

Successful sequential therapy with rituximab and belimumab in patients with active systemic lupus erythematosus: a case series

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ABSTRACT

Objective. *B cells play an important role in the initiation and progression of systemic lupus erythematosus (SLE). Accordingly, B cell-targeted therapy has been suggested as a new rational approach for treating lupus. Belimumab, a human monoclonal antibody directed against B lymphocyte stimulator (BLyS), was reported as the first biological treatment effective in reducing mild-to-moderate SLE disease activity by using different scoring systems and endpoints. Conversely clinical trials with rituximab, a chimeric monoclonal antibody directed against the CD20 expressed by B cells, have failed to achieve primary endpoints in spite of a number of reports showing its beneficial effects. Anecdotal reports have described the sequential use of rituximab and belimumab as a more effective treatment than using the individual drugs alone, without compromising safety.*

Methods. *We report a case series of three patients with active SLE refractory to conventional therapies, who underwent treatment with rituximab followed by belimumab as maintenance therapy.*

Results. *We observed a beneficial effect after sequential treatment with rituximab and belimumab. All patients achieved long-standing remission and could reduce or discontinue corticosteroids. Concomitantly, after rituximab administration we observed a rise in BLyS levels, which were dramatically reduced after belimumab introduction.*

Conclusion. *The modulation of plasma BLyS kinetics in patients undergoing sequential treatment with rituximab and belimumab may represent a possible rationale behind the effectiveness of this combined therapy.*

Introduction

B cells play an important pathogenic role in the induction and progression of systemic lupus erythematosus (SLE). Due to their ability to escape the selection mechanisms that normally lead to the deletion of autoreactive clones, B cells from SLE patients not only produce autoantibodies, but also exert their pathogenic action by presenting autoantigens to T cells and secreting a variety of pro-inflammatory cytokines that perpetuate

the activation of the immune system (1). For this reason, the interest of researchers has been drawn to drugs that are effective in modulating the B cell compartment such as rituximab and belimumab. Rituximab is an anti-CD20 chimeric monoclonal antibody able to deplete CD20 positive B cells. Although it has been shown to have beneficial effects on SLE in a number of observational and retrospective studies, it failed to reach primary endpoints in two clinical trials (EXPLORER and LUNAR) (2, 3). The negative outcome of these clinical trials may depend on a wrong trial design, but the observation that treatment with rituximab triggers a surge in B lymphocyte stimulator (BLyS), could explain the exacerbation of the disease and subsequent failure of the studies (4, 5). BLyS, also called B cell-activating factor (BAFF), is actually fundamental in promoting B cell survival, differentiation and activity, via its interactions with BLyS receptor 3 (BR3), transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI), and B cell maturation antigen (BCMA), all expressed on B cells (6). Belimumab, a fully humanised IgG1 monoclonal antibody directed against BLyS, is the first biological treatment specifically designed for patients with SLE and, unlike rituximab, it has been shown to have beneficial effects in two randomised trials (BLISS-52 and BLISS-76) involving autoantibody-positive SLE patients with persistent disease activity despite standard treatment (7, 8). Belimumab inhibits B cells by blocking BLyS thus reducing disease activity (6).

On the basis of evidence coming from animal models (9) and anecdotal cases (10-13), it has recently been suggested that the combined use of the two B cell-targeted drugs (rituximab as induction and belimumab as maintenance therapy) may be more effective than using the individual drugs alone. We describe three patients with active SLE who achieved disease control after sequential treatment with rituximab and belimumab and we discuss the rationale behind.

Case report 1

This 48-year-old female patient was diagnosed as having SLE in 2007 on

Competing interests: none declared.

the basis of arthritis and renal involvement presenting as nephrotic syndrome (maximum proteinuria 9.4 g/24h), ANA, anti-SSA positivity and high anti-dsDNA levels, hypocomplementaemia and inconstant leukopenia. Remission was achieved after steroid pulses (methylprednisolone 1 g for three consecutive days) and oral/intravenous cyclophosphamide (4 g in total). As maintenance therapy, she was started on hydroxychloroquine (HCQ) 400 mg daily and mycophenolate mofetil (MMF) 2 g daily, with oral prednisone being gradually tapered to 10 mg daily. She experienced remission from 2007 to 2010. In 2010, a mild disease flare was treated by increasing the prednisone dose to 25 mg daily, which led to remission. In 2012, a renal biopsy was performed because of a severe disease relapse with mild leukopenia, hypocomplementaemia, an increase in anti-dsDNA titre, persistent proteinuria (1.4 g/24h) and no urinalysis abnormalities (SLEDAI-2K=13; Fig. 1A and 2A). The renal biopsy showed diffuse proliferative glomerulonephritis (class IVa according to the WHO classification) and so she was treated with steroid pulses (methylprednisolone 1 g for three consecutive days) and two intravenous infusions of rituximab 1 g 14 days apart. MMF therapy was discontinued due to intolerance. Remission (SLEDAI-2K=4) was achieved after the first rituximab cycle, and persisted for more than a year, but she continued taking a medium dose of prednisone (10 mg). Rituximab was therefore discontinued and belimumab 10 mg/kg was started after verifying that CD19⁺ lymphocyte levels were in the normal range. After six months, prednisone was tapered to 2.5 mg daily with no flares. At the 36-month-follow-up the patient was still in remission and she had discontinued steroids while continuing HCQ 400 mg daily (Fig. 1A and 2A). During the course of rituximab treatment, there was a striking reduction in anti-dsDNA antibody titres and an increase in C3 levels (Fig. 1A), which were maintained during belimumab treatment and allowed steroid tapering and stopping (Fig. 2A). Belimumab treatment also led to the complete reso-

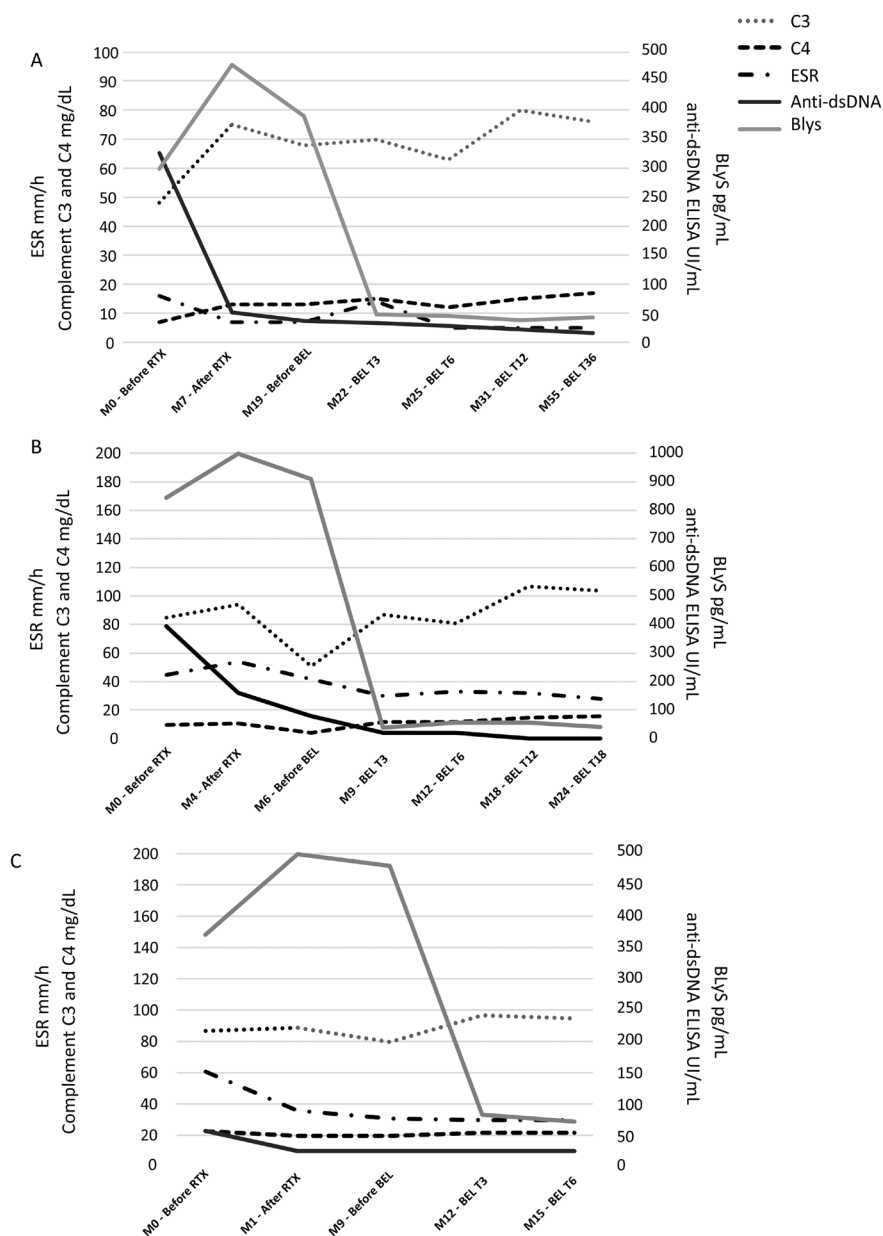


Fig. 1. Overview of the clinical course of case 1 (panel A), case 2 (panel B) and case 3 (panel C). Rituximab (RTX) was administered as two infusions two weeks apart for a total of 1 g and belimumab (BEL) as three 2-weekly infusions followed by monthly infusions of 10 mg/kg. Time scale (months) is reported in the x axis and the time to disease amelioration is illustrated; anti-dsDNA titres, erythrocyte sedimentation rate (ESR), complement (C3 and C4) and BLYS levels are reported. BLYS levels were measured by a specific ELISA kit as previously reported (R&D, #DY124) (16, 19).

lution of proteinuria (Fig. 2A), which had started improving during rituximab treatment. BLYS levels display high values before and particularly after rituximab administration and a strong reduction after belimumab was started (Fig. 1A).

Case report 2

The second case is a 24-year-old female patient. At the beginning of 2011 she experienced polyarthritis and she

was found to be positive for ANA, anti-dsDNA, anti-SSA and anti-SSB. After few months, she developed seizures. No abnormalities were found at central nervous system magnetic resonance imaging. She was diagnosed as having SLE and treated with intravenous methylprednisolone pulses (500 mg) for three consecutive days. Then she was treated with an anti-epileptic drug, azathioprine 100 mg daily and a high dose of prednisone with incom-

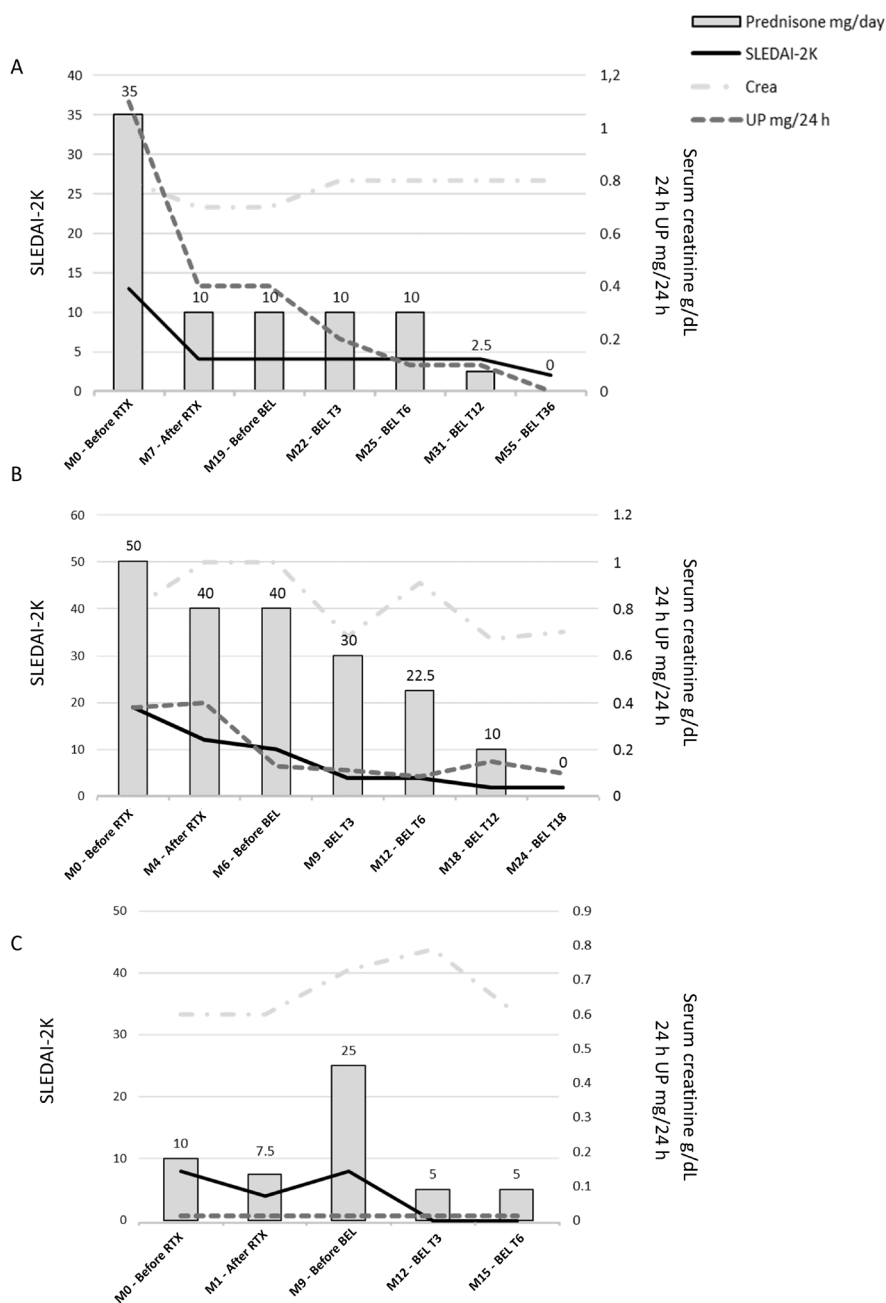


Fig. 2. The equivalent daily prednisone dose, serum creatinine and 24h proteinuria (UP) levels during the follow-up of case 1 (panel A), case 2 (panel B) and case 3 (panel C) with respect to disease activity determined by SLEDAI-2K. Rituximab (RTX) was administered as two infusions two weeks apart for a total of 1 g and belimumab (BEL) as three 2-weekly infusions followed by monthly infusions of 10 mg/kg. Time scale (months) is reported in the x axis and the time to disease amelioration is illustrated.

plete disease control. Over the following two years, she experienced a number of flares involving joints, skin, central nervous system (seizures), associated with anti-dsDNA at high titre, hypocomplementaemia and non-nephrotic proteinuria, which required intravenous pulse steroid treatment. She was treated with different disease-modifying anti-rheumatic drugs, which all turned out to be ineffective

in controlling the disease: azathioprine, MMF, intravenous immunoglobulins, dapsone, and finally chloroquine and cyclosporine. Kidney biopsy was performed during one of these flares, manifesting as severe cutaneous rash, persistent proteinuria and active urinary sediment (severe red blood cell casts), with evidence of class IV and V glomerulonephritis with extracapillary and necrotising components, ac-

ording to WHO classification. After an attempt with MMF (discontinued for diarrhoea), she was treated with oral cyclophosphamide 125 mg/day (cumulative dose 12 g) with a good response. In September 2014, a new flare occurred with fever, polyarthritis and anti-dsDNA positivity (SLEDAI-2K=19; Fig. 1B). Methotrexate 10 mg weekly was reintroduced but soon discontinued because it was not tolerated by the patient. The following month, two intravenous infusions of rituximab 1 g were administered 14 days apart with benefit. In February 2015, a new flare with polyarthritis occurred (SLEDAI-2K=12; Fig. 1B), rituximab was discontinued and belimumab 10 mg/kg monthly was started, after CD19+ cell levels reached the range of normality. This allowed a slow steroid tapering (starting from 40 mg of prednisone), maintaining cyclosporine and chloroquine in therapy. After 18 months of treatment, the patient finally achieved remission (SLEDAI-2K=0; Fig. 1B). Also in this case, we observed a dramatic reduction of anti-dsDNA antibody titre starting after rituximab therapy and persisting during belimumab treatment (Fig. 1B), which also allowed a significant reduction of steroid dosage to 7.5 mg of daily prednisone (Fig. 2B). BLYS levels were elevated before and even more after rituximab administration, but rapidly decreased after treatment with belimumab, showing a behaviour comparable to the case n.1 (Fig. 2B).

Case report 3

The third patient is a 37-year-old woman with a diagnosis of SLE made in 2001 on the basis of thrombocytopenia, leukopenia with lymphopenia, pleurisy, arthritis, triple positivity for anti-phospholipid antibodies, hypocomplementemia and low anti-dsDNA titre. She was initially treated with HCQ and a high dose of steroids, gradually tapered after reaching stable clinical remission. After eight years, a flare occurred with arthritis involving small joints of hands and feet, associated with non-haemolytic anaemia and leukopenia. Clinical remission was achieved thanks to parenteral metho-

trexate up to 15 mg weekly, which was stopped shortly after, due to gastrointestinal intolerance. First azathioprine 150 mg daily, then leflunomide 20 mg daily was added, leading to clinical remission and tapering of steroids, which were eventually discontinued in November 2011. Four years later, an articular flare occurred (SLEDAI-2K=8). After increasing azathioprine and steroid dosage, with no effects, steroid pulses (125 mg for three consecutive days) were therefore administered. Rituximab was started at therapeutic dosage in association with 15 mg/day of oral prednisone, achieving clinical remission (SLEDAI-2K=4; Fig. 1C). After 6 months, rituximab was not repeated due to desire for pregnancy, but an arthritic flare occurred (SLEDAI-2K=8), requiring the steroid dosage to be increased up to 25 mg prednisone daily. Therefore, belimumab was chosen as maintenance therapy in combination with HCQ and azathioprine, after verifying that the CD19+ cells were in the normal range. Belimumab treatment allowed the steroid dose to be reduced to 5 mg daily (SLEDAI-2K=0; Fig. 2C). Again, BLYS levels were high after rituximab and promptly reduced by starting belimumab infusions (Fig. 1C).

Discussion

We here report our experience with the sequential use of rituximab and belimumab in three patients with SLE. All our cases had clinically and serologically active disease (SLEDAI >4) refractory to standard treatments and achieved clinical remission with low dose corticosteroids. Rituximab was initially used because of the failure of any other therapy, in a time when belimumab was only available in phase II trials in Italy. We assume that the clinical improvement of our patients could be due to a synergic effect of rituximab and belimumab on B cell hyperactivation characteristic of SLE (5, 6). Rituximab induces a profound B cell depletion, whereas belimumab has only modest effects on B cell depletion. The rationale for the use of two consecutive B cell-targeted therapies lies in the observation that serum BLYS levels are sig-

nificantly higher and promote disease flares during B cell repopulation following rituximab therapy (5, 14). This may correlate with the incomplete disease control, associated with high risk of flares or requiring high steroid doses as observed in our patients. In line with our findings, Kraaij *et al.* demonstrated that belimumab was able to block the full repopulation of B cells after rituximab treatment in two patients with lupus nephritis (10), an observation confirmed by Simonetta *et al.* (12). Similar observations have also been made in Sjögren's syndrome, where B cell lymphoproliferation and overexpression of BAFF were controlled by a sequential regimen with belimumab followed by rituximab (13).

High BLYS levels after rituximab have been suggested to be associated with a paradoxical expansion of pathogenic B cells, thus possibly explaining the lack of efficacy of rituximab in clinical trials (5). On the other hand, the increase of BLYS levels might be simply the consequence of the reduction of specific receptors expressed on B cells and able to engage it. However, further studies are needed to address this specific issue. In physiological conditions, autoreactive B cells become anergic and undergo apoptosis during their development, whereas in SLE, B cell selection is partially ineffective. This is due in part to increased BLYS levels, which are critical for the rescue and survival of autoreactive clones (1). Flares usually occur 2 months after treatment with rituximab, when B cell levels have risen to $>0.01 \times 10^9$ cells/l (15). In a subset of patients with high anti-dsDNA titre, flares may occur at a considerably lower number of B cells, probably because in these patients BLYS levels are particularly high during flares (14). Accordingly, we observed higher BLYS levels after treatment with rituximab and as expected, a reduction after belimumab treatment (Fig. 1, panels A-C). Although not required by current recommendations, we monitored CD19+ cells in order to rule out possible side effects due to B cell depleting therapy (by rituximab) and before starting a different B cell biologic drug (belimumab). The

fact that BLYS was undetectable after belimumab may be the expression of a competitive binding of belimumab with the same epitope of the ELISA antibody used to detect BLYS serum levels (16). Another hypothesis could be that belimumab accelerates BLYS clearance, but there also is evidence that belimumab directly neutralises BLYS in an *in vitro* model (17).

Furthermore, BLYS supports the survival of plasmablasts, whereas BLYS blockade inhibits the differentiation into plasmablasts of B cells from naïve marginal zone. Human peripheral blood plasmablasts induce via IL-6 the activation of T follicular helper cells, which in turn stimulate differentiation of plasmablasts, thus perpetuating a positive feedback loop (18, 19).

Therefore, the administration of belimumab following rituximab is effective in reducing BLYS levels and consequently may prolong the beneficial effects of the CD20 positive B cell depletion.

Currently, two open-label clinical trials of sequential therapy with rituximab followed by belimumab are ongoing, the CALIBRATE study (Rituximab and Belimumab for Lupus Nephritis, <https://clinicaltrials.gov/ct2/show/NCT02260934>, 2015) and the SYNBI-oSe study (Synergetic B-Cell Immodulation in SLE, <https://clinicaltrials.gov/ct2/show/NCT02284984>, 2015). Finally, a randomised double-blinded clinical trial (BEAT-LUPUS) is currently recruiting in the UK.

Conclusions

Our observations were made in SLE patients refractory to rituximab and treated with belimumab based on their B cell-targeted mechanisms and on previous reports (10-12). We could not follow a regular schedule since the cases were anecdotic, however, we noticed an effective synergistic effect of the sequential treatment in all the three cases suggesting that this might be a useful option in refractory patients. Further studies, and in particular the ongoing randomised double-blind clinical trials will offer the definite demonstration of the effectiveness of such a sequential therapy.

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