LUPUS NEPHRITIS AND B CELL TARGETING THERAPY

Journal: Expert Review of Clinical Immunology

Manuscript ID: ERM-2017-0039.R1

Manuscript Type: Reviews

Keywords: Auto-antibodies, Belimumab, B-cells, Lupus, Lymphocytes, Nephritis, Rituximab
LUPUS NEPHRITIS AND B-CELLS TARGETED CELL TARGETING THERAPY
**ABSTRACT**

**Introduction:** Lupus Nephritis (LN) is a severe manifestation of Systemic Lupus Erythematosus (SLE) with a significant prognostic impact. Over a prolonged course, an exhaustion of treatment alternatives may occur and further therapeutic options are needed. B-cells play a pivotal role in disease pathogenesis and represent an attractive therapeutic target.

**Areas covered:** This review provides an update regarding targeting B-cells in LN. The rational for this approach, as well as currently available and future targets are discussed.

**Expert commentary:** Despite its wide clinical use and the encouraging results from retrospective studies, a role of rituximab in LN has not been prospectively confirmed. Trial design methodologies as well as intrinsic limitations of this approach may be responsible and rituximab use is currently limited as a rescue treatment or in settings where a strong steroid sparing effect is warranted. Despite belimumab now being licensed for use in SLE, the evidence in LN is weak although prospective trials are on-going. The combination of different targeted approaches as well as a focus on new clinical end-points may be strategies to identify new therapeutic options.

**KEY WORDS:** Auto-antibodies, Belimumab, B-cells, Lupus, Lymphocytes, Nephritis, Rituximab.
1. INTRODUCTION

Systemic Lupus Erythematosus (SLE) is a chronic autoimmune disease characterised by a wide spectrum of organ involvement and disease severity; renal involvement (lupus nephritis, LN) may occur in up to 40% of SLE patients (1).

SLE glomerulonephritis is the hallmark of the immune complex disease and it is the most frequent severe manifestation of LN. Among all autoantibodies, anti-dsDNA plays a central role; they are in fact the most represented among the ones eluted from kidney biopsies (2) and may act as clinical biomarker (3). Despite this, they account for only 10-20% of the total auto-antibodies (2), with several others likely to play an important role including anti-C1q (4).

As precursors of autoantibody producing cells, B cells are considered central in LN pathogenesis. However, this is only one of their several roles as suggested by the observation of the lack of development of LN in murine models knocked out for mutant B cells despite the presence of autoantibodies unable to secrete immunoglobulins while still expressing them on the surface (5); interaction with T-cells and cytokine production are other essential activities (6, 7). Interstitial nephritis is common in LN as unique manifestation of renal disease or in association with glomerulonephritis; interestingly SLE interstitial nephritis is usually characterized by an infiltrate rich in T and B cells sometimes organized in aggregates (8, 9) or even germinal center-like structures with evidence of local autoantibody production (9). Tubulointerstitial inflammation is particularly relevant in defining long-term prognosis of LN patients and is correlated with the risk of end-stage renal disease (10). The prompt diagnosis and treatment of LN has a positive impact on survival as well as on risk of end stage renal disease, however its chronic and relapsing course poses several challenges to the overall management. Induction and maintenance of remission are key phases of the staged approach to LN and the identification of the right balance between disease activity, organ damage and degree of immunosuppression is central (11, 12). Corticosteroids alone are not sufficient in the treatment of LN and the association with immunosuppressive drugs is therefore necessary (13); although long term efficacy is well established for cyclophosphamide based regimen, mycophenolate mofetil has shown similar effectiveness at least in short and medium term trials with a more favorable adverse event profile (14).

The availability of biologics with molecular targets is therefore of interest and among several possible targets, the B cell compartment has strong biological rationale. However, evidence supporting the clinical use of available B cell therapies in LN is lacking.

In this paper we discuss the rationale, the current role as well as future prospective of a B cell targeted approach in LN.
2. ANTI-CD20 APPROACH AND RITUXIMAB

CD20 is a B-cell membrane specific antigen involved in B-cell differentiation as well as B-T cell stimulation. This antigen is broadly expressed by every lineage of B cells with the exception of pro-B lymphocytes and plasma cells (13).

On the basis of the central role of B cells in SLE pathogenesis and of the results of the effectiveness of this approach in other autoantibody mediated diseases (14-15, 16, 17), anti-CD20 antibodies have been employed in SLE.

Rituximab (RTX) is a chimeric anti-CD20 monoclonal antibody that was approved in 1997 for the treatment of Non-Hodgkin Lymphoma with its use progressively extended to different autoimmune diseases, such as rheumatoid arthritis and ANCA-associated vasculitis. RTX is a type I monoclonal antibody and its action relies on the ability of redistributing CD20 into lipid rafts and the development of antigen-antibody complexes required to induce CD20+ B-cells death through three mechanisms: complement dependent cytotoxicity (CDC), antibody dependent cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP) (16, 18, 19). A further possible mechanism has been described, that involves direct cell death through activation of pro-apoptotic intracellular signaling although this mechanism seems more relevant for type II anti-CD20 antibodies that act without redistributing CD20 into lipid rafts (17, 18, 20, 21). The first retrospective study published in 2002 by David Isenberg’s group involved six patients with SLE and RTX was co-administered with cyclophosphamide: a reduction of the disease activity score used (British Isles Lupus Assessment Group, BILAG) from 14 (range 9-27) to 6 (range 3-8) was observed as well as improvement of some biomarkers (overall increase of the C3 levels, in some patients reduction of the anti-dsDNA levels); in two patients affected by LN a reduction of the protein-to-creatinine ratio was also described (19). After this study, six retrospective and prospective surveys including more than fifty patients exploring the effectiveness of RTX in SLE and LN were identified (20-25, 23, 26) (Table 1). In all the studies RTX showed overall efficacy in disease control and, where explored, in allowing steroid sparing. The renal response was typically positive and the adverse event rate was relatively low, especially in the context of a population mainly characterized by a relapsing course of the disease and exposure to several immunosuppressive drugs. Only one study (23) reported the outcome of four patients treated with pre-emptive multiple administrations of RTX; interestingly, no flares were observed in this
population during a follow-up of 22 ± 9 months. A larger cohort of LN patients with relapsing or refractory disease, were studied by pooled data analyses of 164 patients (99 obtained via the UK-BIOGEAS registry and 65 via the review of other original articles already published) with LN treated with RTX: in keeping with the reports discussed in Table 1, a positive response rate was observed in 67% of the population at 6 and 12 months with improvements in proteinuria (4.41 g baseline vs 1.31 g post therapy, p=0.006) and serum albumin level (28.55 g baseline vs 36.46 g post therapy, p<0.001) (26,29).

The good results observed in the retrospective reports warranted prospective randomized controlled trials (RCTs) for confirmation. Two main RCTs have been published in 2010 and 2012: EXPLORER and LUNAR with only the latter including patients with LN (27,28,30, 31). Surprisingly, both failed the primary outcome (29). Table 2 summarizes the main characteristics of these studies (32). Trials designs issues may have contributed to the negative results, especially the use of a background corticosteroid and immunosuppressive therapy in both arms, the selection of non-refractory cohorts, relatively short follow-up time, patients’ selection and no inclusion of the corticosteroid dose tapering among the parameters to assess the response to therapy. Despite the failure to demonstrate clinical benefit, both trials found RTX effective in reducing anti-dsDNA levels and improving complement levels.

Despite the positive results of the retrospective studies and continuing physician and patient interest in the use of RTX, B cell depletion is currently only recommended as a rescue treatment for patients that are refractory to first and second line induction-maintenance approaches or in particular settings where a strong steroid sparing approach may be required (25,28,30,33).

2.1 Main limitations of B-cells cell depletion therapy with Rituximab.

The lack of clear evidences supporting the use of RTX in SLE and LN may not only be due to issues related to trials design but also to intrinsic limitations of a B-cells cell depletion approach through anti-CD20 antibodies as well as intrinsic SLE limitations.

SLE has a huge variability in terms of clinical profiles and a B-cells cell depletion approach might be effective only in specific subsets of the disease yet to be identified. RTX has typically been given in addition to an immunosuppressive in LN trials, most commonly mycophenolate mofetil. The BELONG trial (34) tested ocrelizumab in addition to either mycophenolate or cyclophosphamide and a larger treatment effect was noted on a cyclophosphamide background, in part due to a lower placebo response rate with cyclophosphamide. There are theoretical advantages to the
combination of RTX with both mycophenolate or cyclophosphamide by targeting B cells at different levels.

Interestingly, the combination of RTX and cyclophosphamide has shown in small studies positive results (35); although the safety profile does not allow a prolonged cyclophosphamide administration, the combination of these drugs at least in some stages might lead in subgroups of patients to advantages in the disease control.

Moreover, B-cell depletion is not selective leading to the targeting also of subsets that may be of help in controlling autoimmunity (such as regulatory B cells, B-regs) sparing at the same time other subsets able to produce pathogenic autoantibodies (namely plasma cells/plasmacells).

Several studies focused on the use of RTX in autoimmune diseases have shown that the longer duration of B-cell depletion achieved, the better is the clinical response to the drug (3136); however despite also in SLE a deep depletion has been found as associated to clinical response (37), the proportion of patients achieving B-cells depletion after RTX is lower and the timing of B-cell repopulation is quicker (32)/(38). Several factors have been considered responsible, such as, the presence or development of human anti-chimeric antibodies (HACAs) (33, 3439, 40) as well as differences in terms of RTX clearance (3541) as a consequence of a different immunoglobulin metabolism.

It should also be considered that, although we usually assess B-cell depletion in the circulating compartment, this might not reflect what is happening in inflamed tissues. Tissue resident CD20+ B cells may be more resistant to RTX (3642) as a consequence of a low tissue penetration of the drug or local survival factors including cell to cell contact and B cell stimulating cytokines; interestingly it has also been described in ABO-incompatible kidney transplant patients who received a single RTX infusion, that the proportion of CD19+ B cells in lymph nodes did not differ to the ones of untreated controls and their phenotype was more often compatible with that of switched memory B cells (lgD-CD27+), the phenotype most represented in peripheral blood of active SLE and not detectable in SLE patients in remission once treated (37, 3843, 44).

Moreover, as already discussed, RTX is a type I monoclonal antibody and its action involves the gathering of antibody-antigen complexes into lipid rafts of the cells: this facilitates ADCC and CDCC, but induces internalization and inactivation of the drug (30, 4045, 46). Internalization may also be consequence of other factors such as the surface Fcgamma receptor (FcgR) IIb (41, 4247, 48).

Many other factors have been proposed to play a role such as low complement levels and the presence of anti-C1q antibodies as factors impacting negatively on CDCC; moreover, the genetically determined variability of FcgR affinity for the Fc portions of antibodies (42, 4449, 50) as well as hypergammaglobulinemia might cause an alteration of the phagocytosis/ADCC through low affinity for ligand/ligand competition (1220).
Newer anti CD20 antibodies include the fully humanized type I (ocrelizumab and ofatumumab) or type II antibodies (obinutuzumab). For ocrelizumab a randomized controlled trial in LN (BELONG) was suspended due to excessive adverse events (4534) although the drug seems to be effective especially in cases refractory to RTX (51), whereas for ofatumumab there is a limited, although positive, clinical experience on five patients with SLE and LN (46,4752, 53) with further data required before its use may be considered in routine clinical practice. Regarding obinutuzumab (42)(20), a randomized control trial is now running and it will provide important information regarding the feasibility and safety of its use in SLE (NCT02550652). Of note, both LUNAR with RTX and BELONG with ocrelizumab found similar increases in renal response rates of 11-12% when compared to placebo (4534). These were lower than the predicted response rates to show statistical superiority but of a similar order to that found in the belimumab trials, although using a different end-point. The latter studies had very much larger sample sizes and consequently met their end-points and were regarded as positive studies.

3. MODULATING B-CELL SURVIVAL

A suitable alternative to B-cells cell depletion may be the modulation of B-cells cell survival. In SLE B-cells are characterized by a longer lifespan, an anti-apoptotic molecular profile as well as a lower threshold for activation compared to healthy patients (48)(54). Acting on factors inducing these characteristics with the aim of restoring a physiological profile may improve disease control.

A central role in driving SLE B-cell hyperactivity is accounted by two members of the Tumor Necrosis Factor (TNF) ligand superfamily (TNFSF13): the B-cell Activating Factor (BAFF, also known as Blys or TNFSF13B) and A Proliferation-Inducing Ligand (APRIL).

BAFF and APRIL actions are mediated via three receptors: the Transmembrane Activator and Calcium-Modulator and Cytophilin Ligand Interactor (TACI), the B-cell Maturation Antigen (BCMA) and the B-cell cell Activating Factor Receptor (BR3) (4955).

B-cells at different maturation stages express different receptors with TACI mainly promoting T-cell independent antibody responses as well as B-cell regulation and immunoglobulin isotype switching, BCMA mainly promotes plasma-cell survival and BR3 accounts for immature B-cell survival and maturation.(5056).

The rationale for targeting these pathways is provided by the observation that both BAFF and APRIL levels are increased in SLE patients (51-5357-59), moreover, mice models characterized by BAFF over-expression develop an SLE-like disease (5460). Furthermore, BAFF levels have been consistently described as increased after B-cell depletion via
anti CD20 approaches (55, 56, 61, 62) and it has been described as higher in SLE patients experiencing a relapse after RTX administration when compared to patients who maintain disease remission (55, 61); this has been also leading to the hypothesis that BAFF may play a role, at least of some degree, in limiting the effectiveness of B-cell depletion. Whether the BAFF increase reflects simply the loss of BAFF receptors, only found on B cells, or implies a disease stimulating effect of RTX is unclear and there is also evidence that a high BAFF level influences the returning B cell repertoire towards a more autoreactive phenotype (57, 63).

Since the early 2000s drugs targeting BAFF and APRIL have been studied in SLE. Atacicept is a recombinant fusion protein including the extracellular domain of the TACI receptor joined to a human IgG1 Fc domain (58, 64) that has been tested in moderate-severe SLE in a phase II trial, this study was terminated prematurely due to safety concerns (59, 60, 65, 66). Efficacy was observed in the arm treated with the higher dose of the drug tested (150 mg-arm) although the same group experienced two fatal infections. The ongoing ADDRESS II trial (NCT02070978) might be able to shed more light on the possible role of this drug. A LN trial was terminated due to the occurrence of hypogammaglobulinaemia and infection in atacicept treated patients, when administered in addition to mycophenolate mofetil and high dose corticosteroid (61, 67).

More evidence are available for the anti-BAFF humanized monoclonal antibody belimumab: BLISS-52 and BLISS-76 are two large phase III multinational trials, which showed efficacy for belimumab in SLE in reducing disease activity as defined by the SLE responder index, a composite of disease activity scores (Table 3) (62, 63, 68, 69); these data found a favorable long-term safety profile (64) and resulted in this monoclonal antibody being the first biological drug approved for the use in SLE in several countries. However, the evidence for its efficacy is at the moment mainly limited to musculoskeletal and mucocutaneous subsets of the disease and it is still uncertain whether it may have a role also in LN (65, 71). Interestingly a post-hoc analysis of the pooled cohorts of the BLISS trial showed a signal toward a potential role for belimumab in improving some renal outcomes with the limitation of both trials excluding patients with acute renal activity at the moment of the enrollment (66, 72). Retrospective case reports seem to support the hypothesis for a role of belimumab in LN (62, 68, 73, 74), however it will be the BLISS-LN study (NCT01639339), a phase III randomized control trial, the one able to confirm this hypothesis.

Two other anti-BAFF drugs have shown signals for a potential role in SLE: the monoclonal antibody Tabalumab and the fusion protein Blisibimod (75, 76). Despite a signal for the latter in terms of proteinuria reduction, both have now been abandoned and no further studies are ongoing at the moment. Tabalumab efficacy has been explored in two phase III randomized controlled trials, ILLUMINATE-1 and ILLUMINATE-2.
(68, 70) showing a signal for efficacy only in the latter, although limited to the group of patients receiving the drug at the higher dose (120 mg every 2 weeks). A post-hoc analysis of these two studies failed in identifying any effect on renal outcomes (71).

Blisibimod appears to be a candidate of great interest: it is a fusion protein characterized by high potency (72) with the highest affinity among the group of BAFF inhibitors (73). Moreover, it has a long serum half-life (8-10 days) and binds BAFF in both its form (soluble and already membrane-bound) (73).

Blisibimod efficacy has been tested in a phase 2b study (PEARL-SC), conducted on 547 SLE patients with a SELENA/SLEDAI score >6 where a profile for better response compared to placebo was identified in the group treated with the higher dose of the drug; interestingly a significant variation in terms of proteinuria compared to placebo was observed (74). A phase III trial (CHABLIS-SC1, NCT01395745) terminated early due to a lack of efficacy and further development of blisibimod for SLE abandoned.

4. OTHER B-CELL TARGETED THERAPY

4.1 Modulating B-cells cell response

B-cell receptor (BCR) signalling, as well as its intracellular transduction via second messengers, is increased in SLE (75, 76-77, 78) representing therefore a target of potential interest.

The inhibitory transmembrane protein CD22 is a member of the immunoglobulin superfamily that recognizes sialic acid-containing ligands; it is expressed on different lineage of B-cells with the exception of plasma-blasts and plasma-cells (77, 79). Its mechanism of action is not fully understood but for sure it is active at different levels: following BCR stimulation, CD22 gets activated through a phosphorylation process mediated by Lyn, a tyrosine kinase induced by BCR itself, in a feed-forward cycle (78, 80). Once active, CD22 induces intracellular signalling resulting eventually in BCR dephosphorylation and inhibition (78, 81). Interestingly, CD22 mechanisms of action appear to be various including indirect interaction with other intracellular signalling pathways such as the ones mediated by CD40 and Toll-like Receptors as well as clustering with the BCR component CD79a (80, 82). CD22 is therefore central in setting the B-cell threshold of activation.

Epratuzumab is a humanised anti-CD22 monoclonal antibody that acts by inducing CD22 phosphorylation as well and shifting into lipid rafts, this reduces thus reducing intracellular BCR signalling. Internalisation of CD22, and therefore also of the BCR component CD79a, is another proposed mechanism of action of epratuzumab (81) as well as a partial B-cells depletion involving mainly the subset of naive B-cells (82).
Effectiveness for this drug in SLE has been proposed from two phase II trials (ALLEViate 1 and 2) but efficacy has not been confirmed according to the preliminary results of two still unpublished phase III randomised control trials (EMBODY-1 and 2) and the drug has been at the moment abandoned. Despite that, CD22 remains a target of great interest that may be considered for future therapeutic approaches.

4.2 Targeting Intracellular pathways

One of the main limitations of aiming for cytokines, or their receptors, is the presence of physiological mechanisms acting at the limitation of the “single targets approaches” such as the redundancy of the stimulus as well as the molecules’ internalization once bound to their cellular surface targets. Acting directly on the intracellular signals, and therefore on the downstream part of the activation cascade, might therefore represents a way to avoid the downsizes of the single target approach.

The Bruton’s tyrosine kinase (BTK) is a key member of the BCR and FcgR signaling cascade, inducing the stimulation of B-cell survival, differentiation as well as setting the activation threshold and inducing the antibody production. BTK blocking has been explored in several SLE mouse models with signals of effectiveness in terms of preventing and treating renal disease.

The Spleen Tyrosine Kinase (SYK) is a kinase directly activated by the Immunoreceptor tyrosine-based activation motif (ITAM) sequence of BCR co-receptors as well as of other immune and non-immune related receptors such as the T-cell receptor (TCR) and FcgR. SYK has a central pathogenic role in different autoimmune diseases and malignancies as well as in glomerulonephritis. A retrospective study on 120 kidney biopsies of patients with glomerulonephritis showed that SYK expression was increased in proliferative and crescentic glomerulonephritis and correlates with the presenting serum creatinine as well as histological signs of disease activity; interestingly, in the subgroup of LN, SYK showed also a role as biomarker being higher in patients that achieved complete remission at 6 months. SYK inhibitors, such as, fostamatinib, seem an attractive and available option for autoimmune diseases in general and for kidney involvement of autoimmune diseases in particular. A role in LN has been shown in pre-clinical studies on mice; interestingly, the use of this drug was not associated to reductions of anti-dsDNA titers confirming a downstream mechanism of action. Data regarding fostamatinib in human SLE are not available although the encouraging results in terms of potential efficacy and safety in rheumatoid arthritis suggest that this is a treatment worth exploring.

The Janus kinase-Signal Transducer and Activator of Transcription (JAK-STAT) path has been recently tested in SLE and

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LN in two studies. There are four members of the JAK family and this path is particularly active in cytokine receptor signaling influencing DNA transcription and protein synthesis. The first study explored CP690550 showing in murine models improvement of proteinuria, renal function as well as histological lesions of the kidneys; interestingly a reduction of glomerular deposition of anti-dsDNA, of C3 and IgG was observed as well as a reduction in kidney gene expression of inflammatory cytokines (10199). Another potential candidate acting on the JAK-STAT cascade is Tofacitinib characterized by promising results in terms of improvement of the nephritis in mice models (102100).

4.3 Modulating B-T cell interactions

T-cells play a detrimental role in driving and boosting autoimmune responses. Several reports have described dysfunction of the T cell compartment in SLE mainly involving Follicular Helper T cells (Tfh), Regulatory T cells and Th17 (103).

T cells play a detrimental role in driving and boosting autoimmune responses. Several reports have described dysfunction of the T cell compartment in SLE mainly involving Follicular Helper T cells (Tfh), Regulatory T cells and Th17 (103).

Tfh are attractive targets having a central role in conditioning germinal centers, in facilitating expansion of autoreactive clones and in stimulating differentiation of B cells into plasmablasts and plasma cells (104). Tfh actions are mediated via cytokine secretion as well as via B-T cell interactions; the latter is allowed through several co-receptors, such as CD40-CD40L (CD154), OX40-OX40L, ICOS-ICOSL, CTLA4/CD28-CD80/CD86.

CD40L is an important co-stimulatory molecule that is up-regulated in SLE, with higher levels of its soluble form described as well in this disease (103,105,102), and seem to correlate with disease activity in mice models (108,109,106,107); moreover, a single nucleotide polymorphisms (SNP) of CD40 have been associate with disease susceptibility (110). The inhibition of the CD40-CD40L interaction seems a reasonable target and has proven to be effective in preventing disease in murine models (111,112,109,110). A phase II trial of a CD40L inhibitor (BG9588) in lupus nephritis showed a signal in terms of immunological benefit in the patients treated (reduction of the anti-dsDNA antibodies, increase of C3 concentrations, reduction of the hematuria); however, the trial was stopped due to safety concerns related to a high incidence of thromboembolic events (111). This was caused by the occurrence of CD40L on platelets with Fc mediated platelet cross-linking caused by the therapeutic antibody. Encouraging results in terms of safety have now been described with a PEGylated anti-CD40L Fab’ fragment (CDP7657) from a phase I study (114,115,112,113) suggesting that inhibition of this pathway remains a possible therapeutic target in SLE. An alternative is
to target CD40 and this is being pursued by BI655064, an anti-CD40 monoclonal antibody in a Phase II LN trial (NCT02770170).

CTLA4 is a surface molecule with inhibitory functions competing with the activating surface protein CD28 for the binding of CD80/86. A CTLA4-Ig (abatacept) has shown efficacy in patients with non-severe granulomatosis with polyangiitis (GPA) (116-114). The rationale for targeting this pathway in SLE has been developed from mice models where a CTLA4-Ig approach displayed effectiveness in preventing disease onset (117-115) or in reducing signs of activity in LN when used in combination with cyclophosphamide (118). However, a phase IIb randomized controlled trial in non-severe SLE patients (119) and a phase II/III trial in 398 patients with LN failed in meeting the primary end-points although a benefit in terms of reduction of anti-dsDNA titre, improvement of C3 and C4 levels as well as proteinuria was observed (120). More recently no beneficial effects of abatacept were found in the ACCESS trial when added to a background of cyclophosphamide and corticosteroids although a greater effect of abatacept despite effectiveness on anti-dsDNA and complement levels was seen compared with placebo, no beneficial clinical effect has been so far shown (121).

4.4 Proteasome inhibitors

Proteasome inhibitors are an emerging class of drugs targeting mainly plasma-cells. Among proteasome inhibitors, bortezomib is the first one employed in clinical practice with indication at the moment for multiple myeloma. The mechanism of actions of this class of drugs is the inactivation of the Nuclear Factor kappa-light chain enhancer of activated B-cells (NfkB) process eventually able to induce apoptosis (122). Since the high rate of protein synthesis typical of plasma-cells, these are very sensitive to the effects of this drug. For proteasome inhibitors also a role in reducing the interferon (IFN) path activation as well as IFN production has been proposed as further mechanism of action (123).

Due to encouraging results for the use of bortezomib in mice models of SLE and LN (124-126) this drug has been tested also in humans in a study involving 12 patients with benefits in terms of reduction of the autoantibody-producing plasma cells, IFN pathway activation and, more significantly, also in terms of clinical activity of the disease (127). The use of bortezomib was however associated to a reduction of protective IgG levels and necessity of withdrawing the treatment in seven patients due to adverse events. Interestingly, in murine models a quick reconstitution of the plasma cells pool have been observed once the treatment is stopped (128) and a role for co-administration of B-cells depleting treatment has been proposed as strategy for delaying plasma-cell
reconstitution after depletion (128124). An oral proteasome inhibitor, ixasomib with a more favourable safety profile is in clinical trials in LN (NCT02176486).

5. CONCLUSIONS

SLE and LN are chronic diseases that often feature a long disease course with large variability in disease extent and severity. First and second line approaches are not infrequently exhausted in a single patient clinical history and newer options are required for disease management. The toxicity of current regimens and their associated serious adverse event rates directly worsen patient outcomes and safer therapies allowing lower corticosteroid dosing are needed.

The targeting of B-cell is supported by our understanding of pathogenesis, and RTX and belimumab have entered routine use in carefully defined scenarios where standard therapies are failing. The uptake of RTX has affected by the imbalance between positive retrospective cohort studies in largely refractory patients, and the negative results of the two randomised trials. Belimumab has obtained registration for use in SLE although the signals for its utility in LN are still weak and its role at the moment is limited to non-renal SLE; more information will be provided after a prospective trial in LN now ongoing (NCT01639339).

Several targets of the B-cell biology have been identified and for most of them, despite good preliminary results in phase II trials and pre-clinical models, no confirmation has been so far observed in phase III studies.

6. EXPERT COMMENTARY

SLE is a disease with a complex and still partially unknown biology. The success of the conventional immunosuppressive approaches may be due to their low specificity characterized by a widespread effect on the immune system; more selective approaches, although potentially of great interest and safer, may allow only partial benefits due to the lack of inhibition of alternative pathways not known. Based on this rationale, combining different biologics may allow an increase in terms of efficacy; however, such approaches need to be tested carefully considering concerns in terms of safety. The most obvious approach at the current stage, would be the combination use of RTX and belimumab: B-cell depletion via RTX is in fact characterized by a rebound of BAFF levels which might explain, at least in some settings, the lack of efficacy. Preliminary results of subsequent therapy with RTX and belimumab are encouraging (129, 130, 125, 126) and prospective trials are now on-going (Table 4). Furthermore, RTX B-cell killing will be potentiated by BAFF blockade. Two trials are studying this approach, CALIBRATE IN LN, and BEAT-LUPUS in non-renal SLE (Table 4).
Another issue related to RTX, although potentially characteristic of other biologics as well, is that the lack of efficacy may be a consequence of the mode of administration. We know from experience in other autoimmune diseases that retreatment with RTX may improve remission rates as well as prolonging the time to relapse when compared to a single course of the drug although, eventually, at the drug withdrawn the relapse rate remains high (131127).

Moreover, alternative trial designs or the definition of new end-points may facilitate demonstration of drug effectiveness: on average the studies published so far are designed to show a superiority or a non-inferiority of a tested drug compared to the standard of treatment, usually with an add-on philosophy to what considered the standard of care. However, alternative end-points may be of interest such as benefits in terms of tolerability, safety and adverse events rate of a new approach compared to standard of treatment despite similar efficacy. The ongoing RITUXILUP (Table 4) (NCT01773616) in diseases characterized by a chronic relapsing course, frequent treatment changes or dose adjustments are required. In this context the efficacy of a new drug may not necessarily be shown only via a greater clinical benefit when added to the standard of care. Of note, clinical trials are usually characterized by a relatively short follow-up and an intervention that is lacking impact during the explored time span might however unveil significant outcomes in the long term. In the everyday clinical life the standard of care may sometimes not be employed, not tolerated at full dose, refused by the patient or even ineffective. The design of classical clinical trials does not take into account this flexibility and may lead to the abandon of potentially useful drugs. Alternative end-points may be taken into account in order to provide the clinical community with as many options as possible. We should therefore seek for non classical clinical trial designs able to overcome these limitations: examples of alternative strategies might include aiming at showing the ability of a new add-on treatment in allowing a quicker and aggressive drug sparing effect; a better clinical and safety profile in contexts where an optimal dose of the standard of care may not be employed; a better safety or burden of damage in longer follow-up time. Studies with different end-points may be characterized by more difficulties in terms of recruitment and may benefit from the use of alternative strategies of enrollment or treatment allocation including for example adaptive approaches. An example might be the ongoing RITUXILUP (Table 4) (NCT01773616) that is exploring whether the combination of RTX and mofetil mycophenolate (MMF) may allow sparing steroids.

SLE is a complex disease and LN is its most severe manifestation. Research aimed at the identification of new therapeutic targets or biomarkers able to perform effective patient stratification is a high priority task in the research agenda in order to increase our therapeutic options. Moreover, alternative trials design should be developed in order to identify, among all the possible therapeutic targets, the ones that may have a role in the management of this
autoimmune disease.

7. FIVE YEARS VIEW

Despite significant research efforts in the field of pathogenesis and drug development, no major changes have occurred in the management of LN. We do know that MMF and cyclophosphamide appear equal alternatives as first line approaches as induction (132128) and that azathioprine, MMF, and in some settings, methotrexate may be useful for the maintenance stage. Steroids are the cornerstones of the treatment, playing a central role in all phases as well as for the management of minor systemic flares. In this picture is now relatively well established the use of RTX as third line agent for both relapsing-refractory SLE and LN. Moreover belimumab is now approved for the management of SLE with its use mainly limited at the moment to the mucocutaneous and articular manifestations.

The studies published so far taught us that the heterogeneity of SLE manifestation and population, as well as the complexity of the disease, may allow difficulty in the identification of a “superiority” or of a “non-inferiority” profile for new drugs when compared to an optimal standard of care. We feel that in the next years the trial designs will switch to the identification of different end-points such as tolerability, safety and ability of a drug sparing effect for the treatment tested.

The future will probably see also testing different combination of biologics and targeted therapy in order to allow a potential increase in efficacy of the intervention proposed. This will need to happen under strict monitoring from the safety point of view.

Beside B-cells, very promising are the preliminary results of targeting the interferon pathway (133, 134129, 130) but we do feel there may be in the short-term more interesting therapeutic options. Complement plays a central role in SLE and LN pathogenesis and several complement blockade molecules are now available (135131). However, no trials are on-going to test effectiveness of this approach in SLE. Interestingly, complement is also detrimental in mediating the action of several monoclonal antibodies and will be of great interest to see the consequences of combining anti complement drugs and other monoclonal antibodies.

8. KEY ISSUES

- B-cells play a central role in the pathogenesis of SLE and LN therefore representing a therapeutic target of interest.
- Rituximab is an anti-CD20 monoclonal antibody for which retrospective studies and clinical practice have
shown efficacy in SLE and LN; prospective trials failed in confirming this although trial design issues, intrinsic limitation of both prospective studies in SLE and of an anti-CD20 approach may have had a role in these negative findings.

- Belimumab is an anti BAFF monoclonal antibody registered for the use in SLE in several countries although evidences of its effectiveness in LN are still lacking; prospective trials are now on-going and will shed more light on this topic.

- Several other targets have been so far identified and tested at different levels of pre-clinical and clinical development; the more interesting are the SYK inhibitors and the anti-proteasome respectively for the central role of SYK in the renal manifestations of immunological diseases and for the ability of anti-proteasome of targeting plasma-cells and therefore the main producers of auto-antibodies.

- Combination of different targeted drugs as well as the identification of new trial designs and end-points are going to be key aspects of the clinical research agenda in the field of SLE and LN in the near future.
REFERENCES

** Randomized controlled trial exploring the use of rituximab in SLE: the negative results may be partly due to trial design issues and not necessarily to lack of effectiveness of the drug


** Randomized controlled trial exploring the use of rituximab in LN: the negative results may be partly due to trial design issues and not necessarily to lack of effectiveness of the drug


** Randomized controlled trial showing effectiveness of belimumab in SLE patients with mainly musculoskeletal and mucocutaneous disease.


** Randomized controlled trial showing effectiveness of belimumab in SLE patients with mainly musculoskeletal and mucocutaneous disease.


This study shows the possible role of SYK as biomarker in LN providing the rational as therapeutic target.


Mohan C, Shi Y, Laman JD, Datta SK. Interaction between CD40 and its ligand gp39 in the development of


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LUPUS NEPHRITIS AND B CELL TARGETING THERAPY
ABSTRACT

Introduction: Lupus Nephritis (LN) is a severe manifestation of Systemic Lupus Erythematosus (SLE) with a significant prognostic impact. Over a prolonged course, an exhaustion of treatment alternatives may occur and further therapeutic options are needed. B cells play a pivotal role in disease pathogenesis and represent an attractive therapeutic target.

Areas covered: This review provides an update regarding targeting B cell in LN. The rational for this approach, as well as currently available and future targets are discussed.

Expert commentary: Despite its wide clinical use and the encouraging results from retrospective studies, a role of rituximab in LN has not been prospectively confirmed. Trial design methodologies as well as intrinsic limitations of this approach may be responsible and rituximab use is currently limited as a rescue treatment or in settings where a strong steroid sparing effect is warranted. Despite belimumab now being licensed for use in SLE, the evidence in LN is weak although prospective trials are on-going. The combination of different targeted approaches as well as a focus on new clinical end-points may be strategies to identify new therapeutic options.

KEY WORDS:

Auto-antibodies, Belimumab, B cell, Lupus, Lymphocytes, Nephritis, Rituximab.
1. INTRODUCTION

Systemic Lupus Erythematosus (SLE) is a chronic autoimmune disease characterised by a wide spectrum of organ involvement and disease severity; renal involvement (lupus nephritis, LN) may occur in up to 40% of SLE patients (1).

SLE glomerulonephritis is the hallmark of the immune complex disease and it is the most frequent severe manifestation of LN. Among all autoantibodies, anti-dsDNA plays a central role; they are in fact the most represented among the ones eluted from kidney biopsies (2) and may act as clinical biomarker (3). Despite this, they account for only 10-20% of the total auto-antibodies (2), with several others likely to play an important role including anti-C1q (4).

As precursors of autoantibody producing cells, B cells are considered central in LN pathogenesis. However, this is only one of their several roles as suggested by the development of LN in a murine model carrying mutant B cells unable to secrete immunoglobulins while still expressing them on the surface (5); interaction with T-cells and cytokine production are other essential activities (6, 7). Interstitial nephritis is common in LN as unique manifestation of renal disease or in association with glomerulonephritis; interestingly SLE interstitial nephritis is usually characterized by an infiltrate rich in T and B cells sometimes organized in aggregates (8, 9) or even germinal center-like structures with evidence of local autoantibody production (9). Tubulointerstitial inflammation is particularly relevant in defining long-term prognosis of LN patients and is correlated with the risk of end-stage renal disease (10). The prompt diagnosis and treatment of LN has a positive impact on survival as well as on risk of end stage renal disease, however its chronic and relapsing course poses several challenges to the overall management. Induction and maintenance of remission are key phases of the staged approach to LN and the identification of the right balance between disease activity, organ damage and degree of immunosuppression is central (11, 12). Corticosteroids alone are not sufficient in the treatment of LN and the association with immunosuppressive drugs is therefore necessary (13); although long term efficacy is well established for cyclophosphamide based regimen, mycophenolate mofetil has shown similar effectiveness at least in short and medium term trials with a more favorable adverse event profile (14).

The availability of biologics with molecular targets is therefore of interest and among several possible targets, the B cell compartment has strong biological rationale. However, evidence supporting the clinical use of available B cell therapies in LN is lacking.

In this paper we discuss the rationale, the current role as well as future prospective of a B cell targeted approach in LN.

2. ANTI-CD20 APPROACH AND RITUXIMAB
CD20 is a B cell membrane specific antigen involved in B cell differentiation as well as B – T-cell stimulation. This antigen is broadly expressed by every lineage of B cells with the exception of pro-B lymphocytes and plasmacells (15). On the basis of the central role of B cells in SLE pathogenesis and of the results of the effectiveness of this approach in other autoantibody mediated diseases (16, 17), anti-CD20 antibodies have been employed in SLE.

Rituximab (RTX) is a chimeric anti-CD20 monoclonal antibody that was approved in 1997 for the treatment of Non-Hodgkin Lymphoma with its use progressively extended to different autoimmune diseases, such as rheumatoid arthritis and ANCA-associated vasculitis. RTX is a type I monoclonal antibody and its action relies on the ability of redistributing CD20 into lipid rafts and the development of antigen-antibody complexes required to induce CD20+ B cell death through three mechanisms: complement dependent cytotoxicity (CDC), antibody dependent cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP) (18, 19). A further possible mechanism has been described, that involves direct cell death through activation of pro-apoptotic intracellular signaling although this mechanism seems more relevant for type II anti-CD20 antibodies that act without redistributing CD20 into lipid rafts (20, 21). The first retrospective study published in 2002 by David Isenberg’s group involved six patients with SLE and RTX was co-administered with cyclophosphamide: a reduction of the disease activity score used (British Isles Lupus Assessment Group, BILAG) from 14 (range 9-27) to 6 (range 3-8) was observed as well as improvement of some biomarkers (overall increase of the C3 levels, in some patients reduction of the anti-dsDNA levels); in two patients affected by LN a reduction of the protein-to-creatinine ratio was also described (22). After this study, six retrospective and prospective surveys including more than fifty patients exploring the effectiveness of RTX in SLE and LN were identified (23-28) (Table 1). In all the studies RTX showed overall efficacy in disease control and, where explored, in allowing steroid sparing. The renal response was typically positive and the adverse event rate was relatively low, especially in the context of a population mainly characterized by a relapsing course of the disease and exposure to several immunosuppressive drugs. Only one study (26) reported the outcome of four patients treated with pre-emptive multiple administrations of RTX; interestingly, no flares were observed in this population during a follow-up of 22 ± 9 months. A larger cohort of LN patients with relapsing or refractory disease, were studied by pooled data analyses of 164 patients (99 obtained via the UK-BIOGEAS registry and 65 via the review of other original articles already published) with LN treated with RTX: in keeping with the reports discussed in Table 1 a positive response rate was observed in 67% of the population at 6 and 12 months with improvements in proteinuria (4.41 g baseline vs 1.31 g post therapy, p=0.006) and serum albumin level (28.55 g baseline vs 36.46 g post therapy, p<0.001) (29).

The good results observed in the retrospective reports warranted prospective randomized controlled trials (RCTs) for...
confirmation. Two main RCTs have been published in 2010 and 2012: EXPLORER and LUNAR with only the latter including patients with LN (30, 31). Surprisingly, both failed the primary outcome (32); Table 2 summarizes the main characteristics of these studies. Trials designs issues may have contributed to the negative results, especially the use of a background corticosteroid and immunosuppressive therapy in both arms, the selection of non-refractory cohorts, relatively short follow-up time, patients’ selection and no inclusion of the corticosteroid dose tapering among the parameters to assess the response to therapy. Despite the failure to demonstrate clinical benefit, both trials found RTX effective in reducing anti-dsDNA levels and improving complement levels.

Despite the positive results of the retrospective studies and continuing physician and patient interest in the use of RTX, B cell depletion is currently only recommended as a rescue treatment for patients that are refractory to first and second line induction-maintenance approaches or in particular settings where a strong steroid sparing approach may be required (28, 33).

2.1 Main limitations of B cell depletion therapy with Rituximab.

The lack of clear evidences supporting the use of RTX in SLE and LN may not only be due to issues related to trials design but also to intrinsic limitations of a B cell depletion approach through anti-CD20 antibodies as well as intrinsic SLE limitations.

SLE has a huge variability in terms of clinical profiles and a B cell depletion approach might be effective only in specific subsets of the disease yet to be identified. RTX has typically been given in addition to an immunosuppressive in LN trials, most commonly mycophenolate mofetil. The BELONG trial (34) tested ocrelizumab in addition to either mycophenolate or cyclophosphamide and a larger treatment effect was noted on a cyclophosphamide background, in part due to a lower placebo response rate with cyclophosphamide. There are theoretical advantages to the combination of RTX with both mycophenolate or cyclophosphamide by targeting B cells at different levels. Interestingly, the combination of RTX and cyclophosphamide has shown in small studies positive results (35); although the safety profile does not allow a prolonged cyclophosphamide administration, the combination of these drugs at least in some stages might lead in subgroups of patients to advantages in the disease control.

Moreover, B cell depletion is not selective leading to the targeting also of subsets that may be of help in controlling autoimmunity (such as regulatory B cells, B-regs) sparing at the same time other subsets able to produce pathogenic autoantibodies (namely plasmacells).

Several studies focused on the use of RTX in autoimmune diseases have shown that the longer duration of B cell
depletion achieved, the better is the clinical response to the drug (36); despite also in SLE a deep depletion has been
found as associated to clinical response (37), the proportion of patients achieving it is lower and the timing of
repopulation quicker (38). Several factors have been considered responsible, such as, the presence or development of
human anti-chimeric antibodies (HACAs) (39, 40) as well as differences in terms of RTX clearance (41) as a
consequence of a different immunoglobulin metabolism.

It should also be considered that, although we usually assess B cell depletion in the circulating compartment, this
might not reflect what is happening in inflamed tissues. Tissue resident CD20+ B cells may be more resistant to RTX
(42) as a consequence of a low tissue penetration of the drug or local survival factors including cell to cell contact and
B cell stimulating cytokines; interestingly it has also been described in ABO-incompatible kidney transplant patients
who received a single RTX infusion, that the proportion of CD19+ B cells in lymphnodes did not differ to the ones of
untreated controls and their phenotype was more often compatible with that of switched memory B cells (IgD-
CD27+), the phenotype most represented in peripheral blood of active SLE and not detectable in SLE patients in
remission once treated (43, 44).

Moreover, as already discussed, RTX is a type I monoclonal antibody and its action involves the gathering of antibody-
antigen complexes into lipid rafts of the cells: this facilitates ADCC and CDCC, but induces internalization and
inactivation of the drug (45, 46). Internalization may also be consequence of other factors such as the surface Fc
gamma receptor (FcγR) IIb (47, 48).

Many other factors have been proposed to play a role such as low complement levels and the presence of anti-C1q
antibodies as factors impacting negatively on CDCC; moreover, the genetically determined variability of FcγR affinity
for the Fc portions of antibodies (49, 50) as well as hypergammaglobulinemia might cause an alteration of the
phagocytosis/ADCC through low affinity for ligand/ligand competition (20).

Newer anti CD20 antibodies include the fully humanized type I (ocrelizumab and ofatumumab) or type II antibodies
(obinutuzumab). For ocrelizumab a randomized controlled trial in LN (BELONG) was suspended due to excessive
adverse events (34) although the drug seems to be effective especially in cases refractory to RTX (51), whereas for
ofatumumab there is a limited, although positive, clinical experience on five patients with SLE and LN (52, 53) with
further data required before its use may be considered in routine clinical practice. Regarding obinutuzumab (20), a
randomized control trial is now running and it will provide important information regarding the feasibility and safety
of its use in SLE (NCT02550652). Of note, both LUNAR with RTX and BELONG with ocrelizumab found similar increases
in renal response rates of 11-12% when compared to placebo (34). These were lower than the predicted response

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rates to show statistical superiority but of a similar order to that found in the belimumab trials, although using a different end-point. The latter studies had very much larger sample sizes and consequently met their end-points and were regarded as positive studies.

3. MODULATING B CELL SURVIVAL

A suitable alternative to B cell depletion may be the modulation of B cell survival. In SLE B cells are characterized by a longer lifespan, an anti-apoptotic molecular profile as well as a lower threshold for activation compared to healthy patients (54). Acting on factors inducing these characteristics with the aim of restoring a physiological profile may improve disease control.

A central role in driving SLE B cell hyperactivity is accounted by two members of the Tumor Necrosis Factor (TNF) ligand superfamily (TNFSF13): the B cell Activating Factor (BAFF, also known as Blys or TNFSF13B) and A Proliferation-Inducing Ligand (APRIL).

BAFF and APRIL actions are mediated via three receptors: the Transmembrane Activator and Calcium-Modulator and Cytophilin Ligand Interactor (TACI), the B cell Maturation Antigen (BCMA) and the B cell Activating Factor Receptor (BR3) (55).

B cells at different maturation stages express different receptors with TACI mainly promoting T-cell independent antibody responses as well as B cell regulation and immunoglobulin isotype switching, BCMA mainly promotes plasma-cell survival and BR3 accounts for immature B cell survival and maturation.(56).

The rationale for targeting these pathways is provided by the observation that both BAFF and APRIL levels are increased in SLE patients (57-59); moreover, mice models characterized by BAFF over-expression develop an SLE-like disease (60). Furthermore, BAFF levels have been consistently described as increased after B cell depletion via anti CD20 approaches (61, 62) and it has been described as higher in SLE patients experiencing a relapse after RTX administration when compared to patients who maintain disease remission (61); this has been also leading to the hypothesis that BAFF may play a role, at least of some degree, in limiting the effectiveness of B cell depletion.

Whether the BAFF increase reflects simply the loss of BAFF receptors, only found on B cells, or implies a disease stimulating effect of RTX is unclear and there is also evidence that a high BAFF level influences the returning B cell repertoire towards a more autoreactive phenotype (63).

Since the early 2000s drugs targeting BAFF and APRIL have been studied in SLE. Atacicept is a recombinant fusion protein including the extracellular domain of the TACI receptor joined to a human IgG1 Fc domain (64) that has been
tested in moderate-severe SLE in a phase II trial, this study was terminated prematurely due to safety concerns (65, 66). Efficacy was observed in the arm treated with the higher dose of the drug tested (150 mg-arm) although the same group experienced two fatal infections. The on-going ADDRESS II trial (NCT02070978) might be able to shed more light on the possible role of this drug. A LN trial was terminated due to the occurrence of hypogammaglobulinaemia and infection in atacicept treated patients, when administered in addition to mycophenolate mofetil and high dose corticosteroid (67).

More evidence are available for the anti-BAFF humanized monoclonal antibody belimumab: BLISS-52 and BLISS-76 are two large phase III multinational trials, which showed efficacy for belimumab in SLE in reducing disease activity as defined by the SLE responder index, a composite of disease activity scores (Table 3) (68, 69); these data found a favorable long-term safety profile (70) and resulted in this monoclonal antibody being the first biological drug approved for the use in SLE in several countries. However, the evidence for its efficacy is at the moment mainly limited to musculoskeletal and mucocutaneous subsets of the disease and it is still uncertain whether it may have a role also in LN (71). Interestingly a post-hoc analysis of the pooled cohorts of the BLISS trial showed a signal toward a potential role for belimumab in improving some renal outcomes with the limitation of both trials excluding patients with acute renal activity at the moment of the enrollment (72). Retrospective case reports seem to support the hypothesis for a role of belimumab in LN (73, 74), however it will be the BLISS-LN study (NCT01639339), a phase III randomized control trial, the one able to confirm this hypothesis.

Two other anti-BAFF drugs have shown signals for a potential role in SLE: the monoclonal antibody Tabalumab and the fusion protein Blisibimod (75, 76). Despite a signal for the latter in terms of proteinuria reduction, both have now been abandoned and no further studies are ongoing at the moment.

4. OTHER B CELL TARGETED THERAPY

4.1 Modulating B cell response

B cell receptor (BCR) signalling, as well as its intracellular transduction via second messengers, is increased in SLE (77, 78) representing therefore a target of potential interest.

The inhibitory transmembrane protein CD22 is a member of the immunoglobulin superfamily that recognizes sialic acid-containing ligands; it is expressed on different lineage of B cells with the exception of plasmablasts and plasmacells (79). Its mechanism of action is not fully understood but for sure it is active at different levels: following BCR stimulation, CD22 gets activated through a phosphorylation process mediated by Lyn, a tyrosine kinase induced
by BCR itself, in a feed-forward cycle (80). Once active, CD22 induces intracellular signalling resulting eventually in BCR dephosphorylation and inhibition (81). Interestingly, CD22 mechanisms of action appear to be various including indirect interaction with other intracellular signalling pathways such as the ones mediated by CD40 and Toll-like Receptors as well as clustering with the BCR component CD79a (82). CD22 is therefore central in setting the B cell threshold of activation.

Epratuzumab is a humanised anti-CD22 monoclonal antibody that acts by inducing CD22 phosphorylation as well and shifting into lipid rafts, thus reducing intracellular BCR signalling. However its clinical efficacy has not been confirmed and the drug has been at the moment abandoned (83). Despite that, CD22 remains a target of great interest that may be considered for future therapeutic approaches.

4.2 Targeting Intracellular pathways

One of the main limitations of aiming for cytokines, or their receptors, is the presence of physiological mechanisms acting at the limitation of the “single targets approaches” such as the redundancy of the stimulus as well as the molecules’ internalization once bound to their cellular surface targets. Acting directly on the intracellular signals, and therefore on the downstream part of the activation cascade, might therefore represent a way to avoid the downsizes of the single target approach.

The Bruton’s tyrosine kinase (BTK) is a key member of the BCR and FcgR signaling cascade, inducing the stimulation of B cell survival, differentiation as well as setting the activation threshold and inducing the antibody production (84-87). BTK blocking has been explored in several SLE mouse models with signals of effectiveness in terms of preventing and treating renal disease (88-92).

The Spleen Tyrosine Kinase (SYK) is a kinase directly activated by the Immunoreceptor tyrosine-based activation motif (ITAM) sequence of BCR co-receptors as well as of other immune and non-immune related receptors such as the T-cell receptor (TCR) and FcgR. SYK has a central pathogenic role in different autoimmune diseases and malignancies (93) as well as in glomerulonephritis. A retrospective study on 120 kidney biopsies of patients with glomerulonephritis showed that SYK expression was increased in proliferative and crescentic glomerulonephritis and correlates with the presenting serum creatinine as well as histological signs of disease activity; interestingly, in the subgroup of LN, SYK showed also a role as biomarker being higher in patients that achieved complete remission at 6 months (94). SYK inhibitors, such as, fostamatinib, seem an attractive and available option for autoimmune diseases in general and for kidney involvement of autoimmune diseases in particular (95). A role in LN has been shown in pre-clinical studies on
mice; interestingly, the use of this drug was not associated to reductions of anti-dsDNA titers confirming a
downstream mechanism of action (96, 97). Data regarding fostamatinib in human SLE are not available although the
encouraging results in terms of potential efficacy and safety in rheumatoid arthritis suggest that this is a treatment
worth exploring (98).

The Janus kinase-Signal Transducer and Activator of Transcription (JAK-STAT) path has been recently tested in SLE and
LN in two studies. There are four members of the JAK family and this path is particularly active in cytokine receptor
signaling influencing DNA transcription and protein synthesis. The first study explored CP690550 showing in murine
models improvement of proteinuria, renal function as well as histological lesions of the kidneys; interestingly a
reduction of glomerular deposition of anti-dsDNA, of C3 and IgG was observed as well as a reduction in kidney gene
expression of inflammatory cytokines (99). Another potential candidate acting on the JAK-STAT cascade is Tofacitininb
characterized by promising results in terms of improvement of the nephritis in mice models (100).

4.3 Modulating B-T cell interactions

T cells play a detrimental role in driving and boosting autoimmune responses. Several reports have described
dysfunction of the T cell compartment in SLE mainly involving Follicular Helper T cells (Tfh), Regulatory T cells and
Th17 (101).

Tfh are attractive targets having a central role in conditioning germinal centers, in facilitating expansion of
autoreactive clones and in stimulating differentiation of B cells into plasmablasts and plasmacells (102). Tfh actions
are mediated via cytokine secretion as well as via B-T cell interactions; the latter is allowed through several co-
receptors, such as CD40-CD40L (CD154), OX40-OX40L, ICOS-ICOSL, CTLA4/CD28-CD80/CD86.

CD40L is an important co-stimulatory molecule that is up-regulated in SLE, with higher levels of its soluble form
described as well in this disease (103-105), and seem to correlate with disease activity in mice models (106, 107);
moreover, a single nucleotide polymorphisms (SNP) of CD40 have been associate with disease susceptibility (108). The
inhibition of the CD40-CD40L interaction seems a reasonable target and has proven to be effective in preventing
disease in murine models (109, 110). A phase II trial of a CD40L inhibitor (BG9588) in lupus nephritis showed a signal
in terms of immunological benefit in the patients treated (reduction of the anti-dsDNA antibodies, increase of C3
concentrations, reduction of the hematuria); however, the trial was stopped due to safety concerns related to a high
incidence of thromboembolic events (111). This was caused by the occurrence of CD40L on platelets with Fc mediated
platelet cross-linking caused by the therapeutic antibody. Encouraging results in terms of safety have now been
described with a PEGylated anti-CD40L Fab' fragment (CDP7657) from a phase I study (112, 113) suggesting that inhibition of this pathway remains a possible therapeutic target in SLE. An alternative is to target CD40 and this is being pursued by BI655064, an anti-CD40 monoclonal antibody in a Phase II LN trial (NCT02770170).

CTLA4 is a surface molecule with inhibitory functions competing with the activating surface protein CD28 for the binding of CD80/86. A CTLA4-Ig (abatacept) has shown efficacy in patients with non-severe granulomatosis with polyangiitis (GPA) (114). The rationale for targeting this pathway in SLE has been developed from mice models where a CTLA4-Ig approach displayed effectiveness in preventing disease onset (115) or in reducing signs of activity in LN when used in combination with cyclophosphamide (116). However, despite effectiveness on anti-dsDNA and complement levels, no beneficial clinical effect has been so far shown (117).

4.4 Proteasome inhibitors

Proteasome inhibitors are an emerging class of drugs targeting mainly plasma-cells. Among proteasome inhibitors, bortezomib is the first one employed in clinical practice with indication at the moment for multiple myeloma. The mechanism of actions of this class of drugs is the inactivation of the Nuclear Factor kappa-light chain enhancer of activated B cell (NFκB) process eventually able to induce apoptosis (118). Since the high rate of protein synthesis typical of plasma-cells, these are very sensitive to the effects of this drug. For proteasome inhibitors also a role in reducing the interferon (IFN) path activation as well as IFN production has been proposed as further mechanism of action (119).

Due to encouraging results for the use of bortezomib in mice models of SLE and LN (120-122) this drug has been tested also in humans in a study involving 12 patients with benefits in terms of reduction of the autoantibody-producing plasma cells, IFN pathway activation and, more significantly, also in terms of clinical activity of the disease (123). The use of bortezomib was however associated to a reduction of protective IgG levels and necessity of withdrawing the treatment in seven patients due to adverse events. Interestingly, in murine models a quick reconstitution of the plasma cells pool have been observed once the treatment is stopped (124) and a role for co-administration of B cell depleting treatment has been proposed as strategy for delaying plasma-cell reconstitution after depletion (124). An oral proteasome inhibitor, ixasomib with a more favourable safety profile is in clinical trials in LN (NCT02176486).

5. CONCLUSIONS
SLE and LN are chronic diseases that often feature a long disease course with large variability in disease extent and severity. First and second line approaches are not infrequently exhausted in a single patient clinical history and newer options are required for disease management. The toxicity of current regimens and their associated serious adverse event rates directly worsen patient outcomes and safer therapies allowing lower corticosteroid dosing are needed. The targeting of B cell is supported by our understanding of pathogenesis, and RTX and belimumab have entered routine use in carefully defined scenarios where standard therapies are failing. The uptake of RTX has affected by the imbalance between positive retrospective cohort studies in largely refractory patients, and the negative results of the two randomised trials. Belimumab has obtained registration for use in SLE although the signals for its utility in LN are still weak and its role at the moment is limited to non-renal SLE; more information will be provided after a prospective trial in LN now ongoing (NCT01639339).

Several targets of the B cell biology have been identified and for most of them, despite good preliminary results in phase II trials and pre-clinical models, no confirmation has been so far observed in phase III studies.

6. EXPERT COMMENTARY

SLE is a disease with a complex and still partially unknown biology. The success of the conventional immunosuppressive approaches may be due to their low specificity characterized by a widespread effect on the immune system; more selective approaches, although potentially of great interest and safer, may allow only partial benefits due to the lack of inhibition of alternative pathways not known. Based on this rationale, combining different biologics may allow an increase in terms of efficacy; however, such approaches need to be tested carefully considering concerns in terms of safety. The most obvious approach at the current stage, would be the combination use of RTX and belimumab: B cell depletion via RTX is in fact characterized by a rebound of BAFF levels which might explain, at least in some settings, the lack of efficacy. Preliminary results of subsequent therapy with RTX and belimumab are encouraging (125, 126) and prospective trials are now on-going (Table 4). Furthermore, RTX B cell killing will be potentiated by BAFF blockade. Two trials are studying this approach, CALIBRATE IN LN, and BEAT-LUPUS in non-renal SLE (Table 4).

Another issue related to RTX, although potentially characteristic of other biologics as well, is that the lack of efficacy may be a consequence of the mode of administration. We know from experience in other autoimmune diseases that retreatment with RTX may improve remission rates as well as prolonging the time to relapse when compared to a single course of the drug although, eventually, at the drug withdrawn the relapse rate remains high (127).
Moreover, alternative trial designs or the definition of new end-points may facilitate demonstration of drug effectiveness: on average the studies published so far are designed to show a superiority or a non-inferiority of a tested drug compared to the standard of treatment, usually with an add-on philosophy to what considered the standard of care. In diseases characterized by a chronic relapsing course, frequent treatment changes or dose adjustments are required. In this context the efficacy of a new drug may not necessarily be shown only via a greater clinical benefit when added to the standard of care. Of note, clinical trials are usually characterized by a relatively short follow-up and an intervention that is lacking impact during the explored time span might however unveil significant outcomes in the long term. In the everyday clinical life the standard of care may sometimes not be employed, not tolerated at full dose, refused by the patient or even ineffective. The design of classical clinical trials does not take into account this flexibility and may lead to the abandon of potentially useful drugs. Alternative end-points may be taken into account in order to provide the clinical community with as many options as possible. We should therefore seek for non classical clinical trial designs able to overcome these limitations: examples of alternative strategies might include aiming at showing the ability of a new add-on treatment in allowing a quicker and aggressive drug sparing effect; a better clinical and safety profile in contexts where an optimal dose of the standard of care may not be employed; a better safety or burden of damage in longer follow-up time. Studies with different end-points may be characterized by more difficulties in terms of recruitment and may benefit from the use of alternative strategies of enrollment or treatment allocation including for example adaptive approaches. An example might be the ongoing RITUXILUP (Table 4) (NCT01773616) that is exploring whether the combination of RTX and mofetil mycophenolate (MMF) may allow sparing steroids.

SLE is a complex disease and LN is its most severe manifestation. Research aimed at the identification of new therapeutic targets or biomarkers able to perform effective patient stratification is a high priority task in the research agenda in order to increase our therapeutic options. Moreover, alternative trials design should be developed in order to identify, among all the possible therapeutic targets, the ones that may have a role in the management of this autoimmune disease.

7. FIVE YEARS VIEW

Despite significant research efforts in the field of pathogenesis and drug development, no major changes have occurred in the management of LN. We do know that MMF and cyclophosphamide appear equal alternatives as first line approaches as induction (128) and that azathioprine, MMF, and in some settings, methotrexate may be useful for
the maintenance stage. Steroids are the cornerstones of the treatment, playing a central role in all phases as well as for the management of minor systemic flares. In this picture is now relatively well established the use of RTX as third line agent for both relapsing-refractory SLE and LN. Moreover belimumab is now approved for the management of SLE with its use mainly limited at the moment to the mucocutaneous and articular manifestations.

The studies published so far taught us that the heterogeneity of SLE manifestation and population, as well as the complexity of the disease, may cause difficulty in the identification of a “superiority” or of a “non-inferiority” profile for new drugs when compared to an optimal standard of care. We feel that in the next years the trial designs will switch to the identification of different end-points such as tolerability, safety and ability of a drug sparing effect for the treatment tested.

The future will probably see also testing different combination of biologics and targeted therapy in order to allow a potential increase in efficacy of the intervention proposed. This will need to happen under strict monitoring from the safety point of view.

Beside B cells, very promising are the preliminary results of targeting the interferon pathway (129, 130) but we do feel there may be in the short-term more interesting therapeutic options. Complement plays a central role in SLE and LN pathogenesis and several complement blockade molecules are now available (131). However, no trials are on-going to test effectiveness of this approach in SLE. Interestingly, complement is also crucial in mediating the action of several monoclonal antibodies and will be of great interest to see the consequences of combining anti complement drugs and other monoclonal antibodies.

8. KEY ISSUES

- B cells play a central role in the pathogenesis of SLE and LN therefore representing a therapeutic target of interest.

- Rituximab is an anti-CD20 monoclonal antibody for which retrospective studies and clinical practice have shown efficacy in SLE and LN; prospective trials failed in confirming this although trial design issues, intrinsic limitation of both prospective studies in SLE and of an anti-CD20 approach may have had a role in these negative findings.

- Belimumab is an anti BAFF monoclonal antibody registered for the use in SLE in several countries although evidences of its effectiveness in LN are still lacking; prospective trials are now on-going and will shed more light on this topic.
• Several other targets have been so far identified and tested at different levels of pre-clinical and clinical development; the more interesting are the SYK inhibitors and the anti-proteasome respectively for the central role of SYK in the renal manifestations of immunological diseases and for the ability of anti-proteasome of targeting plasma-cells and therefore the main producers of auto-antibodies.

• Combination of different targeted drugs as well as the identification of new trial designs and end-points are going to be key aspects of the clinical research agenda in the field of SLE and LN in the near future.
REFERENCES


** Randomized controlled trial exploring the use of rituximab in SLE: the negative results may be partly due to trial design issues and not necessarily to lack of effectiveness of the drug.


** Randomized controlled trial exploring the use of rituximab in LN: the negative results may be partly due to trial design issues and not necessarily to lack of effectiveness of the drug.


49. Hatschak E, Xie L, Santos DD, Hunter ZR, Ciccarelli BT, Verselis S, et al. Increased natural killer cell expression of CD16, augmented binding and ADCC activity to rituximab among individuals expressing the


** Randomized controlled trial showing effectiveness of belimumab in SLE patients with mainly musculoskeletal and mucocutaneous disease.


** Randomized controlled trial showing effectiveness of belimumab in SLE patients with mainly musculoskeletal and mucocutaneous disease.


* This study shows the possible role of SYK as biomarker in LN providing the rational as therapeutic target.


97. Deng GM, Liu L, Bahjat FR, Pine PR, Tsokos GC. Suppression of skin and kidney disease by inhibition of

* Paper showing the potential role of bortezomib in refractory SLE, further studies are needed in order to confirm this observation.


<table>
<thead>
<tr>
<th>Study</th>
<th>Type of study</th>
<th>Therapy</th>
<th>Follow-up</th>
<th>Disease activity score at study entry</th>
<th>Number of patients assessed (patients with LN)</th>
<th>Overall response* – timing of response assessment after RTX</th>
<th>Renal response</th>
<th>Steroid sparing</th>
<th>Adverse events</th>
<th>Relapse</th>
<th>Response after re-treatment with RTX*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iaccarino L. et al., 2015</td>
<td>Prospective multicentric</td>
<td>RTX 1g two weeks apart, 375mg/m² for four weeks * with background immunosuppressive</td>
<td>NA</td>
<td>ECLAM score – 4.11 ± 1.73</td>
<td>34 (51%)</td>
<td>85.8% - 6y 12 months</td>
<td>74.1%</td>
<td>NA</td>
<td>23.9%</td>
<td>69.5%</td>
<td>84.4%</td>
</tr>
<tr>
<td>Fernandez Nebro A. et al., 2012</td>
<td>Retrospective multicentric</td>
<td>RTX 1g two weeks apart, 375mg/m² for four weeks with background immunosuppressive</td>
<td>26.7 weeks (range, 11.1-42.1)</td>
<td>SELENA-SLEDAI score – 14.6 ± 5.3</td>
<td>16 (46%)</td>
<td>62.9% - 6 months</td>
<td>45.8%</td>
<td>From 32.4 ± 57.3 mg/day to 11.7 ± 11.9 mg/day</td>
<td>32.4/100 patients-years / 05.0/100 patients-years were serious</td>
<td>48.3%</td>
<td>64%</td>
</tr>
<tr>
<td>Aguiar R. et al., 2017</td>
<td>Retrospective monocentric</td>
<td>RTX 1g two weeks apart with 500-750 mg CYC once or twice and two administrations of methylprednisol</td>
<td>46.03 ± 11.10 months after the last RTX cycle.</td>
<td>BILAG score – 18.29 ± 10.62</td>
<td>15 (26%)</td>
<td>67% - 6 months BILAG improvement</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>62%</td>
<td>62%</td>
</tr>
<tr>
<td>Study</td>
<td>Design Type</td>
<td>RTX Dosing</td>
<td>SELENA-SLEDAI Score</td>
<td>Relapse Rate</td>
<td>Maintenance Immunosuppression</td>
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<tr>
<td>1.</td>
<td>Terrier B. et al., 2010</td>
<td>Prospective multicentric</td>
<td>1g two weeks apart, 375mg/m² for four weeks</td>
<td>11.3 ± 8.9</td>
<td>71% ± 6 ± 3 months</td>
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<td>2.</td>
<td>Ramos-Casals et al., 2010</td>
<td>Prospective multicentric</td>
<td>1g two weeks apart, 375mg/m² for four weeks</td>
<td>11.3 (27%)</td>
<td>71% from 30.3 ± 23.6 mg/day to 12.3 ± 10.1 mg/day</td>
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<td>3.</td>
<td>Condon MB et al., 2013</td>
<td>Prospective monocentric</td>
<td>1g two weeks apart with methylprednisolone 500 mg at each administration</td>
<td>107 (46%)</td>
<td>77% ± 12 months</td>
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<tr>
<td>4.</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>77% Reduction of corticosteroid dose in 79% (withdrawal in 14%)</td>
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</table>

**Notes:**
- For Peer Review Only
- Background immunosuppression was suspended until relapse.
- **RTX** = Rituximab
- **SELENA-SLEDAI** = Systemic Lupus Erythematosus Disease Activity Index
- **IQR** = Interquartile Range
LN: lupus nephritis; CYC=cyclophosphamide; BILAG= British Isles Lupus Assessment Group; ECLAM= European Consensus Lupus Activity Measurement; SLEDAI= Systemic Lupus Erythematosus Disease Activity Index; NA: not available; MMF: mycophenolate mofetil; IQR: interquartile range.

*: 99% of the patients received steroids, 76% of the patients received other immunesuppressive

*: including complete and partial response according to study definition.

#: Registry data

*: in ten cases the RTX administration was followed by two further infusions after month 1 and 2 together with two pulses of cyclophosphamide 750 mg and three pulses of methylprednisolone (15 mg/Kg).

£: no background immunesuppressive treatment in 32 patients

&: MMF was started at the dose of 500 mg bid and then gradually titrated up to a maximum dose of 1.5 g bid. No maintenance prednisolone were used.

$: Renal relapses. 12% were reported to have systemic flares.
Table 2. Randomized controlled trials of Rituximab in SLE

<table>
<thead>
<tr>
<th>Patients</th>
<th>Inclusion criteria</th>
<th>Treatment</th>
<th>Major end points</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>EXPLORER (Phase II-III) (2730)</td>
<td>257 Moderate-severe disease activity (≥ 1 BILAG A score or &gt;2 BILAG B score), Exclusion of CNS and renal involvement</td>
<td>RTX 1g on days one, 15, 168 and 182 vs Placebo (background treatment in both arms)*</td>
<td>Response at 52 weeks (BILAG score)</td>
<td>Overall response rate 28.4% (RTX arm) vs 29.6% (placebo arm) (p=0.975)</td>
</tr>
<tr>
<td>LUNAR (Phase III) (2831)</td>
<td>144 Class III or class IV LN</td>
<td>RTX 1g on days one, 15, 168 and 182 vs placebo (MMF based background treatment in both arms)#</td>
<td>Renal response (complete and partial) at 52 weeks</td>
<td>Complete renal response 26% RTX arm vs 31% placebo arm; Partial renal response 31% RTX arm vs 15% placebo arm (p=0.55)</td>
</tr>
</tbody>
</table>

Table 2. Randomized controlled trials of Rituximab in SLE

BILAG= British Isles Lupus Assessment Group; CNS=Central Nervous System; LN=Lupus nephritis; RTX=Rituxumab; MMF=Mycophenolate mofetil

*Background treatment: azathioprine 100-250 mg/day, mycophenolate mofetil 1-4 g/day, methotrexate 7.5-27.5 mg/week. Prednisone was administered at the dose of 0.5 mg/Kg, 0.75 mg/Kg or 1 mg/Kg according to the BILAG score.

#Background treatment: MMF 1.5 g/day in three doses increased to 3 g/day by week four up to week 52. Intravenous methylprednisolone was administered two times on day one and within day three. Oral prednisone at the dose of 0.75 mg/kd/day (maximum 60 mg) was administered until day 16 and then tapered to ≤ 10 mg/day by week 16.
### Table 3. Main Randomized Controlled Trials of Belimumab in SLE

<table>
<thead>
<tr>
<th>Partecipants</th>
<th>Inclusion criteria</th>
<th>Treatment</th>
<th>Major end points</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BLISS-52</strong> (phase III)</td>
<td>SELENA-SLEDAI score &gt;6 and a stable treatment regimen for a minimum of 1 month. Exclusion of patients with lupus nephritis and CNS involvement</td>
<td>1 mg/kg or 10 mg/kg or placebo on days 0, 14, 28, thanthen every 28 days until 48 weeks.</td>
<td>Improvement in SRI at week 52.</td>
<td>SRI improvement in 51, 58 and 44% in belimumab 1, 10 mg/kg and placebo, respectively (p=0.0006)</td>
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<tr>
<td>867</td>
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<tr>
<td><strong>BLISS-76</strong> (phase III)</td>
<td>SELENA-SLEDAI score &gt;6 and a stable treatment regimen for a minimum of 1 month. Exclusion of patients with lupus nephritis and CNS involvement</td>
<td>1 mg/kg or 10 mg/kg or placebo on days 0, 14, 28, thanthen every 28 days until 72 weeks.</td>
<td>Improvement in SRI at week 76.</td>
<td>SRI improvement in 40.6, 43.2, 33.5% in belimumab 1, 10 mg/kg and placebo, respectively (p=0.089 and p=0.017)</td>
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<td>819</td>
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</tbody>
</table>

SELENA-SLEDAI= SELENA-SLE Disease Activity Index; SRI= Systemic Lupus Erythematosus Responder Index; CNS=Central Nervous System
Table 4. On-going clinical trials in lupus nephritis employing biologics drugs.

<table>
<thead>
<tr>
<th>Target</th>
<th>Drug</th>
<th>Trial - ID</th>
<th>Type of study</th>
<th>Phase</th>
<th>Primary Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD20</td>
<td>Rituximab</td>
<td>RITUXILUP - NCT01773616</td>
<td>Randomized: rituximab + IV MP + MMF vs oral prednisolone + IV MP + MMF</td>
<td>III</td>
<td>Non-inferiority of rituximab vs control in the proportion of patients achieving complete renal response at week 52 without the need of steroid prescription.</td>
</tr>
<tr>
<td></td>
<td>Rituximab</td>
<td>RING - NCT01673295</td>
<td>Randomized: rituximab + standard of care vs standard of care</td>
<td>III</td>
<td>Percentage of patients achieving renal complete response at 104 weeks.</td>
</tr>
<tr>
<td></td>
<td>Obinutuzumab</td>
<td>NA - NCT02550652</td>
<td>Randomized: obinutuzumab + MMF vs placebo + MMF</td>
<td>II</td>
<td>Percentage of patients who achieve complete renal response at week 52.</td>
</tr>
<tr>
<td>BAFF</td>
<td>Belimumab</td>
<td>BLISS-LN - NCT01639339</td>
<td>Randomized: belimumab + standard of therapy vs placebo + standard of therapy</td>
<td>III</td>
<td>Number of patients with a renal response at week 104.</td>
</tr>
<tr>
<td></td>
<td>Blisibimod</td>
<td>CHABLIS 7.5 - NCT02514967</td>
<td>Randomized: blisibimod + standard of care vs placebo + standard of therapy</td>
<td>III</td>
<td>Proportion of the responders to the SRI-6 composite responder index at week 52.</td>
</tr>
<tr>
<td>CD20 and BAFF</td>
<td>Rituximab - belimumab</td>
<td>CALIBRATE - NCT02260934</td>
<td>Randomized: rituximab + cyclophosphamide + IV</td>
<td>II</td>
<td>Proportion of patients experiencing at least one grade 3 or higher infectious adverse events up to week 48.</td>
</tr>
<tr>
<td>Initiative</td>
<td>Study Name</td>
<td>Study Design</td>
<td>Primary Endpoint</td>
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<tr>
<td>Rituximab and belimumab.</td>
<td>SYNBioSe - NCT02284984</td>
<td>Non randomized</td>
<td>Reduction of pathogenetic autoantibodies</td>
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<tr>
<td>IFN α/β receptor</td>
<td>Anifrolumab - TULIP-LN1 - NCT02547922</td>
<td>Randomized: anifrolumab at 2 different doses + standard of care vs placebo + standard of care</td>
<td>Relative difference in change from baseline to 52 week proteinuria</td>
<td></td>
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<tr>
<td>Proteasome</td>
<td>Ixazomib - NA - NCT02176486</td>
<td>Randomized: Ixazomib at different doses + standard of care vs placebo + standard of care</td>
<td>Safety up to 28 weeks (Emergent Adverse Event, Serious Adverse Events)</td>
<td></td>
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<tr>
<td>CD40</td>
<td>BI 655064 - NA - NCT02770170</td>
<td>Randomized: BI 655064 at 3 different doses + standard of care vs placebo + standard of care</td>
<td>Proportion of patients with complete renal response at 52 weeks.</td>
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<tr>
<td>NFkB</td>
<td>Iguratimod - NA - NCT02936375</td>
<td>Randomized: Iguratimod + steroids vs cyclophosphamide followed by azathioprine + steroids</td>
<td>Renal remission rate at week 52.</td>
<td></td>
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<tr>
<td>CD74</td>
<td>Milatuzumab</td>
<td>NA - NCT01845740</td>
<td>Randomized: Milatuzumab at 2 different doses vs placebo *</td>
<td>I-II</td>
<td>Safety and efficacy (variation of BILAG score) up to 2 years.</td>
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</tbody>
</table>

RTX=rituximab; CYC=cyclophosphamide; MMF=mycophenolate mophetil; BCDT=B cell Depleting therapy; CR=Complete Response; LN=lupus nephritis; SOC=standard of care; AZA=azathioprine
* not specified if added to standard of care.