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B cell-Targeted Therapies in Systemic Lupus Erythematosus and ANCA-Associated Vasculitis: Current Progress

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Summary:

B cells play a central role in the pathogenesis of systemic lupus erythematosus (SLE) and ANCA-associated vasculitis (AAV). There are various strategies for targeting B cells including depletion, inhibition of survival factors, activation and inhibition of co-stimulatory molecules. Controlled trials in SLE have shown positive results for belimumab, promising results for epratuzumab and negative results for rituximab. The failure of rituximab in controlled trials has been attributed to trial design, sample size and outcome measures rather than true inefficacy. In AAV, rituximab is effective for remission induction and in relapsing disease. However, the optimal long term re-treatment strategy remains to be determined. Over the next 5 years, evidence will be available regarding the clinical efficacy of these novel therapies, biomarkers and their long-term safety.

(120 words)

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic inflammatory disorder characterised by production of auto-antibodies affecting the skin, joints and other internal organs such as brain, lungs, heart and kidneys. Clinical features are heterogeneous and the prognosis is unpredictable. The exact aetiology remains unclear with numerous underlying genetic and environmental factors identified that vary between patients. SLE is characterised by antibodies against nuclear antigens such as Ro, histones and double-stranded deoxyribonucleic acid (dsDNA), and this has been linked to underlying genetic abnormalities in the clearance or innate immune response to apoptotic debris[1]. The subsequent deposition of immune complexes in multiple organs is a key mechanism for resulting inflammation. There is a significant unmet need for therapies that improve efficacy (in life and organ-threatening manifestation), improve quality of life and reduce complications of therapy. For SLE, unlike rheumatoid arthritis (RA), a number of pivotal randomised controlled trials of biologic therapy failed to meet their primary endpoints. This was particularly disappointing as open-label experiences had been promising.

The anti-neutrophil cytoplasmic antibody-associated vasculitides (AAV), defined based on the classification from 2012 revised Chapel Hill Consensus Conference [2], are a group of necrotizing vasculitides, predominantly affecting small vessels and associated with the presence of circulating anti-neutrophil cytoplasmic antibody (ANCA) with few or no immune deposits. AAV includes microscopic polyangiitis (MPO), granulomatosis with polyangiitis (GPA) and eosinophilic granulomatosis with polyangiitis (EGPA). The molecular targets of ANCA are myeloperoxidase (MPO) and proteinase-3 (PR3) and these have recently been shown to closely associate

with both HLA genotype and the mutations in the target antigens [3]. AAV was previously associated with a 2-year mortality rate of 93%, primarily due to renal and respiratory failure [4]. The introduction of treatment with intravenous cyclophosphamide (CYC) and glucocorticoids for remission induction in severe AAV has improved survival times, with 5-year survival rates approaching 80%. However, only 50% of the patients achieve initial remission, many relapse within 2 years of diagnosis, and some may have persistent disease [5]. Repeated treatment leads to cumulative toxicity of CYC and also increases the morbidity from long-term glucocorticoids use. Thus there is an unmet need for agents which are effective and safe.

Our understanding of the pathogenesis of these two auto-immune disorders has improved with the understanding of B cells as the key effectors, responsible for both antibody-dependent and antibody-independent functions in auto-immunity. In SLE, B cells play a central role as sources of autoantibody, as antigen-presenting cells (APC), as initiators and regulators of inflammation through cytokine secretion and lastly are responsible for the organisation of tertiary lymphoid tissue [6]. All these functions may promote the generation and amplification of autoimmune responses in target organs. In terms of the pathogenesis of AAV, the findings of B cells at sites of inflammation, the correlation of B cell activation with disease activity in GPA and the contribution of ANCA to the pathogenesis have provided rationales for B cell-targeted therapy [7]. Hence, targeting the various B cells pathways is an attractive approach for the treatment of SLE and AAV.

The aim of this review article is to provide an update of the clinical efficacy and safety data of B cell-targeted therapies in both SLE and AAV. We will conclude with an expert opinion on the challenges of this approach and key questions to be

addressed in future research. A review of the published literature in English language concerning the clinical efficacy and safety of B cell-targeted agents was undertaken using PubMed, Embase and Cochrane Library databases up to March 2013. The keywords searched include 'ANCA-associated vasculitis,' 'B cells,' 'belimumab,' 'biologics,' 'rituximab' and 'systemic lupus erythematosus.' Abstracts from 2008 to 2012 which were presented at the American College of Rheumatology (ACR) and European League against Rheumatism (EULAR) conferences were searched. Finally, clinical trials that were registered in the national registries were reviewed.

2. Systemic Lupus Erythematosus

2.1. Roles of B cells in SLE

B cells and auto-antibodies have a key role in the pathogenesis of SLE. Abnormal B cell proliferation, maturation and prolonged life-span of auto-reactive clones have been noted along with immune deregulation and tolerance breakdown [8]. The loss of B-cell tolerance is the key to the production of auto-antibodies. Whilst B cell autoreactivity to nuclear antigens is common to most patients, the underlying causes of B cell dysfunction are numerous and probably vary between patients. A proportion of SLE patients have abnormal selection from the immature to early transitional stage due to intrinsic defects of central tolerance [9]. The development of B cell auto-reactivity may, however, also be due to innate immune or other abnormalities. SLE is associated with defects of processing of nucleic acids and apoptotic debris, complement and type 1 interferon activity [1]. These auto-reactive B cells subsequently differentiate into memory and plasma cells leading to the production of auto-antibodies. The most common auto-antibodies are directed

against self-antigens such as deoxyribonucleic acid (DNA) in both the double-stranded (ds) and single-stranded (ss) conformations, ribonucleic acid (RNA)-containing/binding nuclear antigens such as Smith antigen (Sm), ribonucleoprotein (RNP), the Ro/SSA and La/SSB antigens and non-nuclear components like ribosomal P antigen and phospholipids [10]. The prevalence of these auto-antibodies varies and may be associated with individual manifestations of SLE. For instance, antibody to dsDNA antibodies which is present in 30%–70% of the serology of SLE patients correlates with the development and progression of glomerulonephritis [11] whereas antibody to Ro is associated with photosensitivity [12].

In addition to functioning as sources of auto-antibody, B cells are also efficient antigen-presenting cells. Antigen specific B cells have been shown to activate T cells by surface expression of peptide-MHC complex [13]. Spontaneous T cell activation has also been demonstrated *in vivo* confirming the antibody-independent role of B cells [14]. Indeed, murine SLE models in which B cells express surface but not secreted immunoglobulin still develop nephritis [15].

Another potential role of B cells in the pathogenesis of SLE is cytokine secretion. B cells secrete a wide range of cytokines which exhibit both autocrine and paracrine effects [16]. For instance, once an appropriate stimulus is detected by both the B-cell receptor (BCR) and CD40, human B cells secrete pro-inflammatory cytokines such as lymphotoxin-alpha, tumour-necrosis factor alpha (TNF-a) and interleukin-6 (IL-6), which may act in an autocrine manner as growth and differentiation factors and assist in formation of germinal centre structures in inflamed tissues. On the other hand, the stimulation of CD40 without BCR engagement induces B cells to produce regulatory interleukin-10 (IL-10), which has a suppressive effect on other immune

cells. This overproduction of cytokines can lead to amplification of the autoimmune response in SLE [17].

Whilst most B cells can secrete IL-10 in selected situations, animal work suggests there may be distinct populations of IL-10 secreting regulatory B cells [18]. Data for their existence in humans is currently limited. However, the existence of regulatory B cells is of great potential importance in planning B cell targeted therapies. Their depletion by anti-CD20 therapies might be counter-productive and the effect of other means of B cell targeting on such populations is unknown.

2.2. Strategies for B cell Targeted Therapies in SLE

Owing to the multiple pathogenic roles of B cells in SLE, there are various ways in which B cells pathways might be successfully targeted. These are as summarised in Table 1. They include B cell depletion through targeting surface antigens, targeting cytokines in order to limit the B cell activation and differentiation, targeting the interaction between B cells and T cells, development of B cell tolerogens and targeting plasma cells.

2.2.1. B cell depletion or and inhibition:

The B cell antigen CD20 is an activated-glycosylated phosphoprotein which is expressed on the surface of B cells in microvilli with the BCR and CD40 at all stages of development except in the pre-B cells and the terminally differentiated plasma cells stages. This range of expression is regarded as ideal for the treatment of haematological malignancies because B cell repopulation is guaranteed at the end of treatment and plasma cell immune memory is spared. However, these features may

be less beneficial in autoimmune disease where repeat cycles of therapy are required and plasma cells may be pathogenic.

The exact biological function of CD20 remains unclear. CD20 probably functions as a calcium channel since calcium flux on BCR/CD40 stimulation is reduced in CD20 knockout mice and after binding by some experimental antibodies [19]. A recent study showed that the CD20-deficient mice and humans had decreased T cell-independent immune responses [20]. However, CD20 has no known natural ligand. There are various ways in which the anti-CD20 monoclonal antibodies (mAb) may exert therapeutic effects. Boross and Leusen in their comprehensive review illustrated that these are achieved through: 1) cross-linking of multiple CD20 molecules, resulting in cell-death via induction of non-classical apoptosis; 2) activation of complement resulting in complement-dependent cytotoxicity and 3) inducing antibody dependent cell-mediated cytotoxicity (ADCC) in the presence of effector cells [21]. The last of these is generally felt to be most important *in vivo*, although this is difficult to test.

Rituximab was the first licensed anti-CD20 mAb, initially approved in 1997 by the United States Food and Drug Administration (FDA) for the treatment of B cell non-Hodgkin's lymphoma. It has since been licensed for use in follicular and diffuse large B cell lymphomas, chronic lymphocytic leukaemia, refractory rheumatoid arthritis and AAV. There have also been reports of its use in other auto-immune diseases such as auto-immune haemolytic anaemia, primary Sjogren Syndrome[22] and multiple sclerosis in which rituximab was used in patients refractory to the standard treatments [23]. In SLE, a high degree of rituximab efficacy in a wide spectrum of SLE manifestations [24,25] including a systematic review of renal disease [26] was reported in open label studies. Despite the success of these reports, two Phase III

randomised placebo-controlled trials in non-renal lupus, EXPLORER and renal lupus, LUNAR failed to meet their primary endpoints. The key findings of these trials are summarised in Table 2. Rituximab's patent will soon expire in the US and EU and several manufacturers are developing "biosimilars" that should influence the reduction in price although several have recently failed [27].

Ocrelizumab, a humanised anti-CD20 mAb, was tested in SLE in the BELONG trial with a design similar to LUNAR. However in March 2010, Roche (Basel, Switzerland) and Biogen (Cambridge, Mass) decided to suspend the ongoing trials of ocrelizumab in patients with RA and SLE following recommendations of an independent monitoring board. An increase in rates of severe and opportunistic infections, some of which were fatal, was observed[28]. No increase in risk of infection had been noted in trials of rituximab in SLE. Doses of ocrelizumab in BELONG were far higher than usually used with rituximab and it appears to be a more effective B cell depleting agent (authors' unpublished data), which may explain these safety problems. Another humanised anti-CD20 mAb, ofatumumab, with improved antibody-dependent cellular cytotoxicity killing, has demonstrated clinical efficacy in Phase I/II trials in RA [29].

Another option for B cell depletion is targeting of the CD19 molecule. CD19 is a 95 kDa transmembrane glycoprotein, expressed in the development of almost all B cell differentiation stages. Unlike CD20, it has well defined function as a regulator of B cell receptor signalling after binding of antigen. A unique property of anti-CD19 therapy is that it directly targets both autoantibody-secreting plasmablasts and plasma cells as well as early B cell differentiation stages that are spared by anti-CD20 therapy [30]. Currently, a chimeric anti-CD19 antigen receptor antibody is undergoing Phase I/II trials for use in B cell malignancies. However, the use of anti-

CD19 antibody, MDX-1342 in combination with methotrexate (MTX) in RA was discontinued in April 2010 [30]. The use of anti-CD19 mAb in SLE has not been undertaken.

B cell depletion and inhibition can be achieved by targeting the CD22 molecule, another member of the BCR complex. CD22 inhibits BCR intracellular signalling. CD22 is a 140-kDa member of the sialo-glycoprotein expressed on mature B cell lineages. CD22 has a role in the regulation of B cell function, both as lectin-like adhesion receptor and as a component of the B cell activation complex. The function of CD22 through the BCR complex is due to phosphorylation of three tyrosine-based inhibitory motifs (ITIM) on its intracellular tail upon BCR stimulation [31]. Phosphorylation of CD22 leads to recruitment of tyrosine phosphatase 1 (SHP-1) and other effector molecules which in turn limit BCR signalling [32]. Studies in CD22-deficient mice and CD22-negative cell lines indicated CD22 acts as a negative regulatory molecule limiting the intensity of BCR-generated signals through the mechanism of controlling calcium efflux in B cells [33]. Currently, the humanised anti-CD22 mAb, epratuzumab is undergoing two Phase III trials after the success of Phase IIb EMBLEM trial. The results showed clinical improvement in terms of British Isles Lupus Assessment Group (BILAG)-based Combined Lupus Assessment (BICLA) in patients receiving a cumulative dose of 2400 mg epratuzumab/month versus placebo [34]. In terms of safety, the incidence rates of serious adverse events (SAEs) and infusion reactions were comparable across all arms and were not dose-dependent. There was no decrease in immunoglobulin at week 12 in any of the epratuzumab regimens.

2.2.2. Phase III clinical trials of rituximab

The Exploratory Phase II/III SLE Evaluation of Rituximab (EXPLORER) trial was a placebo-controlled, double-blind, multicentre study which recruited 257 SLE patients with moderate to severe non-renal and non-central nervous system (CNS) lupus from North America population [35]. Patients received either a cycle of rituximab, 2 x 1000mg infusion given 2 weeks apart or a placebo with continuation of the background immunosuppressant. All patients also received 0.5–1.0 mg/kg of steroids which were tapered to 10mg daily by week 10.The primary endpoint was to achieve and to maintain clinical response (major, partial or no clinical response) at week 52, assessed using BILAG criteria. No difference in both major clinical responses and partial clinical responses was seen between the placebo group and rituximab group with overall response rates of 28.4% and 29.6% respectively; p = 0.973. Subgroup analysis showed superiority in the rituximab arm for some exploratory or post-hoc endpoints. Rituximab was significantly better in African-American and Hispanic subgroups for the primary endpoint. The rates of BILAG A flares were lower on rituximab [36].

To assess the efficacy and safety of rituximab in lupus nephritis, the Lupus Nephritis Assessment with Rituximab Study (LUNAR) trial, a double-blind, placebo-controlled phase III trial, randomised 144 patients in a 1:1 ratio to receive either rituximab or placebo, both in combination with a background mycophenolate mofetil (MMF) and prednisolone [37]. The primary endpoint at 52 weeks was not met. The overall renal response rates, which included either complete response or partial response, were not statistically different between the rituximab and placebo groups (p = 0.18), mostly driven by partial response rate in the rituximab group.

Despite the failure of both trials in meeting their primary endpoints, there are several lessons to be learned from these trials particularly the issues regarding to the trial

design [38]. Both studies permitted aggressive background immunosuppressant therapy and mandated concurrent high dose of oral prednisolone. In EXPLORER study, more than 50% of the patients in both treatment and control groups had become steroid-dependent, while in LUNAR study patients were also prescribed a high dose of MMF (3g/day) concurrently. The intensity of “standard of care” therapy may have masked the therapeutic benefit of rituximab against placebo. An alternative approach has been to include treatment failure (requirement for increased background immunosuppression) as part of a composite primary endpoint, as in the EMBLEM studies.

The primary endpoint used in EXPLORER required patients to first meet a low disease activity landmark (BILAG C or better in all domains) at 6 months, and then to not flare in the second 6 months. However, the initial landmark was difficult to achieve (only 27% of patients achieved all domains C or better) resulting in lower power to detect a difference in flare rate subsequently. This difficulty may have been because (i) most patients had considerably higher baseline scores than the BILAG 1xA or 2xB required in inclusion criteria, so even a substantial improvement in disease activity was not sufficient and (ii) because BILAG B scores are sometimes poor at differentiating partial responses. For instance, a mild transient malar rash scores the same as deep scarring discoid, or synovitis in a large number of joints (BILAG B). Even in patients who did meet the 6 month endpoint, there was no evidence of a significant rise in BILAG total score again in the placebo arm – a high dose steroid regime may have been sufficient to restore stable disease when given with background immunosuppressant. Overall then, this endpoint relied on passing an initial endpoint in a first phase and then not flaring in a second. However, the first of these was rarely achieved and the second was unnecessary.

Lastly, BILAG may have been scored inappropriately in EXPLORER, both as an inclusion and also a response criterion. The BILAG was originally developed to match the physician's intention to change therapy. Although only around one third of patients achieved major clinical response (MCR) or partial clinical response (PCR), and post treatment mean global BILAG was approximately 8 (on the scale A=9, B=3, C=1), withdrawals due to rituximab inefficacy were relatively low. Around 70% of patients continued the trial for one year as per trial protocol. This might suggest that either residual disease activity was actually felt by treating physicians not to be as severe as the BILAG scores awarded, or that the disease activity present at baseline was not as severe as the BILAG scores suggested, or both.

An alternative means to establish the efficacy of rituximab in SLE has been to correlate clinical outcomes with B cell biomarkers. A study using highly sensitive flow cytometry demonstrated that initial clinical responses were related to the initial degree of B cell depletion and later relapses were strongly associated with memory B cell and plasmablast repopulation. As well as demonstrating a biomarker with potential clinical utility, this observation indicates that the benefits seen in open label series were more likely due to the rituximab than any of the other treatments that may have been used [39].

2.2.3. Targeting cytokines to limit B cell growth and function

Increased understanding of the roles of B-cell activating factor of the TNF family (BAFF, also known as B Lymphocyte Stimulator, BLyS) and its homologue, A proliferation-inducing ligand (APRIL), in B cell survival, maturation and function, has led to development of a new class of biologics for SLE. Human BAFF and APRIL bind to three TNF superfamily receptors: BAFF receptor (BAFF-R), TNF receptor

superfamily member 13b (TACI) and B cell maturation antigen (BCMA), that are expressed on B cells at different developmental stages. BAFF-R binds BAFF strongly, BCMA binds APRIL and TACI binds both BAFF and APRIL. APRIL also has a proteoglycan binding site that facilitates its aggregation on cell surfaces [40]. Each receptor activates its own set of signalling pathways with BAFF-R being the only BAFF receptor to activate the alternative NF-κB pathway [41,42]. Transitional and naïve B cells have dominant expression of BAFF-R and are most dependent on BAFF. Plasma cells predominantly express BCMA and depends on APRIL. TACI appears to be an inducible receptor which may have inhibitory functions and is expressed at the memory and plasmablast stage.

The rationale for targeting BAFF is supported by evidence of increased serum levels of BAFF and APRIL in several B cell-mediated autoimmune diseases including SLE, for which both cytokines appeared elaborated at the site of inflammation [43,44]. In addition, BAFF concentration has been shown to correlate with disease activity and anti-dsDNA antibody titres [45].

To date, three BAFF/APRIL targeted agents that have reached Phase III clinical trials in SLE. Anti-BAFF monoclonal antibody (belimumab) induces selective BAFF blockade, whereas a TACI-Fc decoy receptor (atacicept) is capable of inhibiting both circulating BAFF and APRIL signalling pathways. Another anti-BAFF monoclonal antibody that may have superior blockade of soluble trimers and membrane-bound BAFF, tabalumab is currently recruiting patients in Phase III SLE trial [46]. Belimumab is the only new drug in over 50 years that has gained approval from the US FDA in the treatment of active SLE. The main results of the Phase III trials are summarised in Table 2. However, a phase II trial of atacicept in combination with MMF in lupus nephritis was suspended recently due to a high rate of severe

infections. A phase II/III trial of atacicept for patients with non-renal lupus is currently ongoing [28].

2.2.4. Phase III Clinical Trials of belimumab

Phase II trials of belimumab were negative. Many have attributed this to recruitment of around 30% patients who had negative ANA [47]. This may be particularly relevant for a B cell targeted therapy and ANA positive patients maintained responses better in extension studies [48]. The Study of Belimumab in Subjects with Systemic Lupus Erythematosus (BLISS-52) was designed based on a new composite endpoint derived from the phase II study data and requirement for ANA positivity, as well as a larger population [49]. 867 patients with active disease were recruited from various multi-centres in Latin America, Asia-Pacific and Eastern Europe. The patients were randomised in a 1:1:1 ratio to placebo or belimumab 1 mg/kg or belimumab 10 mg/kg. The primary endpoint was improvement in the Systemic Lupus Erythematosus Responder Index (SRI) at week 52 (reduction ≥4 points in Safety of Estrogens in Lupus Erythematosus National Assessment–SLE Disease Activity Index (SELENA-SLEDAI) score; no new BILAG A organ domain score and no more than 1 new B organ domain score; and no worsening (<0·3 increase) in Physician's Global Assessment (PGA) score). Significantly higher SRI rates were achieved with belimumab 1 mg/kg (51%, p=0·0129) and 10 mg/kg (58%, p=0·0006) than placebo (44%) at week 52. Only the 10 mg/kg dose was efficacious against the placebo in all three SRI components.

BLISS-76 was a second phase III clinical trial of belimumab in SLE that differed only in its longer duration (76 weeks) and region of recruitment (North America and

Europe). 826 patients were recruited [50]. Belimumab 10 mg/kg met the primary endpoint with significantly greater SRI response at week 52 compared with placebo (43.2% versus 33.5%; p = 0.017). There was no major difference among the 3 groups in terms of safety compared to that of BLISS-52. One patient with a background history of high dose steroid use and AZA, suffered from an opportunistic infection with disseminated cytomegalovirus, occurred 3 weeks after the third dose of belimumab 10mg/kg. This resolved after treatment with an antiviral medication. One patient in each treatment group had a grade 3 adverse event of IgG reduction at week 52 but neither was associated with infection.

The BLISS-52 and BLISS-76 trials recruited patients mainly with mucocutaneous and musculoskeletal manifestations, which accounted for about 2/3 of cases. Patients with severe lupus nephritis and neurological manifestation were excluded. Hence, further studies to evaluate the use of belimumab in these vital organ complications and the use of this therapy in refractory, severe SLE manifestations are required.

A considerable interest has also focussed on the use of this newly established SRI, as a composite index used in both BLISS trials. This composite index was developed to ameliorate the limitations of each of the individual index that constitute the SRI. The SRI has some significant differences in outcome from the scores from which it was derived. For example, the SRI discounts a single BILAG B score as being enough to classify someone as a non-responder. The relevance of this index to clinical practice is therefore currently less clear.

Although this endpoint is new, the effect size of belimumab in these studies appears relatively small. Indeed, the magnitude of difference between the treatment and

control groups in LUNAR was comparable with the differences observed in BLISS trials numerically but with ten times the numbers of patients in the latter [51]. Post hoc analyses of the BLISS-52 and -76 data demonstrated a higher degree of efficacy in subgroups of patients with markers of higher disease activity – positive anti-dsDNA antibodies, low complement or higher baseline SLEDAI. For this reason, a marker of high disease activity has been specified in belimumab's license in the European Medicines Agency [52]. However the exact criteria for high disease activity are not defined, and could be serological or clinical.

2.2.5. Targeting the interaction between B cell and T cell

Another option for B cell targeted immunotherapy is to block the co-stimulatory interactions between B and T cells. CD40 ligand (CD40L) is expressed on activated helper T cells, which binds to CD40 on B cell surface. The binding of this antigen-ligand, CD40–CD40L provide signals that drive B cell activation, proliferation and differentiation into plasma cells. Translating this into practice, direct blockade of B cell–T cell interaction via the CD40–CD40L pathway has been shown to reduce inflammation, vasculitis and fibrosis in lupus nephritis and prolonging survival in the murine lupus model [53]. Treatment with BG9588, a humanised anti-CD40L monoclonal antibody, has been shown to reduce IgG anti-dsDNA antibody, increase C3 and decrease haematuria in SLE patients [54]. However, a phase II open-label study was terminated prematurely due to occurrence of thrombo-embolic events [55]. The other anti-CD40L mAb, IDEC-31, which blocks the CD154–CD40 also failed to show significant difference in terms of either SLEDAI or adverse events against placebo at week 20 in the Phase II randomised controlled trial [56].

Activated B cells also express other co-stimulatory molecules such as B7 proteins (CD80/86 or B7.1/B7.2) that bind to CD28 on the surface of T cells. These provide a second signal necessary for T cell activation as well as the cognate antigen first signal. The B7 proteins are members of the immunoglobulin superfamily that bind to CD28 on naive T cells. They also bind to the decoy receptor, CTLA-4, expressed on T cells following activation. CTLA-4 binds B7 molecules with higher avidity than CD28 and transduces a negative signal to the activated T cells in order to limit excessive proliferative response of activated T cells [55]. Abatacept, a fusion protein of the extracellular domain of CTLA4 and the constant region of immunoglobulin, has been developed to block co-stimulation[57]. This drug has shown promising results in murine lupus nephritis by reducing proteinuria and prolonging survival [58]. A phase IIb randomised clinical trial however failed to meet the primary endpoint of reducing lupus flares. Despite this, secondary analysis showed that SLE patients with arthritis significantly improved using this agent [59].

2.2.6. Development of B cell tolerogens

Acknowledging the implication of anti-dsDNA antibodies in the pathogenesis of lupus nephritis, Abetimus sodium (LJP-394) was developed as a B cell tolerogen. It is a synthetic agent that is made up of 4 identical strands of dsDNA, covalently linked to a small molecule platform. The intention is to selectively remove circulating pathogenic antibodies. The results from a Phase III randomised controlled trial showed that Abetimus (dose: 100 mg/week) significantly reduced anti-dsDNA antibody levels but did not significantly prolong time to renal flare when compared with placebo [60]. However, there was a trend towards fewer renal flares in the abetimus group and significantly more patients in this group also experienced reduction of ≥ 50% proteinuria compared to the placebo at 1 year ($p = 0.047$).

Disappointingly, another Phase III trial, ASPEN had to be terminated as continuation of study was deemed futile according to the interim efficacy analysis.

2.2.7. Targeting plasma cells

Elimination of plasma cells and long-lived auto antibodies that are spared by most B cell targeted therapies is also a potentially attractive option. This may be achieved using proteasome inhibitors. Bortezomib is a selective inhibitor of the 26S proteasome that triggers the terminal unfolded protein response, leading to cell-cycle arrest and apoptosis. Other roles of proteasome inhibitors that can provide therapeutic benefits include blocking the nuclear factor κB (nFκB), inhibiting the activated B cells, germinal centre B cells and dendritic cell and rendering the release of nFκB-induced pro-inflammatory cytokines such as TNF, IL-1 and IL-6 from activated T cells [61]. Bortezomib has been approved for the initial treatment of multiple myeloma [62] and has been shown to induce depletion of plasma cells producing dsDNA antibodies in murine lupus [63]. However, its use is limited by toxicity such as painful peripheral neuropathy. The alternative agent, carfilzomib has shown improved safety compared with bortezomib [64]. This selective proteasome inhibitor was approved by the FDA for use in patients with relapsing and refractory multiple myeloma in July 2012. Trials of both proteasome inhibitors in human SLE have not been reported.

3. ANCA Associated Vasculitis

3.1. Roles of B cells in AAV

There is substantial evidence that ANCA is directly pathogenic in AAV [65]. In 1990, ANCA was demonstrated to stimulate respiratory bursts in neutrophils and triggered the release of primary granule constituents [66]. This was also supported by *in vitro* studies which showed ANCA stimulated vascular damage by inducing neutrophil effector function, including cytokine and chemokine release and produced cell lysis through adhesion to cultured endothelial cells [67]. Lepse and colleagues, in their comprehensive review of the immunopathogenesis of AAV, described that the release of antigens PR3 or MPO from activated neutrophils can be internalised and presented by APC to T-helper cells [68]. Current understanding of the pathogenesis of AAV has also suggested contribution of dysregulation of the T lymphocytes; impaired function of regulatory T cells (Tregs) in AAV may facilitate the differentiation of B cells into ANCA-producing plasma cells [69]. Other roles of ANCA include inducing the release of BAFF from neutrophils. BAFF has been demonstrated to promote the survival of auto-reactive B cells and together with Interleukin-21, they synergise in stimulating plasma cell differentiation [70].

In addition to being precursors of ANCA-producing plasma cells, B cells also have other roles in the pathogenesis of AAV. As described earlier in this article, B cells are responsible for producing pro-inflammatory cytokines and present antigens to T cells. They activate T cells and thereby stimulate their proliferation, differentiation and polarisation, thus enhancing the activation of primed T cells [71]. It has also been noted that the frequency of activated B lymphocytes has been associated with both disease activity and disease severity [72]. Moreover, autoantigen-specific B cells are present at inflammation sites where tertiary lymphoid-like organs are produced [73]. All these provide a rationale for using B cell targeted therapies in AAV.

The potential value of therapeutic targeting of B cells was shown around 3 decades ago. A study on mice found that re-expression of B cell surface immunoglobulin was grossly impaired following treatment with CYC, but not with other immunosuppressive agents. This explained the increased susceptibility of B cells to tolerance induction with thymus-independent antigens [74]. Translating this concept to human studies, elevated spontaneous secretion of immunoglobulin by peripheral blood B cells in patients with GPA, was found to suppress back to normal levels during CYC therapy. There was a selective difference in suppressive effect of CYC therapy on the various stages of the B cell cycle including activation, proliferation, and differentiation [75]. Hence, this selective suppression of B cell function at multiple points in the B cell cycle may be responsible for the efficacy of CYC therapy in immune complex-mediated diseases.

3.2. Results from Randomised Controlled Trials of rituximab in AAV

In April 2011, Rituximab became the first biologic to gain approval from US FDA for use in remission induction in severe AAV. This followed 2 successful randomised controlled trials; Rituximab versus Cyclophosphamide for ANCA-Associated Vasculitis (RAVE) and Rituximab versus Cyclophosphamide in ANCA-Associated Renal Vasculitis (RITUXVAS) trials. The main results are summarised in Table 3.

RAVE was a US multicentre, randomised, double-blind, double-dummy, trial comparing rituximab with standard CYC therapy [76]. The primary endpoint was disease remission based on Birmingham Vasculitis Activity Score (BVAS) of 0 without the use of prednisolone at 6 months. 197 patients were recruited and were randomised in a 1:1 ratio to rituximab or a cyclophosphamide-based regimen. Nearly half of the patients in each arm were newly diagnosed with GPA or MPA while the

other half had relapsing diseases. The inclusion criteria were positive serum assays for PR3 or MPO, manifestations of severe disease and a BVAS of 3 or more. Patients with severe alveolar haemorrhage requiring ventilator support and those with advanced renal disease were excluded. The results showed non-inferiority of rituximab against the standard CYC regime for remission induction of severe AAV at 6 months. Subgroup analysis also indicated superiority of rituximab against CYC among patients with relapsing disease after previous remission induction with cylophosphamide. In terms of safety, treatment with rituximab was comparable to the control group with no statistically difference in the numbers of total adverse events, SAEs or non−disease related adverse events. 1 cancer case was diagnosed in each group during the first 6 months. At 12 months, solid tumours developed in 6 of 124 (5%) in rituximab-exposed patients, as compared with 1 of 73 patients without exposure to rituximab (1%, $p = 0.26$). These patients had histories of exposure to at least two medications known to increase the risk of cancer (CYC, azathioprine (AZA), or MTX).

The second study was an open label randomised controlled trial; rituximab versus cyclophosphamide in ANCA associated vasculitis (RITUXVAS) which recruited patients with renal involvement and was powered for superiority [77]. 44 patients recruited from Europe and Australia were randomised in a 3:1 ratio to either a standard glucocorticoid regimen plus rituximab with two intravenous CYC pulses (treatment group) or intravenous CYC for 3 to 6 months followed by AZA (control group). Inclusion criteria included a new diagnosis of AAV, ANCA positivity and renal involvement as evidenced by necrotising glomerulonephritis on biopsy or red cell casts or haematuria (≥30 red cells per high power field) on urinalysis. The primary endpoints were sustained remission rates (BVAS score of 0 for at least 6 months)

and SAEs at 12 months. Although the sustained remission rates were high in both groups, rituximab was not superior to the standard CYC regime; p = 0.68. Among the 9 patients who were dialysis-dependent at study entry, 6 of the 8 patients (75%) in the rituximab group had a sustained remission (5 of whom no longer required dialysis). SAEs occurred in 14 patients in the rituximab group (42%) and 4 patients in the control group (36%) (p = 0.77). At 12 months, 6 of the 33 patients in the rituximab group (18%) and 2 of the 11 patients in the control group (18%) died due to severe infections, cardiovascular events and complications of end-stage renal disease. 2 cancer cases; melanoma and breast cancer developed in the rituximab group at 9 and 14 months respectively.

4. Expert Commentary

There is an unmet need for more effective therapies in SLE and AAV. The high mortality rate, the impairment of quality of life and the complications of therapy are all unsatisfactory with use of conventional therapy. In both conditions, there are clear potential targets for biologic therapies from disease models. Given the licensing of rituximab in RA, implementation of B cell depletion in other indications was predicted to be straightforward. Rituximab is widely used based on open-label case series and it appears highly efficacious and cost-effective. There are several reasons for the failure of B cell depletion in clinical trials in SLE. B cell depletion is more frequently incomplete than in RA and long-lived plasma cells that produce the auto-antibody may not be affected by this anti-CD20 mAb. These factors may perhaps explain why “B cell modulating” treatments such as inhibition of the BAFF system or modification of BCR signalling have had more success in clinical trials. However, the development of biologics in SLE has revealed unexpected problems with the design

and conduct of clinical trials. The failure of rituximab in RCTs has been suggested to be due to inappropriate endpoints, the use of an active comparator, inadequate inclusion criteria for a heterogeneous disease and sample size. Although belimumab has met primary endpoints in phase III clinical trials, these trials, and the negative phase II trials that preceded them, may have been affected by similar issues. We still do not know the best means to establish new therapies in SLE. The number of patients required to demonstrate efficacy for belimumab was very large and the effect size according to the primary endpoint was small. The true effect size is hard to judge as the SRI endpoint is rather intangible in relation to usual clinical practice. Furthermore, the duration of therapy required is unknown due to the unpredictable natural history of SLE. For these reasons, estimation of the cost-effectiveness of belimumab has been problematic.

The key issue underlying the complexity of biologics development in SLE is therefore the heterogeneity of the disease. As therapies become more specific, they may also become more selective in which manifestations they will treat. The spectrum of disease in SLE includes certain manifestations with clear autoantibody or immune complex-mediated inflammation, where B cell targeted agents are likely to be effective. In other manifestations, it may be difficult to judge whether there remains current active inflammation, such as arthralgia and fatigue. Lastly, there may be inflammatory features where the roles of B cells are less clear, and other targets must be blocked, such as type 1 interferons. There is intriguing evidence for this possibility; belimumab is more effective in patients with high titres of autoantibodies and low complement, whilst the anti-interferon agent rontalizumab appears more effective without this abnormal serology [78]. Rituximab is less effective in certain types of cutaneous LE [79]

In AAV, establishing the efficacy of rituximab has been more straightforward. Data from randomised controlled trials has matched those seen in open label series. The rapid implementation of rituximab in the clinic has been due to its advantage over cyclophosphamide in resistant disease and potentially, cumulative toxicity. Future issues concern uncertainty over the long-term management after successful remission induction. Patients unresponsive to other agents may require repeated cycles of rituximab, but cumulative side effects, particularly hypogammaglobulinaemia, may then become more problematic. The duration of response is highly variable which makes determining the best dosing schedule of rituximab long-term difficult. Surprisingly, although there is good evidence for the pathogenicity of ANCA *in vitro*, its levels are only weakly correlated to response and relapse. Most importantly, the mechanism of action of B cell depletion has not been clearly demonstrated. Hence, biomarkers that can guide treatment decisions, developed in RA and SLE, are needed in AAV.

5. 5-year View

We are still in the earliest phases of the development of biologics for SLE and AAV. Several appropriate targets have been identified, including some non-B cell targets. In SLE, most of the early challenges have been related to trial design. Next stage of development will be the assessment of the numerous molecules in pre-clinical and phase I and II studies using lessons learned from the development of rituximab, belimumab and epratuzumab. Studies that guide stratification and personalisation of therapy to individual patients and disease manifestations are required but currently limited. In AAV, the next challenge is to develop the best strategy for long-term use of rituximab. Several studies are planned or in progress to address this.

In both SLE and AAV, the first priority has been to meet the unmet need for treatments that prevent life and organ threatening inflammatory disease, especially in patients resistant to conventional therapies. As this need will be soon met, other challenges will become more prominent particularly in terms of long-term management of these diseases. First, studies should aim to focus on patient-reported outcomes, quality of life, reduce long-term steroid use, improving work instability and social participation. Secondly increased mortality from cardiovascular disease and infection must be improved. It is not yet entirely clear what benefits biologics will have on these outcomes relative to one another or conventional therapy. With respect to infection, it is worth noting that the question of a link between progressive multifocal leucoencephalitis and B cell depletion remains unresolved but has previously led to withdrawal of two licensed biologics in other disease areas.

The patent for rituximab in RA will expire in the next 1-2 years in North America and Europe. Several manufacturers are developing biosimilars. The licensing of these agents in the next few years may greatly influence the cost-effectiveness of rituximab which could become a major factor in choice of biologic. There are important questions to be addressed regarding the extrapolation of efficacy data between indications; all planned biosimilar studies are in RA or malignancy.

(6720 words)

6. Key Issues:

- B cells are responsible for both antibody-dependent and antibody-independent functions in the pathogenesis of SLE and AAV.
- The main strategies for B cell targeted therapies include B cell depletion, targeting cytokines in order to limit the B cell growth and functions, targeting the interaction between B cells and T cells, development of B lymphocytes tolerogens and targeting plasma cells.
- Translating the findings from bench to bedside in the treatment of SLE has proven difficult, as open label experiences did not match results from controlled trials.
- Data from controlled trials in SLE showed positive results for belimumab (BLISS-52 & BLISS-76), promising results for epratuzumab (EMBLEM) and negative results for rituximab (EXPLORER & LUNAR).
- Failure of controlled trials of rituximab was attributed to poor trial design including inappropriate endpoints, the use of an active comparator, inadequate inclusion criteria for a heterogeneous disease and non-powered sample size.
- Data from controlled trials in AAV showed rituximab is effective for remission induction and is superior to cyclophosphamide regimen in relapsing disease (RAVE & RITUXVAS).
- The best long-term re-treatment strategy after remission induction in AAV remains to be determined. The duration of efficacy appears to be highly variable between patients, which makes judicious use of rituximab long term difficult. Thus, biomarkers that can guide treatment decision are needed.

- The future research agenda will focus on better trial design, clinical efficacy of novel B cell targeted therapies with stratification of therapy to disease manifestation and their long-term safety particularly in terms of the risk of severe infection.

Table 1 B cell targeted therapies in clinical trials in SLE

Drug	Molecular Target	Phase	Comments
Rituximab [35,37]	CD20 depletion	III	Both Phase III trials failed to achieve primary and secondary endpoints
Ocrelizumab [28]	CD20 depletion	III	Both Phase III trials were suspended due to safety signals
Ofatumumab [29]	CD20 depletion	N/A	Evidence of clinical efficacy in Phase I/II trial of RA
CD19 chimeric antigen receptor antibody [30]	CD19 depletion	N/A	Currently undergoing Phase I/II trial in B cell malignancies
MDX-1342 [30]	CD19 depletion	N/A	Phase I study in RA was suspended
Epratuzumab [34]	CD22 depletion and inhibition	II	Phase II trial showed clinical efficacy in the 2400 mg cumulative dose/month. Currently undergoing two Phase III trials
Belimumab [49,50]	BAFF blockade	III	Both Phase III trials successfully met their primary endpoints. First biologic approved by US FDA for use in SLE

Drug	Molecular Target	Phase	Comments
Atacicept [28]	BAFF & APRIL blockade	II/III	Phase II trial in renal SLE was suspended. Phase II/III in non-renal SLE is ongoing
Tabalumab [46]	BAFF blockade	III	Phase III trial in SLE is ongoing. Phase III trial in RA was discontinued due to lack of efficacy.
BG9588 [55]	CD40L blockade	II	Phase II trial was terminated due to thrombo-embolic events
IDEC-31 [56]	CD154–CD40 blockade	II	Phase II study failed to achieve primary endpoint
Abatacept [59]	Selective T cell co-stimulation modulator	II	Phase II study failed to achieve primary endpoint. May be beneficial in SLE patients with arthritis predominance
Abetimus [59]	B cell tolerogen to anti-ds-DNA antibody	III	Phase III trial failed to achieve primary endpoints. Another trial was terminated as deemed futile

Drug	Molecular Target	Phase	Comments
Bortezomib [63]	Proteasome inhibitor to eliminate plasma cells	N/A	Approved for multiple myeloma. Showed evidence in murine lupus
Carfilzomib [64]	Proteasome inhibitor to eliminate plasma cells	N/A	Recently approved for multiple myeloma

APRIL: a proliferation-inducing ligand, BAFF: B-cells activating factor of the tumour necrosis factor family, dsDNA: double stranded-deoxyribonucleic acid, N/A: data not available for trials in systemic lupus erythematosus, RA: rheumatoid arthritis, SLE: systemic lupus erythematosus

Table 2 Results of Phase III RCTs in SLE

Characteristic	EXPLORER	LUNAR	BLISS-52	BLISS-76
Author	Merrill J T et al [35]	Rovin B H et al [37]	Navarra S V et al [49]	Furie R et al [50]
Number of patient	267	144	867	819
Follow-up (weeks)	52	52	52	52
Inclusion Criteria	ANA +ve, Active disease (>1 BILAG A or ≥2 BILAG B) and 1 stable DMARDs	ANA +ve history, ≥ Class 3 LN on biopsy and Proteinuria (UPC ratio >1)	ANA/Anti-dsDNA +ve, Active disease (SELENA/SLEDAI ≥6) & stable DMARDs	ANA/Anti-dsDNA +ve, (SELENA/SLEDAI ≥6) & HCQ can be added up to week 16
Exclusion Criteria	Severe CNS or LN and ≤12 weeks recent use of CYC or CAL	> 50% glomerular sclerosis and eGFR <25 ml/minute/1.73 m^2	Severe LN or CNS, prior RTX & <6 months use of CYC	Severe LN or CNS, prior RTX & <1 year use of other biologics
Dosing Schedule	Pb + Pred + DMARDs vs RTX + Pred + DMARDs	Pb + Pred + MMF vs RTX + Pred + MMF	Pb + Pred vs BLB 1mg/kg + Pred vs BLB 10mg/kg + Pred	Pb + Pred vs BLB 1mg/kg + Pred vs BLB 10mg/kg + Pred

Characteristic	EXPLORER	LUNAR	BLISS-52	BLISS-76
Primary Endpoint	Major or Partial or No response based on BILAG	Complete or Partial or No Renal Response Rate	SRI	SRI
Achieved? (Yes/No)	No	No	Yes	Yes
Comments	Secondary endpoints were not achieved. Primary endpoint met in Hispanic & African-American. Anti-dsDNA ↓ Complements ↑	Secondary endpoints were not achieved. Black patients showed more partial response although was not statistically significant	BLB 10 mg/kg was efficacious against the placebo in all three SRI components & reduction of >50% Pred dose from baseline	Only BLB 10 mg/kg statistically met the primary endpoint and was still efficacious despite index threshold increased

ANA: Anti-neutrophil antibody, BILAG: British Isle of Lupus Assessment Group, BLB: Belimumab, CAL: Calcineurin inhibitor, CNS: Central Nervous System, CYC: Cyclophosphamide, dsDNA: Deoxyribonucleic acid antibody, DMARDs: Disease Modifying Anti-Rheumatic Drugs, HCQ: Hydroxychloroquine, LN: Lupus nephritis, Pb: Placebo, Pred: Prednisolone, RTX: Rituximab, SELENA-SLEDAI: Safety of Estrogens in Lupus Erythematosus National Assessment–SLE Disease Activity Index, SRI: Systemic Lupus Erythematosus Responder Index, UPC: Urine Protein Creatinine Ratio

Table 3 Results of Phase III RCTs in AAV

Characteristic	RAVE	RITUXVAS
Reference	Stone J H et al [76]	Jones R B et al [77]
Number of patients	197	44
Follow-up (weeks)	24	52
Inclusion Criteria	PR3 or MPO positivity, manifestations of severe disease and a BVAS of 3 or more. Half of the patients were nearly diagnosed	Newly diagnosed AAV, ANCA positivity and renal involvement evidenced by renal biopsy or red cell casts or haematuria on urinalysis
Exclusion Criteria	Severe alveolar haemorrhage requiring ventilator support and advanced renal dysfunction (serum creatinine >354 µmol/l)	Previous CYC (> two weeks of either oral or IV) and co-existence of another multisystem autoimmune disease e.g. SLE
Dosing Schedule	Pb + oral CYC (2mg/kg) ± AZA if in remission after 3 month vs RTX (375mg/m^2 weekly x 4) ±	IV CYC (3-6 months) + Pred ± AZA if in remission after 3 months vs RTX (375mg/m^2 weekly x 4) along with (2 x IV CYC pulses) +

Characteristic	RAVE	RITUXVAS
	Pb AZA if in remission after 3 month	Pred
Primary Endpoint	BVAS of 0 and successful completion of the prednisone taper	Sustained remission (BVAS score of 0 for at least 6 months) and SAEs at 12 months
Achieved? (Yes/No)	Yes	No
Comments	RTX is non-inferior to CYC in remission induction and maybe superior in relapsing disease. Also efficacious in patients with major renal disease or alveolar haemorrhage	RTX is not superior to CYC in remission induction in renal vasculitis and also in reducing early SAEs. Sustained remission rates were > 90% among survivors in both groups

AAV: ANCA-associated vasculitis, ANCA: Anti-neutrophil cytoplasmic antibody, AZA: Azathioprine, BVAS: Birmingham Vasculitis Activity Score, CYC: Cyclophosphamide, IV: Intravenous, MPO: Myeloperoxidase, Pb: Placebo, Pred: Prednisolone, PR3: Proteinase 3, RTX: Rituximab, SAEs: Serious adverse events, SLE: Systemic Lupus Erythematosus

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