

# B-cell therapy in lupus nephritis: an overview

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## ABSTRACT

Systemic lupus erythematosus (SLE) is an autoimmune multi-system disease that commonly affects the kidneys. It is characterized by persistent autoantibody production that targets a multitude of self-antigens. B-cells, plasmablasts and plasma cells, as the source of these autoantibodies, play a major role in the development of lupus nephritis (LN), and are therefore promising therapeutic targets. To date, however, randomized clinical trials of B-cell therapies in LN have not lived up to expectations, whereas uncontrolled cohort and observational studies of B-cell antagonists have been more promising. In this article, we will review the current experience with B-cell therapy in LN and highlight the pitfalls that may have limited their success. We will conclude by suggesting B-cell-centric approaches to the management of LN based on what has been learned from the overall B-cell experience in SLE.

**Keywords:** BAFF, B-cell, lupus nephritis, rituximab, SLE

## INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by loss of self-tolerance resulting in persistent generation of autoantibodies. These autoantibodies are classically directed against nuclear antigens, but can also target many others including cytoplasmic, cell membrane and phospholipid-associated antigens, blood cells and endothelial cells, with the total number of autoantibody specificities identified exceeding a hundred [1]. Kidney injury is a major morbidity of SLE and most often is caused by immune-complex accumulation in the kidney [lupus nephritis (LN)]. The management of LN usually involves an ‘induction’ phase that aims to decrease inflammation and suppress the autoimmune pathways contributing to it, and a ‘maintenance’ phase that aims to keep those pathways at bay, limiting relapses. The currently available medications for the management of LN are associated with significant adverse effects and have limited success rates, thus the need for new agents is high and urgent.

Our understanding of SLE and LN pathogenesis has evolved over the past few decades, and the multiple and diverse roles of the various effector cells of the immune system have been better

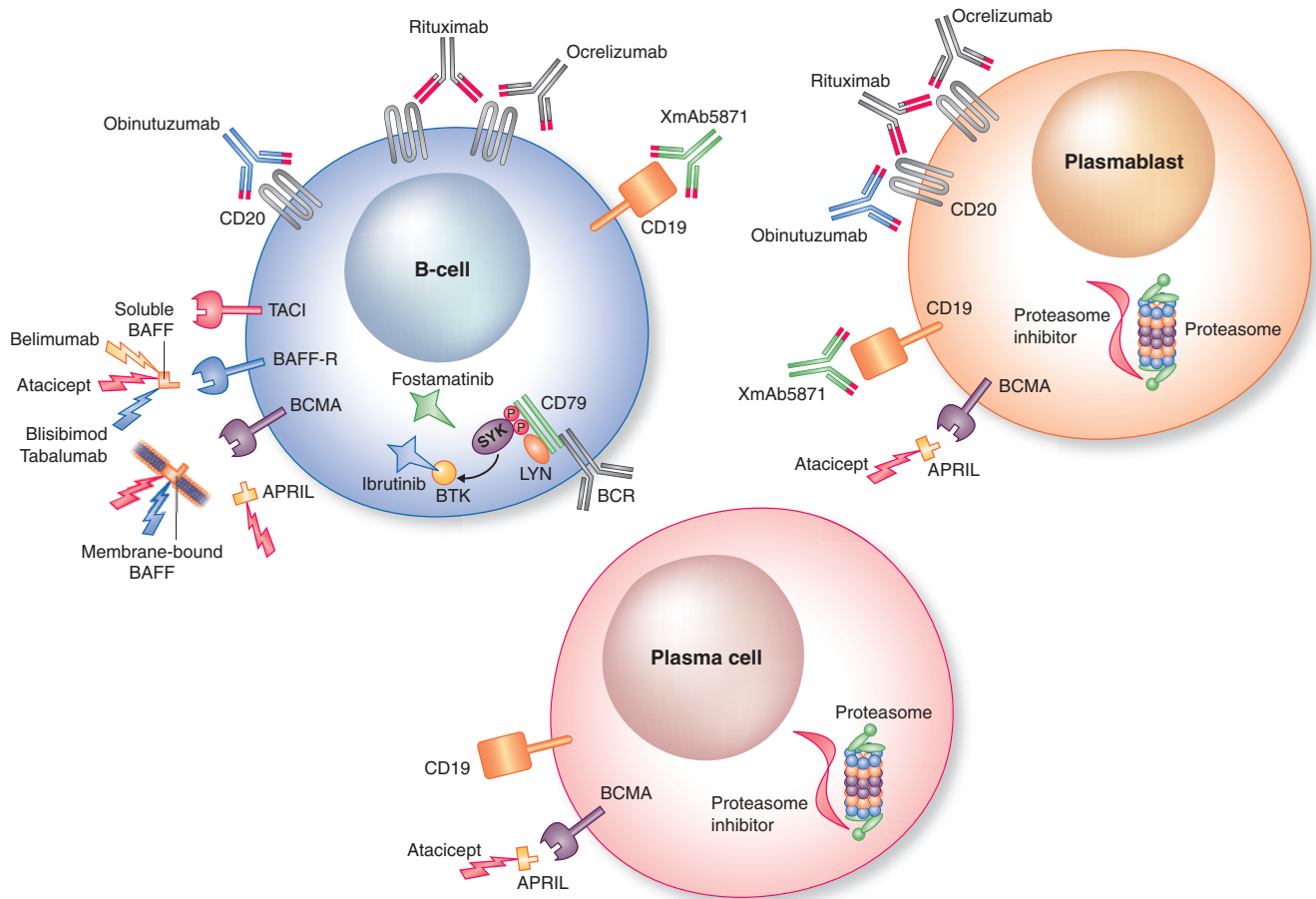
elucidated. In this regard B-cells have received considerable attention. Although an in-depth review of B-cells in the pathogenesis of SLE is beyond the scope of this article, some key studies are important to note. Shlomchik *et al.* [2] and Vlahakos *et al.* [3] demonstrated that lupus-prone mice lacking B-cells did not develop nephritis, and injection of antibodies from lupus mice into normal mice resulted in glomerular immune-complex deposition and development of a lupus-like nephritis. The relevance of B-cells to LN is not limited to autoantibody production. Mice with B-cells that do not secrete antibodies were still able to develop nephritis [4]. B-cells also act as antigen-presenting cells, can promote T-cell activation and produce inflammatory cytokines [5]. These and other data (reviewed in [6–8]) have positioned B-cells as a potentially high-value therapeutic target for the treatment of SLE and LN. The evolution and application of B-cell therapies (Figure 1) to human LN will be reviewed here.

## THERAPEUTIC B-CELL STRATEGIES

### B-cell depletion

CD20 promotes B-cell activation and was one of the first B-cell surface antigens exploited for B-cell depletion. Lupus-prone MRL/lpr mice treated with anti-CD20 monoclonal antibodies (mAbs) had a fall in anti-double-stranded DNA (dsDNA) antibody titers and attenuation of their autoimmune nephritis [9] (Figure 1). Lupus-prone NZB/W F1 mice treated with anti-CD20 mAbs showed a delay in disease onset and decreased progression of nephritis [10]. These findings were not solely autoantibody-mediated as there was a reduction in T-cell activation after B-cells regenerated and antibody levels returned to baseline [10]. B-cell depletion was also shown to impair protein and pathogen CD4<sup>+</sup>-mediated T-cell activation and clonal expansion [11].

Another target antigen that can decrease the number of B-cells when engaged is CD22, a transmembrane protein mainly expressed on mature B-cells (but not plasma cells or memory B-cells) [12, 13]. Lupus-prone mice were found to express functionally deficient CD22 [14], which can contribute to enhanced autoimmunity as CD22 is believed to be an inhibitor of B-cell receptor (BCR) signaling [15]. In addition, polymorphisms in the CD22 gene were identified in human SLE [16].



**FIGURE 1:** A schematic of the current targets of B-cell therapy. LYN, Lck/Yes novel tyrosine kinase; SYK, spleen tyrosine kinase; BTK, Bruton's tyrosine kinase.

Importantly, neither CD20 nor CD22 is expressed on plasmablasts or plasma cells [17], and depletion with an anti-CD20 mAb did not affect plasma cell survival in mice [18]. Plasmablasts and plasma cells produce antibodies and autoantibodies in SLE. Plasma cell depletion resulted in decreased autoantibody production, improved survival and LN in NZB/W F1 and MRL/lpr mice [19–21]. Plasma cells are also thought to be the source of persistent antigen-specific antibodies [22], which decreased when B-cells and plasma cells were depleted at the same time [18], suggesting that targeting B-cells and their progeny, plasma cells, might have a synergistic effect on B-cell-driven autoimmunity.

Another approach to achieve a more comprehensive depletion of B-cells (and plasma cells) is targeting CD19, which has a broader range of B-cell lineage expression including plasmablasts and plasma cells. Its use is currently being evaluated in autoimmune diseases in humans [23]. Alternatively, CD19<sup>+</sup> B-cells can be suppressed (rather than depleted) when CD19 is co-engaged with FcγRIIb [24]. This was demonstrated using XmAb5871, an engineered anti-CD19 mAb that also binds FcγRIIb. XmAb5871 suppressed humoral immunity and

reduced serum immunoglobulin levels without depleting B-cells in severe combined immunodeficiency (SCID) mice engrafted with human SLE peripheral blood mononuclear cells [25].

### B-cell neutralization

B-cell activating factor (BAFF, also known as B-lymphocyte stimulator) and a proliferation-inducing ligand (APRIL) are two members of the tumor necrosis factor (TNF) superfamily that promote B-cell survival and differentiation (Figure 1) [26–28]. They are predominantly secreted by dendritic cells, macrophages and neutrophils. BAFF is known to bind three receptors; BAFF receptor (BAFF-R), transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) and B-cell maturation antigen (BCMA), while APRIL only binds to TACI and BCMA. B-cells express all three receptors, but plasma cells predominantly express BCMA.

There is ample evidence suggesting a role for BAFF in SLE. Circulating BAFF levels are higher in NZB/W F1 and MRL/lpr mice during the onset and progression of SLE, and treatment of NZB/W F1 mice with a soluble TACI-Ig or BAFF-R-Ig fusion

protein prolonged survival and inhibited development of proteinuria [29, 30]. BAFF-deficient NZM lupus-prone mice had less severe proteinuria and reduced mortality [31]. In addition, in patients with SLE, CD19<sup>+</sup> B-cell expression of BAFF and APRIL correlated with disease severity (as measured by the SLE disease activity index) and anti-dsDNA titers, and plasma cells had increased BAFF and APRIL expression [32]. Interestingly, BAFF levels are elevated in African-Americans with SLE compared with patients of European ancestry [33], which may partially explain their greater degree of B-cell activation [34] and possibly a worse prognosis.

B-cell depletion and neutralization strategies have been applied to the treatment of human LN with some mixed but also some promising results. These studies will be discussed in the remainder of this review to provide insight into how B-cell therapies may be best deployed for patients with LN.

### B-CELL DEPLETION FOR LN INDUCTION

The first multinational randomized controlled trial of B-cell therapy to improve renal response rates in LN was the lupus nephritis assessment with rituximab (LUNAR) study [35]. Rituximab is a chimeric IgG mAb directed against CD20, and is currently used in the management of B-cell dyscrasias and autoimmune diseases such as rheumatoid arthritis. LUNAR randomized 144 patients with proliferative LN (ISN/RPS Class III/IV  $\pm$  V), and a mean serum creatinine concentration and urine protein-to-creatinine ratio (uPCR) of  $1.0 \pm 0.5$  mg/dL and  $4.0 \pm 2.9$  mg/mg, respectively, to receive rituximab (four 1 g doses on Days 1, 15, 168, 182) or placebo in addition to standard-of-care [pulse methylprednisolone followed by oral prednisone and mycophenolate mofetil (MMF)]. The study did not demonstrate significantly more complete or partial renal responses than placebo at 52 weeks. The overall renal response (complete plus partial) was 57% for the rituximab group and 46% for the placebo group. However, patients treated with rituximab had more improvements in complement and anti-dsDNA levels, and a larger decrease in proteinuria than the controls.

Ocrelizumab, a humanized anti-CD20 mAb was subsequently evaluated in 223 patients with Class III/IV  $\pm$  V LN in the BELONG trial [36]. Patients were randomized to placebo or one of two ocrelizumab doses plus either MMF or cyclophosphamide as background immunosuppression. Although BELONG was stopped early due to an imbalance in serious infections in the ocrelizumab groups, 148 patients completed >32 weeks of therapy. Reminiscent of the LUNAR trial, the ocrelizumab-treated patients showed a nonsignificant increase (12% over control) in overall renal response, but significant improvement in complement and anti-dsDNA levels.

These negative results were unexpected given the established role of the B-cell in SLE. Several concerns over trial design were raised. These included adding B-cell therapy to intense background therapy with corticosteroids and immunosuppression, insufficient sample size, insufficient sensitivity in determining complete B-cell depletion, failure to account for tissue B-cells that may be harder to kill, lack of effect on plasma cells and insufficient length of follow-up [37]. These concerns have led to

some innovations in LN trial design as outlined below, and importantly, have not discouraged the evaluation of anti-B-cell therapies in LN (Table 1).

Add-on trials in which a novel therapeutic is given with standard-of-care background immunosuppression are common in LN because of the potential for rapid disease progression and the uncertain efficacy of the novel drug being tested. However, the benefits of immunosuppressive therapies beyond those which may be attributed to corticosteroids alone can take >1–2 years to become apparent [38, 39], raising the possibility that any beneficial effects of B-cell therapies added to high-dose corticosteroids may have been masked. For example, in BELONG, ocrelizumab showed no additive improvement for patients receiving >1 g of intravenous methylprednisolone, who had a higher rate of renal response regardless of concomitant therapy, but did appear to have efficacy in patients who had been given less methylprednisolone initially. Rituximab has also been shown to be ‘steroid-sparing’ in the treatment of LN. In a single-center cohort 50 consecutive patients with ISN/RPS Class III, IV or V LN were treated with rituximab (two 1 g doses on Days 1, 15), methylprednisolone (two 500 mg doses on Days 1, 15) and MMF without any oral steroids, and achieved excellent renal responses with 90% complete or partial remission after a median follow-up of 37 weeks [40]. Finally, a small study examined the potential of rituximab to facilitate rapid weaning of corticosteroids and minimization of maintenance immunosuppression in severe SLE and LN. A total of 12 patients with multiorgan involvement, including LN (three patients with Class IV, four with Class III + V and five with Class V), were treated with rituximab administered as four weekly doses of 375 mg/m<sup>2</sup>, followed by two additional doses 1 and 2 months after the last weekly infusion, cyclophosphamide (10 mg/kg on Days 4 and 17) and methylprednisolone (15 mg/kg on Days 1, 4 and 8). Oral prednisone was started at 0.8 mg/kg/day for 2 weeks, but rapidly tapered to 5 mg at 3 months and was maintained afterward. At 12 months all patients were in complete renal remission (defined by 24 h proteinuria <0.5 g/day and GFR within 10% of baseline). After a mean follow-up time of 51.6 months, 9 out of 12 patients remained in remission with minimal maintenance immunosuppression [41]. Taken together, these findings suggest that it may be necessary to design future LN trials with less background corticosteroid.

The concern that LUNAR and BELONG failed because of incomplete B-cell depletion in the periphery, lymphoid tissues or the kidney is being addressed by the NOBILITY trial (NCT02550652). NOBILITY is similar to LUNAR, but the B-cell drug is obinutuzumab, a new-generation humanized anti-CD20 mAb with superior antibody-dependent cellular cytotoxicity and antibody-dependent cellular phagocytosis. In trials of B-cell malignancies, obinutuzumab was more effective at depleting peripheral and lymphoid tissue B-cells than rituximab [42–44]. The expectation of NOBILITY is that the enhanced cytotoxicity will translate into improved outcomes, as the degree of B-cell depletion has been associated with clinical response in LN [45].

The lack of plasma cell depletion with anti-CD20 mAbs might be circumvented by targeting CD19 instead, which is

**Table 1. Current clinical trials evaluating B-cell therapies in SLE and LN**

Target	Drug	Study title	Study status	Disease target	Study phase	Identifier
CD20	Rituximab (anti-CD20 mAb)	Rituximab objective outcome measures trial in SLE	Recruiting	SLE	Phase 2	NCT03054259
		RING	Recruiting	LN	Phase 3	NCT01673295
	Obinutuzumab (anti-CD20 mAb)	A study to evaluate the safety and efficacy of obinutuzumab compared with placebo in participants with LN	Recruiting	LN	Phase 2	NCT02550652
CD19	XmAb5871	A study of the effect of XmAb <sup>®</sup> 5871 in patients with SLE	Active	SLE	Phase 2	NCT02725515
BAFF/APRIL	RC18 (TACI-antibody fusion protein)	Study of RC18 administered subcutaneously to subjects with SLE	Recruiting	SLE	Phase 2	NCT02885610
	Belimumab (anti-BAFF)	Efficacy and safety of belimumab in patients with active LN	Active	LN	Phase 3	NCT01639339
	AMG 570 (bispecific peptibody—BAFF and ICOS ligand)	Single ascending dose study of AMG 570 in healthy subjects	Active	SLE	Phase 1	NCT02618967
Immunoproteasome	KZR-616	A study of KZR-616 in patients with SLE with and without nephritis	Recruiting	SLE	Phase 1/2	NCT03393013
Combination	Rituximab, belimumab	Rituximab and belimumab for LN	Active	LN	Phase 2	NCT02260934
		Synergetic B-cell immunomodulation in SLE	Recruiting	SLE	Phase 2	NCT02284984
		A study to evaluate the efficacy and safety of belimumab administered in combination with rituximab to adult subjects with SLE—BLISS—BELIEVE	Recruiting	SLE	Phase 3	NCT03312907

expressed on B-cells and plasma cells. An anti-CD19 mAb, XmAb5871 is currently under study in human SLE (NCT02725515), but proliferative LN is excluded. Alternatively, plasma cells can be targeted with proteasome inhibitors (PIs). Proteasomes are the major nonlysosomal degradation system for proteins, and proteasome inhibition leads to the accumulation of misfolded proteins within the endoplasmic reticulum. Plasma cells are particularly sensitive to proteasome inhibition due to their high rate of antibody synthesis [46]. PIs have effects other than plasma cell depletion that could also be relevant for the treatment of LN. For example, in mice bortezomib can abrogate Type-I interferon activity [20], probably by inducing apoptosis in Toll-like receptor-activated plasmacytoid dendritic cells [47]. Bortezomib also prevents degradation of I $\kappa$ B $\alpha$ , thus inhibiting the activation of the transcription factor NF- $\kappa$ B [48, 49]. NF- $\kappa$ B plays multiple roles in SLE including B- and T-cell development, maturation of dendritic cells and cytokine secretion [50]. NF- $\kappa$ B mediates the synthesis of TNF- $\alpha$ , interleukin (IL)-1 $\beta$ , IL-6 and IL-8, and is one of the most important regulators of proinflammatory gene expression [51]. Thus PIs may have a role in reducing inflammation in LN.

Preliminary studies have examined PI inhibition in LN. Bortezomib depleted peripheral and bone marrow plasma cells, reduced interferon- $\alpha$  activity, decreased anti-dsDNA antibodies, decreased proteinuria and led to improvement in C3 levels in 12 patients with SLE, 5 of whom had active LN previously treated with cyclophosphamide or MMF [52]. Unfortunately, bortezomib was discontinued in seven patients due to adverse reactions. In five patients with refractory LN and high histological activity on kidney biopsy bortezomib resulted in a partial

renal remission in four patients [53]. Most patients also had an improvement in serological markers. With these encouraging findings, bortezomib is currently being evaluated in a Phase 2 trial for SLE (NCT02102594) and ixazomib, another PI, is being evaluated in LN (NCT02176486). Ixazomib differs from bortezomib as it is a second-generation PI, that is given orally and may have less neurotoxicity [54]. Newer PIs that selectively inhibit the immunoproteasome, but not the constitutive proteasome, are being developed for use in autoimmunity (NCT03393013). Immunoproteasomes are typically expressed in hematopoietic cells but can also be expressed in other cells exposed to an inflammatory milieu. The selectivity of immunoproteasome inhibitors may reduce the risk of adverse effects observed with inhibition of all cellular proteosomal activity [55], and help specifically target sites of inflammation.

### B-CELL NEUTRALIZATION FOR LN INDUCTION

There have been three randomized controlled trials of BAFF antagonists for the treatment of SLE. Although active or severe LN was excluded from these trials some information about the effects of BAFF inhibition on LN can be gleaned from their results. Belimumab is a humanized monoclonal antibody that inhibits the soluble form of BAFF, and was shown to be effective for nonrenal SLE in two large trials (BLISS-52 and BLISS-76) [56, 57]. Although patients with active nephritis (defined as a serum creatinine >2.5 mg/dL or >6 g/day of proteinuria) were excluded, a *post hoc* analysis of patients with a history of LN demonstrated a dose-dependent reduction in renal flares



compared with placebo during follow-up [58]. Belimumab is currently being tested as an add-on agent for LN induction therapy in the BLISS-LN trial (NCT01639339). Given the questions surrounding LUNAR regarding insufficient duration of follow-up, patients in BLISS-LN will be observed for 2 years rather than 1 year.

Two large studies (>2000 patients) of tabalumab, an mAb that binds soluble and membrane-bound BAFF, were done in nonrenal SLE [59, 60]. Patients with severe active LN, defined as an uPCR >200 mg/mmol, creatinine clearance <30 mL/min or by physician assessment were excluded. In contrast to the BLISS trials, the outcome of these studies was not clear cut, and *post hoc* analysis did not demonstrate any difference in renal outcomes for patients who had LN and were treated with tabalumab compared with those treated with placebo [61].

Blisibimod is a fusion protein of antibody Fc region and BAFF binding domains that also binds soluble and membrane-bound BAFF. Blisibimod was evaluated in a Phase 2 trial of predominantly Hispanic patients with moderate-to-severe SLE, but not severe LN (PEARL-SC) [62]. Although treatment with blisibimod did not significantly improve the SLE response index-5, a subgroup analysis of patients with baseline proteinuria (uPCR 1–6 mg/mg) demonstrated a significantly greater reduction in proteinuria in those treated with blisibimod, along with improved levels of C3, C4 and anti-dsDNA. It remains to be seen whether these results hold up in CHABLIS-SC1 (NCT01395745), a recently completed Phase 3 study of blisibimod in patients with SLE.

Taken together, the results of BAFF inhibition in LN are mixed, but seem to support investigation of BAFF inhibitors to prevent LN flares. The blisibimod data may also support induction treatment of LN, a question that will be answered by BLISS-LN.

Another approach to B-cell growth factor inhibition was through atacept, a human recombinant fusion protein of TACI and the Fc portion of human IgG1 (TACI-IgG). Atacept blocks BAFF and APRIL. A Phase 2/3 study adding atacept to corticosteroids plus MMF in LN was terminated early because of a high incidence of hypogammaglobulinemia [63]. A trial of atacept in moderate-to-severe SLE was also terminated early due to a higher incidence of death in the atacept group [64]. A third study of atacept in nonrenal SLE (ADDRESS II NCT01972568) did not show a safety signal, but showed only a nonsignificant trend toward improvement [65].

## B-CELL THERAPY FOR LN MAINTENANCE

The *post hoc* analysis of BLISS-52 and BLISS-76 suggested belimumab could prevent LN flares and may therefore be useful in the maintenance of remission [58]. The CALIBRATE trial (NCT02260934) is the first prospective investigation to test B-cell directed therapies for induction and maintenance in LN. In CALIBRATE, patients initially receive rituximab plus cyclophosphamide, given as two 1 g and 750 mg doses, respectively, 2 weeks apart. Patients are then randomized to placebo or belimumab (10 mg/kg Weeks 4, 6, 8, then every 4 weeks). This unique design tackles the observation that B-cell depletion results in a marked increase in serum BAFF levels [66], and that in SLE patients treated with rituximab, relapse was associated

with significantly elevated serum BAFF levels after B-cell repopulation [67]. Animal studies in nonautoimmune mice not only showed similar results after B-cell depletion with cyclophosphamide, but also demonstrated that repopulation of B-cells in a high BAFF environment led to cells with an autoimmune phenotype and glomerular immunoglobulin deposition [68]. The underlying hypothesis of CALIBRATE is that B-cell repopulation in a BAFF-poor environment may attenuate reactivation of autoimmunity and prevent disease flares.

## B-CELL THERAPY IN REFRACTORY LN

The efficacy of rituximab in refractory LN is currently being evaluated prospectively in a randomized clinical trial: rituximab for lupus nephritis with remission as a goal (RING, NCT01673295). The RING trial is supported by several studies describing the use and utility of rituximab for LN refractory to conventional approaches. For example, an Italian multicenter observational cohort described 145 SLE patients in whom rituximab was added on to 76% of the cohort after they had not responded to at least one other immunosuppressant [69]. LN was present in 68% of patients (mean serum creatinine  $1.09 \pm 0.63$  mg/dL; mean proteinuria  $4.04 \pm 2.91$  g/day). A complete or partial renal response was seen in 94% of patients. Proteinuria decreased from  $4.1 \pm 2.9$  at baseline to  $1.1 \pm 1.9$  g/day ( $P = 0.021$ ) after 12 months.

Additional evidence for rituximab in refractory LN comes from a systematic review of 26 studies that reported the outcomes of 300 refractory LN patients given rituximab [70]. Most (60%) of the patients had Class III/IV  $\pm$  V LN, and all had been treated previously with cyclophosphamide, MMF or both. Complete or partial remission was achieved in 74% after receiving rituximab. However, a cautious interpretation of these results is warranted. Kidney pathology was not available in 30% of patients, the definition of refractory LN was inconsistent and not explained for 30% of patients, the duration of prior immunosuppression was not analyzed, rituximab was added to ongoing immunosuppression in 62% of the patients, and rituximab dosing was not uniform. Rituximab was generally given as four weekly doses of 375 mg/m<sup>2</sup> or two doses of 1 g given 2 weeks apart, but some trials gave two doses of 750 mg/m<sup>2</sup> given 2 weeks apart, and others gave 500 mg every 2 weeks for two to four doses. Assuming depletion of peripheral blood CD19 cells as measured by routine flow cytometry represents the correct biomarker to follow for rituximab efficacy, it is conceivable that rituximab regimens can be personalized for individual patients by using peripheral B-cell counts to determine when to dose and how much to use.

### Future directions

Although there is considerable circumstantial evidence that B-cell-targeted therapies may be effective in the initial treatment of LN, this still needs to be verified in a randomized controlled trial. Based on the presumptive mechanisms of actions of B-cell therapies, and the results of pre-clinical and clinical trials we suggest three regimens that may be worth consideration for future randomized clinical trials (Table 2). As a disclaimer,

**Table 2. Proposed future B-cell-targeted strategies for management of ISN/RPS Class III/IV ± V LN**

Proposed regimen	Target	Timeline	Expected response
1	Proteasome inhibitor	At flare	<ul style="list-style-type: none"> <li>• Deplete plasma cells and plasmablasts</li> <li>• Stop autoantibody production</li> <li>• Attenuate inflammatory cytokine production</li> <li>• Attenuate Type-1 interferon activity</li> </ul>
2	Anti-CD20	At flare	<ul style="list-style-type: none"> <li>• Deplete B-cells and prevent autoreactive plasma cell generation</li> <li>• Prevent autoreactive B-cell repopulation</li> <li>• Stop autoantibody production</li> <li>• Deplete effector B-lineage cells (B-cells, plasma cells, plasmablasts)</li> <li>• Control inflammation</li> </ul>
	Anti-BAFF/APRIL Anti-CD19	Post-peripheral B-cell depletion At flare	
3 <sup>a</sup>	Pulse methylprednisolone	At flare	<ul style="list-style-type: none"> <li>• Prevent autoreactive B-cell repopulation</li> <li>• Deplete B-cells and prevent autoreactive plasma cell generation</li> <li>• Control inflammation</li> <li>• Suppress other immune effector cells (T-cells, monocytes/macrophages)</li> <li>• Provide ongoing generalized immunosuppression</li> </ul>
	Anti-BAFF/APRIL	Post-peripheral B-cell depletion	
	Anti-CD20	At flare	
	Pulse methylprednisolone MMF/cyclophosphamide MMF	At flare At flare Maintenance	

<sup>a</sup>In Regimen 3, if the anti-CD20 agent is given as a loading regimen, that is expected to deplete peripheral B-cells and there is no further anti-CD20 dosing, we suggest MMF be given after anti-CD20 and continued for maintenance; if the anti-CD20 agent is continued for two doses beyond loading we suggest cyclophosphamide be given in between anti-CD20 doses and possibly no maintenance immunosuppression will be needed.

we are not suggesting these regimens be used off-label, but rigorously evaluated in trials.

Regimen 1 is corticosteroid-free. This protocol takes advantage of the anti-inflammatory activities of PIs to eliminate the need for high-dose corticosteroids to immediately attenuate intra-renal inflammation. This may not be sufficient, and one could consider adding a complement inhibitor with the proteasome inhibitor to help control inflammation without corticosteroids, as has been demonstrated recently in vasculitis-associated glomerulonephritis [71]. The single published study of proteasome inhibition in human LN showed a very rapid (within the first 11-day cycle of bortezomib) depletion of peripheral blood plasma cells [52], so one induction course will likely be sufficient. Anti-CD20 therapy would be started mid-proteasome course to ensure that B-cells would be depleted by the time the proteasome inhibitor was stopped, because plasma cells returned within 10 days of withdrawing bortezomib [52]. Notably, a similar regimen was tested in murine LN and was shown to effectively deplete long-lived plasma cells, delay onset of nephritis and improve overall survival [72]. After B-cells have been depleted anti-BAFF therapy could be given to prevent B-cell repletion in an environment conducive to autoreactive cell development. The duration of anti-BAFF therapy is unknown, nor is it clear whether or what type of maintenance immunosuppression would be needed; however, the CALIBRATE trial should answer these questions, as there no maintenance immunosuppression will be given in that trial.

Regimens 2 and 3 (Table 2) incorporate high-dose pulse methylprednisolone to rapidly control inflammation while at the same time depleting B-cells and plasma cells (Regimen 2). Both regimens are otherwise corticosteroid-free, and both offer the possibility of no maintenance immunosuppression. Of note, evaluating corticosteroid-free/reduced regimens in a lupus trial is challenging, possibly because of prevailing attitudes on how lupus must be treated, and the almost ubiquitous use of corticosteroids for anyone with LN. For example, the RITUXILup trial (NCT01773616) [40] inspired Regimen 3, MMF version (Table 2), but failed to enroll adequately due to difficulty in

identifying and recruiting patients that had not been exposed to corticosteroids, and was closed down.

Finally, there are other ways to target B-cells in LN. For example, Bruton's tyrosine kinase and spleen tyrosine kinase are important in BCR signaling, and inhibitors of these tyrosine kinases have shown efficacy in murine LN models. Similarly, an oral JAK2 kinase inhibitor has been shown to ameliorate experimental LN and, among other anti-inflammatory activities, also depleted autoreactive plasma cells [73]. Tyrosine kinase inhibition in glomerular disease has recently been reviewed in this journal [74].

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## CONFLICT OF INTEREST STATEMENT

All of B.H.R.'s potential COIs are managed through the Ohio State University office of research risks and protections; none of B.H.R.'s associations or positions have impacted the current manuscript.

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