Treating lupus: from serendipity to sense, the rise of the new biologicals and other emerging therapies

Elsa Sousa, MD, Rheumatology Trainee a,*, David Isenberg, MD, FRCP, Professor b

a Rheumatology Research Unit, Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa and Hospital de Santa Maria, Av. Professor Egas Moniz, 1649-028 Lisbon, Portugal
b Centre For Rheumatology Department of Medicine, University College London, London, UK

Keywords:
- systemic lupus erythematosus
- new biologics
- B-cell depletion

During the last 10 years our increasing understanding of the immunopathogenesis of systemic lupus erythematosus (SLE) has led to the introduction of several new biological therapies. SLE treatment has moved from the use of conventional drugs such as hydroxychloroquine, corticosteroids, and non-specific immunosuppressants to targeting selective components of the immune system in the hope that they can be more effective and reduce undesired side-effects. These new treatments include B-cell-depleting therapies, antibodies and fusion proteins that block interleukins or the cross-talk between B and T cells, and tolerogens. However, although there are great expectations about new agents, double-blind controlled trials demonstrating safety and efficacy are still awaited, and better instruments for evaluating disease activity need to be developed.

Systemic lupus erythematosus (SLE) is an autoimmune rheumatic disease characterized by a complex deregulation of the immune system leading to multi-organ damage in which autoantibodies play a central role. Until the early 1990s SLE treatment was limited to conventional drugs, including hydroxychloroquine and corticosteroids, and non-specific immunosuppressive drugs such as azathioprine (AZA), cyclophosphamide (CYC), methotrexate (MTX) and mycophenolate mofetil (MMF). Our increased understanding of SLE immunopathogenesis has led to the introduction of several new agents over the last 10 years, a move from therapeutic serendipity to sense.

There is growing evidence that B cells are not only passive producers of antibodies but also secrete interleukins (IL), act as antigen-presenting cells (APCs), contribute to T-cell activation, and modulate...
dendritic cells [1]. Some of these new therapies directly target B cells by inducing cell depletion through monoclonal antibodies (mAbs) that bind to specific surface antigens such as CD20 and CD22. Cytokines that are involved in B-cell activation, differentiation and survival, or that are secreted by them – such as IL-6, IL-10, interferon α (IFN-α), TNF-α, B-lymphocyte stimulator (BlyS) and proliferation-inducing ligand (APRIL) – also appear to be relevant targets, and specific antibodies or fusion proteins have been successfully developed. In addition, inactivation of B- and T-reactive cells via tolerance induction and blocking ‘cross-talk’ between T and B cells through CD28-B7 and CD40-CD40L co-stimulatory molecules have become potential treatment approaches.

Thus, recent advances in SLE treatment have evolved to target selective components of the immune system in the expectation that patients would have fewer side-effects than those related to generalized immunosuppression.

**B-cell-depleting therapies**

Several abnormalities in SLE B-cell populations have been identified, supporting the hypothesis that B cells play a central role in the pathogenesis of this disease. As part of the generalized B-cell hyperactivity, active SLE patients have increased numbers of transitional and memory B cells and plasma cells, while naïve B cells are diminished [2].

**Anti-CD20**

Rituximab is a chimeric monoclonal antibody that targets B-lymphocyte surface marker CD20; it has been used in the treatment of SLE for the last 8 years, with encouraging results. Rituximab was ‘created’ from the fusion of a human IgG1κ constant region and a murine variable region. The mechanisms by which rituximab induces depletion probably include antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC) and apoptosis [3]. Because CD20 is only expressed from the pre-B stage to the mature B cells, rituximab spares haematopoietic stem cells, pro-B and plasma cells, allowing B-cell regeneration [4].

**Efficacy**

Many prospective open-label studies and retrospective cohort studies have stressed the efficacy of rituximab in the treatment of most SLE manifestations, including nephritis, autoimmune cytopenias, central nervous system (CNS) involvement, joint and skin disease, as well as fatigue, serositis and fever [5–19]. However, the heterogeneity of the disease itself, the diversity of treatment regimens (including doses of rituximab, concomitant immunosuppressive drugs), and the use of different disease activity index scores make these data hard to compare.

In 2002 Leandro et al at University College, London, UK, were the first to report the benefits of rituximab in the treatment of active refractory SLE. At 6 months of follow-up, the five evaluable patients showed a reduction in the British Isles Lupus Assessment Group (BILAG) disease activity index from a mean of 14 to 6, with particular improvement of arthralgia/arthritis, fatigue, serositis and skin vasculitis. The treatment protocol consisted of two 500-mg infusions of rituximab plus 750-mg of CYC, given 2 weeks apart, and high-dose corticosteroids for 5 days [6]. Later in 2005, this cohort was extended to 24 patients. The 18 new patients were treated with a higher dose of rituximab (1000 mg) plus CYC 750 mg and methylprednisolone 250 mg, two infusions given 2 weeks apart; 96% of the patients achieved B-cell depletion (<0.005 × 10^9 CD19^+ cells/L) that lasted between 3 and 8 months, and again an improvement in the BILAG score (from 14 to 5 at 6 months) was noticed (mean follow-up of 23 months) [7]. The repeated administration of rituximab (up to three cycles) in seven patients from the same cohort was well tolerated and associated with an increase in the duration of remission (13 versus 7 months) [8]. In a retrospective study at this centre of 50 patients given the same treatment regimen between 2000 and 2007, 42% achieved full remission, judged on the basis of all BILAG A and B scores falling to C or D, and a further 47% reached partial remission (some but not all systems improving from A/B to C/D) at 6 months after the first cycle of rituximab. B cells repopulated (>0.03 × 10^9 CD19^+ cells/L) after a mean of 6 months, although 15 patients remained depleted for longer than 1 year. During a follow-up period of 40 months, 44% of the patients remained free of
disease flare, while 56% experienced a flare, the majority between 6 and 12 months after treatment. Twenty of these patients were retreated, with similar results but showing a tendency to a more sustained remission [9].

Jónsdóttir et al have recently published similar results using rituximab 375 mg/m², once a week for 4 weeks, combined with cyclophosphamide 0.5 mg/m² and methylprednisolone 250 mg, in the first and last infusions, and oral corticosteroids (0.5 mg/kg) during the 4 weeks of treatment, in 16 female patients with severe refractory SLE. At 6 months the mean systemic lupus erythematosus disease activity index (SLEDAI) values decreased from 12.1 to 4.7, and 81% achieved a SLEDAI-50 clinical response. Clinical remission defined as a SLEDAI <3 was obtained in 56% of patients at 3 months, and relapses occurred in 44% after 18 months (mean follow-up of 27 months). All relapses were preceded by B-cell repopulation which occurred at a median of 7 months [10].

Other groups have analysed the efficacy of treatment regimens that do not include CYC. The first, in 2004, was a dose-escalating phase-I/II trial conducted by Looney et al; 12 patients received rituximab, a single infusion of 100 mg/m² (low dose) or 375 mg/m² (intermediate dose) and six received 375 mg/m² (high dose) weekly for 4 weeks, plus 80 mg of oral corticosteroids, in addition to previous baseline therapy: prednisolone (PDN), AZT, MTX or MMF. Effective B-cell depletion was verified in 11/17 patients and was associated with a significant reduction in the systemic lupus activity measure (SLAM) score, which was maintained for 12 months. However, the authors do not mention any differences in efficacy between the subgroups. Clinical response was especially notable for rashes, mucositis, alopecia and arthritis [11]. Recent results from a prospective phase-I/II open-label study, by Albert et al, using rituximab (375 mg/m² weekly for 4 weeks) plus methylprednisolone 100 mg, allowing a low daily dose of PDN (<20 mg), non-steroidal anti-inflammatory drugs (NSAIDs) and hydroxychloroquine, showed that B-cell depletion remained effective for 7 months. The SLEDAI score decreased from 8 to 4 points at 4 months, and similar benefits were observed in the SLAM. A correlation between SLEDAI and B-cell counts was identified, but not between other clinical and laboratory parameters [12].

Several trials have showed promising results in the treatment of lupus nephritis. Sfikakis et al reported a complete remission in 50% and a partial remission in 80% of ten patients with class-III and -IV nephritis, treated with rituximab as monotherapy, 375 mg/m² weekly for 4 weeks, combined with a tapering moderate dose (0.5 mg/kg/day) of corticosteroids for 10 days. The remission was sustained for 12 months in 40% of patients [13]. Vigna-Perez et al demonstrated the benefits of rituximab added to baseline immunosuppressive therapy (AZA, MTX or MMF) in 20 patients with class-III/IV nephritis [14]. The efficacy of rituxumab combined with CYC in the treatment of proliferative nephritis was further verified by Gunnarsson et al, showing that the decrease in the renal activity index was associated with histopathological improvement [15]. Takunaga et al also described favourable results in the treatment of patients with CNS involvement [16].

These results strongly suggest that rituximab is effective in the treatment of SLE by inducing B-cell depletion in the majority of the patients which lasts for about 6 months, with a complete B-cell repopulation in 12 months. Interestingly, two patients treated in different centres, with different regimens of rituximab, both using CYC, have experienced a surprisingly prolonged B-cell depletion of 5 and 7.5 years [17].

The questions of optimal indications, ideal rituximab regimen, and the best monitoring parameters for B-cell depletion remain unanswered. However, our data from the largest single cohort indicates that the combination of rituximab 1000 mg, CYC 750 mg and methylprednisolone 250 mg, given 2 weeks apart, induced complete or partial remission in 89% of SLE patients with severe disease inadequately responding to conventional immunosuppressives. The long-term follow-up assessment suggests that patients can be retreated using the same protocol when there is evidence of relapse and B cells have repopulated, with at least the same efficacy.

Given the generally encouraging results of the above open-labelled studies, it was most disappointing to learn that the EXPLORER study in the USA, comparing rituximab with placebo in non-renal lupus, did not reach its primary endpoints (using the BILAG system). The reasons for this failure are still being discussed, although there are indications (unpublished reports) that relatively large amounts of oral corticosteroids were permitted for patients entering the trial and that this may well have masked the ability of rituximab to show benefit [18].
Safety profile

Rituximab, which was introduced in 1997 for the treatment of non-Hodgkin’s lymphoma, has been generally well tolerated, and short- and long-term safety profiles appear to be favourable.

At University College, London, five of the 50 treated SLE patients experienced serious adverse events; one had a severe serum-sickness-like reaction, one a pneumococcal pneumonia with septicaemia, and one patient died of pancarditis related to active disease (a month after B cells had repopulated). The two other adverse events were considered to be related to CYC infusions: a grand mal seizure secondary to hyponatraemia, and a death due to adult respiratory distress syndrome following the second CYC infusion [9].

In the trial of Looney et al, one patient developed mid-infusion-related bronchospasm, two had infectious events and one a transitory ischaemic accident related to anti-phospholipid syndrome [11]. Four severe adverse events were described by Albert et al; two were reactions to infusions, both associated with high human anti-chimeric antibodies (HACAs) titres, one patient was diagnosed with possible lupus cerebritis and meningitis 8 weeks after the fourth infusion of rituximab, and one patient died due to sepsis and other medical problems that were attributed in part to disease activity [12]. There were no severe adverse events noted in the study by Jönsdóttir et al [10]. Retreatment with additional cycles does not seem to change the safety profile of rituximab, and in a small study of seven patients one had a urinary tract infection and another had an episode of shingles [8]. Two cases of progressive multifocal leucoencephalopathy (PML) have been described in SLE patients treated with rituximab, but the relationship to the treatment remains uncertain as more than 20 other SLE patients not given rituximab have also been reported to have developed PML [19].

Infusion-related reactions can be severe but are reduced by the concomitant use of intravenous corticosteroids and/or antihistamines. Infectious events are a main concern in rituximab-treated patients, and standard vaccination against influenza virus and pneumococcus prior to rituximab treatment has been recommended [9]. Supporting these findings, Cambridge et al have demonstrated that anti-pneumococcal antibodies are preserved after one cycle of rituximab for at least 1 year [20].

Immunoglobulins, antibodies and complement

Relatively few studies have addressed the question of alteration in immunoglobulin, antibody and complement levels, and the results differ. These differences are probably related to the different treatment regimens and patients studied.

Looney et al and Albert et al, using CYC-free protocols, did not note significant changes in the levels of antinuclear antibody (ANA), anti-dsDNA antibody, or complement [11,12]. In contrast, the addition of CYC to rituximab has been associated with lowered anti-dsDNA antibody titres in patients that responded to treatment, while the levels of anti-extractable nuclear antigens (anti-ENA) were generally unchanged [9,10,20]. A non-significant decrease in the levels of IgM and IgG immunoglobulins was described, but not of IgA, and a correlation with infections has not been established [10,11]. It seems to be agreed that pre-existing antibodies to pneumococcal polysaccharides and tetanus remain unchanged for at least 1 year after rituximab, but the response to any of these immunizations is possibly impaired [11,12,20].

Human anti-chimeric antibodies

The development of HACAs has been reported independently of the treatment regimen, but lower doses of rituximab in monotherapy are possibly associated with a higher risk of circulating HACAs.

Looney et al reported an incidence of HACAs in 11 of the 17 treated patients. High HACA titres (≥100 ng/mL) were detected in six patients, more commonly in African Americans. This finding was associated to higher baseline SLAM scores, less effective B-cell depletion, and a rapid drop in rituximab levels [11]. Albert et al also described a high incidence of HACAs: 10 of 24 patients, of whom three did not receive the full dose of rituximab or, in two cases, the methylprednisolone infusion. The development of HACAs was also associated with failure to deplete completely or maintain depletion, implying that HACA formation decreases the drug effect and the extent of B-cell depletion. In addition, two of the patients that developed severe reactions to infusions had detectable HACAs [12].

HACAs, therefore, might be associated with a decrease in rituximab efficacy and infusion reactions. As an alternative, fully humanized anti-CD20 monoclonal antibodies have been developed (including ocrelizumab) and are being studied in clinical trials [21].
Predictors of response

The peripheral-blood B-cell count reflects, to some degree, the biological effects of rituximab, and a failure to achieve even a short-term depletion seems to predict a poor therapeutic response [12]. The clinical improvement, however, is not limited to the period of B-lymphocyte depletion, and even if some patients relapse when repopulation occurs, others remain in remission for longer periods [6,10,22].

The FcγRIIIa genotype that exhibits an enhanced affinity for IgG1 has been associated with a decrease in circulating B cells, but this was not confirmed in another study [12].

Several other factors – including the number of CD19⁺ cells and autoantibody profile – have been associated with clinical response and risk of flare. High baseline CD19⁺ B lymphocyte counts correlate with lower probability of achieving clinical response, longer time to obtain clinical improvement, and shorter depletion [10].

The presence and higher levels of baseline anti-SSA (Ro) and anti-Sm have been reported to be independent predictors of flare, and low C3 to be associated with a shorter period of remission [8,22].

Anti-CD22

In contrast to rituximab, the experience with the use of epratuzumab in SLE is very limited. Epratuzumab is a humanized recombinant monoclonal antibody that targets CD22. CD22 is a co-receptor of the B-cell-antigen receptor (BCR) that links α2,6-sialic acid residues found in many glycoproteins, including IgM [23]. It is detectable in the cytoplasm of pro-B and pre-B cells and on the surface of mature B cells, but is absent from plasma cells. CD22 attenuates BCR-mediated signalling through a down-regulation in calcium efflux in B cells.

Epratuzumab's mode of action seems to be distinct from rituximab. It may act as an immuno-modulatory agent that regulates B-cell function, inducing only partial depletion through ADCC. Epratuzumab does not block the ligand-binding domain but induces a rapid internalization of CD22 followed by tyrosine phosphorylation [24]. Recently, Jacobi et al described a reduction in the activation and proliferation of lupus B cells, and specifically a pronounced reduction in naive and transitional B cells, suggesting that these subsets are preferential targets for epratuzumab [23].

In the only published trial, four doses of 360 mg/m² epratuzumab were administered every other week to 14 patients with moderately active SLE. Dörner et al reported an improvement of at least 50% in the BILAG score in 77%, 71% and 38% of patients at 6, 10 and 18 weeks after treatment, respectively. There was only a mild fall in B-cell count (35%), but no other changes were detected on immunoglobulins, ANA, anti-dsDNA or C3 serum levels. Epratuzumab infusions were generally well tolerated. Five patients developed mild infectious events, and human anti-human antibodies (HAHA) were not detected, suggesting that this antibody may not be immunogenic [25].

B-cell survival factors inhibitors

B-cell maturation, proliferation and survival depend on cell–cell interactions and on the effect of various cytokines, including the B-lymphocyte-stimulator protein (BlyS) and the proliferation-inducing ligand (APRIL). BlyS – also known as B-cell-activation factor (BAFF), TALL-1, THANH or zTNF4 – is a member of the TNF ligand superfamily that binds to different types of B-cell membrane receptors: the B-cell-maturation antigen (BCMA), the BAFF receptor (BAFF-R), and the transmembrane activator and calcium modulating cyclophilin ligand interactor (TACI) [26]. APRIL, on the other hand, binds only to BCMA and TACI. It probably has low isolated biological activity but can form heterotrimers with BlyS (BAHTs), possibly acquiring BlyS-like activity [27].

SLE patients show elevated BlyS levels that correlate with anti-dsDNA titres, disease activity and BAFF-R mRNA expression. TACI expression is also increased compared to controls [28–30].

Belimumab, a fully human monoclonal antibody that neutralizes the activity of soluble BlyS, was first used by Furie et al in a phase-I escalating-dose trial in 57 SLE patients. Only a decrease in B-cell counts (12–47%) was noticed, without any effect on the anti-dsDNA titres or the disease activity; no increased incidence of adverse events was observed [31]. Wallace et al later performed a randomized, double-blind, placebo-controlled, phase-II trial in which 449 SLE patients were treated with three
different intravenous doses (1, 4 and 10 mg/kg) for 52 weeks. Concerns have been expressed about this study as nearly 30% of the patients included were ANA-negative and, although the BILAG system was used, it was inadequately taught to the participants. This trial failed to find significant reduction in disease activity at week 24, but the subset of patients with baseline serological activity (ANA ≥ 1:80 and/or anti-dsDNA ≥ 30 UI) showed an improvement in the SELENA-SLEDAI (Safety of Estrogen in Lupus Erythematosus National Assessment- Systemic Lupus Erythematosus Disease Activity Index) at week 52 [32]. The results remained positive at week 160, and a reduction in SLE flares was reported [33]. A phase-III trial to evaluate the efficacy, safety, tolerability and impact on the quality of life of SLE patients using physicians who have had formal BILAG training is being conducted [34].

Atacicept, a TACI–Ig fusion protein, binds both BlyS and APRIL and is expected to have higher clinical efficacy than an anti-BlyS alone. The only published trial is a phase-I, double-blind, placebo-controlled, dose-escalating study conducted by Dall’Era et al that demonstrated biological activity through a decrease in immunoglobulin levels and in B-cell counts. There were no significant differences in the type or frequency of adverse events comparing to placebo, but atacicept-treated patients experienced mild injection-site reactions [35]. One trial on atacicept in non-renal SLE is ongoing [36].

Anti-cytokine therapy

Anti-IL-6

IL-6 is a crucial cytokine for the differentiation of plasmablasts into mature plasma cells and of T cells into effector cells. It is a growth factor for mesangial cells and epidermal keratinocytes, and a potent inflammatory cytokine inducing fever, anaemia, fatigue and increasing acute-phase proteins [37]. The IL-6 receptor consists of two glycoprotein chains: the IL-6R that is the ligand-specific binding component and the gp130 that mediates intracellular signalling, both found in membranous and soluble forms [38].

In SLE patients, an increase in IL-6 serum levels was detected and associated with disease activity, anaemia, and presence of anti-dsDNA [39,40]. Anti-dsDNA antibodies can also up-regulate IL-6 production in endothelial cells and resting mononuclear cells, supporting a possible reciprocal up-regulation [41]. In lupus nephritis, IL-6 stimulates mesangial cell proliferation, and its urinary levels correlate well with proliferative glomerulonephritis activity [42].

Tocilizumab is a recombinant humanized monoclonal antibody that neutralizes IL-6 receptor, suppressing signalling through both its membranous and soluble forms. It was initially tested in 14 patients with mild to moderated lupus leading to a reduction in acute-phase reactants and activated lymphocyte counts [43]. Illei et al reported an open-label, dose-escalating trial in which 16 SLE patients were treated with three intravenous doses of tocilizumab (2, 4 and 8 mg/kg) bi-weekly for 12 weeks. There was an improvement in the disease activity scores, a decrease in the IgG and anti-dsDNA antibodies, and a dose-related decrease in absolute neutrophil count [44]. A large double-blind controlled trial is needed.

Anti-TNF-α

The evidence of anti-TNF-α efficacy and safety in SLE patients is limited to small open-label trials and case reports, the majority using infliximab. Aringer et al treated six patients with lupus nephritis and/or arthritis, refractory to standard treatment, with four administrations of infliximab 300 mg, in addition to AZA or MTX. There was a significant reduction in proteinuria and a remission of arthritis during the period of treatment [45]. Three patients had urinary tract infections. The levels of anti-dsDNA and anti-cardiolipin antibodies increased in four patients; however, they were not associated with any flare of the disease or thrombotic event. In contrast, Katz et al reported that in nine patients with polyarthritis, treated with infliximab, only one third improved while two thirds experienced infusion reactions [46].

The main concern regarding anti-TNF-α therapies in SLE is related to the potential development of autoantibodies and lupus-like disease that has been recognized with its use in the treatment of rheumatoid arthritis, spondyloarthropathies and Crohn’s disease. Up to two thirds of the patients given
TNF-blockade treatment can develop new-onset ANA. Anti-dsDNA antibodies have also been detected, with an incidence of 9–54% in patients treated with infliximab, even though many of them were IgM subtype and thus non-pathogenic [47]. Nevertheless, lupus-like disease induced by TNF-α blockage seems to be rare, with an incidence of 0.5–1% regardless of the type of anti-TNF-α drug. In these cases, lupus manifestations are usually mild and disappear after the drug is stopped, but there are at least seven reported cases of nephritis that occurred with any of the three anti-TNF agents [48–50]. In addition, anti-phospholipid antibodies can arise during TNF-α blocking therapy and, although uncommon, can lead to major vascular complications [51].

We lack sufficient data to recommend the use of TNF-α blockers in the treatment of SLE. Larger trials are needed, and immunogenicity is still a matter of concern. One study with infliximab in the treatment of active lupus membranous nephritis (WHO class V) in association with azathioprine is being conducted, and another is examining safety and tolerability of etanercept in the treatment of lupus nephritis in combination with standard treatment [52,53].

**Anti-IL-10**

IL-10 is produced in large amounts in SLE patients and correlates with disease activity, although its exact role in SLE pathogenesis has not been completely elucidated [39]. An open-label trial in six corticosteroid-dependent SLE patients showed a reduction in disease activity reported up to 6 months after the administration of an anti-IL-10 murine mAb (B-N10) for 21 days. However, all subjects developed antibodies against B-N10 [54]. Phase-I trials are awaited with a human anti-IL-10 monoclonal antibody.

**Anti-interferons**

The role of interferons (IFNs) in SLE-prone mice is controversial, and there are reports of cases of lupus-like disease induced by IFNα treatment [55]. In fact, patients with active SLE exhibit high levels of IFNα, and IFN-induced gene expression correlates with disease activity [56]. MEDI-545 is a fully human mAb against IFNα whose safety and tolerability in SLE are being analysed in phase-I/II trials [57].

**Inhibitors of costimulation**

The elucidation of the costimulatory interactions between T and B cells and APCs has led to a new range of possible targets for inhibition of pathogenic immune responses like the ones seen in SLE.

**B7-CD28**

Activation of naïve T cells is dependent on the interaction between CD28 on T cells and its ligands B7.1 (CD80) and B7.2 (CD86) on APCs, thus, increasing IL-2 production and T-cell proliferation. Once activated, T cells express an additional surface receptor, the cytotoxic T-lymphocyte-associated antigen 4 (CTLA4), which binds B7 molecules with much more avidity than CD28. CTLA4 has regulatory functions controlling excessive T-cell proliferation through negative intracellular signalling [58].

A recombinant fusion protein encoding regions of the CTLA4 and the Fc region of a human IgG1 (CTLA4Ig) has been developed. It acts as a soluble B7 receptor and reduces the production of pro-inflammatory cytokines by activated T cells and, subsequently, T-cell-dependent antibodies.

The administration of CTLA4Ig to lupus-prone mice prevented autoantibody production and lupus nephritis progression [59]. A marked synergic benefit resulted from the association of CTLA4Ig and cyclophosphamide with faster and sustained decrease of proteinuria and more prolonged survival [60]. Thus, combining CTLA4Ig and other therapeutic agents might be an interesting approach. The disease remission could be induced by a short course of combined treatment and maintenance by CTLA4Ig alone [58]. A sub-therapeutic dose of CTLA4Ig in association with antiCD40L was more effective than using each agent alone in suppressing lupus in NZB/W mice [61]. The results from a major double-blind controlled trial in SLE using abatacept are awaited.
**CD40-CD40 ligand**

B-cell proliferation, activation and immunoglobulin class switching are achieved by multiple costimulatory signals that include the interaction between the B-cell-membrane-bound CD40 and the T-cell CD40 ligand (CD40L or CD154).

The identification of abnormal hyperexpression of CD40L in both murine and human SLE T cells and the evidence of improvement in renal disease in SLE-prone mice with a CD40L antibody encouraged human studies [62,63]. Two humanized anti-CD40L monoclonal antibodies have been created: the IDEC-131 and the BG9588. Despite improvement in proteinuria, haematuria and anti-dsDNA titres, a trial of BG9588 in 28 patients with active proliferative lupus nephritis was stopped due to serious thrombotic events [64]. Phase-I and -II studies of IDEC-131 in patients with mild to moderate SLE did not highlight any safety concerns but failed to demonstrate efficacy [65,66].

**B- and T-cell tolerogens**

Loss of tolerance to self has been considered a major pathogenic mechanism in autoimmune diseases. Treatments that aim to induce tolerance in the immune system have been explored as potential adjuvant therapies in SLE. A good example is the use of tolerogens, synthetic molecules that can bind and cross-link autoantibodies on reactive B-cell surface, promoting B-cell depletion or inactivity.

*Abetimus sodium (LJP-394)*

Abetimus sodium is a synthetic tolerogen composed of four identical strands of double-stranded DNA covalently linked to a non-immunogenic carrier platform. It was developed to target anti-dsDNA receptors on the surface of B-cells, possible leading to B-cell inactivation, limiting its proliferation and antibody production. It also binds soluble anti-dsDNA antibodies, forming small complexes that are then removed from the circulation without activation of the complement system [67].

Abetimus has been used in the investigation of lupus nephritis since early 1990s; it has a good safety profile. It was first demonstrated that abetimus reduced circulating anti-dsDNA antibodies in SLE patients [68]. Later, Alarcon-Segovia et al conducted a phase-II/III trial in which 230 patients received weekly 100 mg followed by 50 mg of intravenous abetimus. Although this trial failed to achieve the primary endpoint in a subgroup of patients with high-affinity antibodies to abetimus DNA epitope, the number of renal flares decreased and the time to flare was prolonged. Generally, 100 mg of abetimus was more effective that the 50-mg dose [69].

In a recently published randomized placebo-controlled study, abetimus 100 mg/weekly was given for up to 22 months to 145 lupus nephritis patients with high-affinity antibodies. Although it did not prolong time to renal flare or initiate immunosuppressive treatment, there were 25% fewer flares in the treatment arm and a significant decrease in anti-dsDNA titres. Other positive effects included a 50% reduction in proteinuria, an improvement in SLEDAI score, and a decrease of 21% in major SLE flares [70]. Single doses of up to 2400 mg have been used without major side-effects, raising the question of whether clinical improvement could be optimized by using higher doses of abetimus. An ongoing trial with lupus nephritis patients using 300 mg and 900 mg of abetimus may answer this question [71].

*Edratide*

Edratide is a peptide synthesized on the basis of the sequence of the first complementarity-determining region (CDR1) of a pathogenic human monoclonal anti-DNA antibody that bears the common idiotype 16/6Id.

CDR1 peptide is able to prevent the development of SLE-like disease in lupus-prone mice and to treat established disease. A reduction in anti-dsDNA antibody levels and an improvement in proteinuria and leucopenia were observed [72]. CRD1 peptide may act by down-regulation of TNF-α and up-regulation of tumour growth factor β (TGF-β) by reducing the T-cell apoptosis rate and by regulating gene expression [72–74].
Two phase-I trials were completed in 2004, showing that edratide was generally safe. A double-blind controlled trial, however, failed to show an improvement in SLEDAI score after 24 weeks of treatment [75].

**Complement blockade**

The activation of the complement system by immune complexes is a well-recognized feature of SLE immunopathogenesis. Both complement pathways converge to C5 activation leading to an amplification of the inflammatory response. In murine models, Bao et al have demonstrated that a C5a receptor antagonist can ameliorate lupus nephritis [76]. Eculizumab, a humanized anti-C5 monoclonal antibody, is currently being investigated in SLE and other autoimmune conditions.

**Immunoablation**

The rationale to use haematopoietic stem-cell transplantation (HSCT) after aggressive immunosuppression in SLE treatment is related to its ability to eliminate pathogenic autoreactive B- and T-cell clones and restore the immune-system self tolerance. Although, effective, HSCT has been associated with a high mortality rate and therefore continues to be considered a rescue therapy for severe refractory organ involvement and should only be performed in experienced centres [77,78].

**Conclusions**

There is some frustration that new therapies introduced for lupus have yet to be shown to be effective in double-blind controlled trials. To date, B-cell depletion seems the most promising biological approach, but there is a need to optimize drug trial design, and we may need to develop a lupus flare index [79].

**Practice points**

- rituximab has been shown to be effective in refractory renal and non-renal lupus in various open-label studies; in our experience, the use of a combined cyclophosphamide protocol induced good remission rates, and retreatment was equally effective
- B-cell-stimulating factors are crucial for B-cell development and survival, but convincing evidence for the use of belimumab and atacicept in SLE treatment is still awaited
- the use of anti-TNF–α therapies has been limited by the risk of developing autoantibodies and possibly lupus-like diseases; therefore, in spite of some early encouraging results in open-label studies, they are not yet widely recommended in clinical practice
- tocilizumab and MEDI-545 are in phase-I/II studies, and a phase-I trial is awaited with a human anti-IL-10
- anti-CD40ligand antibodies have either been associated with the development of thrombotic events or failed to demonstrate efficacy
- reducing the effect of pathogenic anti-DNA antibodies is an attractive approach, especially in renal lupus; abetimus has shown some benefit, but the optimal dose has not yet been defined, while edratide failed to meet its primary endpoints in a small double-blind controlled study

**Research agenda**

- new molecular targets have been identified and directed therapies developed, but evidence of efficacy and safety still needs to be proven in double-blind controlled trials
- drug trial designs and disease activity indexes need to be improved
References

137–54.
systemic lupus erythematosus at University College London Hospital: the first fifty patients. Arthritis Care Res, in press.
systemic lupus erythematosus at University College London Hospital: the first fifty patients. Arthritis Care Res, in press.
[18] US National Institutes of Health. A study of Belimumab, a fully human monoclonal antibody to BLyS in subjects with
prevented by down-regulation of the T cell costimulatory molecule CD40 ligand: an open-label trial. Arthritis Rheum
among serum B lymphocyte stimulator levels, autoantibody profile and clinical response. Arthritis Rheum 2008;67(7):
1011–6.
[33] Gross J, Johnston J, Mudri S, et al. TACI and BCMA are receptors for a TNF homologue implicated in B cell autoimmune
[37] Petri M, Stohl W, Chatham W, et al. Association of plasma B Lymphocytes stimulator levels and disease activity in
[38] Furie R, Stohl W, Ginzler E, et al. Safety, pharmacokinetic and pharmacodynamic results of a Phase I single and double
dose escalation study of lymphostat-B (human monoclonal antibody to BLyS) in SLE patients. Arthritis Rheum 2003;48:
5377.
[40] Furie R, Petri M, Weisman MH, et al. Belimumab (fully human monoclonal antibody to BLyS) improved or stabilized SLE


