B-cell depletion with rituximab in the treatment of primary Sjögren's syndrome: what have we learnt?

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ABSTRACT

Despite the well-established role of B cells in the pathogenesis of primary Sjögren's syndrome (pSS), the beneficial role of B-cell depletion therapy with rituximab remains elusive in this condition, contrary to other autoimmune diseases. Although early, small-scale studies showed promising results, two recent large randomised controlled trials did not meet their primary endpoints. It is evident from most trials that rituximab has a positive impact on B-cell numbers and activity, both in the peripheral blood and in salivary glands, but clinical outcomes vary among studies. We review here the evidence to date of B-cell depletion in pSS, analysing the underlying causes for the discrepancies in different studies and their limitations. We also discuss the potential use of peripheral and salivary gland biomarkers for patient stratification and targeted patient selection. Overall, rituximab remains a plausible treatment for pSS provided future studies address the shortfalls that emerged from our current knowledge of the use of B-cell depletion in this condition.

Introduction

Primary Sjögren's syndrome (pSS) is a systemic autoimmune disease affecting predominantly the exocrine glands but very often associated with systemic manifestations. The salivary and lacrimal glands are preferentially affected with a characteristic lymphocytic infiltration, leading to progressive loss of glandular secretory function and resulting in the clinical manifestations of dry mouth and dry eyes (sicca symptoms). Fatigue and pain are present in all patients and can be disabling; approximately 30–50% of patients will also develop systemic manifestations including renal, pulmonary or nervous system involvement (1-4).

The involvement of B cells in the pathogenesis of pSS is well established. The presence of autoantibodies such as rheumatoid factor (RF), SS-A/Ro (in 60–80% of patients) and SS-B/La (in 30–40% of patients) (3, 5) polyclonal elevation of immunoglobulin levels in the serum (hypergammaglobulinaemia), increased levels of B-cell activating factor (BAFF) (6-8), a key cytokine in B-cell activation, proliferation and survival, are all markers of B-cell hyperactivity. B cells are also found in the inflammatory infiltrate of the salivary glands and they form, progressively with T cells, organised lymphoid tissue which resembles secondary lymphoid organs (9) with evidence of functional germinal centres (GC) (10) as will be discussed later. Moreover, patients with pSS have an increased risk for B-cell lymphoma, this is higher compared with other autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) (11, 12). The commonest type of lymphoma in pSS is the mucosa associated lymphoid tissue (MALT) type affecting the parotid glands, the target tissue of the chronic inflammatory autoimmune process. The evolution of lymphoma in pSS is thought to be a multi-step process whereby B cells undergo an antigen driven hyper-reactivity and selection from polyclonal to oligo-clonal to mono-clonal proliferation (13), highlighting once more the central role of B cells in the pathogenesis of the disease.

Rituximab is a genetically engineered chimeric monoclonal antibody (with human kappa and IgG_1 constant regions and murine light and heavy-chain variable regions) directed against the transmembrane phosphoprotein CD20, a 33-

37kDA protein expressed on the surface of almost all B cells with the exception of stem cells, pro B cells and plasma cells which are therefore not affected by this treatment (14). The mechanism of action of rituximab is mediated largely by antibody dependent cell mediated cytotoxicity, complement mediated lysis and apoptosis also contribute to B-cell killing (15, 16). In autoimmune rheumatic diseases rituximab is usually given as a course of two infusions of a 1000 mg dose 2 weeks apart, or four infusions at weekly intervals of 375 mg/m^2. Patients are usually pre-medicated with 10 mg of chlorphenamine intravenously, 1gr of oral paracetamol and 100 mg intravenous methylprednisolone or other equivalent in order to avoid side effects. However, the pre-medication regimen can vary from centre to centre. Although originally designed for the treatment of B-cell malignancies, rituximab is now an established treatment in several autoimmune diseases such as RA, ANCA-associated vasculitis (AAV) and SLE. In contrast to these conditions, the role of rituximab in the treatment of pSS remains to be established with controversial results on its efficacy published over the years. Some case reports where rituximab was used for the treatment of pSS associated lymphoma showed improvement in the disease symptoms and signs (17, 18). Given the central role of B cells in the disease pathogenesis this is unarguably a justified treatment to be considered. Several small-scale retrospective or open labelled studies followed with promising results, and more recently two large randomised control trials (RCT).

We discuss here the clinical, laboratory and histological findings reported to date from all the clinical studies with rituximab treatment in pSS. Potential biomarkers for prediction of response and patient stratification are also discussed.

Effect on clinical manifestations

Several objective clinical parameters have been used to report on the effect of treatment, these include measurements of glandular function such as the stimulated whole salivary flow rate (SWSF) or the unstimulated whole salivary flow rate (UWSF) for saliva production, and the Schirmer's test for tear production.

Until recently there were no standardised and clinically validated disease activity assessment criteria for pSS; this made comparison of clinical trials, patient classification and stratification very difficult. A consensus was reached on assessing systemic disease activity using the EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI) score whilst patient assessment is done via the EULAR Sjögren's Syndrome Patient Reported Index (ESSPRI) (19). The ESSDAI is a systemic disease activity assessment tool and includes 12 domains or organ systems: cutaneous, articular, muscular, respiratory, renal, peripheral nervous system (PNS), central nervous system (CNS), haematological, glandular, constitutional, lymphadenopathic, biological. A decline in the ESSDAI score by ≥3 points is considered to be clinically meaningful improvement (20) and is currently been used to classify response to treatment. However, most of the rituximab studies in pSS either preceded ESSDAI or did not use ESSDAI for inclusion. The ESSPRI is a patient-reported index of disease activity and comprises three domains: dryness, pain and fatigue.

Subjective measurements of sicca symptoms use visual analogue scales (VAS) for oral and ocular dryness. Fatigue is a common feature of the disease and is measured with VAS, the multidimensional fatigue inventory (MFI) and the Profile of Fatigue and Discomfort (PROFAD).

Effect of rituximab on exocrine function and sicca symptoms

One of the first small scale randomised double-blind controlled trials by Meijer *et al*. reported on the SWSF rate and UWSF rate in 30 patients with significant improvement in the rituximab group compared with placebo (21). Pijpe *et al*. found improvement in the SWSF but not in the UWSF (22); in both these studies the beneficial effect was observed in patients with residual salivary gland function before treatment. Carubbi *et al*. also found improvement in the UWSF in an open labelled study of 41 patients with active disease (23). This study compared rituximab with disease modifying anti-rheumatic drugs (DMARDs). Other trials, however, did not find improvement in the UWS including the large randomised controlled trial by Devauchelle-Pensec *et al*. (also known as TEARS study) (22, 24-27). In the TRACTISS trial the UWSF in the rituximab group remained stable whilst there was deterioration of the UWSF in the placebo group resulting in a significant difference in UWSF after 48 weeks (28).

Most studies used the Schirmer's test to assess ocular gland function; only the open labelled study by Carubbi *et al*. showed significant improvement (23), whereas in the TEARS study there was no deterioration in the Schirmer's test compared with placebo (27). The sensitivity of Schirmer's test to detect change in lacrimal gland function is limited; the most sensitive tests that use ocular surface staining with rose Bengal or lissamin green were only used in the early small scale studies and they did show improvement after treatment with rituximab (21, 22). These tests should be used in future trials to provide a more accurate tool for assessment of treatment on the ocular gland integrity.

Subjective measurements of oral and ocular dryness were used more consistently in most studies to date, mostly via VAS measurements of oral, ocular or global dryness. Several studies showed improvement in oral dryness (21, 22, 24) or global dryness (23, 26, 27) with rituximab. The TEARS study demonstrated significant improvement in the VAS dryness score but this was less than 30mm which was set as a primary end-point for the trial (27). In contrast the TRACTISS study failed to show a significant improvement in the VAS dryness, despite 2 courses of rituximab and similar patient cohort compared with the TEARS study (28). Table I provides a comprehensive summary of all the trials conducted to date with all major findings.

More recently, there has been increasing interest in the validity of ultrasound evaluation of the salivary glands, both for inclusion in the classification criteria and patient stratification and also as

Table I. Clinical trials which investigated the effects of rituximab in pSS.

Study	Year	Baseline characteristics				Effects of Rituximab									
		Study design	Patients treated		Disease duration	Salivary gland function	Tear gland function	Dryness VAS	Fatigue	Pain	Change in ESSDAI	IgG	RF	Anti-Ro/La	SG B cells
			RTX	PbO											
Pijpe *et al.* (22)	2005	Open label	8	0	2±1	UWSF ↔, Stim SM/SL ↑	RB ↓, Schirmer ↔	Oral ↓, Ocular ↔	MFI GF ↓	SF-36 BP ↔	N/A	↔	↓	N/A	↓
Devauchelle-Pensec *et al.* (26)	2007	Open label	16	0	13±10	UWSF ↔,	Schirmer ↔	↓	VAS ↓	VAS ↓	N/A	↔	↔	↓	↓
Dass *et al. (25)*	2008	RCT pilot	8	9	7 (1-18)	UWSF ↔	Schirmer ↔	N/A	VAS ↓	VAS ↔	N/A	↓	↓	N/A	N/A
Meijer *et al. (34)*	2009	Open label (re-treatment)	5	0	N/A	SWSF ↑	N/A	Oral ↓	MFI GF ↓	N/A	N/A	N/A	↓	N/A	N/A
Meijer *et al.* (21)	2010	RCT	20	10	5±4	UWSF ↑, SWSF ↑	LG ↓ Schirmer ↔	Oral ↓	MFI GF ↓	N/A	↓	↓	↓	N/A	↓
Meiners *et al.* (35)	2012	Open label (re-treatment)	28	0	7±4	SWSF ↔	N/A	Oral ↔, Ocular ↔	MFI GF ↓	N/A	↓	↓	↓	↓	N/A
Gottenberg *et al.* (37)	2013	Registry	78	0	12 (3-32)	N/A	N/A	N/A	N/A	N/A	↓	N/A	N/A	N/A	N/A
Carubbi *et al.* (23)	2013	Open label	19	22	1 (1-2)	UWSF ↑	↑	↓	↓	VAS ↓	↓	↔	↔	↔	↓
St Clair *et al.* (24)	2013	Open label	12	0	8 (2-12)	UWSF ↔, SWSF ↔	Schirmer ↔	Oral ↓, Ocular ↔	VAS ↓	VAS ↔	N/A	N/A	↓	↔	N/A
Devauchelle-Pensec *et al.* (TEARS) (27)	2014	RCT	63	57	5±5	UWSF ↔	Schirmer ↔	↓	VAS ↓	VAS ↔	↔	↓	N/A	N/A	↓
Bowman *et al.* (TRACTISS) (28)	2017 ↓	RCT	67	66	5±5	UWSF ↔	Schirmer ↔	↔	VAS ↓	VAS ↔	↓	↓	↓	N/A	

ESSDAI: EULAR SS disease activity index; RTX: rituximab; PbO; placebo; LG: Lissamine Green test; MFI-GF: Multidimensional Fatigue Index; RB: Rose Bengal test; RCT: randomised control trial; SF-36BP: bodily pain domain; SWSF: stimulated whole salivary gland flow; UWSF; unstimulated whole salivary gland flow; VAS: Visual Analogue Scale; SG: salivary glands; N/A: not available.

a useful tool for monitoring response to treatment (29). In the TEARS study there was an improvement of the salivary gland echostructure with rituximab (30) whereas in the TRACTISS study rituximab resulted in improvement of the total ultrasound score compared with placebo (31). Further research into the standardisation, prognostic value and therapeutic monitoring of this method will be required to establish its utility in pSS.

Overall, one could conclude from the data so far available that rituximab stabilises, if it does not improve, sicca symptoms in pSS in a selected cohort of patients with residual exocrine function.

Effect on systemic manifestations

Fatigue is very common in pSS (approximately 70% of patients) and can be debilitating with major impact on quality of life, posing a challenge for patient management and assessment as it can only be measured subjectively (32, 33). The VAS score for fatigue has been used by most authors, with more complex and detailed tools such as the MFI and the PROFAD used only in selected studies.

Most studies did show improvement in fatigue with rituximab (21-23, 25, 34, 35) including one of the two large randomised controlled studies, the TEARS (27). In the TEARS study fatigue improved at early assessment points (weeks 6 and 16 post treatment) whereas in the TRACTISS study there was no improvement at the first time point (week 16) or at subsequent assessments. The difference between these two studies is that in the TEARS study patients received one cycle of treatment whereas in the TRACTISS study patients had a repeat cycle of treatment at week 24 from baseline, therefore one would have expected a more profound effect.

The recent introduction of ESSDAI for systemic disease assessment provided an important tool to use in clinical trials which has been shown to be sensitive to change (35) with a clinically meaningful improvement score agreed of at least 3 points or more (36). The ESSDAI was not obviously assessed in earlier studies; it was first used in an open label prospective study of 28 patients and was shown to improve with rituximab (35). Similarly a pSS registry from France reported efficacy of rituximab in systemic disease manifestations on 78 patients with a median decrease in ESSDAI from 11 before treatment to 7.5 after treatment (37). One more open label trial from Italy showed improved systemic disease activity using the ESSDAI on a cohort of 42 patients (23) and a smaller randomised control study by Moerman *et al*. of 30 patients (38) showed similar results. In contrary the two more recent large double blind control trials did not show significant improvement in ESSDAI; this could be partially explained by the relatively low baseline disease activity and therefore low ESSDAI in both trials (mean baseline score 5.7±4.5 in the TRACTISS trial and 10±7 in the TEARS trial) (27, 28). In addition in the TEARS study the baseline involvement of the 4

domains that show higher sensitivity to change, namely the glandular, articular, haematological and biological domains (39) was lower compared with the studies by Carubbi *et al*. (23) and Meiners *et al*. (35), indicating disease variability with regards to systemic involvement among studies. The beneficial effect of rituximab in pulmonary disease in the pSS French registry (37) and in articular disease (40) and its established role in the treatment of systemic vasculitis probably provides a reasonable rational for the use of this therapy in specific clinical settings, which is reflected in the recent guideline (41) of the Sjögren's Syndrome Foundation.

Effect on peripheral B cells and autoantibodies

Given the central role of B cells in driving the immunopathogenesis of the disease, the effect of rituximab on this compartment of the immune system is of great interest with regards not only to their absolute numbers but also markers of their activity. The latter includes autoantibody production, immunoglobulins, serum free light chain (FLC), β2-microglobulin and BAFF.

The most well described change in B-cell subsets in the peripheral blood of patients with pSS is a reduction of memory B cells, defined by the CD27 marker and an increase in naïve (IgD+CD27-) B cells in numerous studies (42-48). This has been attributed to the migration of this subset of B cells in the inflammed salivary glands (43) or shedding of the CD27 molecule from the cell surface (44). Several studies investigated the changes not only of the total B-cell number but also alterations of B-cell subsets after treatment with rituximab.

With regards to the effect on B-cell number, treatment with rituximab leads to a nearly complete depletion in the peripheral blood for at least 3 months to a maximum of 9 months in most patients (21, 22, 49). What constitutes complete B-cell depletion in peripheral blood has not been established in pSS with reports using a cut-off for B-cell depletion of 0.1%-1% of total lymphocytes. Time of repopulation is highly variable, usually occuring at 36-48 weeks (26), but earlier repopulation at 16 weeks has also been described (50). Earlier studies with rituximab in other disease settings (51, 52) and a more recent study by Abdulahad *et al*. suggested that the first cells to re-appear are transitional B cells, indicating replenishment of the newly emerging B cells from the bone marrow (42). Plasmablasts and memory B cells representing autoreactive B cells were seen at repopulation in one study from a French cohort of 15 patients (49).

Most studies measured serum levels of RF before and after treatment, Meijer *et al*. (21) observed consistently and significantly reduced levels of RF 5-36 weeks post-rituximab treatment compared to baseline, similar results were obtained by several investigators (22, 25, 34, 53, 54). Rising levels of RF at repopulation seem to predict relapse in two studies (35, 54). Measurement of the disease specific autoantibodies Ro/La were only performed in a few trials and only one of them, in a Dutch cohort of patients (55), found substantial reduction after B-cell depletion therapy with the rest of the investigators failing to produce similar results (23, 24, 26).

The large RCT by Devauchelle-Pensec *et al*. (27) and two further studies showed a significant reduction of the total serum IgG level 24 weeks after treatment or later (21, 53). Significant reduction in β2-microglobulin levels was observed earlier, at week 16 after treatment, in the same study by Devauchelle-Pensec *et al*. (27) and in an earlier study by Seror *et al*. (54). Verstappen *et al*. evaluated the more sensitive measurement of immunoglobulin production by serum FLC and showed a reduction from 5 weeks onwards after treatment with rituximab (56). Both β2-microglobulin and serum FLC were shown to correlate positively with ESSDAI scores in a French cohort of patients as well as in a large UK pSS cohort and they may be useful markers for monitoring disease activity (3, 57).

BAFF levels are increased in pSS and measurement of this cytokine in relation to treatment is of great interest. Serum BAFF levels increase following B-cell depletion (58). This was attributed to lack of available BAFF receptors on B cells following B-cell depletion and could potentially facilitate the survival of autoreactive B cells as it has been demonstrated in other disease settings such as autoimmune haemolytic anaemia (59). Additionally, baseline BAFF levels closely predict the time of B-cell repopulation after rituximab (49).

In contrast to BAFF the levels of other cytokines such as IL-6, IL-10, TNF-α and GMCSF are reduced by rituximab and it is not clear if this is the direct effect of treatment on B cells or indirect effect of B-cell depletion on other cytokine producing cells (60). Increased level of IL-21 is observed in the sera of pSS patients (61), and a reduction has been described by Verstappen *et al*. in association with T follicular helper (Tfh) cells as discussed in the next section (55).

Effect on T cells

An important role for the B/T cell cross-talk in the pathogenesis of pSS has been widely accepted (9). Plasmablasts, through the secretion of IL-6, are able to induce the differentiation of Tfh cells in the periphery, which in turn via IL-21 secretion are able to stimulate the differentiation and upregulation of germinal-centre (GC) B cells. It has been reported that pSS patients have increased levels of circulating Tfh cells supporting the generation of peripheral autoreactive plasma cells (55). Furthermore, several animal models of pSS have shown that IL-17-producing T cells are critical in sustaining the chronic inflammation observed in the disease (62, 63). The percentage of Th17 cells, a subset of T helper cells able to secrete inflammatory IL-17, was also found to be increased in the peripheral blood of pSS patients compared to healthy controls (55). Verstappen *et al*. found a decrease in both Tfh and Th17 cells following rituximab therapy and the decrease in circulating Tfh cells correlated with lowering of ESSDAI scores (55). Similarly, Alunno *et al*. reported a clear reduction of circulating Th17 cells after B-cell depletion in pSS, mostly related to a subset of double negative (CD3+CD4-CD8-) peripheral Th17 cells expressing CD20 (64).

Effect of rituximab on salivary gland histopathology

The histopathological characteristic of pSS is a focal immune cell infiltrate observed preferentially in salivary and lachrymal glands, leading to their impaired function. B/T cell infiltrates within the glandular tissue organise around the ductal epithelium with the co-operation of stromal cells (65). These have the ability to transform into more organised ectopic lymphoid structures (ELSs) with compartmentalised T and B-cell zones and follicular dendritic cells resembling GCs (66). The maintenance of ELSs is critically dependant on the chronic ectopic expression of inflammatory molecules by a variety of cells (9), hence characterising SGs before and after treatment could help us understand the disease pathogenesis.

There is now good evidence that rituximab reduces the total number and proportion of infiltrating B cells in the salivary glands, both labial and parotid, of pSS patients (18, 23, 26, 49, 50, 67) and interferes with the formation of GC-like structures (23, 67), whilst T cells remain largely unaffected in number. Rituximab also reduces the number of FcRL4+ B cells (68), a unique subset of intraepithelial B cells that are thought to contribute to the formation of the intraepithelial infiltration of the striated ducts and are thought to be precursors of MALT lymphoma. The disruption of ELSs within the SG is likely due not only to direct depletion of infiltrating B cells, but also to the reduction of new B cells migrating into the gland via regulation of chemokines such as CXCL12 and CXCL13 (23).

It is anticipated that plasma cells, which lack CD20 expression, are not affected by rituximab but there is a paucity in the literature on the effect of rituximab on salivary gland plasma cells with the exception of a report on a small cohort of patients (69).

Peripheral and salivary gland predictors of response to rituximab in pSS

The clinical efficacy of B-cell depletion therapy with rituximab in pSS remains controversial. It is apparent from all the studies described above that rituximab does reduce the number of B cells in the peripheral blood and salivary glands and ameliorate several parameters of B-cell hyperactivity but correlation with clinical outcomes and identification of patients who will possibly benefit from this treatment remains to be proven.

High pre-treatment levels of BAFF are associated with reduced clinical response (50) and early repopulation of B cells (49). On the other hand, BAFF levels also increase following B-cell depletion (58, 70) which may lead to promotion of autoreactive B cells as discussed earlier.

Cornec *et al*. reported on data from two prospective trial cohorts (including the TEARS study) showing that higher serum levels of anti-SSA antibodies are associated with good response to rituximab using the SSRI-30 score for clinical response (50).

Devauchelle-Pensec *et al*. investigated the validity of gene expression profiling in salivary glands at baseline as a prognostic tool of response to B-cell depletion in a small group of 15 patients and found the presence of IFN-gamma signature in conjunction with B-cell related transcripts (71) but these results need confirmation.

With regards to B-cell depletion with rituximab, in contrast to what has been suggested for RA and SLE where the degree of B-cell depletion has been associated with a clinical response (72, 73), this has not been the case in pSS. Neither has a specific peripheral blood immunophenotype been linked to response to treatment as it has been shown in RA where low number of CD27+ memory B cells and low number of plasmablasts are associated with good clinical response (74, 75).

The histopathology of the diseased tissue, *i.e*. the salivary glands, has been studied in pSS with regards to prediction of response to treatment. The results published from two groups to date have been contradictory. Deli *et al*. showed that a high absolute number and proportion of B cells in the parotid gland are associated with good clinical response as defined using the ESSDAI score (67, 76). Their results are in contrast with those of Cornec *et al*. who showed that a high proportion of infiltrating B cells and higher focus score in labial salivary gland biopsies can predict poor response to treatment (50, 77) which was defined with the use of SSRI-30 score.

Possible explanations for this discrepancy include difference in baseline disease activity, the nature of tissue biopsied (parotid *vs*. minor salivary glands), different ways of histological assessments (numbers *vs*. proportions of B cells) and different methods with which the clinical assessments were performed (76, 78).

Safety of rituximab

Rituximab is generally safe in pSS. Reported side effects from the studies include infusion related reactions, immediate or delayed, less frequent infections, respiratory symptoms and gastrointestinal symptoms (21, 27, 28). Devauchelle-Pensec *et al*. and Meijer *et al*. reported similar infection rates between rituximab and placebo groups (21, 27).

In the TRACTISS study there were more adverse events in the rituximab group compared with placebo but serious adverse events were equal between the two groups (10 and 10) (28). In the TEARS study there were 2 cases of cancer diagnosed on routine screening in the rituximab group (squamous cell carcinoma of the skin, breast cancer, the latter patient died 1 year after inclusion) and one in the placebo group (basal cell carcinoma) (27).

Limitations of the current studies and scope for future work

Although initial smaller studies in pSS demonstrated promising results for the role of B-cell depletion therapy in the disease, the two large RCT of rituximab in pSS, TEARS and TRACTISS (27, 28) did not meet their primary outcomes. It is important to notice that both studies had low baseline disease activity of patient cohorts as assessed by ESSDAI. A considerable reason for the conflicting data among studies is the study design, single *versus* multicentre recruitment and the variability in the tools used for patients inclusion and clinical assessments, both objective and subjective. The ESSDAI was only

recently introduced and not used in the earlier studies for clinical assessment. Similarly, TEARS and TRACTISS did not include baseline ESSDAI as an inclusion criteria, resulting in low average ESSDAI at study entry. Although a formal assessment of each individual domain of ESSDAI after rituximab has not be reported, it is likely that several components of the ESSDAI may not be amenable to change after B-cell depletion and therefore only selected ESSDAI domains could be used for inclusion in future studies. Additionally, considering that there is poor correlation between patient-reported symptoms and objective measurement of dryness in pSS (79), new composite outcome measures, such as those recently proposed may prove beneficial in assessing combined objective measurements and subjective symptoms (80). Large consortia such as the EU-funded Innovative Medicines Initiative "Necessity" had a definition of novel clinical endpoints in pSS at the core of their objectives which will be validated in future trials.

Despite the significant variation of clinical results from all the studies, it is clear that rituximab does impact on the underlying disease process with improvement on B-cell hyperactivity and glandular histology in some patients. Thus, an important aspect for future work is the validation of peripheral and salivary gland biomarkers of B-cell hyperactivity for patient stratification and assessment of response. However, in the case of histological assessment of the salivary glands, the methods used vary considerably and a consensus is needed as we have recently advocated (66).

The time-window of disease activity should also be considered; data emerging from all the studies performed suggest that rituximab is more beneficial in early disease and before permanent loss of glandular function develops as well as in patients with evidence of high disease activity and systemic disease.

Finally, it is entirely plausible that several mechanisms underlying the pathophysiological abnormalities in the B-cell compartment in pSS patients will inevitably impact on resistance and/or relapse to B-cell depletion resulting in poor response rates. In this regard, promising combination therapies, such as the combination of rituximab and other biologics which target B-cell pathways active in pSS such as the anti-BAFF antibody belimumab, are currently undergoing clinical testing and the results of the ongoing clinical trials are eagerly awaited.

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