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 normalization 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 randomized 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 registry 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 cryoglobulinemia (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/BR3), transmembrane activator and calcium modulator and cytoplitin ligand inductor (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 characterized by the presence of hypergammaglobulinemia, 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 patho-
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, SLE 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 assessment 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) S S Disease Activity Index (ESSDAI) and EULAR S S 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 transplantation 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 adjuction 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.

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 randomized 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, antinuclear, 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 high immunosuppressant background
Double anti-B cell and anti-BAFF targeting for the treatment of pSS / S. Gandolfo & S. De Vita

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, randomized, double-blind, placebo-controlled study on RA (NCT00701812), 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 employed 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.
**A. Anti-BAFF and anti-CD20 double therapy**

1. **BELIMUMAB** | **RITUXIMAB**

2. **BELIMUMAB** | **RITUXIMAB**

**b. SUBSEQUENT THERAPY**

1. **RITUXIMAB**

2. **BELIMUMAB**

**B. Anti-BAFF-R therapy**

**IANALUMAB**

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**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].

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 re-conducted 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 leukemia (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 neutralizing 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 characterized 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, finally leading 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 depletion.

Parenthetically, the difference in the “BAFFing” 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 characterizing each patient subset, reinforces the concept that a disease stratification, mainly to better address different needs in terms of treatment, is crucial.

The “BAFFing” 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 of 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 target ed 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 preapprative 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 pathologic 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).

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