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List of abbreviations

APC: Antigen-presenting cells

APRIL: A proliferation-inducing ligand

Auto-Ab: Autoantibodies

BAFF: B-cell activating factor

BCR: B-cell receptor

Bm: mature B cell

BR3: B-cell activating factor receptor 3

DC: Dendritic cell

FDC : follicular dendritic cell

GC: germinal center

GWAS: Genome-wide association studies

HPA: Hypothalamic pituitary-adrenal

IC: immunocomplex

IFN-I: Type I interferon

IL: interleukin

ILC2: Type 2 innate lymphoid cell

IL-1Ra: IL-1 receptor antagonist

iNKT: invariant natural killer T cell

LF: Lymphocitic Foci

LT: lymphotoxin

mAb: Monoclonal antibody

MZ: Marginal zone B cell

NK: Natural killer

NOD : non-obese diabetic mouse

OAS1: Oligoadenylate synthetase

PC: Plasma cell

pDC: plasmacytoid DC

PRR: Pattern recognition receptor

RA: Rheumatoid arthritis

RCT: Randomized controlled trial

RF: Rheumatoid factor

SGEC: Salivary gland epithelial cell

SSDAI: Sjogren's Syndrome Disease Activity Index

Sjs: Sjogren Syndrome

SLE: Systemic lupus erythematosus

T2: Transitional type 2 B cell

TACI: calcium modulator and cyclophylin ligand interactor

T_{FH}: Follicular helper T cell

T_H: helper T cell

TLR: Toll-like receptor

T_{reg}: regulatory T cell

TSLP: Thymic stromal lymphopoietin

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ABSTRACT

Primary Sjögren syndrome (SjS) is a systemic autoimmune disease that may affect 1 in 1000 people (overwhelmingly women) and that can be a serious disease with excess mortality due to severe organ-specific involvements and the development of B cell lymphoma; systemic involvement clearly marks the disease prognosis, and strongly suggests the need for closer follow-up and more robust therapeutic management. Therapy is established according to the organ involved and severity. As a rule, the management of systemic SjS should be organ-specific, with glucocorticoids and immunosuppressive agents limited to potentially-severe involvements; unfortunately, the limited evidence available for these drugs, together with the potential development of serious adverse events, makes solid therapeutic recommendations difficult. The emergence of biological therapies has increased the therapeutic armamentarium available to treat primary SjS. Biologics currently used in SjS patients are used off-label and are overwhelmingly agents targeting B cells, but the most recent studies are moving on into the evaluation of targeting specific cytokines involved in the SjS pathogenesis. The most recent etiopathogenic advances in SjS are shedding some light in the search for new highly-selective biological therapies without the adverse effects of the standard drugs currently used (corticosteroids and immunosuppresants drugs). This review summarizes the potential pharmacotherapeutic options targeting the main cytokine families involved in the etiopathogenesis of primary SjS and analyzes potential insights for developing new therapies.

KEY WORDS: Sjögren syndrome, cytokines, pathogenesis, therapy, biological agents

1. Introduction

Sjögren syndrome (SjS) is a systemic autoimmune disease in which the exocrine glands are the main tissue damaged (1). The histological hallmark of SjS is focal lymphocytic infiltration of the glands, which is mainly diagnosed by biopsy of the minor labial salivary glands (2). The lymphocytic foci (LF) consist of mainly T cells, a lesser amount of B cells and a smaller number of natural killer (NK) and dendritic cells (DC), and are often associated with acinar epithelial cell atrophy, progressive fibrosis and the presence of adipocytes. Clinically, patients overwhelmingly present with sicca symptoms caused by autoimmune exocrine gland involvement, although general features (fatigue and chronic pain) and systemic extraglandular involvement (including lymphoma) are also frequent (3). Autoantibodies (auto-Ab) are key serological markers of autoimmune disease and, in patients with SjS, may be present up to 20 years before the disease diagnosis (4): SjS patients may present a broad spectrum of circulating auto-Ab, of which antinuclear antibodies are the most frequently detected, anti-Ro/SS-A the most specific, and cryoglobulins the main prognostic marker. An early diagnosis of SjS is especially important in patients in whom systemic manifestations are the presenting feature and this requires a multi-step sequential diagnostic process using a close multidisciplinary workup (5).

The treatment of primary SjS is based on the symptomatic management of sicca manifestations and the use of immunosuppressive agents for systemic disease, with very limited scientific evidence on the balance between efficacy and the side effects associated with their use (6). The emergence of biological immunotherapies targeting etiopathogenic pathways has increased the armamentarium available to treat primary SjS. Currently, they are largely centered on targeting B cells (7), but their use is still limited due to the lack of licensing (8). More recently, studies are moving on to the evaluation of therapeutic interventions on specific cytokines involved in SjS pathogenesis (9). This review summarizes the potential pharmacotherapeutic options targeting the main cytokine families involved in the etiopathogenesis of primary SjS and discuss their potential for developing new and more effective therapies.

2. Etiopathogenesis: a brief overview

The pathogenesis of SjS remains unclear, as it is a complex, multifactorial disease in which many cell types, including DC, T and B lymphocytes, may ultimately participate. Recent genome-wide association studies (GWAS) and broad multi-omic analyses have led to the identification of novel SjS disease modifiers (10,11). Although different components are involved, there is not yet agreement on the contribution of each molecule or cell type behind the disease mechanisms and the network connection between them (12). The most-widely currently accepted etiopathogenic model of SjS is based on the development of autoimmune epitheliitis, characterized by lymphocytic infiltration of the exocrine epithelium, as the key pathogenic underlying scenario (1). Early stages of this process involve enhanced activation of the type I interferon (IFN-I) system, probably induced by external damaging factors (13) such as infections (mainly viruses). These pathogenic factors are believed to trigger autoimmunity through interaction with pattern recognition receptors (PRRs) such as toll-like receptors (TLR), thus allowing the recruitment and activation of DC and lymphocytic cells. The response of autoreactive T and B cells to unmodified or altered self-antigens abnormally expressed by the epithelium of the exocrine glands (e.g., Ro and La ribonucleoproteins) promotes the release of pro-inflammatory cytokines (e.g., IFN-I, interleukin (IL)-17 and B-cell activating factor -BAFF-) and chemokines, as well as the increased expression of adhesion molecules, apoptosis-related factors, co-stimulatory molecules, autoantigens and functional innate immune receptors. This cascade leads to chronic inflammatory damage of the exocrine glands and the progressive loss of their physiological function (1,14–16) **(Figure 1).** Within this inflammatory context, antigenic presentation, mediated both by professional antigen-presenting cells (APC; namely DC) and/or activated epithelial cells, is of particular relevance, since it may initiate and/or maintain the autoimmune response.

Besides autoimmune epitheliitis, other etiopathogenic processes have been proposed, such as those related to neuroendocrine mechanisms, which would explain why some SjS patients present with severe sicca symptoms and no (or limited) inflammatory histopathological features (17–20). In this regard, hypoactivity of the hypothalamic-pituitary-adrenal (HPA) axis has been associated with either pituitary defects or with

adrenal gland dysfunction (17). Inadequate secretory function has been linked to neural innervation destruction in the residual gland, the absence of acetylcholine receptors on the glandular cells, and the release of inflammatory cytokines by lymphocytes and glandular cells (19). Other etiopathogenic factors recently associated with sialoadenitis include autophagy, the role of aromatase (also named estrogen synthase) and, especially, epigenetic mechanisms (1,21).

3. The main etiopathogenic cellular players

3.1. Epithelial cells

The epithelial cells of the exocrine glands play a central role in the etiopathogenesis of SjS and various studies have investigated how salivary gland epithelial cells (SGEC) work. SGEC constitutively express a plethora of molecules associated with the recruitment, homing, activation, differentiation and proliferation of lymphocytes (14,22). Accordingly, SGEC can activate $CD4^+$ T cells *in vitro* and mediate their differentiation into follicular-helper T-cells (T_{FH}) (23) which, in turn, enhance B-cell survival (14,22). In addition, SGEC may interact directly with B-cells, promoting their differentiation into antibody (Ab)-secreting cells (plasma cells; PC) (1,24).
Epigenetic changes and/or viral infections may initiate epithelial activation in patients with SjS (14,25). A key role for the IFN-I-related pathway has been proposed, based on enhanced expression of IFN-I inducible genes (the so-called IFN-I signature) in salivary gland tissue and blood from patients with primary SjS (26) (**Figure 1**). The mechanisms involved are still not well understood, but triggers such as viruses or immune complexes (IC) (27) may play a role at the beginning of the process. Li et al., (28) have recently identified 2'-5'-oligoadenylate synthetase 1 (OAS1) as a risk locus for SjS, supporting a genetic pathophysiological background for potential defective viral clearance. Activated SGEC overexpress MHC class I and II, TLR and costimulatory molecules, and may present self-antigens and produce pro-inflammatory cytokines, especially in individuals with a specific gene susceptibility (e.g., carriers of specific risk alleles from *IRF5* and *STAT4*) (29). SGEC also secrete IL-7 following TLR engagement, with levels correlating with local inflammation and T-cell infiltration (30). In contrast, thymic stromal lymphopoietin (TSLP), also produced by SGEC, is reduced in primary SjS

patients' salivary glands and inversely correlated with T-cell infiltration and inflammation. Apparently, TSLP may promote a protective T_H2 milieu at mucosal sites and thus mediate tissue homeostasis (31). In addition, under the influence of a high IFN glandular milieu and through TLR ligation, BAFF is produced by epithelial cells and, together with self-antigen presentation on salivary gland epithelial cells, stimulates the adaptive immune system.

3.2. Dendritic cells

DC are the main professional APC and have been recognized as key regulators of the immune responses, integrating both stimulatory and inhibitory effectors (32). The mucosal surfaces of the oral cavity contain a unique distribution of DC subsets. In addition to tissue-specific properties, mucosal tissue-resident DC are essential for transferring immune responses against microbial exposure to regional lymph nodes and colonization of the oral cavity (33).

TLR7 and TLR8 engagement in plasmacytoid DC (pDC) is involved in the earliest phases of SjS pathology, as it results in enhanced IFN-I production, making pDC the major source of such cytokines (34). In patients with primary SjS, activated pDC are detected in minor salivary gland biopsies (35), but are reduced in peripheral blood, possibly due to glandular tissue accumulation (36). In addition, pDC are triggered by abnormal continuous synthesis of IFN-I by self-antigens from apoptotic cells, and by NK and B cells following stimulation by IC (37). Therefore, B-cell-derived auto-Ab stimulate pDC to produce IFN-I, thus closing a feed-forward loop that includes both innate and adaptive immune cell effectors (38). DC are also essential for the development of ectopic/tertiary lymphoid tissue, which consists of periductal clusters of T and B lymphocytes, the differentiation of high endothelial venules and networks of stromal follicular dendritic cells (FDC) (39). The formation and maintenance of these tertiary lymphoid structures is regulated by the ectopic expression of lymphotoxins (LT) and lymphotropic chemokines (CXCL12/13 and CCL19/21) (40,41).

3.3. B lymphocytes

B-cells are central in the pathogenesis of primary SjS. B-cell hyperactivity is the consequence of the coordinated and integrated action of B-cell receptors (BCR), CD40 and TLR signals in the presence of appropriate cytokines (42). Like DC and T cells, B lymphocytes are detected in certain regions within the ectopic lymphoid structures, probably due to increased ectopic chemokine expression (e.g., CXCL13) by SGEC (43). The etiopathogenic role of cytokines involved in the homeostasis of B cells, especially BAFF and the A proliferation-inducing ligand (APRIL), is now considered essential for B-cell proliferation and survival from immature B cells to the development of PC and the permanence of autoreactive B cells. Accordingly, overexpression of IFN-I and BAFF on the one hand, and IL-6 and IL-21 on the other hand, is critically involved in the marked presence of PC in primary SjS patients (42). In addition, BAFF induce $CD4^+$ T-cell activation and sustain follicular dendritic cell-networks (44), thus impairing B-cell tolerance. Moreover, excess BAFF has been related to an abnormal composition of circulating mature B (Bm)-cell subsets, and the abnormal accumulation of transitional type 2 (T2), marginal zone (MZ) and memory B cells within the exocrine glands. Due to the membrane expression of IgD and CD38, Bm may be subdivided into sequential stages (from Bm1 to Bm5). Once activated in secondary lymphoid organs, naïve Bm1 (IgD^+CD38^-) become Bm2 (IgD^+CD38^+) and progress to germinal centre (GC) founder Bm2′ cells (IgD^+CD38^{++}). There, they evolve into Bm3 centroblasts and Bm4 centrocytes (IgD^-CD38^+), which differentiate into either PC or early (eBm5) and late memory Bm5 (IgD^-CD38^+ and IgD^-CD38^-, respectively). In contrast to other rheumatic diseases, patients with primary SjS present an increased percentage of Bm2 and Bm2' cells, and reduced eBm5 and Bm5 cells, and a ratio of Bm2+Bm2' to eBm5+Bm5 of >5 is considered a key feature in primary SjS (45). These abnormalities may reflect the migration of active memory B cells into the exocrine glands (45). Excess BAFF is also associated with B-cell functional abnormalities, such as the internal synthesis of BAFF, and a default mechanism that promotes auto-Ab production in ectopic GC (46).

3.4. T lymphocytes

Active $CD4^+$ cells are the main T-cell subset infiltrating the salivary glands (47), and contribute to disease pathogenesis by both releasing pro-inflammatory cytokines and recruiting B cells. Historically, SjS was thought to be a T_H1-driven disease, due to the predominance of $CD4^+$ T lymphocytes in focal lymphocytic sialadenitis. In addition, the robust link between certain MHC class I and II alleles and SjS pathogenesis strongly suggests T cell involvement. Furthermore, IFN-γ and IL-17 secreting T cells have been associated with tissue damage and found within inflamed salivary glands (30,48). $CD4^+$ T cells seem to be responsive to self-antigens from apoptotic cells, such as Ro and La antigens or cytoskeletal antigens (α-fodrin). Accordingly, physical injury to ocular surfaces may also lead to T-cell mediated responses to self-antigens and perpetuate disease. T-cell responsiveness in the salivary glands is further promoted by the abnormal capacity of salivary epithelial tissue to provide co-stimulation and enhanced antigen presentation. Cytokines are key mediators of the T-cell contribution to SjS etiopathogenesis (15). Beyond their role in inducing B-cell hyperactivity and Ab secretion, T cells are involved in promoting glandular destruction through Fas- and perforin-mediated mechanisms. As previously mentioned, the key histopathological marker of the disease (focal lymphocytic sialadenitis) consists of periductal aggregates, referred to as LF, which occasionally appear as GC-like structures. An important and dynamic role for helper T cells, specifically T_H1, T_H17 and T_{FH}, has been linked to the formation and organization of these LF (49). Over-expressed cytokines (IL-7, IL-17, IL-21, IL-22, IL-23) contribute to the diversification of T cell and B cell responses and may increase the risk of lymphoid neogenesis (13,15,50). T_H1 cells (the key main producers of IFN-γ and IL-2) together with T_H17 cells (secretors of IL-17 and TNF-α) have suggested to be key player (51). Recent data have implicated T_H17 in the stimulation of B cells, mediated by IL-21 produced by T_{FH} cells (52). An elevated ratio of T_{FH} cells and increased IL-21 production have been linked to a more severe disease course (53). Moreover, T_{FH} cell expansion correlates with serum IgG levels, IC and auto-Ab. A recent study has also reported that the main biological therapy currently used in severe cases of systemic SjS (B-cell depletion using rituximab) induces a significant decrease in circulating T_{FH} cells, together with a reduction in IL-17 and IL-21 serum levels (54).

Whereas the role of other cell components involved, such as T_H2, which is increased in the salivary glands, remains unclear, new evidence seems to implicate the T regulatory (T_{reg})-cell subset in SjS. Interestingly, a recently-described suppressive T_{reg} subset ($CD4^+CD25^{low}GITR^+$) is expanded in peripheral blood from patients with primary SjS together with reduced $CD4^+CD25^+$ T cells (55). The authors suggest that $CD4^+CD25^{low}GITR^+$ cells in primary SjS may act as a countermeasure to fight autoimmune-driven inflammation and may ultimately become a putative target for upcoming therapies. The involvement of T_{reg} in SjS pathophysiology has also been recently shown in mice, with RNA-binding self-antigens such as La/SS-B involved in the positive selection of T_{reg} during their thymic maturation (56).

The IL-7 cytokine has been shown to stimulate effector T cells and induce murine sialoadenitis (57). Interestingly, IL-7 is over-expressed in the salivary glands of primary SjS patients as a result of DC, endothelial or epithelial cell production (13,30), which may be triggered by innate signaling (e.g., TLR-mediated activation).

4. Cytokine-based therapeutic approaches

Although novel players in the pathophysiology of SjS have recently been identified, the key pathogenic mechanism of the disease remains unclear, thus blurring the identification of putative therapeutic targets for SjS. Cytokines are a key component that seems to contribute to SjS etiopathogenesis by communicating the different cell types (epithelial, DC and B/T cells) (**Figure 1**). Cytokines constitute a complex signaling network that modulates cell behavior and homeostasis through their interaction with high-affinity cell surface receptors (58). Their main general characteristics are potency, pleiotropism, and redundancy, and they may act in autocrine, paracrine and juxtacrine ways. There are up to 38 interleukins (ILs) identified so far, grouped into different families susceptible to therapeutic targeting. The current evidence on the use of cytokine-targeted therapies in primary SjS is summarized in **Table 1** (59–67).

4.1. Targeting the TNF family

The main members of the TNF family of ligands are TNF-α, LT-α/TNF-β, LT-β/TNF-C, CD40L, CD27L, CD30L, TRAIL, FasL, APRIL and BAFF. The promising results of TNF-

targeted therapies in rheumatoid arthritis (RA) led to their testing in patients with primary SjS. A prospective open-label study in 16 patients found significant improvements in subjective and objective measures after the administration of infliximab, although the authors retracted the manuscript 12 years ago (68). In 2004, Mariette et al., conducted a randomized controlled trial (RCT) in 103 patients and found no significant clinical efficacy of infliximab with respect to symptoms, salivary flow rates, ocular tests, quality of life parameters or salivary biopsy results, though improvements in fatigue and some analytical parameters were observed compared with placebo (59). Two studies using etanercept (one RCT, one prospective) found no significant improvements in the main sicca signs and symptoms (60,61), but improvement in some analytical parameters. The interest in TNF-α blockade as a therapeutic SjS target has gradually decreased in proportion to the growing interest in other TNF members closely involved in B cell survival (69).

The B-cell activating factor axis comprises two ligands (BAFF and APRIL) and three receptors (BCMA, TACI, BR3) (70). BAFF (also named BLyS or TNFSF13B) is a critical cytokine involved in the survival of circulating B cells (71). Its binding to the corresponding receptors inhibits intracellular apoptotic pathways and provides survival signals to B cells (15). Quartuccio et al., (72) found higher soluble BAFF levels in patients with primary SjS, which was closely associated with the main immunological markers, systemic disease activity and lymphoproliferation (monoclonal lymphocytic infiltration, myoepithelial sialoadenitis and lymphoma). Other studies have described aberrant BAFF expression in B cells infiltrating the salivary glands (20) and have reported BAFF as a key component of the formation of ectopic GC in SjS (14). Increased levels of BAFF also improve the survival of self-reactive B cells and facilitate abnormal tissue infiltration into follicle/marginal zone niches (20).

Encouraging results have emerged from the BELISS trial (63), an open-label study carried out in 30 primary SjS patients with systemic or early-onset disease treated with belimumab, a human IgG1λ monoclonal antibody (mAb) binding to soluble BAFF (10 mg/kg at weeks 0, 2 and 4, and then every 4 weeks until week 24). The primary end-point (improvement of ≥ 2 of the following items: dryness, fatigue, musculoskeletal pain, physician systemic activity and reduction in biomarkers) was evaluated at week

28 and was achieved in 18 (60%) patients, with a high rate of response in patients with early disease onset (73%) or parotid enlargement (77%). No improvement was observed in two patients with low-grade parotid lymphoma. The ESSDAI score decreased from 8.8 to 5.6 and the ESSPRI score from 6.4 to 5.6. With respect to adverse events, one patient developed pneumococcal meningitis. In an extension of the study, 19 patients were followed for 52 weeks after therapy (50). Thirteen (87%) of the 15 responders at week 28 maintained the response, the improvement in the ESSDAI score (especially in the glandular, lymphadenopathy and articular domains), and the decrease in B-cell biomarkers, while diagnostic tests (salivary flow, Schirmer's test, focus score) did not change (64). Interestingly, treatment with belimumab restored B-cell frequency and subset composition, and normalized BAFF receptor expression after 24 weeks of therapy, which was maintained until the end of the therapeutic protocol (65). A recent study has reported an increase in the ESSDAI score and higher serum levels of rheumatoid factor (RF), IgM and BAFF (66). in the follow-up of 13 SjS patients after the end of belimumab treatment. Other anti-BAFF mAbs are under investigation, mainly in RA and systemic lupus erythematosus (SLE), including the recombinant glycoproteins, atacicept (TACI-Ig) and briobacept (BR3-Fc), blisibimod (which binds to both cell membrane-expressed and soluble BAFF) and tabalumab (a human IgG_4 monoclonal antibody that binds to and neutralizes membrane and soluble BAFF) (73).

The LT pathway has been implicated in the development and maintenance of lymphoid structures and some studies in murine models have reported improvement in salivary and lachrymal gland function by targeting LT (74,75). St Clair et al. (67) have reported the preliminary results of a 24-week RCT using baminercept (a LT-β receptor fusion protein). Of 52 eligible subjects, 33 and 19 subjects were randomized to receive subcutaneous injections of baminercept (100 mg) or placebo, respectively. Baminercept was no more effective than placebo in increasing salivary flow or reducing ocular dryness, although patients treated with baminercept showed a statistically-significant reduction in the ESSDAI score after 24 weeks of therapy, suggesting a potential therapeutic effect on systemic disease.

4.2. Targeting the IFN family

Interferons (IFNs) were recognized 50 years ago as anti-viral proteins. Nevertheless, current knowledge suggests they also regulate multiple non-viral biological processes (cell proliferation and survival, inflammation and immunity). There are three IFN families: the type I IFN (IFN-I) family includes 13 IFN-α subtypes, IFN-β and IFN-ω, -κ, and –ε; the type II IFN (IFN-II) family has a sole member (IFN-γ) (76) and the type III (IFN-III) encompasses three members in humans (IFN-λ1, -λ2 and -λ3; so-called IL-29, IL28A and IL-28B, respectively) (56,57,58).

In view of the central role of IFN-I in at least the initiation of the pathogenesis of SjS, their blockade may be a rational therapeutic approach (80). Genetic studies have confirmed the key role of the IFN-I signature in the etiopathogenesis of primary SjS (81), thus supporting the therapeutic potential. Moreover, there is a close link between IFN-α and BAFF, since BAFF expression is directly induced by IFN-I via IRF1 and IRF2, whereas IRF4 and IRF8 are negative regulators of BAFF expression, suggesting that IFN-I blockade could lead to downregulation of BAFF and, in consequence, a reduction in autoreactive B cell clones and auto-Ab (82). Recent studies have also focused on the role of IFN-III in SjS pathogenesis. Ha et al., (83) found enhanced IFN-λ expression in the salivary glands of primary SjS patients which could be involved in glandular inflammation through direct and indirect regulation of the expression of BAFF and CXCL10 in salivary gland epithelium, while Coursey et al., (84) studied goblet cells (specialized secretory cells that produce mucins and other ocular proteins) in the conjunctiva of patients with SjS. These cells are highly sensitive to IFN-γ, suggesting that therapies targeting IFN-γ could increase the synthesis and ecretion of protective goblet cell mucins on the ocular surface.

Biological therapies targeting INFs include mAbs binding to IFN-α (sifalimumab, rontalizumab), IFN-α receptor (anifrolumab), IFN-α kinoid (inactivated IFN-α molecules coupled to the keyhole limpet haemocyanin protein) and IFN-γ (AMG 811). Several trials are currently underway in RA and SLE (85).

4.3. Targeting the IL-1 family

The main members of the IL-1 family are IL-1, IL-18, IL-33, IL-36, IL-37 and IL-38. IL-1 has been implicated in the pathogenesis of primary SjS, since it seems to mediate immune responses in the targeted tissues (86). In 2010, Chen et al., reported that IL-1R blockade did not attenuate lymphocytic infiltration of the lachrymal gland but significantly reduced ocular surface keratinization (87). Norheim et al., carried out the first RCT using anakinra (a non-glycosylated recombinant version of the human IL-1 receptor antagonist, IL-1Ra) in 26 patients with primary SjS who were randomized to receive either anakinra or placebo for four weeks and found no significant reduction in the primary outcome evaluated (fatigue) (62), although a significantly-higher number of patients treated with anakinra reached a post-hoc-defined endpoint (50% reduction in fatigue). Anakinra also showed therapeutic benefits as a topical treatment for aqueous-deficient dry eye in a mouse model (88).

IL-18 is produced locally by acinar cells, intraducts and $CD68^+$ macrophages (89,90). Several studies have reported raised serum levels of IL-18 in patients with SjS, which was associated with IgG_1 and IL-17 production (89,91). Chen et al., (92) have linked higher levels of IL-18 (both circulating and free forms) with disease activity, while another recent study (93) found increased IL-18, IL-18BP and IL-37 serum levels in patients with primary SjS, especially in those carrying anti-Ro/SSA and/or anti-La/SSB auto-Ab.

IL-33 was identified in 2005 as the ligand of T1/ST2 (94), an IL-1R expressed on different structural cells (epithelial, endothelial and smooth muscle cells), and on T_H2, mast cells and type 2 innate lymphoid cells (ILC2) (95). IL-33 coordinates tissue homeostasis, injury, and repair mechanisms (96), bridging innate and adaptive immune responses. Recent studies have described IL-33 as an alarmin cytokine at barrier sites, with emerging roles in obesity, viral and tumor immunity (97). In addition, Awada et al., (98) reported that serum IL-33 and sST2 levels were increased in primary SjS patients, although recent studies have not confirmed these results. IL-33 and its soluble receptor ST2 have recently been analyzed in 15 primary SjS patients, in whom circulating IL-33 levels were detectable only in two (13%). Nevertheless, significant serum hyperexpression of sST2 was found in patients compared with controls,

together with a significant correlation between sST2 levels and the SSDAI score (99). Riviere et al., (100) have suggested there may be technical pitfalls in detecting IL-33 with different ELISA kits.

IL-36α, another IL-1 family member, has also been reported to be significantly over-expressed in the serum and salivary glands of primary SjS patients, with $\alpha\beta^+$ $CD3^+$ and $CD68^+$ T cells being the major source of IL-36α in minor salivary glands. A higher expression of IL-36α and IL-36R was also demonstrated in γδ T cells isolated from primary SjS compared with controls (101).

The most-recently identified member of the IL-1 family, IL-38, shares structural features with IL-1Ra and IL-36Ra. IL-38 binds to IL-36R and acts as a partial receptor antagonist of IL-36 (IL-38 and IL-36Ra function as antagonists at high concentrations, but inhibit the binding of co-receptors at low concentrations). IL-38 inhibits the production of T-cell cytokines, IL-17 and IL-2, as well as IL-8 release induced by IL-36γ, and is thus being considered as a potential target for the inhibition of inflammatory and immune responses (102).

4.4. Targeting the IL-2 family

The IL-2 family comprises IL-2, IL-4, IL-9, IL-15 and IL-21. As widely reported, IL-2 is an essential regulator of immune response homeostasis as it guarantees T_{reg} cell growth and function, but also enhances T and B-cell effector proliferation and survival. Moreover, like IL-4, IL-2 enhances BAFF-stimulated cell viability/survival by activating Erk1/2 and S6K1 signaling in neoplastic lymphoid B-cells (103). These contrasting roles as either immune-suppressor or stimulator may both be exploited therapeutically, as low IL-2 doses are beneficial in the context of autoimmunity, whereas high IL-2 doses potentiate anti-tumor immune responses (104).

Administration of low-dose IL-2 therapy may compensate for potential IL-2 deficiency and thus restores the physiological role of T_{reg} and its ability to efficiently counteract autoimmunity. This therapeutic approach has been tested in patients with type 1 diabetes mellitus (105) but also in patients with systemic autoimmune diseases, including SLE (106) and HCV-related cryoglobulinemia (107). On the other hand, newly-developed IL-2-based therapies include immune complexes of IL-2, non-blocking anti-

IL-2 mAb (which allows the use of lower IL-2 doses with a longer half-life) and molecular IL-2 variants (so-called muteins) that selectively enhance the stimulatory (but not the inhibitory) IL-2 function (108).

Recent studies have focused on another member of the IL-2 family, IL-15. Sisto et al., (109,110) reported a higher expression of IL-15 in SGEC from patients with primary SjS, with strong expression in both the acinar and ductal epithelial cells, and with TLR2 activation being involved in promoting IL-15 SGEC expression through activation of the NF-kB intracellular pathway.

The other IL-2 member that has been implicated in the pathogenesis of primary SjS is IL-21, due to its pleiotropic effects on the INF-I signaling pathway, the generation of T_{FH} and T_H17 cells, and the differentiation of PC (111). A recent study in 30 patients with primary SjS reported significantly-higher levels of IL-21 and IL-21 gene expression in the tears of SjS patients compared with controls. Moreover, IL-21 levels correlated significantly with ocular surface stain scores and Schirmer test results (112). Papp et al.,(113) reported increased IL21-R expression on $CD19^+CD5^+$ B cells and a higher expression of IL-21 on invariant natural killer T cells (iNKT) from patients with primary SjS, suggesting a potential role of IL-21 in regulating B cell functions.

4.5. Targeting the IL-6/IL-12 family

The members of the IL-6/IL-12 family are IL-6, IL-11, IL-12, IL-23, IL-27, and IL-35. IL-6 blockade is now considered a promising therapeutic option, since IL-6 induces the polarization of T_{FH} cells and participates in IL-21 induction (1). Zhou et al., reported that a neutralizing anti-IL-6 mAb inhibits the apoptosis of exocrine gland tissues and exerts a tissue-protective effect in a murine model of SjS (114). In addition, tocilizumab (a humanized mAb targeting both the soluble and membrane-bound forms of IL-6 receptor α , IL-Rα) has shown successful results in two SjS patients: one with refractory organizing pneumonia (115), and another with neuromyelitis optica spectrum disorder refractory to corticosteroids, plasma exchange and cyclophosphamide (116) (**Table 2**) (115–121). Currently, a phase III RCT is actively recruiting patients (NCT01782235).

IL-27 is another member of this family with both pro- and ant-inflammatory properties: it favors T_H1 responses and inhibits T_H17 responses. The latter ability could

then be exploited to reduce the severity of T_H17-mediated autoimmune disorders. Accordingly, exogenous administration of IL-27 has proven efficacious in a mouse model of SjS (122), thus supporting its potential therapeutic value.

4.6. Targeting the IL-10 family

The members of the IL-10 family are IL-10, IL-19, IL-20, IL-22, IL-24 and IL-26. At the beginning of this Century, several studies suggested a key role of IL-10 in the etiopathogenesis of primary SjS, including a higher frequency of circulating B cells secreting IL-10 (123) and the association of some genetic polymorphisms with both susceptibility and clinical expression (124–129). Several trials have been carried in both systemic and organ-specific autoimmune diseases, although some authors suggest that the therapeutic target of the IL-10 response should be considered with caution due to its role of key switcher of immunity responses (130,131).

IL-22 is a pleiotropic cytokine involved in both adaptive and innate immune responses, and has a dual role as a protective or a pro-inflammatory cytokine. At mucosal sites, IL-22 is mainly produced by $CD4^+$ T cells and a subset of NK cells expressing the activating NKp44 receptor (132). A prominent role of pro-inflammatory events has been proposed in SjS, which would contribute to the early etiopathogenic stages and facilitate self-perpetuation of autoimmune damage (133). Ciccia et al., reported that IL-22 (together with IL-23 and IL-17) were significantly increased, at both the protein and mRNA levels, in salivary glands of patients with primary SjS (132). Higher IL-22 serum levels have been associated with hyposalivation, anti-Ro/La auto-Ab, hypergammaglobulinemia and RF in patients with SjS (134). Levels of IL-17 and IL-22 were significantly increased in tears of patients with SjS and positively correlated with questionnaire and keratopathy scores but negatively correlated with tear film break-up time and Schirmer test (135).

Functional *in vitro* studies in human salivary gland cells treated exogenously with IL-22, reported a direct effect on cell cycling by activating STAT3 and, therefore, specifically reducing cell proliferation in the G_2-M phase (136). Moreover, as exhibited in a virus-induced autoimmune murine model (137), IL-22 also regulated lymphoid chemokine production and the assembly of tertiary lymphoid structures, thus providing an

etiopathogenic link between mucosal infection, B-cell recruitment, and humoral autoimmunity. The same authors reported that IL-22 receptor engagement promotes the differential expression of CXCL12/SDF-1 (stromal derived factor 1) and CXCL13/BCA-1 (B cell-attracting chemokine 1) in epithelial and fibroblast stromal cells which, in turn, are pivotal for B-cell recruitment and the organization of tertiary lymphoid structures (137). The role of IL-22 in SjS is supported by observations on cells of hematopoietic origin from patients with primary SjS, in which IL-18-dependent aberrant expression of IL-22R1 has been reported (138). Therefore, blockade of the aberrant IL-18/IL-22 pathway may be a useful therapeutic strategy in primary SjS.

4.7. Targeting the IL-17 family

T_H17 cells have been implicated in many autoimmune diseases, including SjS, although the ultimate role played by T_H17 in SjS pathogenesis remains ill-defined. Previous studies stated that IL-17 impaired the blood-brain and blood-testis barriers by down-regulating occluding and disrupted the intestinal barrier through up-regulation of claudin-1 and -2 (139–141) . A recent study in non-obese diabetic (NOD) mice (142) found that IL-17 derived from infiltrating lymphocytes impairs the integrity of tight junctions. Moreover, mice showing greater changes in tight junctions seemed to have lower saliva production, which may indicate that IL-17 ultimately contributes to salivary gland dysfunction. Significantly-elevated IL-17 has been found in tears (143) and serum (144) and Alunno et al., (145) detected increased serum IL-17 levels in 15/50 (30%) primary SjS patients. In addition, IL-17 protein expression progressively increases with higher salivary biopsy focus scores, and double negative ($CD4^-CD8^-$) T cells are major producers of IL-17 in salivary glands and are expanded in peripheral blood (146). These double negative T cells co-express surface CD20 and, though *in vitro* resistant to the effects of corticosteroids, are sensitive to rituximab (145,146).
In an attempt to unravel the T_H17 contribution in SjS, Lin et al., (147) induced an experimental mouse model of SjS by immunization with salivary gland proteins. Whereas IL-17A-deficient mice were resistant to this immunization and showed no evidence of disease symptoms, wild-type mice had reduced saliva secretion, elevated serum auto-Ab production, and tissue destruction with lymphocytic infiltration in the

submandibular gland. In addition, adoptively transferred T_H17 cells reverted the phenotype observed in immunized IL-17A-deficient mice, resulting in enhanced glandular inflammation and auto-Ab production (147).

With respect to therapeutic approaches, several anti-IL17A mAbs (secukinumab, ixekizumab, brodalumab, ustekinumab) are currently being evaluated in the treatment of RA, inflammatory bowel disease and psoriasis (148).

5. Future prospects

The therapeutic management of SjS is centered on the control of the main symptoms, sicca features, using substitutive and oral muscarinic agents. However, systemic involvement clearly marks the disease prognosis, and strongly suggests the need for closer follow-up and more robust therapeutic management. As a rule, the management of systemic SjS should be organ-specific, with glucocorticoids and immunosuppressive/biological agents limited to potentially-severe involvements and, due to their off-label use, always with a reasonable assessment of the risk of serious adverse events *versus* the benefits of treatment (149).

The most recent etiopathogenic advances in SjS are shedding some light in the search for new highly-selective biological therapies without the adverse effects of the standard drugs currently used (corticosteroids and immunosuppresants). The drugs tested in ongoing trials in SjS included in the clinicaltrials.gov database (accessed July 24, 2017) are heterogeneous (**Figure 2**): of the 35 therapeutic trials not labeled as "completed", 13 tested topical/local interventions and the remaining 22 systemic drugs that targeted cytokines (n=6), intracellular pathways (n=5), T-cells (n=4), pDC (n=1), and miscellaneous (n=6). Surprisingly, no current trial is testing direct B-cell depletion, which is the main biological approach used now in daily practice, and only one ongoing trial is testing a combination of rituximab and belimumab. Five trials testing biologics (efalizumab, antiBAFF-R, lulizumab, baminercept and abatacept) are labeled as terminated or withdrawn.

5.1. Current data and ongoing cytokine-based trials

Current evidence on the use of cytokine-targeted therapies in primary SjS remains limited and includes seven isolated case reports (patients with severe, refractory systemic involvement) (**Table 2)**, two prospective studies (with three additional extension studies) and four RCTs (**Table 1**). After discarding the three studies with negative results using TNF-targeted therapies, there remains only one RCT testing anakinra, one RCT testing baminercept and one prospective study using belimumab (with three additional extension studies): all studies analyzed a small number of patients, ranging from 26 to 52. No solid conclusions can be drawn from these data, and therefore the promising results obtained in the belimumab studies should be confirmed by future RCTs. From a safety point of view, the use of anakinra was mainly related to local injection site reactions, belimumab with infections, and baminercept with liver toxicity (**Table 3**).

Regarding ongoing trials, the main interest is centered on the BAFF pathway, with three studies investigating the effect of mAbs targeting BAFF-R, and one testing the association between B-cell depletion and BAFF inhibition. Recently, de Vita et al.,(64) have reported the successful use of sequential therapy with belimumab followed by rituximab in a patient with severe, refractory parotid low-grade B-cell MALT lymphoma and cryoglobulinemic vasculitis, while Cornec et al., (150) have reported that nearly half of the 45 SjS patients who received a single course of rituximab displayed intense BAFF-driven B-cell activation that correlated with a lack of response to B-cell depletion, suggesting a potential role for BAFF-targeted therapies in rituximab-refractory patients. Out of the BAFF pathway, two ongoing trials are testing the use of other cytokine-based therapies including tocilizumab and human recombinant Il-2.

The other point of interest in the ongoing trials is the targeting of intracellular pathways. Three ongoing trials are exploring the PI3K/Akt/mTOR pathway, which has been considered a feasible therapy in solid cancers (151) and is being investigated in some hematological neoplasias (152). However, the first clinical trials with PI3K inhibitors in monotherapy have been disappointing in solid cancers, and current trials in this area are investigating co-treatments with intra-/extracellular signaling molecules, nuclear hormone receptors, DNA damage repair enzymes, and immune

modulators. Indeed, combination strategies targeting different etiopathogenic pathways are currently the gold standard approach in trials in hematological and solid cancers, and this approach may well arrive soon in the field of systemic autoimmune diseases. It may be anticipated, however, that these strategies would increase the risk of adverse events, as recently reported in patients with melanoma (153).

5.2. Personalized molecular-based therapeutic approach

The clinical and immunological heterogeneity of systemic autoimmune diseases such as SLE, vasculitis and SjS may be one of the most important explanations of the negative results often obtained in RCTs not including large number of patients. In SLE, the positive results reported for the use of belimumab were obtained from two trials including nearly 1,000 patients each. In primary SjS, published RCTs have included between 100 and 150 patients per study, a relatively small number of patients that could contribute to the non-significant results for the primary outcomes in these trials. Therapeutic research in SjS should probably be reconsidered in order to search for a more personalized therapeutic approach based on genetic, clinical, immunological and/or histopathological characteristics. Patients with sicca-limited disease are totally different from those with systemic disease, as are immunonegative patients from those carrying Ro autoantibodies or cryoglobulins. Immunopositive patients seem to have different genetic and epigenetic etiopathogenic profiles, as suggested by a recent study that reported that methylation alterations in B cells were more frequent in patients carrying Ro/La autoantibodies (154). In addition, recent etiopathogenic studies are beginning to divide SjS patients according to the genetic profile between those with or without a predominant IFN-I gene expression signature (155,156). Sensitivity analyses searching for a differentiated response to therapies in these subsets of patients (sicca-limited vs. systemic; Ro+ vs. Ro-; positive vs. negative salivary gland biopsy; positive vs. negative IFN-I signature) could be useful in better delineating the therapeutic effect of a drug tested in primary SjS, although this would require a greater number of randomized patients than those included in past trials. Finally, the potential role of circulating anti-cytokine Ab (157) in patients with primary SjS (a

disease especially characterized by a wide spectrum of circulating auto-Ab) would need further evaluation in trials testing anticytokine therapies.

6. Conclusions

The advent of cytokine-targeted therapies has been a pioneer in the management of inflammatory rheumatic, digestive and dermatological diseases, offering robust confirmation of the pathogenic role played by cytokines (148). The history of the use of biological agents in SjS started with cytokine-targeted therapies at the beginning of the current century. Nevertheless, the results were disappointing and, in this context, the interest in investigating the therapeutic potential of cytokines in SjS vanished. Recent research centered on elucidating the etiopathogenic role of the complex cytokine network in SjS (158,159) has reawakened interest in the usefulness of anti-cytokine therapies. The current scenario, as shown by the *clinicaltrials.gov* webpage, is that the biologic therapeutic approach overwhelmingly used in SjS until now (targeting B-cell depletion) has shifted towards the evaluation of biologics targeting cytokines, T-cells and intracellular signaling pathways. Better knowledge of cytokine activities in SjS, which may be pivotal to sustaining chronic autoimmune damage in glandular and extraglandular tissues, together with the elucidation of the cytokine redundancies that may exist in the blood and targeted tissues, could aid the optimization of the choice of cytokines as molecular targets for future drug developments in this complex systemic autoimmune disease.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

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Figure 1. Etiopathogenesis of primary SjS: intercellular networks and main cytokines involved. External/internal factors inducing activation, stress and/or death of epithelial cells from exocrine glands promote presentation and/or release of self (Ro, La, α-fodrin, DNA) and/or foreign (e.g., viral) antigens and their further recognition by PRRs (e.g., TLRs) from innate cells (epithelial, DC, pDC, MØ) or clonotypic receptors (e.g., TCR, adn BCR) from self-reactive adaptive (T, B) immune cells. Activated epithelium initiates the release of pro-inflammatory cytokines (e.g., IFN-I, IL-7 and BAFF) and chemokines (e.g., CXCL13/BCA-1 and CXCL12/SDF-1) and the expression of adhesion molecules, apoptosis-related factors, and co-stimulatory molecules allowing the recruitment and further activation of DC, pDC, self-reactive T and B lymphocytes and macrophages (MØ). The pro-inflammatory cytokine milieu enhances differentiation of naïve T cells (mainly to T_H1, T_H17 and T_{FH}) and B cells (to PC), thus promoting the formation of ectopic lymphoid tissue and amplifying the activation cascade, as well as the production of cell-death mediators (e.g., perforin and FasL) and autoantibodies (auto-Ab). The ultimate consequence of this altered context is direct damage to the exocrine gland epithelium which, in turn, feeds and perpetuates the inflammatory response. Colored dots represent the main cytokines involved, each color belonging to a different family. CTL, Cytotoxic T lymphocyte; GC, Germinal Center; IC, Immune-complexes; PC, plasma cell; pDC, plasmacytoid dendritic cell; RLR, RIG-like receptor; TSLP, thymic stromal lymphopoietin.

Figure 2. Drugs tested in the ongoing trials in SjS included in the clinicaltrials.gov database (accessed July 24, 2017). ID = immunodepressant agents; IM: immunomodulatory agents; pDCs: plasmacytoid dendritic cell.

TABLE 1. Main studies targeting cytokines in patients with primary SjS*

Author (year)	N (female)	Mean age (yrs.)	Study design (duration)	Drug (number of patients)	Main results (p value)
Mariette et al (2004)	103 (ND)	54	RCT-d 22w	Infliximab infusions (5 mg/kg) (n=54) Placebo (n=49) Weeks 0, 2, 6	Primary endpoint (NS) Decreased IgM mg/dl (0.001)
Sankar et al (2004)	28 (26)	55	RCT-d 12w	Etanercept 25 mg Placebo (n=14) Twice-weekly (n=14)	Primary endpoint (NS)
Zandbelt et al (2004)	15 (14)	48	Prospective 12 w	Etanercept 25 mg twice per week (n=15)	Primary endpoint (NS) Reduction fatigue VAS at week 8 (<0.05) Reduction CRP at week 12 (0.05)
Norheim et al (2012)	26 (19)	55	RCT-d 4w	Anakinra 100 mg/day w0, w4 (n=13) Placebo (n=13)	Primary endpoint (NS) Post-hoc 50% reduction in fatigue VAS (0.03)
Mariette et al (2015)	30 (30)	49.5	Prospective 24 w	Belimumab, 10 mg/kg, Week 0, 2, 4 and then every 4 weeks to W24 (n=30)	Dryness VAS (p=0.0021) ESSDAI (p=0.0015) ESSPRI (p=0.0174)
De Vita et al (2015)	19 (19)	40	Prospective 52 w (extension study, Mariette 2015)	Belimumab, 10 mg/kg, Week 0, 2, 4 and then every 4 weeks to W52 (n=19)	ESSDAI 28w vs 52 w (p=<0.0001) ESSPRI (p=0.01)
Pontarini et al (2015)	10 (10)	49	Phase II open-label 52 w (extension study, Mariette 2015)	Belimumab, 10 mg/kg, Week 0, 2, 4 and then every 4 weeks to W52 (n=10)	Significant reduction in transitional and naive B cell subsets to levels similar to those observed in healthy donors. Normalized BAFF-R expression in all B subsets comprised within the memory compartment. Decreased serum levels of Ig, RF, and ANA; increased of C4 complement factor
Quartuccio et al (2016)	13 (13)	54	Phase II open-label 52 w (extension study, Mariette 2015)	Belimumab, 10 mg/kg, Week 0, 2, 4 and then every 4 weeks to W52 (n=13)	Increased at month 12 after the end of the trial: - ESSDAI (p=0.003) - RF level (p=0.008) - IgM level (p=0.04) - Serum BLyS levels (p=0.04)
St.Clair et al (2015)	52 (ND)	ND	RCT-d 24 w	Baminercept 100 mg weekly (SC) (n=33) Placebo (n=19)	SWSF, UWSF, Schirmer-I-test, ocular staining, fatigue, joint pain, and overall dryness (NS) ESSDAI (p=0.043) Lymphocyte numbers (p < 0.0001) Serum levels of CXCL13, LIGHT, IP10, and BAFF (NS)

RCT: randomized controlled trial; -d: double-blind; pSjS: primary Sjögren syndrome; m: months; n: number; w: weeks; yrs.: years; IV: intravenous; SC: subcutaneous; NS: no significant differences; ND: not detailed; mg: miligrams; Kg: kilogram; rd: third; th: sixth; VAS: visual analogue scale; TBUT: break-up time; UWSF: unstimulated whole salivary flow; SWSF: Stimulated whole salivary flow; CRP: C-reactive protein; ESSPRI: European League Against Rheumatism (EULAR) Sjögren's Syndrome Patient Reported Index; ESSDAI: EULAR Sjögren's Syndrome Disease Activity Index; OSDI: Ocular Surface Disease Index; CXCL13: C-X-C motif chemokine ligand 13; LIGHT: Lymphotoxin; IP 10: Interferon-γ-inducible protein 10; BAFF; B cell activating factor; R: receptor; Ig: Immunoglobulins; RF: rheumatoid factor; ANA: Anti-nuclear antibodies: C: complement; BLyS: anti-B

*lymphocyte Stimulator.***Two small open-label studies reporting therapeutic benefits of the use of infliximab in primary SjS patients published in 2001 and 2002 were retracted by the authors in 2013.*

TABLE 2. Case reports targeting cytokines in patients with primary SjS

Author (year)	N (female)	Study design	Age	Drug	Treatment indication	Adverse events	Outcomes
Caroyer et al (2002)	1 (F)	Case report	68 yrs..	Infliximab 3 mg/kg at weeks 0, 2, 6, and every 12 weeks thereafter	Severe sensory neuronopathy	None	Recovered. At week 12, sensory examination was normal and tendon reflexes were 2/4. Romberg sign and gait were normal. Nerve conduction studies showed reappearance of sensory potentials.
Pessler et al (2006)	1 (F)	Case report	11 yrs.	Infliximab 3 mg/kg every 4 weeks Etanercept 25 mg SC twice weekly	Refractory arthritis to MTX Refractory arthritis to Infliximab	Severe hypokalemic renal tubular acidosis None	Failed. SjS flare. Arthritis improved from 6/10 to 1/10 Patient's xerostomia, uveitis and bicarbonate and potassium supplementation for the RTA have remained unchanged. Her vision remains limited to detection of hand motion.
Haridas et al (2017)	1 (F)	Case report	43 yrs.	Etanercept Topical treatment of etanercept: 10 drops of etanercept were put on the whole ulcerated area (dose: 1 mg/2 x 2 cm area)	Pyoderma gangrenosum	None	At 2 months the wound size was reduced by more than 70%.
Tursi et al (2012)	1 (F)	Case report	48 yrs.	Adalimumab 160 mg at week 0 and 80 mg at week 2, followed by 40 mg every 2 weeks	Active ileal Crohn's disease	None	Clinical remission of both Crohn's disease and SjS.
De Vita et al (2014)	1 (F)	Case report	48 yrs.	Belimumab 10 mg/ kg at day 0, +14, +28 and then every month.	Parotid low-grade B-cell MALT lymphoma and cryoglobulinaemic vasculitis	None	Failed. Skin ulcer worsened No decrease in parotid swelling. Serum cryoglobulins (-), RF and BAFF decreased C4 remained unchanged
Komai et al (2013)	1 (F)	Case report	38 yrs.	Tocilizumab 8mg/kg day 60, 90, and 120, then every month.	Neuromyelitis optica Longitudinal extensive transverse myelitis	None	Gradual amelioration of her neurological symptoms. Motor disability and sensory deficits were gradually improved. No relapse was observed during the clinical course.
Justet et al (2015)	1 (F)	Case report	55 yrs.	Tocilizumab 8 mg/kg, 785 mg/month	Refractory organizing	None	Significantly improved.

					pneumonia		Cs tapered to 5 mg/day. ESSDAI was stable at 4. CTscan and pulmonary function tests were normalised.

mg: milligram; kg: kilograms; n: number; w: weeks; yrs.: years; cm: centimeters; F: female; AEs: adverse events; Cs: Corticosteroids; SjS: Sjögren syndrome; ESSDAI: EULAR Sjögren's Syndrome Disease Activity Index; CTscan: Tomography computerized; MALT: Marginal Zone Lymphoma; BAFF; B cell activating factor; C: complement; RF: rheumatoid factor; SC: subcutaneously; RTA: renal tubular acidosis.

Table 3. Adverse events reported in controlled and uncontrolled studies using cytokine-targeted therapies in patients with primary SjS.

Author (year)	Study design Drug	Related to infusion	Infection	Cancer	Autoimmune-related	Others
Mariette et al (2004)	RCT-d Infliximab	Infusion related (n=2) Isolated cutaneous facial eruption (n=1)	Pneumococcal septicemia (n=1)	Breast cancer (n=1)	Autoimmune hepatitis (n=1)	-
Sankar et al (2004)	RCT-d Etanercept	Injection-site reaction (n=2)	-	-	-	-
Zandbelt et al (2004)	Prospective Etanercept	-	-	-	-	-
Norheim et al (2012)	RCT-d Anakinra	Severe injection site reaction (n=1) (Discontinued) Mild injection site reactions (n=7)	Gastroenteritis (n=1)	-	-	Neutropenia (n=1)
Mariette et al (2015)	Prospective Belimumab	-	Pneumococcal meningitis (n=1) Pneumonia (n=1) Sinusitis (n=1) Rhinitis/pharyngitis (n=7) Bronchitis (n=1) Herpes labialis (n=1) Urinary tract infection (n=2) Gastroenteritis/diarrhea (n=2)	Breast cancer (n=1)	Scleroderma (n=1) Oral aphtosis (n=1)	Headache (n=9) Neutropenia (n=5)
De Vita et al (2015)	Prospective Belimumab	-	Rhinopharingitis (n=2) Gastroenteritis (n=1) Urinary tract infection (n=1) Pneumonia (n=1) Vaginal fungal infection (n=1) Non-complicated cutaneous infection (n=1)	-	-	Headache at the end of the infusion (n=1) Mild transient neutropenia (n=2)
Quartuccio et al (2016)	Phase II open-label Belimumab	-	-	Development of B-cell lymphoma from non-neoplastic parotid sialadenitis (n=2) two years after the end of the trial. Progression of lymphoma after belimumab suspension (n=1)	-	-
St.Clair et al (2015)	Prospective	-	-	-	-	Grade 3 hepatic injury without

	Baminercept					sequelae (n=2) Transaminase abnormalities (>ULN) (n=10 subjects [30%], 15 events)

RCT: randomized controlled trial; -d: double-blind; n: number; ULN: upper limit of normal.

TABLE 4. Ongoing trials in patients with primary SjS targeting cytokines included in the web ClinicalTrials.gov (last accessed 23/APRIL/2017)

Drug	Cytokine targeted	Sponsor (year)	ClinicalTrials.gov Identifier:	Status	Study design	Arms	Inclusion Criteria	Primary outcome
SC Belimumab + IV RTX co-administration	Anti-CD20 (RTX) and BAFF blockade (Belimumab)	GlaxoSmith Kline (April 2017)	NCT02631538	Recruiting	RCT-d Phase 2	**Placebo Comparator:** Placebo Subjects will receive belimumab placebo weekly SC injections to w52 and RTX placebo infusions at W 8 and 10. **Experimental:** Belimumab monotherapy Subjects will receive 200 mg weekly SC injections of belimumab to w 52 and placebo RTX infusions at w 8 and 10. **Experimental:** Belimumab and RTX co-administration therapy Subjects will receive belimumab 200 mg SC weekly for 24 w followed by weekly placebo belimumab injections to w 52 with RTX 1000 mg IV at w 8 and 10. **Active Comparator:** RTX monotherapy Subjects will receive 1000 mg IV RTX infusions at w 8 and 10 and weekly SC injections of placebo belimumab to w 52.	Age >=18 years Documented pSjS by AECG criteria including: either anti-SS-A or anti-SS-B positive. Baseline UWF >0.0 mL/min or evidence of glandular reserve function. Symptomatic oral dryness (>=5/10 on subject completed numeric response scale). ESSDAI >=5 points.	Number of participants with SAEs [Time Frame: 104 w] Number of participants with AESIs [Time Frame: 104 w]
VAY736	human IgG1/κ anti-BAFF-R mAb	Novartis (March 2017)	NCT02149420	Active, not recruiting	RCT-d Phase 2	**Experimental:** VAY736 dose 1 **Experimental:** VAY736 dose 2 **Placebo Comparator:** Placebo	Fulfilled revised European US consensus criteria for pSjS 18 to 75 yrs. ESSDAI value ≥ 6 ANA (≥ 1:160) Seropositive for anti-SSA and/or anti-SSB antibodies SWSF rate of > 0 mL/min.	Change in ESSDAI between baseline and w 12. The effect of VAY736 on clinical disease activity will be measured by the change in ESSDAI between baseline and w 12.
VAY736	human IgG1/κ anti-BAFF-R mAb	Novartis Pharmaceuticals (April 2017)	NCT02962895	Not yet open for participant recruitment	RCT-d Phase 2	**Experimental:** VAY736 dose 1 VAY736 low **Experimental:** VAY736 dose 2 VAY736 medium **Experimental:** VAY736 dose 3	8 to 75 yrs. Fulfilled revised European US consensus criteria for pSjS Seropositive at screening for anti-Ro/SSA antibodies	Dose response [Time Frame: 24 w] Dose response measured by change multi-dimensional disease activity as assessed by the physician.

						VAY736 high **Placebo Comparator:** Placebo Placebo control	Documented salivary/lacrimal gland biopsy result confirming pSjS diagnosis prior to the baseline visit	
Tocilizumab	IL-6 receptor blockade	University Hospital, Strasbourg, France (March 2014)	NCT01782235	Unknown	RCT-d Phase 2 Phase 3	**Experimental:** Tocilizumab arm Tocilizumab arm will receive tocilizumab. **Placebo Comparator:** Placebo arm Placebo arm will receive placebo.	18 to 80 yrs. pSjS according to the AECG criteria. Presence of anti-SSA or of anti-SSA and anti-SSB antibodies ESSDAI score ≥ 5.	Improvement in ESSDAI score ≥ 3 points compared with enrollment. [Time Frame: 24 w] Improvement in ESSDAI score ≥ 3 points compared with enrollment, with no new domain with high activity of the ESSDAI compared with enrollment, and no clinical worsening according to the clinician (no worsening compared with enrollment > 1 point of the Systemic Activity 0-10 VAS according to the physician.
Recombinant Human IL-2	Human recombinant IL-2	Peking University People's Hospital (June 2015)	NCT02464319	Recruiting	RCT-d Phase 2	**Active Comparator:** **Experimental:** hrIL-2 active **Intervention:** Add hrIL-2 according to the protocol to original treatment. HrIL-2 active: 1 million U doses of hrIL-2 SC injection. **Placebo Comparator:** hrIL-2 placebo 1 million U doses of placebo SC injection	Diagnosis of pSjS 18 to 75 yrs. ESSDAI score ≥ 6 Liver values above 1.5 ULN Stable low dose systemic use of Cs（ <=7.5mg) in the last 4 w before begin with study medication.	Examination of the therapeutic effects (improvement in ESSDAI) of low dose IL-2 in patients with pSjS [Time Frame: 24 w]
CDZ173	selective PI3K δ inhibitor	Novartis (February 2017)	NCT02775916	Recruiting	RCT-d Phase 2	**Experimental:** CDZ173 Capsule **Placebo Comparator:** Placebo Capsule matching Placebo	Diagnosis of pSjS 18 to 75 yrs. ESSDAI score ≥ 6	ESSPRI change from baseline to 12 w
UCB5857	PI3K inhibitor	UCB Celltech (April 2017)	NCT02610543	Recruiting	RCT-d Phase 2	**Experimental:** UCB5857 UCB5857 once daily for 12 w **Placebo Comparator:** Placebo Placebo once daily for 12 w	18 to 75 yrs.. Women of childbearing potential must agree to use a highly effective method of birth control during the study Subject must meet the 2002 AECG criteria for pSjS. Subject must have a serum test positive for anti-SSA/Ro (Ro-52 and Ro-60) and/or anti SSB/La autoantibodies.	Change from baseline to w 12 in the ESSDAI
LY3090106	PI3K δ inhibitor	Eli Lilly and Company	NCT02614716	Recruiting	RCT-d Phase 1	**Experimental:** LY3090106 LY3090106 given SC in escalating	18 yrs. to 65 yrs. AECG criteria with active disease (at	Number of participants with one or more AE (s) considered by the investigator to be

		(December 2016)				dose cohorts once every 2 or 4 w for 16 w. **Placebo Comparator:** Placebo Placebo given SC once every 2 or 4 w for 16 w.	any level), as per judgment of the investigator Seropositive for auto-antibodies associated with SjS (anti-SSA or anti-SSB)	related to study drug administration [Time Frame: Baseline through Study Completion (Day 197)]
Iguratimod (T-614)	Inhibition of NF-κB activation and transcription of pro-inflammatory cytokines	Peking Union Medical College Hospital (January 2017)	NCT03023592	Recruiting	Single-center, self-control, open-label study Phase 1 and 2	**Experimental**: Iguratimod patients treated with Iguratimod 25 mg twice a day for 24 w.	A diagnosis of pSjS according to the revised AECG criteria 18 to 75 yrs. Positive dry eyes and (or) dry mouth symptoms Hyperglobulinemia	ESSDAI improvement [Time Frame: w 24] ESSPRI improvement [Time Frame: w 24]
RO5459072	Cathepsin S inhibitor	Hoffmann-La Roche (November 2016)	NCT02701985	Recruiting	RCT-d Phase 2	**Placebo Comparator:** Placebo 200 mg daily, for up to 12 w **Experimental:** RO5459072 200 mg daily, for up to 12 w	A diagnosis of pSjS according to the revised AECG criteria 18 to 75 yrs. ESSDAI score >=5 ESSPRI score >=5 Elevated serum titers of anti-SSA and/or anti-SSB antibodies	Percentage of participants with a clinically relevant decrease in ESSDAI score [Time Frame: 12 w]

RCT: randomized controlled trial; -d: double-blind; mg: milligrams; n: number; g: gram; L: liter; U: unit; IL: Interleukin; kg: kilograms; ml: milliliters; min: minute; w: weeks; yrs.: years; SC: subcutaneous; IV: intravenous; Ig: immunoglobulins; BAFF; B cell activating factor; NF-kB, nuclear factor kappa B; mAb: monoclonal antibody; US: United States; ANA: Antinuclear antibodies; SWSF: Stimulated whole salivary flow; VAS: visual analogue scale; EULAR: European League Against Rheumatism; ESSPRI: European League Against Rheumatism (EULAR) Sjögren's Syndrome Patient Reported Index; ESSDAI: EULAR Sjögren's Syndrome Disease Activity Index; pSjS: primary Sjögren's syndrome; Cs: corticosteroid; RTX: rituximab; UWS, unstimulated whole salivary flow rate; hrIL: Human recombinant interleukin; RF: Rheumatoid Factor; AECG: American-European Consensus Group; anti-SSA: anti-Sjögren's-syndrome-related antigen A; anti-SSB: anti-Sjögren's-syndrome-related antigen B; MSG: minor salivary glands; ULN: upper limit of normal; ACR: American College of Rheumatology; AE: adverse event; SAE: serious adverse event; AESI: Adverse event of special interest; PI3K: Phosphoinositide 3-kinase.

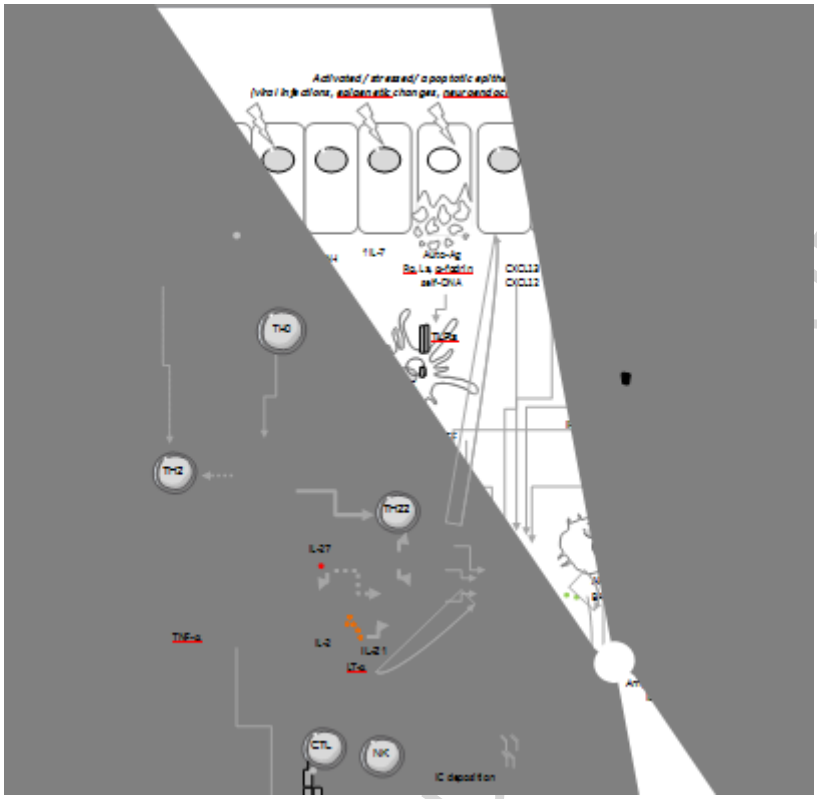

Fig. 1

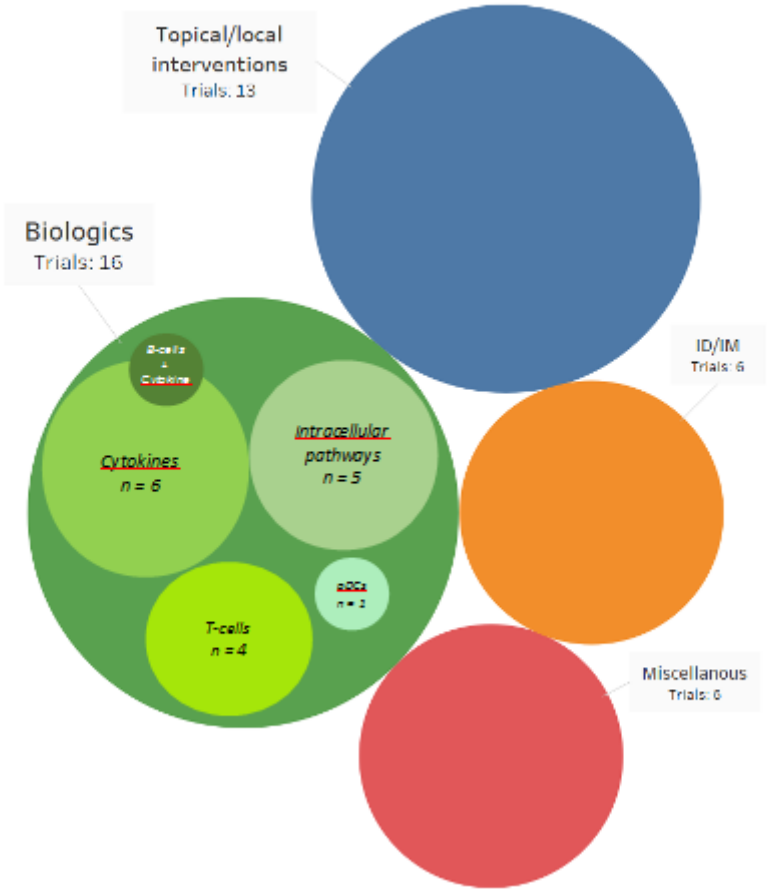

Fig. 2