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REVIEW

Rituximab in primary Sjögren's syndrome: a ten-year journey

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Primary Sjögren's syndrome (pSS) is an autoimmune disorder affecting exocrine glands and characterized in most cases by a rather mild clinical picture. However, a subgroup of pSS patients experience systemic extraglandular involvement leading to a worsening of disease prognosis. Current therapeutic options for the treatment of pSS are mainly empirical, often translated by other autoimmune diseases, and recent systematic reviews have highlighted the lack of evidence-based recommendations for most of the drugs commonly employed in the spectrum of extraglandular involvement. Because of the well-established role of B-lymphocytes in the pathogenesis of pSS, a B-cell targeting therapy may represent a new and intriguing therapeutic approach; in this context, growing evidence suggests that B-cell depletion by rituximab (RTX) is also effective in pSS. Of interest, besides clinical efficacy, RTX also showed biologic effects, consistently affecting the inflammation and the lymphoid organization that occur in target tissue. Moreover, the good results observed in the published trials after RTX treatment in pSS should represent the starting point to develop evidence-based guidelines for the use of biologic therapy in this disease. *Lupus* (2014) **23,** 1337–1349.

Key words: Primary Sjögren's syndrome; B-cells; biological therapies; rituximab

Introduction

Primary Sjögren's syndrome (pSS) is a chronic systemic autoimmune disease characterized by mucosal dryness and, in at least one-third of patients, by extraglandular involvement, with musculoskeletal, cutaneous, renal, pulmonary or neurological manifestations.[1]

In pSS, both T and B cells contribute to disease pathogenesis, infiltrating the exocrine glands and other target tissues, showing evidence of clonal expansion in the affected tissues as well as in the circulation.[2–6]

The therapeutic approach to sicca symptoms is mainly aimed at reducing the discomfort and preventing local complications. Topical medications, including saliva substitutes and eyedrops, are widely employed as are topical cyclosporine and/or corticosteroids for refractory keratoconjunctivitis sicca.[7] In patients with a certain degree of residual glandular function, the employment of systemic secretagogues such as pilocarpine and cevimeline should be considered. However, solid scientific evidence of efficacy is still lacking and adverse events frequently lead to therapy discontinuation.[8] Recent systematic reviews have highlighted the lack of evidence-based recommendations for most of the drugs commonly employed in the spectrum of extraglandular involvement and, ultimately, pSS may be still considered an orphan disease.[1,7–10] Besides conventional immunosuppressive compounds, efficacy of targeted therapies in other systemic autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus (SLE) have suggested their possible use in pSS as well. Indeed, the treatment with immunosuppressive and biologic agents in pSS is mainly based on their efficacy in the aforementioned conditions, expert opinion and uncontrolled studies. Although many clinical manifestations may be shared among different systemic autoimmune diseases, it should be kept in mind that the underlying pathogenic mechanisms should be different. These data point out the need of randomized clinical trials (RCTs) to investigate the safety and efficacy of these drugs in pSS, thus providing solid scientific

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evidence. Taken together, the difficulty of building therapeutic recommendations in pSS may be related to the heterogeneity of the clinical picture, the frequent failure of first-line treatments, the lack of scientific evidence for drugs licensed for other diseases and, finally, the lack of innovative therapeutic compounds.

Growing evidence has pointed out that B cells play a central role in the development, maintenance and progression of the disease, with multiple roles at different levels in pSS pathophysiology.[11] In particular, B-lymphocyte hyperactivity, minor salivary gland (MSG) infiltration and development of B-cell follicles containing germinal center (GC)-like structures represent the hallmark of the disease.[12] Although the peculiar role of B cells in autoimmunity is the production of autoantibodies, several data suggest that B cells may also exert additional pivotal functions such as antigen presentation and release of specific cytokines with immune regulatory, proinflammatory, polarizing and tissue-organizing functions.[13,14] Excessive B-cell activation is responsible for a number of the extraglandular manifestations and serological features of pSS, including hypergammaglobulinemia, cryoglobulinemia, elevated levels of free light chains and β2-microglobulin, presence of autoantibodies to the autoantigens SSA/Ro and SSB/La, or rheumatoid factor (RF), hypocomplementemia, hypergammaglobulinemic purpura, arthritis, vasculitis, neuropathy, and glomerulonephritis.[15] Moreover, prolonged B-cell survival and aberrant B-cell activity may lead to the development of non-Hodgkin lymphomas (NHL) in 5% of pSS patients; in these cases the extranodal marginal zone B-cell lymphoma of the mucosa-associated lymphoid tissue (MALT) represents the most common subtype.[6,11,16]

Because of the well-established role of B lymphocytes in the pathogenesis of pSS, a B-cell targeting therapy may represent a new and intriguing therapeutic approach in this disease.

The aim of this review article is to discuss the currently available literature concerning B-cell depleting therapy with anti-CD20 monoclonal antibody rituximab (RTX) in pSS, separately considering the two main emerging aspects related to this treatment, such as the biological and the clinical effects, and underscoring their reciprocal influence.

Rituximab in pSS: biological effects

RTX is a chimeric murine/human monoclonal antibody targeting the CD20 molecule (human B-lymphocyte-restricted differentiation antigen, Bp35) found on the surface of most B cells, including pre-B and mature B lymphocytes, but not on stem cells, pro-B-cells, normal plasma cells or other normal tissues.[17] There are at least four postulated mechanisms of action for RTX: complement-mediated cytotoxicity, antibody-dependent cell-mediated cytotoxicity, induction of apoptosis and saturation of the Fc receptors of effector cells.[18]

The first prospective, open-label study that investigated RTX effect in 16 pSS patients showed a complete B-cell depletion by immunohistochemistry in all patients treated with RTX at week 12.[19] Subsequently, in 2009, Pijpe et al. reported the first histological evidence of a reduction in glandular inflammation, combined with signs of partial glandular restoration in five pSS patients treated with RTX.[20] Following RTX treatment, the lymphocytic infiltrate was reduced, showing a decreased B:T cell ratio with a disappearance of GCs. Of note, the histological findings paralleled the increase of parotid salivary flow and the normalization of the salivary sodium content, pointing out that the efficacy of B-cell depletion may also induce a potential glandular restoration in pSS.

Pers and colleagues analyzed the recovery of B-cell subsets after RTX treatment in pSS patients.[21] The B-cell repopulation seemed to recapitulate B-cell ontogeny. The first B cells appearing in peripheral blood are transitional type 1 (T1) B cells ($CD19^{+}CD5^{+}IgD^{+}CD38^{++}$) and plasmablasts ($CD19^{+}CD5^{-}IgD^{-}CD38^{++}$), followed by a further increase of naive B cells migrating from the bone marrow (BM). Memory B cells were early detected during repopulation, first in peripheral blood and only later in MSGs, although the number of memory B cells remained relatively lower both in PB and MSGs. Sequential MSG biopsies revealed that B cells were absent in these glands for 12 months but they colonized the affected glands 24 months after RTX treatment. Memory and T1 B cells were the first B cells identified locally and, to note, the GCs previously seen in the MSG were no longer present after B-cell recovery. The sole difference among the treated patients was in the timing of B-cell reappearance and, in this regard, the authors concluded that higher baseline serum levels of B-cell activating factor (BAFF) inversely correlated with the duration of B-cell depletion, resulting in the reconstitution of the preexisting abnormalities in PB.

BAFF and a proliferation-inducing ligand (APRIL) are involved in B-cell survival and humoral immune responses and play a critical role in B-cell homeostasis; these molecules are

produced by a variety of cells, mainly innate immune cells, such as monocytes, macrophages, dendritic cells and neutrophils and their expression is increased in the presence of type I interferon (IFN), type II IFN, other cytokines and Toll-like receptor (TLR) ligands, as well as virus-infected cells.[22,23] Type I IFN and type I IFN-induced genes and proteins are overexpressed in pSS,[24] and pSS patients display elevated levels both of BAFF and APRIL in serum and saliva, correlating with the amount of immune infiltrates in MSGs, serum immunoglobulin (Ig)G levels and autoantibody titers.[11,25] BAFF and APRIL may bind both the B-cell maturation antigen, expressed by plasma cells and memory/GC B-cells, and the "transmembrane activator and calcium modulator and cyclophilin ligand interactor" (TACI), expressed on $CD27^+$ memory B cells, activated B cells and plasma cells. Moreover, BAFF, but not APRIL, binds BAFF receptor (BAFF-R), widely expressed by human peripheral B cells.[26]

Abdulahad and colleagues confirmed that, following RTX treatment, circulating $CD19^+$ B cells started to reappear at week 24 and were partially or fully reconstituted 36–48 weeks after treatment.[27] The vast majority of the B cells that reappeared had a phenotype of transitional B cells and, interestingly, they did not derive from mature naive or memory peripheral B cells that were not depleted by RTX, but were newly generated B cells in the BM. These authors found that the percentages and the absolute numbers of Treg cells and effector T cells, as well as the ratio between effector T/Treg cells, did not significantly change after RTX treatment.

Although different dosing schedules for RTX were used in different studies, it can be concluded that RTX produces effective depletion of circulating B cells in pSS patients with similar kinetics and B-cell subset reconstitution pattern, independent of therapeutic strategies and different dosages.[21,27,28]

RTX treatment might favorably modify the disease course in pSS by depleting pathogenic B-cell clones, which may contribute both to autoimmunity and lymphoma, resetting the B-cell repertoire. However, the local persistence of clonally related Ig-producing B cells in SGs and PB has been reported despite RTX treatment, suggesting the lack of a full restoration of the B-cell repertoire to a pre-disease state.[29,30] Indeed, persistence of B-cell clones may explain the occurrence of relapses after treatment, possibly triggered by additional pathologic stimuli. In fact, it has been shown that B-cell depletion therapy is followed by an increase of serum BAFF levels, inversely correlated to the B-cell number after repopulation, and highlighting the role of BAFF in B-cell homeostasis both in health and B-cell diseases. Of note, serum APRIL levels seem not to be affected by RTX.[31] This datum may be explained by the notion that APRIL receptors, TACI and B-cell maturation agent, are selectively expressed by activated B cells, whose number is generally low in pSS, and by plasma cells, which are unaffected by RTX.

The B-cell depletion following RTX treatment may modify the immunological micro-environment of inflamed SGs, reducing antigen presentation from B to T cells, and inducing apoptotic depletion of other cell subsets, including mast cells. In this setting, a reduction of glandular interleukin (IL)-17 and IL-22 has been reported in pSS patients.[32,33] Moreover, RTX is able to reduce serum levels of different molecules, including granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-1Ra, IL-6, IL-10, IFNα, tumor necrosis factor (TNF)α, CCL4 and CXCL9 and, of note, some of these (GM-CSF, IL-6, IL-10, TNFα) may be produced by activated B cells.[34] The levels of these molecules significantly decreased after five to 12 weeks of RTX treatment, when B cells are virtually absent in PB, suggesting that their decreased levels may be an indirect effects of B-cell depletion.

Finally, an in vitro system of pSS-SG epithelial cells co-cultured with pSS lymphocytes provided evidence that RTX treatment induces a significant decrease of inflammation proteins, cytokines and growth factors released by epithelial cells, as shown by the decreased NF-κB DNA binding activity in these cells, due to an upregulation of the Raf-1 kinase inhibitor protein (RKIP).[35] These data suggest a link between the downregulation of NF-κB activity on the one hand, and the inhibition of proinflammatory mediators observed in vivo following RTX treatment on the other.

We recently concluded the first prospective, 120-week follow-up study performed in a large cohort of pSS patients receiving six courses of RTX as first-line treatment for early active pSS, and MSG biopsies were performed at baseline and at 120 weeks.[36] As far as the extent of glandular inflammation is concerned, our results confirmed the aforementioned studies, showing a reduction of lymphocytic foci and the Chisholm and Mason score, in the majority of patients treated with RTX. MSGs of these patients presented, after 120 weeks of treatment, a non-specific chronic sialadenitis pattern or a full restoration of glandular architecture, associated with the disappearance of GC structures. Our study also provided additional insights into the biologic effects of RTX.

We simultaneously evaluated the mRNA expression of a variety of cytokines and chemokines in an attempt to provide evidence about the modulation exerted by RTX on different molecules.

The formation and maintenance of tertiary lymphoid structures are critically dependent on ectopic expression of lymphotoxins (LTα and LTβ), homeostatic chemokines such as CXCL13, CXCL12, CCL19, CCL21 and their interactions with specific receptors, CXCR5, CXCR4 and CCR7, respectively.[37,38] These interactions are involved in B-cell deregulation, such as the preferential migration of CXCR4 and CXCR5 expressing $CD27^+$ memory B cells, the homing of CCR7-expressing naive B cells into the inflamed salivary glands, and the development of ectopic GC-like structures as well as the peripheral B-cell abnormalities.[39–41]

In this setting, we observed a consistent reduction at mRNA levels of CXCR4 and CXCR5, associated with a parallel increase of the CXCL12 and CXCL13 mRNAs, following anti-CD20 therapy. Although Ltα and Ltβ were markedly reduced by RTX, BAFF was not affected by the biological treatment. Finally, our study showed at the histological and molecular level the strong effect of RTX in dissolving the immunological organization of the affected tissues, which was not observed with disease-modifying anti-rheumatic drug (DMARD) therapy.[36]

Furthermore, we also showed that pSS patients who develop GC-like infiltrates display not only a different clinical and serological profile, but also a different histological and glandular cytokine profile, when compared to those without GC structures or healthy controls.[12]

In conclusion, RTX treatment seems to interfere with ectopic lymphoneogenesis not only by depleting B cells but also by tuning the delicate equilibrium between cells, molecules and receptors, partially affecting the pro-B-cell inflammatory milieu.

Rituximab in pSS: clinical effects

RTX was first tested in several open-label studies in pSS that suggested an improvement of fatigue, sicca symptoms, glandular enlargement and extraglandular manifestations (Table 1). However, the duration of the clinical effects was rather variable among the studies and these effects partially overlapped PB B-cell depletion. Furthermore, retreatment with RTX resulted in a clinical and biological response comparable to the initial treatment.[42]

The first open-label study enrolling a consistent number of pSS patients with active disease was published in 2007.[19] Sixteen patients were followed up for 36 weeks and RTX treatment induced a significant improvement in fatigue, sicca symptoms and joint involvement from week 12, still detectable at week 36, and the treatment showed a significant improvement in patients' quality of life.[58] Two subsequent small, double-blind randomized studies showed a certain efficacy of RTX in patients both with recent and active disease. The first, including 18 patients, showed an improvement of fatigue in the RTX-treated group, after six months, without any significant change in objective and subjective sicca symptoms.[48] The second study, comprising 30 patients, showed an improvement in sicca symptoms and extraglandular manifestations.[50] Of note, the strongest improvement was observed between weeks 12–36, following RTX treatment, but at week 48 the positive effects were lost in all the patients.

On this basis, two larger placebo-controlled, double-blind studies were planned. Recently, only the results of the French Tolerance and EfficAcy of Rituximab in primary Sjögren syndrome (TEARS) study have been published.[57] A total of 122 pSS patients with recent-onset disease, with higher clinical or serological disease activity, or alternatively, extraglandular manifestations, were enrolled. Although the study failed to reach the primary endpoint (improvement of at least 30 mm on two of four visual analog scales (VAS) exploring global activity, fatigue, pain and dryness, between weeks 0 and 24), several secondary evaluation criteria (dryness and fatigue scores, salivary flow rate, laboratory response) were significantly improved in patients treated with RTX. The authors concluded that the efficacy of RTX in pSS is not sufficient to allow its prescription in a large population of patients, but, at the same time, they pointed out that this failure may be related to several limitations regarding their trial. In fact, it is still debatable what might be the best outcome measure for assessing the efficacy of treatment in pSS and, since this study included patients with and without systemic manifestation, the EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI), validated to assess systemic pSS activity, might fail to correctly assess patients with only a glandular involvement. Moreover, the best interval for assessing treatment efficacy in pSS is unclear.

The Trial of anti-B-cell Therapy in Patients with Primary Sjögren's Syndrome (TRACTISS)

Table 1 Main studies evaluating the clinical effects of rituximab treatment in pSS patients

Source (first author)	*Type of study*	*No patients and RTX indication (disease duration, mean)*	*RTX dose schedule (patients, no)*	*Corticosteroids*	*Controls (patients, no)*	*Follow-up*	*Primary outcomes*	*Secondary outcomes*	*Results*	*Adverse events (patients, no)*
Gottenberg, 2005[43]	Retrospective, multicenter, open-label study	Four pSS/two pSS-MALT lymphoma (8.6 years)	375 mg/m^2/week for four weeks (5), for two weeks (1)	Variable dose of methylprednisolone in five patients	None	Six to 11 months	Not well defined	Salivary/lacrimal function, extra-glandular manifest-ations, VAS dryness and fatigue	Improvement of extraglandular manifestations, sicca complaints and fatigue, decrease RF levels	IRR (1), SSR (1)
Pijpe, 2005[44]	Prospective, single center, open-label phase II study	Eight pSS with early disease (2.3 years), seven pSS-MALT lymphoma (6.6 years)	375 mg/m^2/week for four weeks (15)	Prednisolone 25 mg i.v. (pretreatment)	None	12 weeks	Not well defined	Salivary/lacrimal function, serum parameters, VAS dryness, MFI, SF-36; remission rate for MALT lymphoma	Increase in salivary function in patients with residual saliv-ary flow (SWS flow rate > 0.1 ml/min at baseline), improve-ment in lacrimal function, in many domains of MFI and SF-36, decrease in RF levels; pSS-MALT patients: complete remission in three patients, stable disease in three and progres-sion in one.	SSR (3), HACA pres-ence (4), No adverse effects in pSS-MALT lymphoma
Meijer, 2009[42]	Prospective, extended follow-up study (Pijpe, 2005)	Seven pSS with early disease (<4 years) Five pSS	375 mg/m^2/week for four weeks (7) 375 mg/m^2/week for four weeks (5)	Prednisolone 25 mg i.v. (pretreatment)	None	48 week (first course) 48 week (second course)	Not well defined	Salivary and lacrimal function, serum parameters, VAS dryness, MFI, SF-36	Similar response for (re)treatment as reported by Pijpe, 2005; improvements returned to baseline at 36–48 weeks after (re)treatment	No adverse effects SSR (1)
Pijpe, 2009[20]	Prospective study, histological analysis (Pijpe, 2005)	Five pSS with early disease (<4 years)	375 mg/m^2/week for four weeks (5)	Prednisolone 25 mg i.v. (pretreatment)	None	12 weeks	Salivary function and parotid biopsy evaluation (baseline and at 12 weeks)		Increase in parotid flow rate, normal-ization of salivary sodium content, reduced glandular inflammation and salivary gland restoration	No adverse effects
Devauchelle-Pensec, 2007[19]	Prospective, open-label pilot study	16 pSS with active disease (13.3 years)	375 mg/m^2/week for two weeks (16)	No concomitant corticosteroids	None	36 weeks	Safety and biologic effects (B-cell depletion in PB and labial SG)	Salivary/lacrimal func-tion, serum param-eters, tender and swollen joint and point counts, VAS scores for dryness, global disease activity, pain and fatigue, SF-36	Rapid depletion of B-cells in PB and SG. No changes in salivary/lacrimal function, significant improvement of tender joint and tender point count, VAS scores and SF-36, decrease in IgA-RF levels. Efficacy on	IRR (2), suspected SSR (1)

(continued)

Table 1 Continued

Source (first author)	*Type of study*	*No patients and RTX indication (disease duration, mean)*	*RTX dose schedule (patients, no)*	*Corticosteroids*	*Controls (patients, no)*	*Follow-up*	*Primary outcomes*	*Secondary outcomes*	*Results*	*Adverse events (patients, no)*
									pulmonary manifestations	
Jousse-Joulin, 2007[45]	Prospective study, ultrasound assessment of salivary glands (Devauchelle-Pensec, 2007)	16 pSS with active disease (13.3 years)	375 mg/m^2/week for two weeks (16)	No concomitant corticosteroids	Healthy volunteers (9)	12 weeks	Not well defined	Ultrasound features (parenchymal homogeneity and gland size) of SG and Doppler waveform analysis of the transverse facial artery of parotid glands (blood inflow response to salivary stimulation)	Significant size reductions of the parotid and submandibular glands and significant increase of blood inflow response to salivary stimulation	Not specified
Devauchelle-Pensec, 2011[43]	Prospective study, quality of life assessment (Devauchelle-Pensec, 2007)	16 pSS with active disease (13.3 years)	375 mg/m^2/week for two weeks (16)	No concomitant corticosteroids	None	36 weeks	Physical function and quality of life		At 12 weeks, improvement of SF-36 (mental and physical component summary scores). Further improvements occurred from week 12 to week 24 and most gains were sustained at week 36. However, improvements in both scores failed to correlate with improvements in VAS scores	NR
Seror, 2007[46]	Retrospective, multicenter, open-label study	11 pSS with active disease/five pSS-lymphoma (9.5 years)	375 mg/m^2/week for four weeks (14), for six weeks (1), twice 1 g with an interval of two weeks (1); five patients were re-treated	Methylprednisone 100 mg (pretreatment); variable dose of daily oral prednisone	None	Two to 48 months	Not well defined	Tolerance, salivary/lacrimal function, extraglandular manifestations, corticosteroid-sparing effects, serological markers; remission rate for lymphoma	Sicca symptoms improved in a minority of patients, extraglandular manifestations improved in most patients, corticosteroid dose was reduced in 11 patients, decrease in RF, Iγs, β2-microglobulin levels, increase in BAFF concomitantly with B cell depletion; complete remission of lymphoma in four patients and partial remission in one	Adverse effects (3), HACA and SSR (1)
Galarza, 2008[47]		Eight pSS (8.4 years)	375 mg/m^2/week for four weeks or twice		None	Up to 24 months	Not well defined		Half of the patients responded, both for	IRR (3)

(continued)

Table 1 Continued

Source (first author)	*Type of study*	*No patients and RTX indication (disease duration, mean)*	*RTX dose schedule (patients, no)*	*Corticosteroids*	*Controls (patients, no)*	*Follow-up*	*Primary outcomes*	*Secondary outcomes*	*Results*	*Adverse events (patients, no)*
	Retrospective, multicenter, open-label study		1 g with an interval of two weeks (8)	Methylprednisolone 40 mg (pretreatment)				Extraglandular manifestations and sicca symptoms	extraglandular manifestations and for sicca symptoms	
Dass, 2008[48]	Randomized, double-blind, placebo-controlled pilot study	17 pSS with VAS fatigue >50 mm (7.3 years for RTX group and 8.3 years for placebo group)	Twice 1 g with an interval of two weeks (8)	Methylprednisolone 100 mg i.v. (pretreatment); variable dose of daily oral prednisolone	Placebo group (9)	Six months (12 months for safety purposes)	Improvement > 20% in VAS fatigue	Salivary/lacrimal function, serum parameters, PROFAD, FACIT-F and SF-36	Improvement of fatigue and SF-36 at six months, sicca symptoms (objective and subjective) did not improve, decrease in RF levels	IRR (2), SSR (1)
Ramos-Casals, 2010[49]	Retrospective, multicenter, open-label study (BIOGEAS registry)	Nine pSS/six pSS-lymphoma	375 mg/m^2/week for four weeks or twice 1 g with an interval of two weeks (9)	Not specified	None	12 months	Not well defined	Complete/partial/no response	67% complete, 20% partial and 13% no response	Adverse effects (2)
Meijer, 2010[50]	Prospective, single center, randomized, double-blind, placebo-controlled trial	30 pSS with a rate of secretion of SWS of ≥0.15 ml/min (5.3 years for RTX group and 5.6 years for placebo group)	Twice 1 g with an interval of two weeks (20)	Methylprednisolone 100 mg i.v. (pretreatment); variable dose of daily oral prednisone	Placebo group (10)	48 weeks	Increased SWS flow rate (ml/min)	Salivary/lacrimal function and immunologic parameters, subjective variables (MFI, SF-36, VAS dryness), extraglandular manifestations	Improvement of SWS flow rate, lacrimal gland function, laboratory parameters (B-cell and RF levels), subjective parameters (MFI, SF-36 and VAS dryness), and extraglandular manifestations. Most significant improvements in the endpoints associated with RTX were observed between 12 weeks and 36 weeks following treatment and, by week 48, most improvements returned to baseline	SSR (1)
Meiners, 2012[51]	Prospective single-center study, part of a long-term follow-up study of (re)treatment with RTX (Meijer, 2010)	28 pSS (5.3 years)	Twice 1 g with an interval of two weeks (28) as first (8), second (15), third (3) or fourth (2) course of RTX	Methylprednisolone 100 mg i.v. (pretreatment); variable dose of daily oral prednisone	None	60 weeks	Responsiveness of ESSPRI and ESSDAI in pSS patients treated with RTX		ESSPRI and ESSDAI are sensitive measures of change in disease activity after RTX. The responsiveness of ESSDAI was greater than that of ESSPRI	NR
Terrier, 2010[52]	Retrospective study (AIR registry)	Nine pSS with cryoglobulinemia vasculitis	375 mg/m^2/week for four weeks (8) or twice 1 g with an interval of 15 days (1)	Variable dose of daily oral prednisone	None		Not well defined	Safety, clinical response, laboratory (serum cryoglobulin and/or C4 complement	Nine clinical complete response, six immunologic complete response, three immunologic partial response	Minor events (2)

(continued)

Table 1 Continued

Source (first author)	*Type of study*	*No patients and RTX indication (disease duration, mean)*	*RTX dose schedule (patients, no)*	*Corticosteroids*	*Controls (patients, no)*	*Follow-up*	*Primary outcomes*	*Secondary outcomes*	*Results*	*Adverse events (patients, no)*
								fraction level) response		
Tony, 2011[53]	Retrospective study (GRAID registry)	Six pSS	RTX mean (SD) dose (mg): 2271 (995)	NR	None	Zero to 25.1 months	Not well defined	Response rate	Two complete responses, two partial responses	NR
Mekinian, 2012[54]	Retrospective study (AIR registry)	11 pSS with CNS involvement (nine years)	375 mg/m^2/week for four weeks (9) or twice 1 g with an interval of 15 days (2)	NR	None	Six to 58 months	Neurological response		No neurological change occurred in nine patients. Two patients improved. No effective in progressive multiple sclerosis-like manifestations of patients with pSS-CNS involvement	NR
Mekinian, 2012[55]	Retrospective study (AIR registry)	17 pSS with PNS involvement, divided into two groups: patients with (group 1; 10) or without (group 2; seven) cryoglobulinemia/vasculitis	375 mg/m^2/week for four weeks (9) or twice 1 g with an interval of 15 days (8)	In 15 cases at a median daily dose of 10 mg (5–80 mg)	None	Seven to 77 months	Safety and efficacy in pSS-PNS involvement (neurological response, partial or complete, clinical and/or electrophysiological)		Effective, at three months, in neurological involvement in nine of 10 patients in group 1 and in two of seven patients in group 2	Adverse events (6), IRR (1)
Gottenberg, 2013[56]	Prospective study (AIR registry)	78 pSS with systemic involvement (74) or severe glandular involvement (4) (11.9 years)	375 mg/m^2/week for four weeks (11) or twice 1 g with an interval of 15 days (67); 41 patients were retreated: two cycles: $n=21$; three cycles: $n=8$; four cycles: $n=3$; five to 12 cycles: $n=9$	Methylprednisolone 100 mg i.v. (pretreatment); variable dose of oral corticosteroids in in 29 patients with a median dosage of 17.6 mg/day (5–60)	None	Six to 81.4 months	Efficacy (assessed six months after the first cycle, according to the global opinion of the physician) and safety		At six months, efficacy in 47 patients (60%) after the first cycle of RTX, improvement of ESSDAI. Efficacy in 16 of 21 patients treated with two cycles, seven of eight patients treated with three cycles and 11 of 12 patients treated with four cycles or more	IRR (4), SSR (1)
Carubbi, 2013[36]	Prospective, multicenter, follow-up study	41 pSS with early, active disease (13 months for RTX group and 14 months for DMARDs group)	Twice 1 g with an interval of 15 days (19). All patients in RTX arm received six courses of therapy	Methylprednisolone 40 mg i.v. (pretreatment); stable dose of prednisone 12.5 mg daily	22 pSS with early, active disease treated with DMARDs	120 weeks	Safety and significant variation in the reduction of the ESSDAI	Salivary/lacrimal function, subjective variables and biologic effects in minor SGs	Faster and pronounced decrease of ESSDAI and other clinical parameters in RTX vs DMARDs group. Reduction in glandular infiltrate and germinal centers in minor SGs, and reduction in expression of chemokines/	No adverse effects

(continued)

Table 1 Continued

Source (first author)	*Type of study*	*No patients and RTX indication (disease duration, mean)*	*RTX dose schedule (patients, no)*	*Corticosteroids*	*Controls (patients, no)*	*Follow-up*	*Primary outcomes*	*Secondary outcomes*	*Results*	*Adverse events (patients, no)*
									cytokines after RTX treatment	
St.Clair, 2013[28]	Prospective, open-label, single-arm, phase I study	12 pSS with active disease (eight years)	Twice 1 g with an interval of two weeks (12)	Methylprednisolone 100 mg i.v. (pre-treatment); three patients received oral prednisone 10 mg/day	None	52 weeks for safety purposes and 26 weeks for clinical and biological purposes	Safety	Clinical and biologic (lymphocyte subsets, serum autoantibody, BAFF levels, analysis of gene expression) efficacy	Modest improvements at week 26 in patient-reported symptoms of fatigue and oral dryness, no significant improvement in the objective measures of lacrimal and salivary gland function. While blood B cell depletion was associated with an increase in serum BAFF levels, no significant changes were observed in the levels of autoantibodies or in the blood interferon signature.	Serious adverse events (2)
Devauchelle-Pensec, 2014[57]	Randomized, double-blind, placebo-controlled, parallel group trial (TEARS)	122 pSS with recent-onset biologically active or systemic disease (4.6 years for RTX group and 5.5 years for placebo group)	Twice 1 g with an interval of two weeks (63); 58 patients received two infusions of RTX and five patients received one infusion	Methylprednisolone 100 mg i.v. (pretreatment); 34 patients received steroid therapy	Placebo group (59)	24 weeks	Improvement of at least 30 mm in two of four VAS (global disease, pain, fatigue, and dryness) by week 24	Salivary and lacrimal function, subjective variables, ESSDAI, laboratory response and biologic (BAFF level) effects	No significant difference between groups in the primary endpoint; however, proportion of patients with at least 30-mm decreases in at least two of the four VAS scores was higher in the RTX group at week 6. Improvement in fatigue from baseline to week 24 was greater with RTX. No significant effect on systemic pSS, by ESSDAI. At week 24, improvement of serum IgG, IgA, IgM, and β2-microglobulin in RTX group.	Few patients had IRR with no difference between the RTX and placebo groups except for respiratory disorders and purpura.

pSS: primary Sjögren's syndrome; RTX: rituximab; MALT: mucosa-associated lymphoid tissue; RF: rheumatoid factor; IRR: infusion-related reaction; SSR: serum sickness-like reaction; VAS: visual analog scale; HACA: human anti-chimeric antibody; MFI: multidimensional fatigue inventory; PROFAD: profile of fatigue and discomfort; SF-36: short form 36; SWS: stimulated whole saliva; PB: peripheral blood; SG: salivary gland; i.v.: intravenous; FACIT-F: Functional Assessment of Chronic Illness Therapy-Fatigue; Ig: immunoglobulin; PNS: peripheral nervous system involvement; CNS: central nervous system; ESSDAI: Sjögren's Syndrome Disease Activity Index; DMARDs: disease-modifying anti-rheumatic drugs; BIOGEAS: Spanish Study Group of Biological Agents in Autoimmune Diseases; GRAID: German Registry of Autoimmune Diseases; AIR: AutoImmunity and Rituximab; TEARS: Tolerance and EfficAcy of Rituximab in primary Sjögren syndrome; NR: not reported.

(a randomized, double-blind, placebo-controlled clinical trial of anti-B-cell therapy in patients with primary Sjögren's syndrome, ISRCTN65360827) study in the United Kingdom (UK) completed the enrollment in December 2013 and currently includes 110 pSS patients.[59] The study design was intended to be closely aligned to that of the French study, in order to allow subsequent data meta-analysis.

As far as systemic involvement is concerned, an RCT reported a reduction in extraglandular manifestations in patients treated with RTX, when compared to placebo.[50] Similar results were also observed in uncontrolled studies, especially for articular, vasculitic, pulmonary and neurological involvement (Table 1). Among all pSS extraglandular manifestations, peripheral nervous system (PNS) involvement is still a great challenge for treatment. A concise report analyzed the effect of RTX in 17 pSS patients with PNS involvement.[55] Three months after RTX treatment, 65% of patients reported neurological improvement and the benefits were maintained after nine months, with a statistically significant improvement in the ESSDAI.

Seror et al. reported a decrease in the daily dose of corticosteroids in pSS patients with systemic involvement after RTX treatment, highlighting implications for reduction of the risk of steroid-associated adverse events.[46] Several studies reported significant reductions in analytical parameters, such as erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), cryoglobulinemia, RF, β2-microglobulin, and immunoglobulin levels.[19,28,43,44,46,48–50,55,57]

We recently performed a prospective, multicenter, follow-up study including 41 pSS patients with early (ranging from six to 21 months) and active disease receiving either RTX (19) or DMARDs (22) plus a stable dose of prednisone.[36] Disease activity was assessed by ESSDAI (arbitrarily chosen ≥ 6) and by values >50 mm for two of four VAS (0 to 100 mm) such as: global disease activity (including extraglandular manifestations), pain, sicca symptoms and fatigue. The primary endpoints were the evaluation of the safety of RTX infusion and a significant variation in the reduction of the ESSDAI in the RTX arm during the study period of 120 weeks. Secondary endpoints were measurement of salivary/lacrimal function, subjective variables and biologic effects of RTX in MSGs, compared with patients treated with DMARDs. Unlike previous studies, pSS patients included in the RTX arm received six courses of therapy (twice 1 g with an interval of 15 days, every six months). In both treatment groups, no adverse events were reported during the study and the RTX infusions repeated over time were generally well tolerated. RTX treatment, already from the second course of therapy, displayed a stronger and significant effect in decreasing the ESSDAI, when compared with the DMARDs arm, and this effect was observed throughout the study period. These data are partially due to a rapid and consistent score reduction of constitutional, lymphadenopathy, glandular, articular and cutaneous domains. The response curves for VAS global disease activity, VAS pain, VAS fatigue and physician global assessment mirrored the pattern of ESSDAI. These items significantly decreased in both groups, and again the RTX arm showed a better performance when compared with the DMARDs population. Such decrease was progressive up to week 72 and then reached a plateau until week 120.

At present, some concerns about the efficacy of RTX therapy in pSS still need to be addressed, for example the wide range of immunological effects associated with B-cell depletion as well as the reason underlying unresponsiveness to this compound in some pSS patients. It may be speculated that, at least in part, the contradictory results on the real efficacy of RTX in pSS may be related to the lack of reliable clinimetric measures. Indeed, some aspects of the disease including the definition of early disease and activity may not be fully covered. Although pSS patients with early, active disease and extraglandular manifestations seem to be most likely to benefit from RTX treatment, the lack of such validated measures leads to a large clinical heterogeneity in the study populations, which may account for these conflicting results.[60] In recent years, several activity scores have been developed in order both to catch the evolution of pSS and to be ultimately used in clinical trials. The development and validation of the objective ESSDAI and patient-reported European League Against Rheumatism (EULAR) Sjögren's Syndrome Patient Reported Index (ESSPRI) offered important tools for assessing clinical outcome and responses to treatment.[51,61,62] However, these indexes include different domains related to the large variability of clinical and laboratory aspects of the disease. It should be kept in mind that although patients included in clinical trials display the same scores, this does not reflect an overlapping clinical and serological picture. In fact, an active pSS patient with severe cytopenia may be different from an active pSS patient with arthritis or salivary gland swelling. Taken together, it could be considered that the inclusion of different clinical

subsets of patients in the same study cohort may introduce a critical bias in the understanding of the real therapeutic effect of RTX.

Taken into account that RTX effect is transient and disease relapses parallel B-cell repopulation in the PB, pSS patients should be treated either by monitoring the circulating B-cell number or at fixed time-points.

As far as the safety of RTX treatment is concerned, many studies reported that RTX is a safe therapeutic strategy in pSS patients,[19,28,46,52,55,56] and our data, although in a small cohort of patients, suggest that this safety may still be maintained after six courses of repeated B-cell depletion therapy.[36]

In our experience, RTX monotherapy was not sufficient in the majority of pSS patients with severe extraglandular manifestations, such as vasculitis, nephritis or polyneuropathy; conversely, we obtained better results when we combined RTX with long-term steroid treatment.[36] Theoretically, combining RTX with other biologics that be an important future option, not only to decrease the activity of disease, but also to control the mechanisms which seem to be involved in the flare after RTX discontinuation. Our paper confirmed what has already been observed by other authors concerning the potential role of BAFF in B-cell repopulation and in the occurrence of flares. Hypothetically in pSS patients, after B-cell depletion, targeting BAFF might interfere with the mechanisms that lead to reactivation of the disease.[63]

Conclusions

pSS encompasses several subsets of patients with different genetic background, pathophysiological pathways, demographic features, and different responses to proposed therapies. Despite the acknowledged role of B cells in pSS, mechanisms leading to their abnormal activation and their contribution to pSS pathogenesis are not fully elucidated. In this setting, the development of ''treat-to-target'' strategies in pSS still needs a full knowledge of the molecular mechanisms involved in the disease manifestations, which may change among patients.

Although in recent years important progress in the understanding and management of pSS has been reached, many concerns still remain regarding the evaluation of the disease activity in this slowly progressing disease, the discrimination between activity and damage markers, and the clinical and laboratory heterogeneity of pSS patients, thus limiting our ability to understand the real effects of proposed therapies.

In the era of biologics, randomized, double-blind, controlled trials represent the most powerful tool to obtain comparable results and provide the rationale to build solid therapeutic recommendations in pSS. In particular, in addition to CD20, a variety of additional B-cell-associated surface molecules could be targeted for therapeutic purposes as well as soluble mediators involved in B-cell activation and expansion. In this setting, the good results observed in the published trials after RTX treatment in pSS should represent the starting point to develop evidence-based guidelines for the use of biologic therapy in this disease.

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Conflict of interest statement

The authors have no conflicts of interest to declare.

References

1 Mavragani CP, Nezos A, Moutsopoulos HM. New advances in the classification, pathogenesis and treatment of Sjögren's syndrome. *Curr Opin Rheumatol* 2013; 25: 623–629.
2 Christodoulou MI, Kapsogeorgou EK, Moutsopoulos HM. Characteristics of the minor salivary gland infiltrates in Sjögren's syndrome. *J Autoimmun* 2010; 34: 400–407.
3 Sarigul M, Yazisiz V, Bassorgun CI, *et al*. The numbers of Foxp3 + Treg cells are positively correlated with higher grade of infiltration at the salivary glands in primary Sjögren's syndrome. *Lupus* 2010; 19: 138–145.
4 Alunno A, Bistoni O, Bartoloni E, *et al*. IL-17-producing CD4-CD8- T cells are expanded in the peripheral blood, infiltrate salivary glands and are resistant to corticosteroids in patients with primary Sjögren's syndrome. *Ann Rheum Dis* 2013; 72: 286–292.
5 Alunno A, Carubbi F, Bistoni O, *et al*. $CD4^{-}CD8^{-}$ T-cells in primary Sjögren's syndrome: Association with the extent of glandular involvement. *J Autoimmun* 2014; 51: 38–43.
6 Nocturne G, Mariette X. Advances in understanding the pathogenesis of primary Sjögren's syndrome. *Nat Rev Rheumatol* 2013; 9: 544–556.
7 Ramos-Casals M, Tzioufas AG, Stone JH, *et al*. Treatment of primary Sjögren syndrome: A systematic review. *JAMA* 2010; 304: 452–460.
8 Ramos-Casals M, Brito-Zerón P, Sisó-Almirall A, Bosch X, Tzioufas AG. Topical and systemic medications for the treatment of primary Sjögren's syndrome. *Nat Rev Rheumatol* 2012; 8: 399–411.

9 Brito-Zerón P, Sisó-Almirall A, Bové A, Kostov BA, Bamos-Casals M. Primary Sjögren syndrome: An update on current pharmacotherapy options and future directions. *Expert Opin Pharmacother* 2013; 14: 279–289.
10 Fazaa A, Bourcier T, Chatelus E, *et al*. Classification criteria and treatment modalities in primary Sjögren's syndrome. *Expert Rev Clin Immunol* 2014; 10: 543–551.
11 Kroese FG, Abdulahad WH, Haacke E, Bos NA, Vissink A, Bootsma H. B-cell hyperactivity in primary Sjögren's syndrome. *Expert Rev Clin Immunol* 2014; 10: 483–499.
12 Carubbi F, Alunno A, Cipriani P, *et al*. Is minor salivary gland biopsy more than a diagnostic tool in primary Sjögren's syndrome? Association between clinical, histopathological, and molecular features: A retrospective study. *Semin Arthritis Rheum*, Epub ahead of print 15 May 2014. DOI: 10.1016/j.semarthrit.2014.05.
13 Anolik JH, Looney RJ, Lund FE. Insights into the heterogeneity of human B cells: Diverse functions, roles in autoimmunity, and use as therapeutic targets. *Immunol Res* 2009; 45: 144–158.
14 Tatouli IP, Tzioufas AG. Pathogenetic aspects of humoral autoimmunity in Sjögren's syndrome. *Lupus* 2012; 21: 1151–1154.
15 Voulgarelis M, Tzioufas AG. Pathogenetic mechanisms in the initiation and perpetuation of Sjögren's syndrome. *Nat Rev Rheumatol* 2010; 6: 529–537.
16 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.
17 Pescovitz MD. Rituximab, an anti-CD20 monoclonal antibody: History and mechanism of action. *Am J Transplant* 2006; 6(5 Pt 1): 859–866.
18 Perosa F, Prete M, Racanelli V, Dammacco F. CD20-depleting therapy in autoimmune diseases: From basic research to the clinic. *J Intern Med* 2010; 267: 260–277.
19 Devauchelle-Pensec V, Pennec Y, Morvan J, *et al*. Improvement of Sjögren's syndrome after two infusions of rituximab (anti-CD20). *Arthritis Rheum* 2007; 57: 310–317.
20 Pijpe J, Meijer JM, Bootsma H, *et al*. Clinical and histologic evidence of salivary gland restoration supports the efficacy of rituximab treatment in Sjögren's syndrome. *Arthritis Rheum* 2009; 60: 3251–3256.
21 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: 1464–1477.
22 Mackay F, Schneider P. Cracking the BAFF code. *Nat Rev Immunol* 2009; 9: 491–502.
23 Lahiri A, Pochard P, Le Pottier L, *et al*. The complexity of the BAFF TNF-family members: Implications for autoimmunity. *J Autoimmun* 2012; 39: 189–198.
24 Wildenberg ME, van Helden-Meeuwsen CG, van de Merwe JP, Drexhage HA, Versnel MA. Systemic increase in type I interferon activity in Sjögren's syndrome: A putative role for plasmacytoid dendritic cells. *Eur J Immunol* 2008; 38: 2024–2033.
25 Youinou P, Pers JO. Disturbance of cytokine networks in Sjögren's syndrome. *Arthritis Res Ther* 2011; 13: 227.
26 Darce JR, Arendt BK, Wu X, Jelinek DF. Regulated expression of BAFF-binding receptors during human B cell differentiation. *J Immunol* 2007; 179: 7276–7286.
27 Abdulahad WH, Meijer JM, Kroese FG, *et al*. B cell reconstitution and T helper cell balance after rituximab treatment of active primary Sjögren's syndrome: A double-blind, placebo-controlled study. *Arthritis Rheum* 2011; 63: 1116–1123.
28 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: 1097–1106.
29 Hamza N, Bootsma H, Yuvaraj S, *et al*. Persistence of immunoglobulin-producing cells in parotid salivary glands of patients with primary Sjögren's syndrome after B cell depletion therapy. *Ann Rheum Dis* 2012; 71: 1881–1887.
30 Hershberg U, Meng W, Zhang B, *et al*. Persistence and selection of an expanded B cell clone in the setting of rituximab therapy for Sjögren's syndrome. *Arthritis Res Ther* 2014; 16: R51.
31 Pollard RP, Abdulahad WH, Vissink A, *et al*. Serum levels of BAFF, but not APRIL, are increased after rituximab treatment in patients with primary Sjögren's syndrome: Data from a placebo-controlled clinical trial. *Ann Rheum Dis* 2013; 72: 146–148.
32 Ciccia F, Guggino G, Rizzo A, *et al*. Rituximab modulates IL-17 expression in the salivary glands of patients with primary Sjögren's syndrome. *Rheumatology (Oxford)* 2014; 53: 1313–1320.
33 Ciccia F, Giardina A, Rizzo A, *et al*. Rituximab modulates the expression of IL-22 in the salivary glands of patients with primary Sjögren's syndrome. *Ann Rheum Dis* 2013; 72: 782–783.
34 Pollard RP, Abdulahad WH, Bootsma H, *et al*. Predominantly proinflammatory cytokines decrease after B cell depletion therapy in patients with primary Sjögren's syndrome. *Ann Rheum Dis* 2013; 72: 2028–2050.
35 Sisto M, Lisi S, D'Amore M, Lofrumento DD. Rituximab-mediated Raf kinase inhibitor protein induction modulates NF-κB in Sjögren's syndrome. *Immunology*, Epub ahead of print 21 March 2014. DOI: 10.1111/imm.12288.
36 Carubbi F, Cipriani P, Marrelli A, *et al*. Efficacy and safety of rituximab treatment in early primary Sjögren's syndrome: A prospective, multicenter, follow-up study. *Arthritis Res Ther* 2013; 15: R172.
37 Aloisi F, Pujol-Borrell R. Lymphoid neogenesis in chronic inflammatory diseases. *Nat Rev Immunol* 2006; 6: 205–217.
38 Pitzalis C, Jones GW, Bombardieri M, Jones SA. Ectopic lymphoid-like structures in infection, cancer and autoimmunity. *Nat Rev Immunol* 2014; 14: 447–462.
39 Hansen A, Reiter K, Ziprian T, *et al*. Dysregulation of chemokine receptor expression and function by B cells of patients with primary Sjögren's syndrome. *Arthritis Rheum* 2005; 52: 2109–2119.
40 Hansen A, Lipsky PE, Dörner T. B cells in Sjögren's syndrome: Indications for disturbed selection and differentiation in ectopic lymphoid tissue. *Arthritis Res Ther* 2007; 9: 218.
41 Pereira JP, Kelly LM, Cyster JG. Finding the right niche: B-cell migration in the early phases of T-dependent antibody responses. *Int Immunol* 2010; 22: 413–419.
42 Meijer JM, Pijpe J, Vissink A, Kallenberg CG, Bootsma H. Treatment of primary Sjögren's syndrome with rituximab: Extended follow-up, safety and efficacy of retreatment. *Ann Rheum Dis* 2009; 68: 284–285.
43 Gottenberg JE, Guillevin L, Lambotte O, *et al*. Tolerance and short term efficacy of rituximab in 43 patients with systemic autoimmune diseases. *Ann Rheum Dis* 2005; 64: 913–920.
44 Pijpe J, van Imhoff GW, Spijkervet FK, *et al*. Rituximab treatment in patients with primary Sjögren's syndrome: An open-label phase II study. *Arthritis Rheum* 2005; 52: 2740–2750.
45 Jousse-Joulin S, Devauchelle-Pensec V, Morvan J, *et al*. Ultrasound assessment of salivary glands in patients with primary Sjögren's syndrome treated with rituximab: Quantitative and Doppler waveform analysis. *Biologics* 2007; 1: 311–319.
46 Seror R, Sordet C, Guillevin L, *et al*. Tolerance and efficacy of rituximab and changes in serum B cell biomarkers in patients with systemic complications of primary Sjögren's syndrome. *Ann Rheum Dis* 2007; 66: 351–357.
47 Galarza C, Valencia D, Tobón GJ, *et al*. Should rituximab be considered as the first-choice treatment for severe autoimmune rheumatic diseases? *Clin Rev Allergy Immunol* 2008; 34: 124–128.
48 Dass S, Bowman SJ, Vital EM, *et al*. Reduction of fatigue in Sjögren syndrome with rituximab: Results of a randomised, double-blind, placebo-controlled pilot study. *Ann Rheum Dis* 2008; 67: 1541–1544.
49 Ramos-Casals M, García-Hernández FJ, de Ramón E, *et al*. Off-label use of rituximab in 196 patients with severe, refractory systemic autoimmune diseases. *Clin Exp Rheumatol* 2010; 28: 468–476.
50 Meijer JM, Meiners PM, Vissink A, *et al*. Effectiveness of rituximab treatment in primary Sjögren's syndrome: A randomized, double-blind, placebo-controlled trial. *Arthritis Rheum* 2010; 62: 960–968.
51 Meiners PM, Arends S, Brouwer E, Spijkervet FK, Vissink A, Bootsma H. Responsiveness of disease activity indices ESSPRI and ESSDAI in patients with primary Sjögren's syndrome treated with rituximab. *Ann Rheum Dis* 2012; 71: 1297–1302.

52 Terrier B, Launay D, Kaplanski G, *et al*. Safety and efficacy of rituximab in nonviral cryoglobulinemia vasculitis: Data from the French Autoimmunity and Rituximab registry. *Arthritis Care Res (Hoboken)* 2010; 62: 1787–1795.
53 Tony HP, Burmester G, Schulze-Koops H, *et al*. Safety and clinical outcomes of rituximab therapy in patients with different autoimmune diseases: Experience from a national registry (GRAID). *Arthritis Res Ther* 2011; 13: R75.
54 Mekinian A, Ravaud P, Larroche C, *et al*. Rituximab in central nervous system manifestations of patients with primary Sjögren's syndrome: Results from the AIR registry. *Clin Exp Rheumatol* 2012; 30: 208–212.
55 Mekinian A, Ravaud P, Hatron PY, *et al*. Efficacy of rituximab in primary Sjögren's syndrome with peripheral nervous system involvement: Results from the AIR registry. *Ann Rheum Dis* 2012; 71: 84–87.
56 Gottenberg JE, Cinquetti G, Larroche C, *et al*. Efficacy of rituximab in systemic manifestations of primary Sjögren's syndrome: Results in 78 patients of the AutoImmune and Rituximab registry. *Ann Rheum Dis* 2013; 72: 1026–1031.
57 Devauchelle-Pensec V, Mariette X, Jousse-Joulin S, *et al*. Treatment of primary Sjögren syndrome with rituximab: A randomized trial. *Ann Intern Med* 2014; 160: 233–242.
58 Devauchelle-Pensec V, Morvan J, Rat AC, *et al*. Effects of rituximab therapy on quality of life in patients with primary Sjögren's syndrome. *Clin Exp Rheumatol* 2011; 29: 6–12.
59 Brown S, Navarro Coy N, Pitzalis C, *et al*. The TRACTISS protocol: A randomised double blind placebo controlled clinical trial of anti-B-cell therapy in patients with primary Sjögren's syndrome. *BMC Musculoskelet Disord* 2014; 15: 21.
60 Seror R, Theander E, Bootsma H, *et al*. Outcome measures for primary Sjögren's syndrome: A comprehensive review. *J Autoimmun* 2014; 51: 51–56.
61 Moerman RV, Arends S, Meiners PM, *et al*. EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI) is sensitive to show efficacy of rituximab treatment in a randomized controlled trial. *Ann Rheum Dis* 2014; 73: 472–474.
62 Seror R, Theander E, Brun JG, *et al*. Validation of EULAR primary Sjögren's syndrome disease activity (ESSDAI) and patient indexes (ESSPRI). *Ann Rheum Dis*, Epub ahead of print 2014. DOI: 10.1136/annrheumdis-2013-204615.
63 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 overexp[r]ession of BAFF: Evidence for long-term efficacy. *Clin Exp Rheumatol* 2014; 32: 490–494.