Double anti-B cell and anti-BAFF targeting for the treatment of primary Sjögren's syndrome

S. Gandolfo, S. De Vita

Rheumatology Clinic, Udine University Hospital, Department of Medical Area, University of Udine, Italy.

Saviana Gandolfo, MD
Salvatore De Vita, MD

Please address all correspondence to:
Prof. Salvatore De Vita,
Clinica di Reumatologia,
Ospedale Universitario
Santa Maria della Misericordia,
Piazzle S. Maria della Misericordia 15,
33100 Udine, Italy.
E-mail:
salvatore.devita@asuiud.sanita.fvg.it

Received on July 2, 2019; accepted in revised form on July 8, 2019.

Clin Exp Rheumatol 2019; 37 (Suppl. 118): S199-S208.

Key words: Sjögren's syndrome, belimumab, rituximab, BAFF, therapy

Competing interests: S. De Vita has acted as Consultant Expert for Roche, Human Genome Science, Glaxo Smith Kline and Novartis. S. Gandolfo has declared no competing interests.

ABSTRACT

Primary Sjögren's syndrome (pSS) is an autoimmune connective tissue disease characterised by an enhanced lymphoproliferative status, with a greater risk of hesitating in malignant lymphoma. The pathological hallmark of pSS is the mucosa-associated lymphoid tissue (MALT) arising in chronically inflamed tissues, mainly in salivary glands (SG), where inflammation, autoimmunity and lymphoproliferation coexist. In the microenvironment of MALT, the B lymphocyte activating factor (BAFF or BLys) is one of the main actors contributing to B cell survival and hyperactivity in pSS.

Due to such a lymphoproliferative background, targeting directly and/or indirectly B lymphocytes has become a cornerstone of developing therapeutic strategies for pSS.

The simultaneous and direct targeting of the BAFF axis and of B cells represents a promising new treatment approach for pSS and other immune-mediated diseases, but only investigational at present. Immunobiological evidences support a sequential scheme of administration with belimumab preceding rituximab, aiming to firstly target the microenvironmental BAFF to improve the success of the subsequent depleting treatment in the MALT pathologic tissue with rituximab.

In a real pSS case, the sequential therapy with belimumab alone followed by rituximab alone successfully led to a long-term clinical remission of lymphoma and cryoglobulinaemic vasculitis, together with the persistent normalisation of B cell hyperactivity and the disappearance of persistent SG swelling and cryoglobulinaemia, which are strong predictors of lymphoma in pSS. Hopefully, further trials will assess if improvement in B-cell targeting will lead to the decrease in SG lymphoid infiltrate as well as to a possible reduction of lymphoma development in pSS.

Introduction

Primary Sjögren's syndrome (pSS) is an autoimmune connective tissue disease, mainly characterised by sicca and systemic constitutional symptoms, such as fatigue and pain, variably combined to a wide range of possible extraglandular manifestations, and by an enhanced lymphoproliferative status, with a greater risk of hesitating in malignant lymphoma (1-4).

The pathological hallmark of pSS is the mucosa-associated lymphoid tissue (MALT) arising in chronically inflamed tissues, mainly in salivary glands (SG), where inflammation, autoimmunity and lymphoproliferation coexist, creating a complex biological and immunological substratum that fuels autoreactive B lymphocytes persistence and promotes their proliferation, towards a clonal selection and a possible lymphoma development (2, 3, 5, 6).

In the microenvironment of MALT, the B lymphocyte activating factor (BAFF or BLys) is one of the main actors contributing to B cell survival and hyperactivity in pSS (7-9).

Due to this lymphoproliferative background, targeting directly and/or indirectly B lymphocytes has become a cornerstone of developing therapeutic strategies for pSS (10-13).

Rituximab is a B cell-depleting anti-CD20 monoclonal antibody that, although failed to reach primary endpoints in large randomised trials in pSS, mainly due to the suboptimal trial design and to the lack of stratification of pSS patients, remains a valid therapy in the pSS treatment armamentarium for the management of some extraglandular manifestations, as supported by experts recommendation, several open and reg-

istry studies, and case series/reports (10-12, 14, 15). However, as firstly noticed almost 20 years ago (16) in pSS patients with myoepithelial sialadenitis, rituximab monotherapy might not effectively deplete B cells within the SG. Experimental research showed that this occurs due to the presence, within the MALT microenvironment, of factors which can mediate B-cell resistance to depletion by rituximab, such as BAFF (17). Thus, heavy SG inflammation in pSS (18), *e.g.* that associated with persistent glandular swelling and cryoglobulinaemia (2, 19-21), which are also the main adverse predictors of malignant lymphoma in pSS (22), might not respond to rituximab monotherapy.

The efficacy and safety of belimumab, a fully human recombinant monoclonal IgG1λ antibody targeting soluble human BAFF, approved for the treatment of active, autoantibody-positive systemic lupus erythematosus (SLE), were investigated in pSS by the bicentric (Udine, Italy and Paris, France) BELISS open label prospective phase II trial (23, 24), resulting in achievement of primary endpoint in 60% of patients and also in a long-term maintenance of efficacy, with a good safety profile. Importantly, however, after the suspension of belimumab, pSS worsened in some cases and BAFF greatly increased (25).

The last few years saw growing research interest in unravelling the immunobiological mechanisms that relate B cell depletion to BAFF axis perturbance and *vice versa*. Evidence supporting a double therapeutic approach with an anti-CD20 and an anti-BAFF agent, acting in a complementary and/or synergistic manner, and possibly more effective than each monotherapy, has been then provided in different diseases, including pSS.

The immunobiological rationale supporting this double therapy will be herein reviewed. The starting points will be the early evidence of the inefficacy of rituximab alone in pSS (16, 17), and the description of the first and only one patient with pSS complicated by a MALT lymphoma and refractory cryoglobulinemic vasculitis, successfully treated with belimumab followed by rituximab (18). Of note, the same rationale is applicable not only to pSS, but also to other diseases. An update about the current ongoing clinical trials including this investigational regimen will be also provided in pSS.

BAFF axis, belimumab, and rituximab in pSS and in other immune-mediated diseases

BAFF axis

BAFF is a protein member of the TNF ligand superfamily expressed under physiological conditions on cell surface, which can be cleaved by a furin protease and consequently released as a soluble and biologically active molecule (26). Both haematopoietic and non-haematopoietic cells produce BAFF. In steady-state conditions, monocytes, macrophages, neutrophils, dendritic cells (DCs) and follicular DCs are the main producers of BAFF (27, 28). Bacterial components such as lipopolysaccharide (LPS), toll-like receptors (TLRs)-mediated pathways, type I interferons (IFNs), transforming growth factor (TGF)-β, interleukin (IL)-10, and granulocyte colony-stimulating factor (G-CSF) can upregulate BAFF production (29, 30). BAFF can also be produced by T cells, activated B cells, natural killer (NK) cells, mast cells, and by several other cytotypes including stromal cells, SG epithelial cells in pSS, airway system epithelial cells, placental cytotrophoblasts, fibroblast-like synoviocytes in rheumatoid arthritis (RA), adipocytes, osteoclasts in multiple, astrocytes in multiple sclerosis, and several lines of cancer cells (7, 31-41).

In inflamed tissues, BAFF overproduction significantly contributes to create, in tissue niches, a milieu that can induce and perpetuate inflammation, promoting lymphoproliferation (42).

BAFF exerts a broad spectrum of biological effects on both innate and adaptive immune system cells, mainly in regulating the B cell development and function (26, 30), by binding three receptors: BAFF receptor (BAFF-R/BR-3), transmembrane activator and calcium modulator and cytophilin ligand interactor (TACI), and B cell maturation antigen (BCMA) (43). BR3, predominantly expressed on naïve and memory B cells, promotes survival and development of transitional and mature B cells. BR3 (but not TACI and BCMA) is also expressed on the surface of effector memory T cells, costimulating T-cell proliferation and cytokines secretion as well (40, 44-46). TACI is expressed mainly on memory B cells and on naïve B cells at steady state (47). TACI signal regulates B-cell proliferation and promotes long-lived plasma cells survival. BCMA signal, finally, promotes the survival of plasmablasts and plasma cells, including long-lived plasma cells (45, 48), and mediates the reverberating loop of interaction between the T helper follicular cells (TFH), which can produce BAFF, and the plasma cells themselves (49, 50).

Animal studies demonstrated that the BAFF transgenic mice develop a SLE/pSS-like syndrome characterised by the presence of hypergammaglobulinaemia, high levels of rheumatoid factor (RF), circulating immune complexes, anti-dsDNA autoantibodies and immunoglobulin (Ig) deposition in the kidneys, an enlarged B-cell compartment (centrally in the bone marrow and peripherally in secondary lymphoid organs) (51), and that these mice are more prone to develop lymphoma (52). This phenotype suggested that dysregulation of BAFF expression may be a critical element in the chain of events leading to autoimmunity, besides lymphoproliferation.

In SLE, BAFF plays a well-recognised central role in the pathogenesis and in the course of the disease (53). SLE-prone mice tend to have increased serum BAFF levels, and BAFF blockade reduces disease manifestations (54). Human studies show that serum BAFF is elevated in SLE patients (55, 56), and positively correlates with disease activity, the titres of serological markers, such as anti-dsDNA autoantibodies, the number of autoantibody-secreting plasma cells, the cumulative organ damage over time, and with higher rates of disease relapses (57-59).

Several data show that BAFF is also significantly involved in the pathogenesis of pSS and of pSS-related lymphoma (52, 60). In pSS, BAFF is overproduced in the context of the SG MALT, during chronic inflammation. The epithelial cells are the main sources of BAFF in

the SG MALT microenvironment in pSS (7, 8), activated both directly and indirectly (*via* an IFN α-dependent stimulation by plasmacytoid DCs) by unknown exogenous antigens and/or autoantigens. Myeloid DCs and B cells themselves, above all, are also BAFF-producers (61, 62).

High levels of BAFF are found in saliva, sera and in SG tissues of pSS patients (63, 64), and resulted increased to a greater level in pSS patients with lymphoma or pre-lymphomatous conditions, such as myoepithelial sialoadenitis (MESA) and cryoglobulinaemic vasculitis, if compared to pSS patients without lymphoproliferative lesions (8, 9, 18).

Belimumab

The anti-BAFF monoclonal antibody, named belimumab, is a biologic agent currently approved for the treatment of SLE, its efficacy and safety being demonstrated by two large phase III randomised controlled trials, the BLISS-52 and BLISS-76 trials (65, 66), and by several additional studies over time. Overall, belimumab-treated SLE patients experienced fewer disease flares, a significant reduction in steroid intake and in long-term organ damage accrual compared to patients receiving the standard of care therapy (65-68).

From an immunobiological perspective (69), SLE patients treated with belimumab showed significant sustained reductions in autoantibodies, with greater conversion rates to negativity for anti-dsDNA, anti-Smith, anticardiolipin, and antiribosomal P positivity, and normalisation of hypergammaglobulinaemia and of low C3 and/or C4 than in the placebo group. Belimumab-treated patients experienced also a significant decrease in naïve ($CD20^+CD27^-$) and activated ($CD20^+CD69^+$) B cells, as well as plasma cells, whereas memory B cells and T cell population numbers were overall preserved (69).

Recently, it has been demonstrated that belimumab therapy was also able to restore T regulatory (Treg)/Th17 balance in patients with refractory SLE, and that the Treg numerically increased after belimumab therapy were fully functional (70).

In pSS, the efficacy and safety of belimumab were evaluated in the BELISS open label prospective phase II trial (23, 24). This included pSS patients with systemic complications, SG enlargement, early disease (<5 years), or increased biomarkers of B cell activation. Belimumab 10 mg/kg, was given at weeks 0, 2 and 4 and then every 4 weeks to week 24, to 30 enrolled patients. The primary endpoint, assessed at week 28, was the improvement in at least two of five items: reduction in ≥30% in dryness score on a visual analogue scale (VAS), ≥30% in fatigue VAS score, ≥30% in VAS pain score, ≥30% in systemic activity VAS assessed by the physician and/or >25%, and improvement in any B cell activation biomarkers. Among 30 patients included, the primary endpoint was achieved in 18 (60%). Furthermore, the mean European League Against Rheumatism (EULAR) SS Disease Activity Index (ESSDAI) and EULAR SS Patient Reported Index (ESSPRI) significantly decreased. 19 patients continued then the study until its completion, with 52 weeks of belimumab therapy (24). This long-term treatment with belimumab led to a further improvement at week 52 in both ESSPRI and ESSDAI scores, mainly in peculiar domains (glandular, lymphadenopathy, biological and articular). Biomarkers of B cell activation persisted decreased until week 52, with RF decreasing further. In long-term treated patients also some fatigue-related parameters, unchanged at week 28, improved. Salivary flow, Schirmer's test and the focus score of salivary biopsies did not change. Safety of treatment was good (24).

Ten BELISS participating patients were then selected to study the immunological peripheral B cell phenotype and BAFF-R expression (71). Therapy with belimumab induced a significant reduction in peripheral blood transitional and naïve B cell subsets to levels similar to those observed in healthy donors, and normalised BAFF-R expression in all B subsets. This was shown both after 24 weeks of therapy and until the end of the therapeutic protocol at week 52. Further analysis on belimumab-treated pSS patients showed that type I IFN type signature, evaluated in peripheral blood mononuclear cells (PBMCs), affected the magnitude of biological effect of belimumab (72).

Besides SLE and pSS, belimumab effects have been also investigated in other diseases, providing interesting insights for further broader applications. Of particular interest is the study (NCT01536379) about belimumab as a useful adjunct to standard of care immunosuppression in kidney transplantation, where beneficial effects on rejection rates were reported in patients receiving belimumab, with no major increased risk of infection (73). Besides clinical aspects strictly related to renal transplantation condition, this study (73) provided novel data on the effects that belimumab exerts on regulatory and memory B cell compartments and autoantibody production, potential relevant also in autoimmune diseases. In the kidney transplant setting, B cells are not only precursor of antibody-secreting plasma cells, but are also involved in T cell-mediated rejection, by acting as antigen-presenting cells, and by producing cytokines, mainly IL-6, able to activate T cells (74). On the other hand, some B cell subpopulations, *e.g.* IL-10-producing regulatory B cells, however, are crucial for immune tolerance and their impairment, *e.g.* after rituximab therapy, could be likewise harmful (75, 76). This represents in fact one of the reasons why rituximab has been associated with increased T-cell mediated rejection and cardiac allograft vasculopathy (77, 78).

In transplant recipients, high serum BAFF levels are associated with the development of *de novo* donor-specific HLA-specific IgG antibodies and higher frequencies of rejection (73). Although belimumab adjunction did not significantly reduce the number of naïve B cells, possibly due to an already impaired number of this subpopulation at baseline because of the high immunosuppressant background in these patients (79), belimumab effect was observed on the transitional B cell compartment, where belimumab induced an expansion of IL-10-producing regulatory B cells and a reduction of IL-6-producing inflammatory B cells (73). Another point

of strength of belimumab therapy was the reduction of circulating activated ($CD95^+$) memory B cells among an increased number of total memory B cells mobilised by belimumab, suggesting a rationale for belimumab as a longer-term adjuvant in sensitised transplant recipients with pre-existing donor-specific memory B cells given in addition to a lymphocyte depleting agent, targeting these memory B cells after mobilisation (73). This strategy is under investigation in a combination trial including, besides belimumab, rituximab, bortezomib, and plasma exchange (NCT02500251). Furthermore, belimumab led to a significant reduction of plasmablasts and of *de novo* IgG antibody formation, including those directed against kidney-specific antigens. Finally, despite no difference was seen in the number of circulating T cells after belimumab adjunction, transcriptomic analysis of circulating $CD4^+$ T cells revealed a reduced expression of cell-cycle genes in belimumab-treated patients, suggesting a possible belimumab effect also on T cell proliferation (73). Taken together, evidence coming from kidney transplant condition supports the notion that belimumab therapy modulates both clinical and immunobiological aspects in different human disorders, by influencing subtle small balances between and inside B cell subpopulations potentially leading to huge consequences. Further studies are definitely worthwhile also in other fields of medicine.

Belimumab therapy was evaluated also in other immune-mediated diseases, here summarised.

In a phase II, randomised, double-blind, placebo-controlled study on RA (NCT00071812), belimumab demonstrated efficacy and was generally well tolerated in patients with RA who had failed previous therapies (80).

Belimumab therapy was investigated also in patients with early diffuse cutaneous systemic sclerosis (dcSSc), treated with background mycophenolate mofetil, by a small pilot, double-blind, placebo-controlled trial (NCT01670565). In this study, the safety profile was confirmed to be good. The improvement in modified Rodnan skin thickness score (MRSS) was greater in the belimumab-treated group of patients than in placebo group, though a statistical significance was not achieved. Interestingly, a significant decrease in expression of B cell signalling and profibrotic genes and pathways was however observed in patients with improved MRSS in the belimumab group but not in the placebo group, suggesting the utility of additional studies with belimumab in this condition (81).

In another multicentre randomised controlled trial (NCT01663623), patients with antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAVs) in remission were randomised to receive intravenous belimumab or placebo alongside azathioprine (2 mg/kg/day) and low-dose oral glucocorticoids (<=10 mg/day), to evaluate the safety and efficacy of belimumab as adjunctive therapy to maintain the remission in AAV. The primary endpoint of reducing the risk of relapse in AAV was not achieved (82).

The adjunction of belimumab did not significantly provide evidence of improvement also in patients with generalised myasthenia gravis (MG) who remained symptomatic despite the standard of care therapy (NCT01480596) (83).

Finally, a trial with belimumab monotherapy is ongoing in idiopathic inflammatory myositis (NCT02347891).

Rituximab

Rituximab is a chimeric monoclonal antibody targeting CD20 molecule expressed on the surface membrane of B cells, from pro-B to memory B-cells. Following the pioneering work by Edwards and Cambridge in RA (84), for the last decades, rituximab-induced B-cell depletion has been increasingly used to treat several autoimmune conditions besides RA, such as immune thrombocytopenia (ITP) and ANCA-associated and cryoglobulinaemic vasculitis, among others (85, 86).

Rituximab therapy was used in pSS for the first time about 20 years ago (16). Thereafter, several studies investigated efficacy and safety of rituximab therapy in pSS with encouraging results, but large randomised-controlled trials in pSS patients (87, 88), as well as occurred in SLE (89, 90), failed to achieve study endpoints. For some peculiar pSS extra-glandular disease manifestations, rituximab use is, however, currently recommended according to available guidelines (10-12, 91), but overall its clinical and biological efficacy in pSS, remains ill defined (16, 92). Besides the limited efficacy of rituximab on B cells directly in pSS tissue lesions, the inadequate patient stratification, endpoints, and evaluation of disease activity may be considered in previous in clinical trials.

Double belimumab and rituximab therapy: possible schemes of administration

Initial therapy must be distinguished by subsequent therapy (Fig. 1). As initial therapy, two different administration schemes of sequential therapy with belimumab and rituximab are currently available in different immune-mediated disease, while clinical data on initial combination starting at the same time are lacking. Subsequent therapy may also vary. Of note, the rationale of each therapy may vary in each different disease and, possibly, in patient subsets with the same disease:

Initial therapy

1. *Sequential therapy*: the drugs are sequentially administered in monotherapy:
   1.1 Belimumab followed by rituximab
   1.2 Rituximab followed by belimumab.
2. *Combination therapy*: the drugs are simultaneously administered *ab initio*.

Subsequent therapy

In presently available data, therapy with either belimumab or rituximab alone may follow initial therapy, while the combination of the two drugs is not reported, at present, as subsequent therapy.

As more extensively detailed in the "immunobiological rationale" section below, biologic evidence and current clinical practice support the usefulness of a sequential therapy scheme, as the initial therapy for pSS, where anti-BAFF therapy represents the first therapy given (*i.e.* Scheme 1.1). This scheme is currently included in ongoing clinical trials in pSS (NCT02631538), and in SLE (named BLISS-BELIEVE, NCT03312907) (93). Double therapy

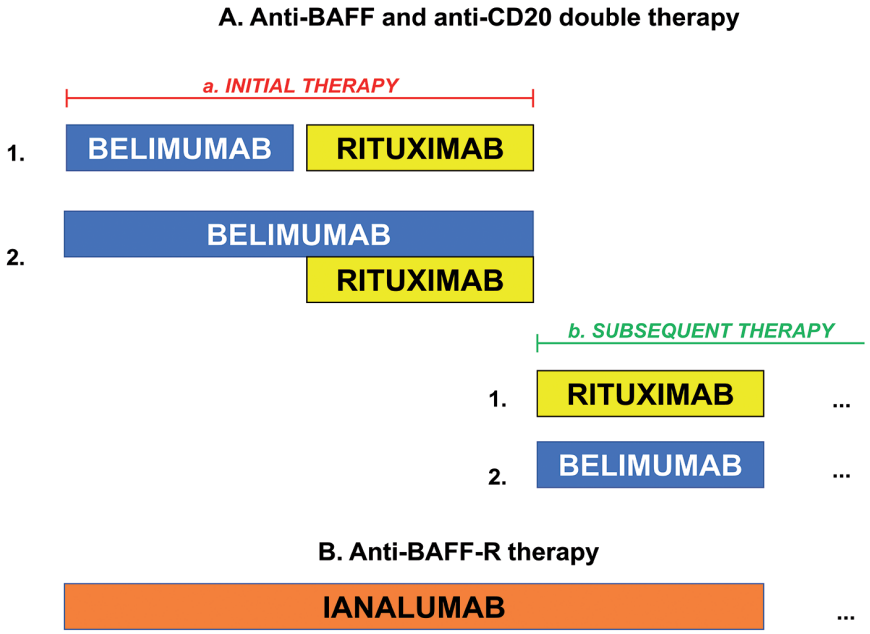

Fig. 1. Current investigational treatments of pSS with direct targeting of both the BAFF axis and the B cells. In double therapy with belimumab and rituximab, the use of sequential therapy, as initial treatment, is available up to now [(18) and NCT02631538], while monotherapy with either of these two drugs is used subsequently [(18) and NCT02631538]. Furthermore, a study is in course in pSS with a monoclonal antibody anti-BAFF-R (Ianalumab), which exerts a simultaneous and direct anti-BAFF anti-B-cell action [(96) and NCT02962895].

is also under investigation in systemic sclerosis (NCT03844061) and in PR3 ANCA-associated vasculitis (COMBIVAS, EUDRACT number: 2017-004645-24). In systemic sclerosis the combination of rituximab and mycophenolate, which may also contribute to B cell inhibition, has been successfully used (94).

By contrast, an initial sequential therapy where rituximab is given before belimumab (*i.e.* Scheme 1.2), is not substantiated at present in pSS [(18) and NCT02631538], and has an apparently weaker rationale. Such a scheme is indeed supported, however, by a different rationale in different diseases, *e.g.* in SLE, where belimumab is used in the long term after the preliminary induction therapy with rituximab (95). Two clinical trials, CALIBRATE (NCT02260934) and BEAT LUPUS (ISRNCTN47873003) are currently ongoing in lupus nephritis and in SLE in general.

Data about a simultaneous targeting of BAFF and CD20 from the beginning of initial therapy in pSS (*i.e.* Scheme 2, Combination therapy) are currently missing. This scheme might however share a common rationale with scheme 1.1., even if belimumab monotherapy is not exactly preceding rituximab.

Figure 1 shows the current investigational treatments of pSS with direct targeting of both the BAFF axis and the B cells. In double therapy with belimumab and rituximab, the use of sequential therapy, as initial treatment, is available up to now [(18) and NCT02631538], while monotherapy with either of these two drugs is used subsequently [(18) and NCT02631538]. Furthermore, a study is in course in pSS with a monoclonal antibody anti-BAFF-R (Ianalumab), which exerts a simultaneous and direct anti-BAFF anti-B-cell action [(96) and NCT02962895)] as discussed below in a separate paragraph.

Early biological evidence supported a sequential approach of belimumab monotherapy preceding rituximab monotherapy to successfully treat a severe pSS patient

In 2002 it was highlighted that the anti-CD20 therapy with rituximab might not deplete the B cell infiltrates in pSS MALT sites (16). In 2005, Gong *et al.* demonstrated in the mouse model that the local production of BAFF was a crucial local factor mediating the resistance to B cell depletion by rituximab in MALT (17). Only when anti-BAFF therapy was combined to anti-CD20 therapy, B cell depletion could be achieved.

Starting from this biologic rationale (17), a pioneer therapeutic approach based on a sequential treatment with belimumab shortly followed by rituximab has been successfully adopted nine years ago to treat a patient, the only one described in literature up to now, affected by pSS complicated by a lymphoma of MALT type of the parotid gland and by a severe and refractory cryoglobulinaemic vasculitis (18). This patient had undergone several therapies, including different immunosuppressors and cyclophosphamide, plasma exchange, rituximab monotherapy for two times (repeated in a prolonged schedule), with and without high doses of glucocorticoids, and belimumab monotherapy, always without response. Bilateral parotidectomy provided a transient response, with relapse concomitantly with parotid lymphoma relapse. Of interest, even if BAFF was overexpressed both in the serum and in the SG tissue of this patient (97), belimumab monotherapy failed. Finally, when rituximab was administered for the third time, but shortly after the failure of belimumab, a marked and persistent clinical and biologic efficacy was noticed: a biopsy-proven lymphoma remission and a progressive complete recovery of skin ulcers, together with a decrease in serum BAFF levels, cryoglobulins and RF (then become negative), were obtained (18). After initial sequential therapy with belimumab followed by rituximab (Scheme 1.1) in a maintenance therapy with rituximab was given every 6 months for one year, while belimumab was never administered again (18). Importantly, a very long-term follow-up of this pSS patient is now available, *i.e.* more than nine years, showing ongoing and stable remission of both lymphoma and of cryoglobulinaemic vasculitis, accompanied with an optimal safety profile. Peripheral B cells are still depleted, cryoglobulins and rheumatoid factor persist not-detectable, and serum BAFF remains negative.

In 2016, based also on the encouraging results provided by this real case, an arm with a double belimumab and rituximab therapy was included in an ongoing multi-national, multi-centre-double-blind, randomised, placebo-controlled trial in subjects with active pSS, also investigating the safety, tolerability and efficacy profile of belimumab monotherapy (NCT02631538). Results from this trial are soon expected.

Double belimumab and rituximab therapy: immunobiological rationale

BAFF mediates the resistance to rituximab-induced B cell depletion in target tissues

Even if rituximab treatment results in a rapid and almost complete depletion of circulating B cells, relatively high numbers of resistant B cells have been demonstrated to persist in target tissues after rituximab (98-101), according to evidence from animal models of B cell depletion showing the persistence of a large fraction of B cells (mainly represented by marginal zone B cells) in the tissues, despite a complete depletion of circulating B cells (17, 102).

In pSS, the biologic explanation of the variable response to rituximab therapy can be reconducted to the overexpression of the microenvironmental BAFF in lymphoid biological niches where the key disease processes take place, *i.e.* in the SG MALT, at least in some patient subsets (18, 97).

The main rituximab resistance-mediating factor has been identified in BAFF also in other diseases, due to pre-existing BAFF overexpression by the disease itself, or to upregulation of BAFF after rituximab treatment. In chronic lymphocytic leukaemia (CLL), NK cells ability to lyse the malignant cells and to mediate antibody-dependent cellular cytotoxicity (ADCC) upon Fc receptor stimulation is impaired. NK in CLL have been demonstrated to produce BAFF (103), both spontaneously and as a consequence of anti-CD20 treatment. BAFF, in turn, enhances the metabolic activity of CLL cells and confers to the malignant cells a resistance to both direct and rituximab-induced lysis. In allogeneic and autologous experimental systems, belimumab showed the ability of restore the susceptibility of malignant CLL cells to both direct and rituximab-induced killing (103). Furthermore, in ITP, rituximab therapy induced the appearance of pathogenic long-lived plasma cells in the spleen, which were not present before treatment or in non-autoimmune conditions, by altering the splenic microenvironment with an excessive production of BAFF demonstrated in the spleen of rituximab-treated patients (104).

A double therapeutic approach employing a BAFF neutralising drug before rituximab (18), may be able to lower the B cell resistance to depletion in the key local milieu in pSS, *i.e.* the SG MALT.

The "BAFFling" effect of rituximab

The "BAFFling" effect of rituximab, *i.e.* the increase in BAFF serum levels following rituximab-induced B cell depletion (105), has been observed in different disease, such as SLE, RA and pSS (106-109). How much these elevated BAFF serum levels are really harmful and contribute to the development of a disease flare remains, however, uncertain.

Initial reports on SLE and evidence coming from rituximab-treated patients with RA indicated that, after a first phase of BAFF increase immediately following the rituximab therapy, BAFF serum levels reverted to pre-treatment values upon B-cell repopulation, without a correlation with disease relapse (106, 107). In subsequent studies, it has been reported that repeated courses of rituximab in SLE patients, but not in RA, led to further progressive increases in BAFF serum levels at the onset of each relapse, potentially worsening the long-term outcome for some SLE patients, despite a temporarily rituximab-mediated improvement of the disease (110). Subsequent studies on SLE showed that elevated BAFF serum levels after rituximab treatment can persist high limitedly to the subgroup of patients who relapse with high anti-dsDNA antibodies levels (110).

From a biological perspective, in the specific subset of anti-dsDNA positive SLE patients, the disease flares are characterised by an excess of plasmablasts, despite an overall lymphopenia induced by rituximab (111). Plasmablasts can mediate the flare of the disease by producing high amount of IL-6, which is the main inducer of the differentiation of TFH cells, which in turn produce BAFF and stimulate plasmablasts in a reverberating loop, leading finally to the overt SLE flare (112-114). Increased BAFF serum levels observed after rituximab therapy in SLE, thus, is not only the expression of the "mechanical" removal of the large amount of "BAFF-users", *i.e.* the circulating B cells (115), but also and possibly more importantly (106) of an increased production of BAFF by TFH, enhanced by the excess of plasmablasts, which are protected from rituximab due to the lack of CD20 expression.

In pSS, serum BAFF levels have been shown to increase after B-cell depletion therapy, and then gradually return to the pre-existing abnormalities along with the reconstitution of the circulating B cell pool after rituximab suspension (109). Higher pre-rituximab serum levels of BAFF correlated inversely with a shorter duration of B cell depletion (108).

As observed in SLE, also in pSS a number of plasmablasts can persist in the peripheral blood after rituximab treatment, lacking of CD20 expression and due to BAFF-driven survival (108). Thus, a double therapy that targets both BAFF and CD20 can be particularly effective. In the pSS patient reported above (18), the sequential therapy of belimumab followed by rituximab was not accompanied by the "BAFFling" effect of rituximab in the long term. In fact, BAFF serum levels, after a slight and transient increment following the first administration of rituximab after belimumab, progressively decreased, even if the patient received two additional maintenance courses of rituximab for one year (one course every 6 months) following the sequential therapy. BAFF levels, finally, became negative and still persist undetectable at present after several years, without any further treatment (18).

Taken together, currently available evidence supports a rationale for the use of belimumab given before rituximab to lower the BAFF-mediated survival

loops of B cells, particularly those enhanced by plasmablasts, and to exert a sort of "preparatory conditioning" action to increase the chances of a more effective subsequent rituximab therapy, potentially reducing or abrogating also the serum BAFF rebound after depleting treatment.

Parenthetically, the difference in the "BAFFling" effect of rituximab between different subgroups of the same disease, as observed in anti-dsDNA positive or negative SLE (110), by likely mirroring peculiar pathogenetic mechanisms characterising each patient subset, reinforces the concept that a disease stratification, mainly to better address different needs in terms of treatment, is crucial.

The "BAFFling" effect of belimumab suspension

An increase in BAFF serum levels has been reported after belimumab suspension in 13 pSS patients followed after the end of the BELISS study. Furthermore, 2 pSS patients developed a MALT lymphoma during the follow-up after this suspension (25).

In the setting of kidney transplantation, where the adjunction of belimumab reduces the risk of rejection, belimumab suspension again led to BAFF serum increase, with hypothetical harmful rebound effects on rejection rates (73).

Both these evidences raise the issue about the necessity for a long-term belimumab therapy, in order to prevent possible severe complications due to suspension.

Of note, in the pSS case report above described (18), the administration of rituximab after belimumab was accompanied by a decrease and, finally, a normalisation in BAFF levels together with the clinical remission and with the stable disappearance of two strongest lymphoma predictors in pSS, *i.e.* SG swelling and cryoglobulinaemic vasculitis (2, 22, 116).

Thus, sequential therapy with belimumab followed by rituximab deserves further attention as an effective alternative to a long-term belimumab therapy also to prevent complications potentially linked to the BAFF rebound due to belimumab suspension (25).

Overall, this approach (13) could also be effective to treat those pSS patients with higher risk of lymphoma development, directly targeting the burden of disease activity in the biological substratum of pSS, *i.e.* the MALT (6, 116), and potentially lowering the risk of lymphoma evolution, without significant toxicity. Whether it could also be useful to treat other MALT-related specific pSS symptoms, such as dryness, is awaited from trial results.

Double belimumab and rituximab therapy: summary and schemes in pSS

The rationale of a double belimumab and rituximab therapy, mainly in the sequential scheme of belimumab followed by rituximab, can be summarised as follows, and peculiar schemes of therapy have a stronger rationale in pSS:

- Belimumab, by targeting tissue BAFF, removes a crucial local microenvironmental factor of B cell resistance to rituximab, rendering tissue-resident B cells more susceptible to be depleted by rituximab itself.
- Belimumab is able to mobilise memory B cells from tissues, resulting in an increase of the number of B cells pushed outside of the "comfort zone" represented by the lymphoid niches of survival, thus becoming available to be depleted by rituximab.
- Belimumab given at first, by interfering with the reverberating loop of activation mediated by TFH and plasmablasts which are not targeted by rituximab due to the lack of CD20, but express BCMA, might potentially reduce or completely abrogate the serum BAFF excess observed after rituximab therapy, and consequently might also exert a preparatory effect to limit and/or to postpone the B cell reconstitution after rituximab.
- The rebound in the serum concentration of BAFF following the cessation of belimumab (25, 73) raises the question of whether belimumab should be given for a long period rather than to be stopped, or whether such BAFF rebound could be limited by giving rituximab after belimumab (18).
- Belimumab followed by rituximab was able to induce the stable disappearance of the strong lymphoma predictors in pSS, *i.e.* SG swelling and cryoglobulinaemic vasculitis, in a real pSS difficult patient (18). It represents a possible effective therapy.

Overall, integrated clinical and immunobiological investigation in larger number of stratified pSS patients are definitely worthwhile.

Data coming from trials in course in pSS and in other immune-mediated diseases will contribute in the future to define the best scheme of administration of the double belimumab and rituximab therapy in the particular disease. In addition, the stratification of patients in well-characterised subgroups and the renewal of clinical trial design, by defining novel inclusion criteria and outcome measures, remain essential unmet needs to be hopefully solved before drawing definitive conclusions and performing clinical trials which may fail despite potential success.

Other simultaneous anti-B-cell and anti-BAFF depleting strategies for pSS

The BAFF axis has become in the last years object of focus of developing novel therapies for pSS. A rapid and profound B cell depletion of long-lasting duration has been described after a single infusion of the monoclonal antibody named ianalumab, a B cell-depleting BAFF-R blocker, which showed encouraging therapeutic effects in a double-blind, placebo-controlled, phase 2, single-centre study in pSS patients (NCT02149420) (96). A larger trial in pSS has been then planned and is currently ongoing (NCT02962895).

Conclusions

The simultaneous and direct targeting of the BAFF axis and of B cells represents a promising new treatment approach for pSS and other immune-mediated diseases, but only investigational at present. Immunobiological evidences support a sequential scheme of administration with belimumab preceding rituximab, aiming to firstly target the microenvironmental BAFF to improve the success of the subsequent depleting treatment in the MALT path-

ologic tissue with rituximab. This approach is currently included in clinical trials in pSS, and also in other diseases. A simultaneous therapeutic approach combining belimumab and rituximab *ab initio* could be also effective and deserves investigation.

In a real pSS case (18) the sequential therapy with belimumab alone followed by rituximab alone successfully led to a long-term clinical remission of lymphoma and cryoglobulinaemic vasculitis, together with the persistent normalisation of B cell hyperactivity and the disappearance of persistent SG swelling and cryoglobulinaemia, which are strong predictors of lymphoma in pSS. Hopefully, new studies will also start to investigate whether the risk of lymphoma in pSS might be reduced.

A deep clinical and immunobiological characterisation of different subgroups of patients together with a modification of clinical trial design and endpoints should better address novel therapies in pSS (117). To this end, a stratification of pSS patients and new tools for clinical trials are currently under development in the HarmonicSS EU Project (European Union Grant 731944; https://harmonicss.eu) (118).

References

1. BRITO-ZERON P, BALDINI C, BOOTSMA H *et al*.: Sjögren's syndrome. *Nat Rev Dis Primers* 2016; 2: 16047.
2. DE VITA S, DE MARCHI G, SACCO S, GREMESE E, FABRIS M, FERRACCIOLI G: Preliminary classification of nonmalignant B cell proliferation in Sjögren's syndrome: perspectives on pathobiology and treatment based on an integrated clinico-pathologic and molecular study approach. *Blood Cells Mol Dis* 2001; 27: 757-66.
3. ANDERSON LG, TALAL N: The spectrum of benign to malignant lymphoproliferation in Sjögren's syndrome. *Clin Exp Immunol* 1972; 10: 199-221.
4. ARGYROPOULOU OD, VALENTINI E, FERRO F *et al*.: One year in review 2018: Sjögren's syndrome. *Clin Exp Rheumatol* 2018; 36 (Suppl. 112): S14-26.
5. TZIOUFAS AG, KAPSOGEORGOU EK, MOUTSOPOULOS HM: Pathogenesis of Sjögren's syndrome: what we know and what we should learn. *J Autoimmun* 2012; 39: 4-8.
6. MOUTSOPOULOS HM: Sjögren's syndrome: autoimmune epithelitis. *Clin Immunol Immunopathol* 1994; 72: 162-5.
7. ITTAH M, MICELI-RICHARD C, ERIC GOTTENBERG J *et al*.: B cell-activating factor of the tumor necrosis factor family (BAFF) is expressed under stimulation by interferon in salivary gland epithelial cells in primary Sjögren's syndrome. *Arthritis Res Ther* 2006; 8: R51.
8. DARIDON C, DEVAUCHELLE V, HUTIN P *et al*.: Aberrant expression of BAFF by B lymphocytes infiltrating the salivary glands of patients with primary Sjögren's syndrome. *Arthritis Rheum* 2007; 56: 1134-44.
9. QUARTUCCIO L, SALVIN S, FABRIS M *et al*.: BLyS upregulation in Sjögren's syndrome associated with lymphoproliferative disorders, higher ESSDAI score and B-cell clonal expansion in the salivary glands. *Rheumatology* (Oxford) 2013; 52: 276-81.
10. CARSONS SE, VIVINO FB, PARKE A *et al*.: Treatment Guidelines for Rheumatologic Manifestations of Sjögren's Syndrome: Use of Biologic Agents, Management of Fatigue, and Inflammatory Musculoskeletal Pain. *Arthritis Care Res* 2017; 69: 517-27.
11. GIACOMELLI R, AFELTRA A, ALUNNO A *et al*.: International consensus: What else can we do to improve diagnosis and therapeutic strategies in patients affected by autoimmune rheumatic diseases (rheumatoid arthritis, spondyloarthritides, systemic sclerosis, systemic lupus erythematosus, antiphospholipid syndrome and Sjögren's syndrome)? The unmet needs and the clinical grey zone in autoimmune disease management. *Autoimmun Rev* 2017; 16: 911-24.
12. RAMOS-CASALS M, TZIOUFAS AG, STONE JH, SISO A, BOSCH X: Treatment of primary Sjögren's syndrome: a systematic review. *JAMA* 2010; 304: 452-60.
13. TORRES-RUIZ J, FAZ-MUNOZ D, HERNANDEZ-DIAZ N, HERNANDEZ-MOLINA G: Use and withdrawal of immunosuppressors in primary Sjögren's syndrome. *Clin Exp Rheumatol* 2018; 36 (Suppl .112): S177-81.
14. FASANO S, ISENBERG DA: Present and novel biologic drugs in primary Sjogren's syndrome. *Clin Exp Rheumatol* 2019; 37 (Suppl. 118): S167-74.
15. TZIOUFAS AG, GOULES AV: Limited efficacy of targeted treatments in Sjögren's syndrome: why? *Clin Exp Rheumatol* 2018; 36 (Suppl. 112): S27-8.
16. DE VITA S, DE MARCHI G, SACCO S, ZAJA F, SCOTT C, FERRACCIOLI G: Treatment of B-cell disorders of MALT in Sjögren's syndrome with anti-CD20 monoclonal antibody. *Proceedings of the 8th International Symposium on Sjögren's syndrome Kanazawa, Japan*. 2002; 51, P8-2.
17. GONG Q, OU Q, YE S *et al*.: Importance of cellular microenvironment and circulatory dynamics in B cell immunotherapy. *J Immunol* 2005; 174: 817-26.
18. DE VITA S, QUARTUCCIO L, SALVIN S *et al*.: Sequential therapy with belimumab followed by rituximab in Sjögren's syndrome associated with B-cell lymphoproliferation and overexpression of BAFF: evidence for long-term efficacy. *Clin Exp Rheumatol* 2014; 32: 490-4.
19. QUARTUCCIO L, BALDINI C, PRIORI R *et al*.: Cryoglobulinemia in Sjögren's Syndrome: A Disease Subset that Links Higher Systemic Disease Activity, Autoimmunity, and Local B Cell Proliferation in Mucosa-associated Lymphoid Tissue. *J Rheumatol* 2017; 44: 1179-83.
20. TZIOUFAS AG, BOUMBA DS, SKOPOULI FN, MOUTSOPOULOS HM: Mixed monoclonal cryoglobulinemia and monoclonal rheumatoid factor cross-reactive idiotypes as predictive factors for the development of lymphoma in primary Sjögren's syndrome. *Arthritis Rheum* 1996; 39: 767-72.
21. QUARTUCCIO L, ISOLA M, BALDINI C *et al*.: Clinical and biological differences between cryoglobulinaemic and hypergammaglobulinaemic purpura in primary Sjögren's syndrome: results of a large multicentre study. *Scand J Rheumatol* 2015; 44: 36-41.
22. QUARTUCCIO L, ISOLA M, BALDINI C *et al*.: Biomarkers of lymphoma in Sjögren's syndrome and evaluation of the lymphoma risk in prelymphomatous conditions: results of a multicenter study. *J Autoimmun* 2014; 51: 75-80.
23. MARIETTE X, SEROR R, QUARTUCCIO L *et al*.: Efficacy and safety of belimumab in primary Sjögren's syndrome: results of the BELISS open-label phase II study. *Ann Rheum Dis* 2015; 74: 526-31.
24. DE VITA S, QUARTUCCIO L, SEROR R *et al*.: Efficacy and safety of belimumab given for 12 months in primary Sjögren's syndrome: the BELISS open-label phase II study. *Rheumatology* (Oxford) 2015; 54: 2249-56.
25. QUARTUCCIO L, SALVIN S, CORAZZA L, GANDOLFO S, FABRIS M, DE VITA S: Efficacy of belimumab and targeting of rheumatoid factor-positive B-cell expansion in Sjögren's syndrome: follow-up after the end of the phase II open-label BELISS study. *Clin Exp Rheumatol* 2016; 34: 311-4.
26. SCHNEIDER P, MACKAY F, STEINER V *et al*.: BAFF, a novel ligand of the tumor necrosis factor family, stimulates B cell growth. *J Exp Med* 1999; 189: 1747-56.
27. MACKAY F, SCHNEIDER P: Cracking the BAFF code. *Nat Rev Immunol* 2009; 9: 491-502.
28. STRIZ I, BRABCOVA E, KOLESAR L, SEKERKOVA A: Cytokine networking of innate immunity cells: a potential target of therapy. *Clin Sci* (Lond) 2014; 126: 593-612.
29. HUARD B, ARLETTAZ L, AMBROSE C *et al*.: BAFF production by antigen-presenting cells provides T cell co-stimulation. *Int Immunol* 2004; 16: 467-75.
30. MACKAY F, SCHNEIDER P, RENNERT P, BROWNING J: BAFF AND APRIL: a tutorial on B cell survival. *Annu Rev Immunol* 2003; 21: 231-64.
31. KATO A, TRUONG-TRAN AQ, SCOTT AL, MATSUMOTO K, SCHLEIMER RP: Airway epithelial cells produce B cell-activating factor of TNF family by an IFN-beta-dependent mechanism. *J Immunol* 2006; 177: 7164-72.
32. LANGAT DL, WHEATON DA, PLATT JS, SIFERS T, HUNT JS: Signaling pathways for B cell-activating factor (BAFF) and a proliferation-inducing ligand (APRIL) in human placenta. *Am J Pathol* 2008; 172: 1303-11.
33. ALSALEH G, MESSER L, SEMAAN N *et al*.: BAFF synthesis by rheumatoid synoviocytes is positively controlled by alpha5beta1 integrin stimulation and is negatively regulated by tumor necrosis factor alpha and Toll-like receptor ligands. *Arthritis Rheum* 2007; 56: 3202-14.
34. OHATA J, ZVAIFLER NJ, NISHIO M *et al*.: Fibroblast-like synoviocytes of mesenchy-

mal origin express functional B cell-activating factor of the TNF family in response to proinflammatory cytokines. *J Immunol* (Baltimore) 2005; 174: 864-70.
35. PELEKANOU V, KAMPA M, KAFOUSI M *et al*.: Expression of TNF-superfamily members BAFF and APRIL in breast cancer: immunohistochemical study in 52 invasive ductal breast carcinomas. *BMC Cancer* 2008; 8: 76.
36. ABE M, KIDO S, HIASA M *et al*.: BAFF and APRIL as osteoclast-derived survival factors for myeloma cells: a rationale for TACI-Fc treatment in patients with multiple myeloma. *Leukemia* 2006; 20: 1313-5.
37. GEFFROY-LUSEAU A, JEGO G, BATAILLE R, CAMPION L, PELLAT-DECEUNYNCK C: Osteoclasts support the survival of human plasma cells in vitro. *Int Immunol* 2008; 20: 775-82.
38. KRUMBHOLZ M, THEIL D, DERFUSS T *et al*.: BAFF is produced by astrocytes and up-regulated in multiple sclerosis lesions and primary central nervous system lymphoma. *J Exp Med* 2005; 201: 195-200.
39. CHU VT, ENGHARD P, RIEMEKASTEN G, BEREK C: *In vitro* and *in vivo* activation induces BAFF and APRIL expression in B cells. *J Immunol* 2007; 179: 5947-57.
40. MACKAY F, LEUNG H: The role of the BAFF/APRIL system on T cell function. *Semin Immunol* 2006; 18: 284-9.
41. SUZUKI K, SETOYAMA Y, YOSHIMOTO K, TSUZAKA K, ABE T, TAKEUCHI T: Effect of interleukin-2 on synthesis of B cell activating factor belonging to the tumor necrosis factor family (BAFF) in human peripheral blood mononuclear cells. *Cytokine* 2008; 44: 44-8.
42. GOENKA R, SCHOLZ JL, SINDHAVA VJ, CANCRO MP: New roles for the BLyS/BAFF family in antigen-experienced B cell niches. *Cytokine Growth Factor Rev* 2014; 25: 107-13.
43. MACKAY F, MACKAY CR: The role of BAFF in B-cell maturation, T-cell activation and autoimmunity. *Trends Immunol* 2002; 23: 113-5.
44. SCHNEIDER P: The role of APRIL and BAFF in lymphocyte activation. *Current Opin Immunol* 2005; 17: 282-9.
45. NG LG, SUTHERLAND AP, NEWTON R *et al*.: B cell-activating factor belonging to the TNF family (BAFF)-R is the principal BAFF receptor facilitating BAFF costimulation of circulating T and B cells. *J Immunol* 2004; 173: 807-17.
46. YE Q, WANG L, WELLS AD *et al*.: BAFF binding to T cell-expressed BAFF-R costimulates T cell proliferation and alloresponses. *Eur J Immunol* 2004; 34: 2750-9.
47. BARBOSA RR, SILVA SL, SILVA SP *et al*.: Reduced BAFF-R and increased TACI expression in common variable immunodeficiency. *J Clin Immunol* 2014; 34: 573-83.
48. BENSON MJ, DILLON SR, CASTIGLI E *et al*.: Cutting edge: the dependence of plasma cells and independence of memory B cells on BAFF and APRIL. *J Immunol* 2008; 180: 3655-9.
49. COQUERY CM, LOO WM, WADE NS *et al*.: BAFF regulates follicular helper t cells and affects their accumulation and interferon-gamma production in autoimmunity. *Arthritis Rheumatol* 2015; 67: 773-84.
50. GOENKA R, MATTHEWS AH, ZHANG B *et al*.: Local BLyS production by T follicular cells mediates retention of high affinity B cells during affinity maturation. *J Exp Med* 2014; 211: 45-56.
51. MACKAY F, WOODCOCK SA, LAWTON P *et al*.: Mice transgenic for BAFF develop lymphocytic disorders along with autoimmune manifestations. *J Exp Med* 1999; 190: 1697-710.
52. BATTEN M, FLETCHER C, NG LG *et al*.: TNF deficiency fails to protect BAFF transgenic mice against autoimmunity and reveals a predisposition to B cell lymphoma. *J Immunol* 2004; 172: 812-22.
53. VINCENT FB, MORAND EF, SCHNEIDER P, MACKAY F: The BAFF/APRIL system in SLE pathogenesis. *Nat Rev Rheumatol* 2014; 10: 365-73.
54. KAMAL A, KHAMASHTA M: The efficacy of novel B cell biologics as the future of SLE treatment: a review. *Autoimmun Rev* 2014; 13: 1094-101.
55. DILLON SR, HARDER B, LEWIS KB *et al*.: B-lymphocyte stimulator/a proliferation-inducing ligand heterotrimers are elevated in the sera of patients with autoimmune disease and are neutralized by atacicept and B-cell maturation antigen-immunoglobulin. *Arthritis Res Ther* 2010; 12: R48.
56. STOHL W, METYAS S, TAN SM *et al*.: B lymphocyte stimulator overexpression in patients with systemic lupus erythematosus: longitudinal observations. *Arthritis Rheum* 2003; 48: 3475-86.
57. PETRI M, STOHL W, CHATHAM W *et al*.: Association of plasma B lymphocyte stimulator levels and disease activity in systemic lupus erythematosus. *Arthritis Rheum* 2008; 58: 2453-9.
58. CHU VT, ENGHARD P, SCHURER S *et al*.: Systemic activation of the immune system induces aberrant BAFF and APRIL expression in B cells in patients with systemic lupus erythematosus. *Arthritis Rheum* 2009; 60: 2083-93.
59. MCCARTHY EM, LEE RZ, NI GABHANN J *et al*.: Elevated B lymphocyte stimulator levels are associated with increased damage in an Irish systemic lupus erythematosus cohort. *Rheumatology* (Oxford) 2013; 52: 1279-84.
60. NEZOS A, PAPAGEORGIOU A, FRAGOULIS G *et al*.: B-cell activating factor genetic variants in lymphomagenesis associated with primary Sjögren's syndrome. *J Autoimmun* 2014; 51: 89-98.
61. LAVIE F, MICELI-RICHARD C, ITTAH M, SELLAM J, GOTTENBERG JE, MARIETTE X: B-cell activating factor of the tumour necrosis factor family expression in blood monocytes and T cells from patients with primary Sjögren's syndrome. *Scand J Immunol* 2008; 67: 185-92.
62. GROOM J, KALLED SL, CUTLER AH *et al*.: Association of BAFF/BLyS overexpression and altered B cell differentiation with Sjögren's syndrome. *J Clin Invest* 2002; 109: 59-68.
63. MUMCU G, BICAKCIGIL M, YILMAZ N *et al*.: Salivary and serum B-cell activating factor (BAFF) levels after hydroxychloroquine treatment in primary Sjögren's syndrome. *Oral Health Prev Dent* 2013; 11: 229-34.
64. MARIETTE X, ROUX S, ZHANG J *et al*.: The level of BLyS (BAFF) correlates with the titre of autoantibodies in human Sjögren's syndrome. *Ann Rheum Dis* 2003; 62: 168-71.
65. FURIE R, PETRI M, ZAMANI O *et al*.: A phase III, randomized, placebo-controlled study of belimumab, a monoclonal antibody that inhibits B lymphocyte stimulator, in patients with systemic lupus erythematosus. *Arthritis Rheum* 2011; 63: 3918-30.
66. NAVARRA SV, GUZMAN RM, GALLACHER AE *et al*.: Efficacy and safety of belimumab in patients with active systemic lupus erythematosus: a randomised, placebo-controlled, phase 3 trial. *Lancet* 2011; 377: 721-31.
67. BRUCE IN, UROWITZ M, VAN VOLLENHOVEN R *et al*.: Long-term organ damage accrual and safety in patients with SLE treated with belimumab plus standard of care. *Lupus* 2016; 25: 699-709.
68. STOHL W, SCHWARTING A, OKADA M *et al*.: Efficacy and safety of subcutaneous belimumab in systemic lupus erythematosus: a fifty-two-week randomized, double-blind, placebo-controlled study. *Arthritis Rheumatol* 2017; 69: 1016-27.
69. STOHL W, HIEPE F, LATINIS KM *et al*.: Belimumab reduces autoantibodies, normalizes low complement levels, and reduces select B cell populations in patients with systemic lupus erythematosus. *Arthritis Rheum* 2012; 64: 2328-37.
70. PRETE M, LEONE P, FRASSANITO MA *et al*.: Belimumab restores Treg/Th17 balance in patients with refractory systemic lupus erythematosus. *Lupus* 2018; 27: 1926-35.
71. PONTARINI E, FABRIS M, QUARTUCCIO L *et al*.: Treatment with belimumab restores B cell subsets and their expression of B cell activating factor receptor in patients with primary Sjögren's syndrome. *Rheumatology* (Oxford) 2015; 54: 1429-34.
72. QUARTUCCIO L, MAVRAGANI CP, NEZOS A, GANDOLFO S, TZIOUFAS AG, DE VITA S: Type I interferon signature may influence the effect of belimumab on immunoglobulin levels, including rheumatoid factor in Sjögren's syndrome. *Clin Exp Rheumatol* 2017; 35: 719-20.
73. BANHAM GD, FLINT SM, TORPEY N *et al*.: Belimumab in kidney transplantation: an experimental medicine, randomised, placebo-controlled phase 2 trial. *Lancet* 2018; 391: 2619-30.
74. INABA A, CLATWORTHY MR: Novel immunotherapeutic strategies to target alloantibody-producing B and plasma cells in transplantation. *Curr Opin Organ Transplant* 2016; 21: 419-26.
75. BLAIR PA, NORENA LY, FLORES-BORJA F *et al*.: CD19(+)CD24(hi)CD38(hi) B cells exhibit regulatory capacity in healthy individuals but are functionally impaired in systemic Lupus Erythematosus patients. *Immunity* 2010; 32: 129-40.
76. IWATA Y, MATSUSHITA T, HORIKAWA M *et al*.: Characterization of a rare IL-10-competent B-cell subset in humans that parallels mouse regulatory B10 cells. *Blood* 2011; 117: 530-41.
77. CLATWORTHY MR, WATSON CJ, PLOTNEK G *et al*.: B-cell-depleting induction therapy

and acute cellular rejection. The *New Engl J Med* 2009; 360: 2683-5.
78. CHANDRAKER A, KOBASHIGAWA J, STEHLIK J *et al*.: Rituximab induction in cardiac transplantation is associated with accelerated coronary artery vasculopathy: CTOT11. *Am J Transplant* 2016; 16 (Suppl. 3): 403.
79. AHMED S: Belimumab in kidney transplantation. *Lancet* 2019; 393: 874.
80. STOHL W, MERRILL JT, MCKAY JD *et al*.: Efficacy and safety of belimumab in patients with rheumatoid arthritis: a phase II, randomized, double-blind, placebo-controlled, dose-ranging Study. *J Rheumatol* 2013; 40: 579-89.
81. GORDON JK, MARTYANOV V, FRANKS JM *et al*.: Belimumab for the treatment of early diffuse systemic sclerosis: results of a randomized, double-blind, placebo-controlled, pilot trial. *Arthritis Rheumatol* 2018; 70: 308-16.
82. JAYNE D, BLOCKMANS D, LUQMANI R *et al*.: Efficacy and safety of belimumab and azathioprine for maintenance of remission in antineutrophil cytoplasmic antibody- associated vasculitis: a randomized controlled study. *Arthritis Rheumatol* 2019; 71: 952-63.
83. HEWETT K, SANDERS DB, GROVE RA *et al*.: Randomized study of adjunctive belimumab in participants with generalized myasthenia gravis. *Neurology* 2018; 90: e1425-e34.
84. EDWARDS JC, CAMBRIDGE G: Sustained improvement in rheumatoid arthritis following a protocol designed to deplete B lymphocytes. *Rheumatology* (Oxford) 2001; 40: 205-11.
85. GODEAU B, PORCHER R, FAIN O *et al*.: Rituximab efficacy and safety in adult splenectomy candidates with chronic immune thrombocytopenic purpura: results of a prospective multicenter phase 2 study. *Blood* 2008; 112: 999-1004.
86. SPECKS U, MERKEL PA, SEO P *et al*.: Efficacy of remission-induction regimens for ANCA-associated vasculitis. *New Engl J Med* 2013; 369: 417-27.
87. BOWMAN SJ, EVERETT CC, O'DWYER JL *et al*.: Randomized controlled trial of rituximab and cost-effectiveness analysis in treating fatigue and oral dryness in primary Sjögren's syndrome. *Arthritis Rheumatol* 2017; 69: 1440-50.
88. DEVAUCHELLE-PENSEC V, MARIETTE X, JOUSSE-JOULIN S *et al*.: Treatment of primary Sjögren's syndrome with rituximab: a randomized trial. *Ann Intern Med* 2014; 160: 233-42.
89. MERRILL JT, NEUWELT CM, WALLACE DJ *et al*.: Efficacy and safety of rituximab in moderately-to-severely active systemic lupus erythematosus: the randomized, double-blind, phase II/III systemic lupus erythematosus evaluation of rituximab trial. *Arthritis Rheum* 2010; 62: 222-33.
90. ROVIN BH, FURIE R, LATINIS K *et al*.: Efficacy and safety of rituximab in patients with active proliferative lupus nephritis: the Lupus Nephritis Assessment with Rituximab study. *Arthritis Rheum* 2012; 64: 1215-26.
91. DE VITA S, QUARTUCCIO L, ISOLA M *et al*.: A randomized controlled trial of rituximab for the treatment of severe cryoglobulinemic vasculitis. *Arthritis Rheum* 2012; 64: 843-53.
92. QUARTUCCIO L, FABRIS M, SALVIN S, MASET M, DE MARCHI G, DE VITA S: Controversies on rituximab therapy in Sjögren syndrome-associated lymphoproliferation. *Int J Rheumatol* 2009; 2009: 424935.
93. TENG YKO, BRUCE IN, DIAMOND B *et al*.: Phase III, multicentre, randomised, double-blind, placebo-controlled, 104-week study of subcutaneous belimumab administered in combination with rituximab in adults with systemic lupus erythematosus (SLE): BLISS-BELIEVE study protocol. *BMJ Open* 2019; 9: e025687.
94. FRATICELLI P, FISCHETTI C, SALAFFI F *et al*.: Combination therapy with rituximab and mycophenolate mofetil in systemic sclerosis. A single-centre case series study. *Clin Exp Rheumatol* 2018; 36 (Suppl. 113): S142-5.
95. GUALTIEROTTI R, BORGHI MO, GEROSA M *et al*.: Successful sequential therapy with rituximab and belimumab in patients with active systemic lupus erythematosus: a case series. *Clin Exp Rheumatol* 2018; 36: 643-7.
96. DORNER T, POSCH MG, LI Y *et al*.: Treatment of primary Sjögren's syndrome with ianalumab (VAY736) targeting B cells by BAFF receptor blockade coupled with enhanced, antibody-dependent cellular cytotoxicity. *Ann Rheum Dis* 2019; 78: 641-7.
97. QUARTUCCIO L, FABRIS M, MORETTI M *et al*.: Resistance to rituximab therapy and local BAFF overexpression in Sjögren's syndrome-related myoepithelial sialadenitis and low-grade parotid B-cell lymphoma. *Open Rheumatol J* 2008; 2: 38-43.
98. REHNBERG M, AMU S, TARKOWSKI A, BOKAREWA MI, BRISSLERT M: Short- and long-term effects of anti-CD20 treatment on B cell ontogeny in bone marrow of patients with rheumatoid arthritis. *Arthritis Res Ther* 2009; 11: R123.
99. TENG YK, LEVARHT EW, TOES RE, HUIZINGA TW, VAN LAAR JM: Residual inflammation after rituximab treatment is associated with sustained synovial plasma cell infiltration and enhanced B cell repopulation. *Ann Rheum Dis* 2009; 68: 1011-6.
100. THURLINGS RM, VOS K, WIJBRANDTS CA, ZWINDERMAN AH, GERLAG DM, TAK PP: Synovial tissue response to rituximab: mechanism of action and identification of biomarkers of response. *Ann Rheum Dis* 2008; 67: 917-25.
101. TENG YK, LEVARHT EW, HASHEMI M *et al*.: Immunohistochemical analysis as a means to predict responsiveness to rituximab treatment. *Arthritis Rheum* 2007; 56: 3909-18.
102. AHUJA A, SHUPE J, DUNN R, KASHGARIAN M, KEHRY MR, SHLOMCHIK MJ: Depletion of B cells in murine lupus: efficacy and resistance. *J Immunol* 2007; 179: 3351-61.
103. WILD J, SCHMIEDEL BJ, MAURER A *et al*.: Neutralization of (NK-cell-derived) B-cell activating factor by Belimumab restores sensitivity of chronic lymphoid leukemia cells to direct and Rituximab-induced NK lysis. *Leukemia* 2015; 29: 1676-83.
104. MAHEVAS M, MICHEL M, WEILL JC, REYNAUD CA: Long-lived plasma cells in autoimmunity: lessons from B-cell depleting therapy. *Front Immunol* 2013; 4: 494.
105. EHRENSTEIN MR, WING C: The BAFFling effects of rituximab in lupus: danger ahead? *Nat Rev Rheumatol* 2016; 12: 367-72.
106. CAMBRIDGE G, ISENBERG DA, EDWARDS JC *et al*.: B cell depletion therapy in systemic lupus erythematosus: relationships among serum B lymphocyte stimulator levels, autoantibody profile and clinical response. *Ann Rheum Dis* 2008; 67: 1011-6.
107. CAMBRIDGE G, STOHL W, LEANDRO MJ, MIGONE TS, HILBERT DM, EDWARDS JC: Circulating levels of B lymphocyte stimulator in patients with rheumatoid arthritis following rituximab treatment: relationships with B cell depletion, circulating antibodies, and clinical relapse. *Arthritis Rheum* 2006; 54: 723-32.
108. PERS JO, DEVAUCHELLE V, DARIDON C *et al*.: BAFF-modulated repopulation of B lymphocytes in the blood and salivary glands of rituximab-treated patients with Sjögren's syndrome. *Arthritis Rheum* 2007; 56: 1464-77.
109. ST CLAIR EW, LEVESQUE MC, PRAK ET *et al*.: Rituximab therapy for primary Sjögren's syndrome: an open-label clinical trial and mechanistic analysis. *Arthritis Rheum* 2013; 65: 1097-106.
110. CARTER LM, ISENBERG DA, EHRENSTEIN MR: Elevated serum BAFF levels are associated with rising anti-double-stranded DNA antibody levels and disease flare following B cell depletion therapy in systemic lupus erythematosus. *Arthritis Rheum* 2013; 65: 2672-9.
111. LAZARUS MN, TURNER-STOKES T, CHAVELE KM, ISENBERG DA, EHRENSTEIN MR: B-cell numbers and phenotype at clinical relapse following rituximab therapy differ in SLE patients according to anti-dsDNA antibody levels. *Rheumatology* (Oxford) 2012; 51: 1208-15.
112. CHAVELE KM, MERRY E, EHRENSTEIN MR: Cutting edge: circulating plasmablasts induce the differentiation of human T follicular helper cells via IL-6 production. *J Immunol* 2015; 194: 2482-5.
113. DE WIT J, JORRITSMA T, MAKUCH M *et al*.: Human B cells promote T-cell plasticity to optimize antibody response by inducing coexpression of T(H)1/T(FH) signatures. *J Allergy Clin Immunol* 2015; 135: 1053-60.
114. ZHANG X, LINDWALL E, GAUTHIER C *et al*.: Circulating $CXCR5^{+}CD4^{+}$helper T cells in systemic lupus erythematosus patients share phenotypic properties with germinal center follicular helper T cells and promote antibody production. *Lupus* 2015; 24: 909-17.
115. SCHOLZ JL, CANCRO MP: Resolve, revise, and relax: the 3 Rs of B cell repertoire adjustment. *Immunol Lett* 2012; 143: 2-8.
116. DE VITA S, GANDOLFO S, ZANDONELLA CALLEGHER S, ZABOTTI A, QUARTUCCIO L: The evaluation of disease activity in Sjögren's syndrome based on the degree of MALT involvement: glandular swelling and cryoglobulinaemia compared to ESSDAI in a cohort study. *Clin Exp Rheumatol* 2018; 36 (Suppl. 112): S150-6.
117. GANDOLFO S, DE VITA S: Emerging drugs for primary Sjögren's syndrome. *Expert Opin Emerg Drugs* 2019; 24: 121-32.
118. PEZOULAS VC, KOUROU KD, KALATZIS F *et al*.: Medical data quality assessment: On the development of an automated framework for medical data curation. *Comput Biol Med* 2019; 107: 270-83.