immunoglobulin reduction and increase the risk of infection. A comparison of the clinical effects of administration at reduced doses showed similar clinical effects at a reduced dose (one infusion of 1,000 mg or two doses of 500 mg) after a first course of rituximab at standard doses (10, 11). In addition, a recent real-world study on the use of rituximab showed that on-demand administration maintains good clinical responses (12).

Retreatment options for rituximab include regular retreatment at fixed intervals (e.g., every 6 months), treatment of flare, or treatment with any deterioration or treatment-to-target (13). This study aimed to investigate the patterns of use of rituximab in patients with RA in the real world, using the KOrean Rheumatology BIOlogics registry (KOBIO). Furthermore, we analysed the persistence rates of rituximab treatment and identified the factors associated with extending the administration intervals and with treatment failure.

METHODS

Study Design and Population

The KOBIO registry is a nationwide, multi-centre, prospective, observational cohort formed by the Korean College of Rheumatology Biologics Registry and launched in 2012 (14). The KOBIO RA registry consisted of the biologic group and the control group [(patients treated with conventional synthetic disease-modifying anti-rheumatic drugs (csDMARDs)]. The biologic group involved patients aged over 18 years who have been diagnosed with RA and are initiating, restarting, or changing to a new biologic agent. The purpose of the registry was to evaluate the clinical outcomes and adverse events of patients. All treatments, including the selection of biological agents, dose, and duration of treatment, were determined by the treating rheumatologists. Evaluations were performed every visit after obtaining consent from each participant. In this study, we used only the biologic RA group (patients treated with biologic DMARDs or targeted synthetic DMARDs).

The present study included patients with RA who were registered in the KOBIO registry or those who had been treated with rituximab at Ajou University Hospital, but not registered with KOBIO. There was no difference of the data collection and baseline characteristics of patients between KOBIO registry and Ajou University Hospital (**Supplementary Table 1**). All patients fulfilled the 1987 American College of Rheumatology (ACR) or 2010 ACR/European League Against Rheumatism classification criteria for RA diagnosis (15, 16). Patients with a low-dose rituximab regimen for retreatment were excluded, considering that other regimens may result in biassed outcomes. A total of 82 patients registered in the KOBIO from its launch to 2020 ($n = 55$) and patients treated at Ajou University Hospital between 1999 and 2020 ($n = 27$) were included.

The data collection form and study protocol for current study was approved by the institutional review board of Ajou University Hospital (AJIRB-MED-MDB-21-055) or local ethics committees at each participating centre, and was conducted in compliance with the principles of the Declaration of Helsinki. All patients provided written consent to participate in the registry.

Data Collection and Assessment of Disease Activity

Medical information was collected through data uploaded to the KOBIO web server (http://www.rheum.or.kr/kobio/). At the time of registration, individual investigators at each centre obtained information through structured interviews or using medical chart records including clinical information, laboratory tests, and radiologic imaging. Data for each patient were updated annually using a standardised case report form, and all data are transferred to the web server. Clinical information, such as age, sex, body mass index, alcohol consumption, smoking habits, extra-articular manifestations, previous or current medications, and concomitant diseases, was collected primarily from health questionnaires and interviews. Laboratory tests included rheumatoid factor (RF), anti-citrullinated protein antibody (anti-CCP Ab), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP). All radiographs were evaluated by radiologists, and bone erosion was defined as the presence of erosion of at least one proximal interphalangeal joint, metacarpophalangeal joint, wrist, and metatarsophalangeal joint on plain radiographs of the hand and foot. Disease activity was evaluated according to the number of tender and swollen joints, visual analogue scales for pain, patient's and physician's global assessment, and Disease Activity Score in 28 joints (DAS28)-ESR and DAS28-CRP.

Rituximab Protocol

All patients received 1,000 mg of rituximab intravenously on days 1 and 15 according to the standard regimen for RA as the first cycle of treatment with rituximab (17). All patients were evaluated for disease activity and adverse events 4 months after

the date of starting rituximab according to Korean National Health Insurance reimbursement criteria. And it was evaluated to be effective in 4-month evaluation, and if the disease worsened again, it could be re-administered after 6 months. Further cycles were repeated with the same regimen in patients with physician-confirmed aggravation of disease activity. Previous or current concomitant csDMARDs, corticosteroids, number of previous biologic agents, dates of onset and withdrawal, and treatment intervals were also collected. In case of treatment failure, the reasons such as lack of efficacy, adverse events, and others, were also identified. As for rituximab's efficacy, all patients are evaluated for disease activity and adverse events 4 months after the date of starting rituximab according to Korean National Health Insurance reimbursement criteria. And it is evaluated to be effective in 4-month evaluation, and if the disease worsens again, it could be re-administered after 6 months.

Statistical Analysis

The baseline characteristics were analysed using descriptive statistics, and data were presented as the mean ± standard deviation. Categorical variables were compared using the chi-square test or Fisher's exact test, while continuous variables were compared using the independent *t*-test. Survival curves of persistence on rituximab were generated using the Kaplan-Meier method. Univariate and multivariate linear regression analyses were used to determine the variables associated with extending the time interval between retreatment courses. Binary logistic regression analysis was performed to identify the risk factors of treatment failure. The results of linear regression analyses were expressed as β coefficients, while those of logistic regression analyses were expressed as odds ratios (OR) with 95% confidence intervals (CI). All statistical analyses were conducted using SPSS version 25.0 (IBM Corporation, Armonk, NY, USA). *P*-values < 0.05 were considered statistically significant.

RESULTS

Clinicodemographic Patient Characteristics

The mean age at the first rituximab cycle was 55.2 ± 13.4 years, and almost all patients were female (81.7%). The clinicodemographic patient characteristics are shown in **Table 1**. The most common comorbidity was hypertension (31.7%). The mean disease duration was 7.9 ± 6.0 years. There were 74 (90.2%) patients who were RF positive and 55 (67.1%) patients who were anti-CCP Ab positive. The mean DAS28-ESR and DAS28-CRP were 5.87 ± 1.02 and 4.83 ± 1.12, respectively. Majority of the patients had received csDMARDs before receiving rituximab, with 56 (68.3%) patients having at least two csDMARDs. The most commonly used csDMARDs were MTX (91.5%), followed by leflunomide (32.9%) and sulfasalazine (25.6%), respectively. All patients, except 3 patients, were taking corticosteroids, and the mean corticosteroid dose was 5.61 ± 3.57 mg prednisone-equivalent. There was little change in the number of patients using csDMARDs during concomitant treatment at the time of the first rituximab cycle, but there was a difference in the number of medications. Most of the patients were taking one

TABLE 1 | Baseline characteristics of patients with RA at time of first cycle of rituximab.

Variable	RA patients ($n = 82$)
Demographics	
Age, mean (years)	55.2 ± 13.4
Sex	
Female, N. (%)	67 (81.7)
Male, N. (%)	15 (18.3)
BMI, mean	22.9 ± 3.96
Smoking, N. (%)	16 (19.5)
Alcohol, N. (%)	7 (8.5)
Comorbidities, N. (%)	
Diabetes mellitus	7 (8.5)
Hypertension	26 (31.7)
Cardiovascular disease	1 (1.2)
Cancer	6 (7.3)
Disease status	
Disease duration (years)	7.88 ± 5.97
RF positivity, N. (%)	74 (90.2)
Anti-CCP Ab positivity, N. (%)	55 (67.1)
Tender joint count	10.3 ± 7.46
Swollen joint count	7.6 ± 5.53
ESR, mm/hr	58.9 ± 30.4
CRP, mg/dL	3.65 ± 7.76
DAS28-ESR	5.87 ± 1.02
DAS28-CRP	4.83 ± 1.12
Patient pain intensity, VAS (mm)	57.8 ± 21.0
Radiographic erosions, N. (%)	44 (53.7)
RA associated ILD, N. (%)	7 (8.5)
Medication	
Previous treatments	
Prior use of methotrexate, N. (%)	75 (91.5)
Prior use of sulfasalazine, N. (%)	21 (25.6)
Prior use of leflunomide, N. (%)	27 (32.9)
Prior use of csDMARDs, N. (%)	79 (96.3)
One csDMARD received, N. (%)	23 (28.0)
Two or more csDMARDs received, N. (%)	56 (68.3)
Corticosteroid use before rituximab treatment, N. (%)	79 (96.3)
Dosage, mean, mg/day (prednisone-equivalent)	5.61 ± 3.57
Concomitant treatments	
Methotrexate, N. (%)	66 (80.5)
Sulfasalazine, N. (%)	3 (3.7)
Leflunomide, N. (%)	14 (17.1)
Number of csDMARDs used, N. (%)	77 (93.9)
One csDMARD received, N. (%)	44 (53.7)
Two or more csDMARDs received, N. (%)	33 (40.2)
Corticosteroid use after rituximab treatment, N. (%)	76 (92.7)
Dosage, mean, mg/day (prednisone-equivalent)	4.63 ± 2.85
Prior use of biologic agents, N. (%)	80 (97.6)
Number of prior biologic agents, median (IQR)	2 (2, 3)
Prior use of ≥ 2 anti-TNF agents, N. (%)	55 (67.1)
Originator, N. (%) (vs. biosimilar)	77 (93.9)

RA, rheumatoid arthritis; BMI, body mass index; RF, rheumatoid factor; Anti-CCP Ab, anti-citrullinated protein antibody; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; DAS, disease activity score; VAS, visual analogue scale; ILD, interstitial lung disease; csDMARDs, conventional synthetic disease modifying anti-rheumatic drugs; IQR, inter-quartile range; TNF, tumour necrosis factor.

TABLE 2 | Treatment outcome of rituximab in patients with RA.

Variable	RA patients ($n = 82$)
Total follow-up period from the first cycle, mean (months)	46.1 ± 37.5
Achieving biologic-free remission after first cycle, N. (%)	10 (12.2)
Patients still receiving rituximab at end of follow-up, N. (%)	57 (69.5)
Number of retreatment courses, mean	2.56 ± 2.31
Time interval between two courses, mean (months)	16.3 ± 8.56
Treatment persistence every year after first cycle, N. (%)	
1 ($n = 79$)	77 (97.5)
2 ($n = 55$)	49 (89.1)
3 ($n = 46$)	39 (84.8)
4 ($n = 36$)	28 (77.8)
5 ($n = 29$)	21 (72.4)
Treatment failure, N. (%)	15 (18.3)
Lack of efficacy, N. (%)	7 (46.7)
Adverse effect, N. (%)	2 (13.3)
Physician/patient decision, N. (%)	1 (6.7)
Death, N. (%)	5 (33.3)

RA, rheumatoid arthritis.

csDMARD, with 44 patients (53.7%) and 33 patients (40.2%) taking at least two csDMARDs. The proportion of patients taking corticosteroids was approximately the same, and the mean dose was 4.63 ± 2.85 mg prednisone-equivalent, which was lower than the dose before receiving rituximab. In total, 80 of the 82 patients had previously experienced other biologic agents and the median number of prior biologic agents was two. Among them, 55 patients (68.8%) received two or more anti-tumour necrosis factor (anti-TNF) agents.

Treatment Outcomes

The treatment outcomes after the mean follow-up of 46.1 months are shown in **Table 2**. Sixty-seven patients sustained treatment without failure, and 10 of them achieved biologic-free remission after the first cycle. Biologic-free remission was defined as a state in which low disease activity was maintained only with csDMARDs without the use of biologic agents after the first cycle of rituximab. In the remaining 57 patients, the retreatment schedule was adjusted according to the judgement of the physician based on disease activity. The median number of rituximab cycles was 2, and the mean time interval of the retreatment courses was 16.3 ± 8.7 months. The probability of persistence for rituximab according to Kaplan-Meier analysis is presented in **Figure 1**. In total, 97.5, 89.1, 84.8, 77.8, and 72.4% of the patients continued rituximab each year until 5e years after the first cycle of rituximab. Each persistence rate was calculated as the percentage of patients who were maintained in the population, excluding those with follow-up loss or a short follow-up period. Treatment failure occurred in 15 patients (18.3%). The most common reason for rituximab discontinuation was lack of efficacy [7 (46.7%) patients], followed by death after rituximab administration [5 patients (33.3%)]. The causes of death were infection and malignancy. The other reasons for discontinuation were adverse effects (13.3%) and the patient's decision (6.7%).

Predictive Factors of Good Response to Rituximab

We analysed the clinical factors associated with achieving biologic-free remission after the first cycle of rituximab (**Table 3**) and found that only the previous use of two or more anti-TNF agents was significant. Disease activities, comorbidities, type or number of concomitant csDMARDs, and the corticosteroid dose did not significantly affect the achievement of biologic-free remission. Multivariable linear regression analysis of the factors that extend the time interval between the retreatment courses showed that prior (β = 5.386; 95% CI: 0.86–9.911, $p = 0.021$) or concomitant use of two or more csDMARDs (β = 4.672; 95% CI: 0.089–9.255, $p = 0.046$) and concomitant use of corticosteroids (β = 7.602; 95% CI: 0.924–14.28, $p = 0.026$) were significant factors (**Table 4**).

Factors Associated With Treatment Failure

The results of the logistic regression analysis for the risk factors of rituximab failure are described in **Table 5**. Univariate logistic regression analysis indicated that anti-CCP Ab positivity was significantly associated with treatment failure, with an OR of 0.157 (95% CI: 0.028–0.875, $p = 0.035$). This means that the probability of failing rituximab treatment is 0.15 times or 85% lower in patients with anti-CCP Ab than in patients without anti-CCP Ab. After multivariate analysis, anti-CCP Ab positivity remained as an independent factor associated with treatment failure of rituximab (OR, 0.184; 95% CI: 0.031–0.709, $p = 0.016$). Bone erosions, the presence of interstitial lung disease, concomitant medications, and the use of biosimilars did not influence treatment failure.

DISCUSSION

On-demand rituximab administration has been reported to achieve outcomes similar to fixed-interval administration. In this study, 57 patients among the 82 patients were treated with on-demand rituximab administration without failure, and 10 of them achieved biologic-free remission after the first cycle. The mean time interval of the retreatment courses was 16.3 ± 8.7 months. Rituximab has a longer-lasting effect on the host immune system than other biologic agents approved for the treatment of RA owing to its depletion of peripheral B cells from about 90% to almost 100% (18). However, although the long-lasting effect is highly advantageous for patients who prefer a convenient lifestyle, there are concerns that early use of new biologic agents may pose additional safety risks even if the effect of rituximab is insufficient.

Given that it is difficult to cope with insufficient efficacy, it is necessary to select subjects who are predicted to be good

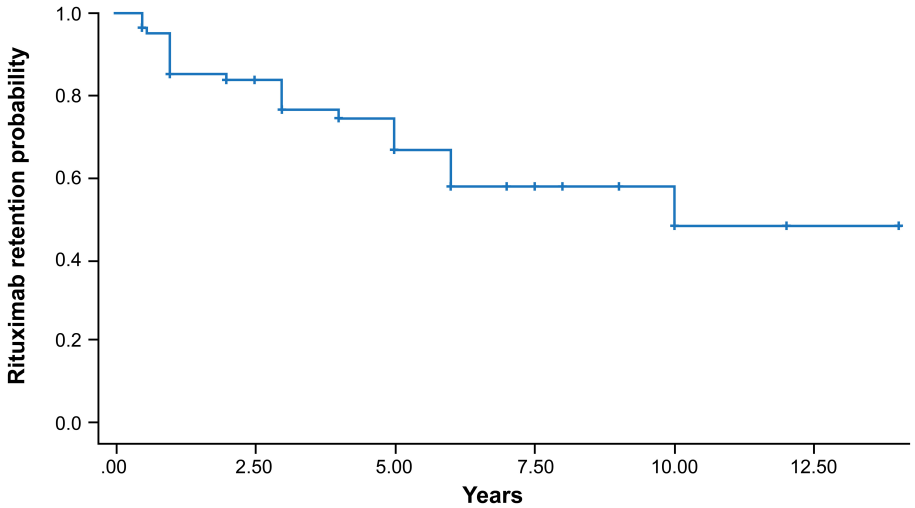

FIGURE 1 | Kaplan-Meier survival curve of the persistence of rituximab in patients with RA. The cumulative persistence rates were 97.5, 89.1, 84.8, 77.8, and 72.4% every year until 5 years after the first cycle of rituximab.

responders to rituximab. As such, the significance of this report is emphasised as, to our best knowledge, it is the first to evaluate the treatment response to rituximab using the nationwide registry in Korea. In our study, 72.4% of patients with RA continued rituximab after 5 years, indicating higher effectiveness and tolerability than previously reported in other cohorts. The rate of persistence in most previous reports ranged from 50-60% after 4 years of rituximab (19–21). The reason for this trend is unclear because there are no differences in disease activity, seropositivity, or number of prior biologic agents between our study and other studies (19, 20).

In the present study, the time interval for patients maintaining rituximab was significantly longer than the fixed retreatment schedule of 6 months. Retreatment was performed on demand rather than on a fixed schedule, as the retreatment schedule in Korea depends highly on the Korean National Health Insurance (KNHI) reimbursement criteria. Unlike other biologic agents that are administered at regular intervals even without flare, rituximab is reimbursed only at the time of flare at least 6 months after administration of the previous course under the KNHI system. A total of 12% of patients in this study achieved biologic-free remission after 1 cycle of rituximab. Given that existing data are mainly on patients who achieved biologic-free remission following treatment with anti-TNF agents, there is limited evidence on whether biologic-free remission is sustained after the discontinuation of rituximab (22).

In a previous study, the sustained rate of biologic-free remission ranged from 14 to 60% within a short follow-up period of 2 years, and thus further studies to select the most appropriate treatment strategies are needed (23). Compared with previous studies, our study is advantageous in that it includes a fairly long follow-up period, and we found a novel result that the use of two or more anti-TNF agents prior to rituximab is the only significant factor influencing biologic-free remission. This is in contrast to previous results that a higher number of biological agents prior to rituximab experience leads to a shorter duration of the clinical response (24, 25). A study by La et al. (26) verified that prolonged exposure to anti-TNF agents could increase B-cell survival factors to induce resistance to rituximab, and this is related to the overall duration of previous anti-TNF agents, rather than the number of anti-TNF failures. In summary, instead of long-term maintenance, the anti-TNF agent should be switched in patients who do not adequately respond to the treatment.

Further, other biologic agents with a different mechanism of action, such as rituximab, should be selected if the patient does not respond to treatment with two or more anti-TNF agents. Furthermore, RF negativity, not smoking and minimal radiographic damage which have been proven to be related to biologic free remission in previous studies, have not shown significant results in this study (27). The lack of significance of baseline disease characteristics for predicting biologic-free remission after treatment implies that other factors such as genetic differences in drug metabolism may affect the response to rituximab (28, 29).

Increasing evidence shows that on-demand, rather than fixed regular retreatment, is a reasonable schedule for long-term maintenance treatment of rituximab in patients with RA (30). The time interval for the average rituximab treatment course in our patients was 16 months, which was longer than in published literature (12, 20, 31). In several real-life observational studies, the average response duration of rituximab ranged from 7.8 months to 13 months. Observational studies also demonstrated that fixed regular retreatment and on-demand retreatment with rituximab showed comparable efficacy in patients who had a good response after the first cycle of the standard regimen (30). As such, on-demand retreatment is a more favourable option with respect to safety and cost saving than fixed regular retreatment. Several studies on low-dose rituximab as retreatment to reduce side effects have proven the non-inferiority of its efficacy; however, the on-demand

TABLE 3 | Clinical characteristics of patients who achieved and did not achieved biologic-free remission after first cycle.

Variable	Achieved ($n = 10$)	Not achieved ($n = 72$)	*P* value
Demographics			
Age, mean (years)	51.1 ± 18.0	55.73 ± 12.7	0.449
Sex, female, N. (%)	8 (80.0)	59 (81.9)	0.882
BMI, mean	23.5 ± 3.25	22.8 ± 4.06	0.648
Smoking, N. (%)	4 (40.0)	12 (16.7)	0.083
Alcohol, N. (%)	2 (20.0)	5 (6.9)	0.169
Comorbidities, N. (%)			
Diabetes mellitus	1 (10.0)	6 (8.3)	0.861
Hypertension	2 (20.0)	24 (33.3)	0.399
Cardiovascular disease	0 (0)	1 (1.4)	0.709
Cancer	2 (20.0)	4 (5.6)	0.102
Disease status			
Disease duration (years)	9.53 ± 5.19	7.65 ± 6.06	0.352
RF positivity, N. (%)	8 (80.0)	66 (91.7)	0.175
Anti-CCP Ab positivity, N. (%)	6 (60.0)	49 (68.1)	0.345
DAS28-ESR	5.81 ± 1.0	5.87 ± 1.03	0.858
DAS28-CRP	4.91 ± 0.81	4.82 ± 1.16	0.827
Radiographic erosions, N. (%)	5 (50.0)	39 (54.2)	0.806
RA associated ILD, N. (%)	1 (10.0)	6 (8.3)	0.861
Medication			
Previous treatments			
Prior use of methotrexate, N. (%)	10 (100)	65 (90.3)	0.306
Prior use of leflunomide, N. (%)	4 (40.0)	23 (31.9)	0.614
Prior use of sulfasalazine, N. (%)	5 (50.0)	16 (22.2)	0.061
Two or more csDMARDs received	8 (80.0)	48 (66.7)	0.399
CS dose before rituximab treatment, mean, mg/day (prednisone-equivalent)	4.89 ± 3.75	5.70 ± 3.75	0.525
Concomitant treatments			
Methotrexate, N. (%)	7 (70.0)	59 (81.9)	0.375
Leflunomide, N. (%)	1 (10.0)	13 (18.1)	0.528
Sulfasalazine, N. (%)	1 (10.0)	2 (2.8)	0.275
Two or more csDMARDs received	5 (50.0)	28 (38.9)	0.505
CS dose after rituximab treatment, mean, mg/day (prednisone-equivalent)	3.25 ± 2.06	4.83 ± 2.90	0.101
Number of prior biologic agents, median (IQR)	2 (2, 3)	2 (2, 3)	0.899
Prior use of ≥2 anti-TNF agents, N. (%)	10 (100)	45 (62.5)	0.019
Originator, N. (%) (vs. biosimilar)	1 (10.0)	4 (5.6)	0.584

BMI, body mass index; RF, rheumatoid factor; Anti-CCP Ab, anti-citrullinated protein antibody; DAS, disease activity score; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; VAS, visual analogue scale; RA, rheumatoid arthritis; ILD, interstitial lung disease; csDMARDs, Conventional synthetic disease modifying anti-rheumatic drugs; CS, corticosteroid; IQR, inter-quartile range; TNF, tumour necrosis factor.

TABLE 4 | Factors related to extending the time intervals between rituximab treatment.

Variable	β coefficient	95% CI	*P* value
Age	−0.068	−0.26–0.124	0.481
BMI	−0.274	−0.835–0.288	0.333
Smoking	−1.914	−8.495–4.668	0.563
Alcohol	−5.215	−17.598–7.167	0.402
Disease duration	0.114	−0.272–0.5	0.558
RF positivity	2.998	−6.014–12.01	0.508
Anti-CCP Ab positivity	−3.062	−12.537–6.414	0.519
DAS28-ESR	0.263	−1.859–2.386	0.804
DAS28-CRP	−0.273	−2.147–1.602	0.772
Patient pain intensity, VAS (mm)	0.058	−0.057–0.173	0.319
Radiographic erosions	4.309	−0.127–8.746	0.057
RA associated ILD	−0.2	−8.306–7.906	0.961
Prior use of methotrexate	3.669	−3.737–11.075	0.325
Prior use of leflunomide	1.257	−3.744–6.258	0.616
Prior use of sulfasalazine	−0.26	−6.07–5.55	0.929
Prior use of csDMARDs yes (vs. no)	2.057	−8.197–12.312	0.689
Prior use of csDMARDs ≥ 2	5.386	0.86–9.911	0.021
Prior use or corticosteroid	3.246	−9.185–15.678	0.603
Prior use of corticosteroid dose	−0.172	−0.753–0.409	0.555
Concomitant use of methotrexate	5.194	−0.445–10.833	0.07
Concomitant use of leflunomide	2.841	−3.716–9.398	0.389
Concomitant use of sulfasalazine	−5.377	−22.783–12.029	0.538
Concomitant use of csDMARDs yes (vs. no)	1.648	−7.318–10.614	0.714
Concomitant use of csDMARDs ≥ 2	4.672	0.089–9.255	0.046
Concomitant use of corticosteroid yes (vs. no)	7.602	0.924–14.28	0.026
Concomitant use of corticosteroid dose	−0.317	−1.133–0.499	0.44
Prior use of biologic agent yes (vs. no)	−0.744	−13.205–11.717	0.905
Prior biologic agent number	−3.514	−9.246–2.219	0.225
Prior use of ≥ 2 anti-TNF agents	−0.897	−5.696–3.901	0.709
Originator (vs. biosimilar)	−3.147	−19.588–13.294	0.704

BMI, body mass index; RF, rheumatoid factor; Anti-CCP Ab, anti-citrullinated protein antibody; DAS, disease activity score; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; VAS, visual analogue scale; RA, rheumatoid arthritis; ILD, interstitial lung disease; csDMARDs, Conventional synthetic disease modifying anti-rheumatic drugs; TNF, tumour necrosis factor.

retreatment of low-dose rituximab has insufficient efficacy (32, 33).

Considering the current tendency to pursue on-demand retreatment globally and the convenience of extending the retreatment time intervals, it is reasonable to find ways to extend the time interval between retreatment courses rather than attempting to reduce the dose. Our study identified that prior or concomitant use of two or more csDMARDs and concomitant use of corticosteroids are associated with the extension of the time interval. Previous studies have reported that concomitant treatment with csDMARDs improves the clinical response of rituximab, and most of these studies are on rituximab in combination with a single csDMARD (34–36). To our best knowledge, we are the first to report that the concomitant use of two or more csDMARDs and/or corticosteroids with rituximab plays a critical role in maintaining

TABLE 5 | Binary logistic regression analysis of risk factors associated with treatment failure in patients with RA after treatment with rituximab.

Variable	Univariate		Multivariate	
	Odds ratio (95% CI)	P value	Odds ratio (95% CI)	*P* value
Age	1.021 (0.977–1.067)	0.365		
BMI	0.987 (0.854–1.140)	0.857		
Female gender	0.873 (0.213–3.578)	0.850		
Smoking	1.667 (0.453–6.138)	0.442		
Alcohol	3.937 (0.78–19.881)	0.097		
Disease duration	0.998 (0.908–1.098)	0.971		
RF positivity	1.4 (0.156–12.578)	0.764		
Anti-CCP Ab positivity	0.157 (0.028–0.875)	0.035	0.184 (0.031–0.709)	0.016
DAS28-ESR	1.042 (0.598–1.815)	0.884		
DAS28-CRP	1.241 (0.739–2.082)	0.414		
Patient pain intensity; VAS (mm)	1.01 (0.983–1.039)	0.47		
Radiographic erosions	2.833 (0.819–9.796)	0.1		
RA associated ILD	0.726 (0.081–6.523)	0.775		
Prior use of csDMARD ≥ 2	2.091 (0.536–8.163)	0.288		
Prior use of corticosteroid dose	0.996 (0.848–1.170)	0.962		
Concomitant use of csDMARD ≥ 2	1.38 (0.447–4.259)	0.576		
Concomitant use of corticosteroid dose	1.177 (0.979–1.416)	0.083		
Prior biologic agent number	1.049 (0.751–1.467)	0.778		
Prior use of ≥ 2 anti-TNF agents	0.486 (0.155–1.523)	0.216		
Originator (vs. biosimilar)	0.118 (0.117–10.852)	0.919		

BMI, body mass index; RF, rheumatoid factor; Anti-CCP Ab, anti-citrullinated protein antibody; DAS, disease activity score; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; VAS, visual analogue scale; RA, rheumatoid arthritis; ILD, interstitial lung disease; csDMARDs, Conventional synthetic disease modifying anti-rheumatic drugs; TNF, tumour necrosis factor.

clinical good responses for long periods. It is quite noteworthy in that it has an acceptable safety profile when compared to concomitant use of csDMARD monotherapy (data not shown). This finding indicates that maintaining low disease activity to prevent disease flare following rituximab treatment is important for extending the time interval between treatment courses. The persistent rates of rituximab were high in our cohort owing to the high proportion of patients taking concomitant csDMARDs or corticosteroids.

The treatment failure rate in this study was 18.3%, which is lower than that in other studies (19, 20). However, the most common reasons for treatment failure (i.e., lack of efficacy, death, and adverse events) were similar to those in previous studies (19). Binary logistic regression analysis showed that only anti-CCP Ab positivity is a significant associated factor of treatment failure. RA patients who are RF or anti-CCP Ab positive are more likely to respond better to rituximab treatment than autoantibody-negative patients. However, studies on each antibody as independent factors have reported conflicting results (24, 37–39). Some studies reported that anti-CCP Ab positivity was associated with a good response and that higher anti-CCP Ab titres predict good responses (40, 41). Meanwhile, other study reported that it is RF positivity, rather than anti-CCP Ab positivity, that is related to the good response to rituximab (25). Thus, the most reliable antibody to predict treatment response is yet to be established.

In addition, other factors, such as the B cell phenotype, have been recently reported to influence the treatment response to rituximab (42, 43). Plasmablasts, for example, was supplemented CD20 positive B cells despite being CD20 negative, becoming a potential biomarker for identifying B cell depletion after treatment with rituximab (44). However, except for anti-CCP Ab, no useful biomarkers predictive of treatment failure have been identified. Although factors other than autoantibody positivity may affect treatment failure in rituximab, rituximab should not be considered as the primary treatment option in autoantibody-negative patients.

The strength of our study is that to our best knowledge, it is the first to analyse the factors that extend the time interval during on-demand retreatment with rituximab in RA patients with a good clinical response. In addition, this is the first study to investigate the treatment outcomes of rituximab in RA patients, using data from a nationwide registry. However, our study also has some limitations. First, the observational study design can lead to an underestimation of events by relying on passive reporting such as adverse events and deaths. Further, the rate of loss to follow-up is also higher than in clinical trials. Second is the possibility of selection bias from the assignment of biological agents because the decision to use rituximab was made by the treating rheumatologist. Finally, the data from the KOBIO registry are not representative of the entire population of RA patients treated with rituximab. Given that data were mainly from outpatients, only a small portion of the patients may have been included in the registry because national guidelines require admission for the

intravenous administration of biological agents. This limitation may be overcome with recruitment of additional patients from multicentre.

CONCLUSIONS

RA patients treated with rituximab in Korea show high persistence rates. Further, the time interval between the retreatment courses was longer than in other countries. Concomitant use of two or more csDMARDs and concomitant use of corticosteroids with rituximab are significant influencing factors of extending the retreatment time interval. Importantly, an extended interval is safe and cost-efficient. These findings should be considered when selecting rituximab as a treatment for patients with RA.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board of Ajou University Hospital (AJIRB-MED-MDB-21-055). The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

J-WK, J-YJ, KS, C-HS, and H-AK contributed to the study design and data collection, analysis, and interpretation. J-WK, KS, and H-AK contributed to the data collection and/or data interpretation. All authors revised the manuscript and gave final approval for submission.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.765535/full#supplementary-material

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