



Review

Optimizing conventional DMARD therapy for Sjögren's syndrome☆☆☆

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ABSTRACT

Primary Sjögren's syndrome (pSS) is an auto immune disorder characterized by exocrine dysfunction as a result of chronic inflammation of the glands. Part of the patients also develops inflammation in other organs. In a complex interplay of different cell types such as T-cells, B-cells, dendritic cells, monocytes/macrophages and NK cells and their effector molecules, all contribute to one of the ultimate hallmarks of pSS: B-cell hyperactivity, subsequent autoantibody production and eventually formation of germinal center-like structures in the salivary gland. Effective treatment options for this disease are currently lacking.

Biological DMARDs (bDMARDs) including those targeting B-cells or B-cell activation (directly or indirectly) have been studied, so far with limited efficacy. Besides that, their high costs provide a major drawback for implementation. Relatively inexpensive conventional DMARDs (cDMARDs) with well-known safety profiles have been shown efficacious in numerous clinical studies in multiple (rheumatic) diseases. cDMARDs target several pathways that are crucial in pSS immunopathology and some have proven to effectively inhibit B-cell hyperactivity and immune activation when given to patients. However, strong conclusions about potential efficacy are hampered by lack of standardization of inclusion criteria and outcome measures, dosing and validated biomarkers for patient selection. Proper implementation of these could help to optimize the use of cDMARDs in pSS treatment. In analogy with effective treatment strategies in for example rheumatoid arthritis, combination of two cDMARDs targeting different dysregulated pathways might result in additive or synergistic inhibition of immune activation. In view of this and the unique and potent mechanisms of action to target immunopathology in pSS, optimizing cDMARDs for treatment of pSS is worthwhile.

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Abbreviations: 6-MP, 6-mercaptopurine; 6-T-GTP, 6-thioguanine triphosphate; ABT, abatacept; Ach, acetylcholine; ALAT, alanine amino transferase; AZA, azathioprine; BAFF, B cell activating factor; Bcl-6, B cell lymphoma 6; bDMARDs, biological disease modifying antirheumatic drugs; BLM, belimumab; CCL, C-C motif chemokine ligand; CD, cluster of differentiation; CCR, C-C chemokine receptor; cDC, conventional dendritic cell; cDMARDs, conventional disease modifying antirheumatic drugs; clinESSDAI, clinical ESSDAI; CRP, C-reactive protein; CXCL, C-X-C motif ligand; CXCR, C-X-C chemokine receptor; CyA, cyclosporin A; DC, dendritic cell; DcIR, dendritic cell immunoreceptor; DHODH, dihydroorotate dehydrogenase; ESR, erythrocyte sedimentation rate; ESSDAI, EULAR Sjögren's syndrome disease activity index; FcγR, Fc gamma receptor; fDC, follicular dendritic cell; GC, germinal centre; GM-CSF, granulocyte-macrophage colony-stimulating factor; GTP, guanosine triphosphate; GWAS, genome wide association study; HCQ, hydroxychloroquine; HLA, human leukocyte antigen; IBD, inflammatory bowel disease; ICAM, intracellular adhesion molecule; ICOS, Inducible T cell co-stimulator; IFN, interferon; Ig, immunoglobulin; IL, interleukin; IL-1R, interleukin receptor; JAK-STAT, Janus-Kinase – Signal transducer and activator of transcription; LEF, leflunomide; LFS, lymphocytic focus score; LPS, lipopolysaccharide; LSG, labial salivary gland; MFI, multidimensional fatigue inventory; MHC, major histocompatibility complex; MS, multiple sclerosis; MTX, methotrexate; MZ, marginal zone; NFAT, nuclear factor of activated T cells; NFRB, Nuclear factor kappa-light-chain-enhancer of activated B cells; NHL, non-Hodgkin lymphoma; NI, no information; NK, natural killer; nSS, non-Sjögren sicca; PBMC, peripheral blood mononuclear cell; PD1, programmed cell death protein 1; pDC, plasmacytoid dendritic cell; pSS, primary Sjögren's syndrome; RA, rheumatoid arthritis; RF, rheumatoid factor; ROR-γt, retinoic acid related orphan receptor gamma t; RTX, rituximab; SGEc, salivary gland epithelial cell; SLE, systemic lupus erythematosus; SOCE, store-operated calcium entry; SSZ, sulfasalazine; STIM, stromal interaction molecule; TCR, T cell receptor; Th, T follicular helper cells; Th, T helper; TLR, toll-like receptor; TNF, tumor necrosis factor; Treg, regulatory T cell; VCAM, vascular cell adhesion protein.

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1. Introduction

Primary Sjögren's syndrome (pSS) is the second most prevalent systemic rheumatic auto-immune disease, after rheumatoid arthritis (RA), with a prevalence of 0.5% [1] affecting mainly women (female/male ratio 9:1) [2]. Hallmark of the disease is exocrine dysfunction leading to severe dryness of eyes and mouth. Although other mechanisms have been suggested, dryness to a considerable extent is the result of chronic inflammation, associated with B-cell hyperactivity, a key feature of the disease. As a consequence, B-cell non-Hodgkin lymphoma (NHL), especially Marginal Zone (MZ) B-cell lymphoma of the MALT-type (mucosal-associated lymphoid tissue), develop in 5–10% of the pSS patients.

Although chronic inflammation of the exocrine glands and dryness are key features of pSS, approximately one third of the patients also exhibits extraglandular manifestations, and involvement of practically every organ is possible [3]. In addition, invalidating fatigue is a common feature of pSS. The severe dryness with its accompanying complications and the debilitating fatigue make pSS a disease with a great impact on social, professional, and private life.

Despite an extensive search for a clinically effective treatment for common symptoms such as dryness, fatigue, myalgia and arthralgia, to this day symptomatic treatment consisting of saliva- and tear substitutes and supportive advice concerning lifestyle is the best that can be offered (except in cases of serious complications of internal organs that require strong immunosuppression). Various curative options that target the immune activation seen in pSS have been evaluated in the past, all with relatively disappointing results. Biologicals (bDMARDs, targeting specific molecules) were expected to have great potential and their efficacy is the focus of a large amount of research effort. In recent years numerous studies have been initiated to test the effects of biologicals targeting B-cells directly or indirectly by interfering with immune cells that drive B-cell hyperactivity or soluble factors that mediate their activation [4–12]. However, besides showing limited and inconsistent efficacy, lack of long-term safety assessments and their high costs provide major drawbacks in their clinical applicability in pSS.

Considering the high costs of biologicals (€10,000–€15,000 per year) versus the low costs of conventional DMARDs (cDMARDs) (in general some hundreds of euros per patient per year), evaluation of cDMARD therapy for pSS and comparison with bDMARDs using the same clinical measures and inclusion criteria should be further explored. cDMARDs have been shown efficacious in numerous clinical studies in multiple (rheumatic) diseases and detailed safety profiles are available. Several

of these drugs target pathways that are crucial in pSS immunopathology. However, their potential use in pSS is under debate yet not well studied.

This paper reviews our current knowledge on cDMARD function and use in rheumatic disease and pSS. It aims to describe the potential of cDMARD therapy for treatment of pSS, and discusses the current lack of knowledge and potential therapeutic strategies that might be applied.

2. (Targeting) immunopathology in pSS

2.1. pSS immunopathology

pSS is considered to be a multifactorial disease, in which environmental factors trigger inflammation in genetically prone individuals [1]. The pathological process is the resultant of the involvement of both the innate and the acquired immune system. The exact pathophysiological mechanisms of the disease have not been fully elucidated, but significant progress has been made in the understanding of the complex interplay between the different cell types involved and the way they become activated. Characteristic for pSS is focal infiltration of target organs with mononuclear cells, mainly CD4 and CD8 T-cells, B-cells and to a lesser extent dendritic cells (DC's), monocytes/macrophages and natural killer (NK) cells [13]. In a complex interplay these different cell-types all contribute to one of the hallmarks of pSS: B-cell hyperactivity, subsequent autoantibody production and ultimately formation of germinal center-like structures in the salivary gland [14] [15].

Genome Wide Association Studies (GWAS) in Sjögren's syndrome [16,17] support the involvement of multiple components of both the innate and adaptive immune systems. Most significantly associated with pSS is the HLA/MHC region. However, polymorphisms in six other, non-HLA loci proved to be associated with pSS. These loci are involved in the IFN pathway, TLR signaling, activation of B and T-cells and dysregulation of the NFκB pathway, which supports the existing knowledge on the pathogenesis of pSS.

The exact origin of the pathological process in pSS remains to be elucidated, but various mechanisms that initiate the inflammation in pSS patients have been suggested and experimentally supported. Thus, tissue cells and cells of the innate and adaptive immune system have been shown to be critical players in the initiation and perpetuation of the inflammatory processes in pSS. Their potential roles are discussed below (see also Fig. 1).

2.2. Epithelial cells

Instead of being passive victims in the inflammatory process of pSS, salivary gland epithelial cells (SGECs) are indicated to play an active role in the pathophysiology. Epithelial cells in pSS patients express toll-like receptors (TLRs) and in response to TLR ligands produce a variety of pro-inflammatory mediators enhancing inflammation in the glands. Thus, it is assumed that epithelial cells in the salivary gland become activated by certain environmental factors such as viruses and chemical compounds [1]. Indeed, these cells were shown to overexpress MHC class II, adhesion and co-stimulatory molecules (e.g. CD40 to costimulate Th cells). Also, they secrete a variety of chemokines (CXCL13, CCL17, CCL19, CCL21 and CCL22) to recruit inflammatory cells and activate and orchestrate the inflammation.

SGECs are able to produce IL6, and in this way proved to contribute to differentiation of Tfh cells. Furthermore, the expression of ICOSL by SGECs is involved in IL21 production by Tfh cells [18]. Ultimately, epithelial cells produce cytokines such as IL-7 [19], and BAFF [20,21] known to contribute to germinal center formation [20]. All together epithelial cells are indicated to be involved in recruitment of a variety of innate and lymphoid cells and by cytokine secretion, antigen presentation and co-stimulation contribute to activation and differentiation of innate and acquired immune cells.

2.3. Innate immunity in pSS

Recent studies have shown that the interferon pathway is involved in the pathophysiology of pSS [22–25]. More than half of the patients systemically exhibit a type 1 interferon signature, an upregulation of several interferon type I inducible genes in whole blood or isolated cells such as monocytes. Locally, an upregulation of interferon type II inducible genes was demonstrated [26]. The presence of an interferon signature is associated with a higher disease-activity (ESSDAI scores), auto-antibody titers and serum IgG levels and with lower C3 levels, absolute lymphocyte and neutrophil counts [27]. The trigger for activation of the interferon pathway has not yet been identified, although some studies point towards viral infection. Epstein-Barr virus, hepatitis C virus, retroviruses and Coxsackie A virus have all been suspected, however no clear association between these viruses and pSS could be demonstrated [28]. Possibly this is due to the fact that it takes some years before clinically manifested disease has developed, whereby the original stimulus is no longer demonstrable at a later timepoint. Viral infection leading to epithelial activation and injury may lead to a release of pSS specific ribonucleoprotein auto-antigens (Ro/SSA and La/SSB) and chemokines resulting in migration of DCs and pDCs to the infected glands [3]. Viral RNA and DNA might activate TLRs and subsequent production of type I interferon by pDCs and mDCs and other myeloid cells.

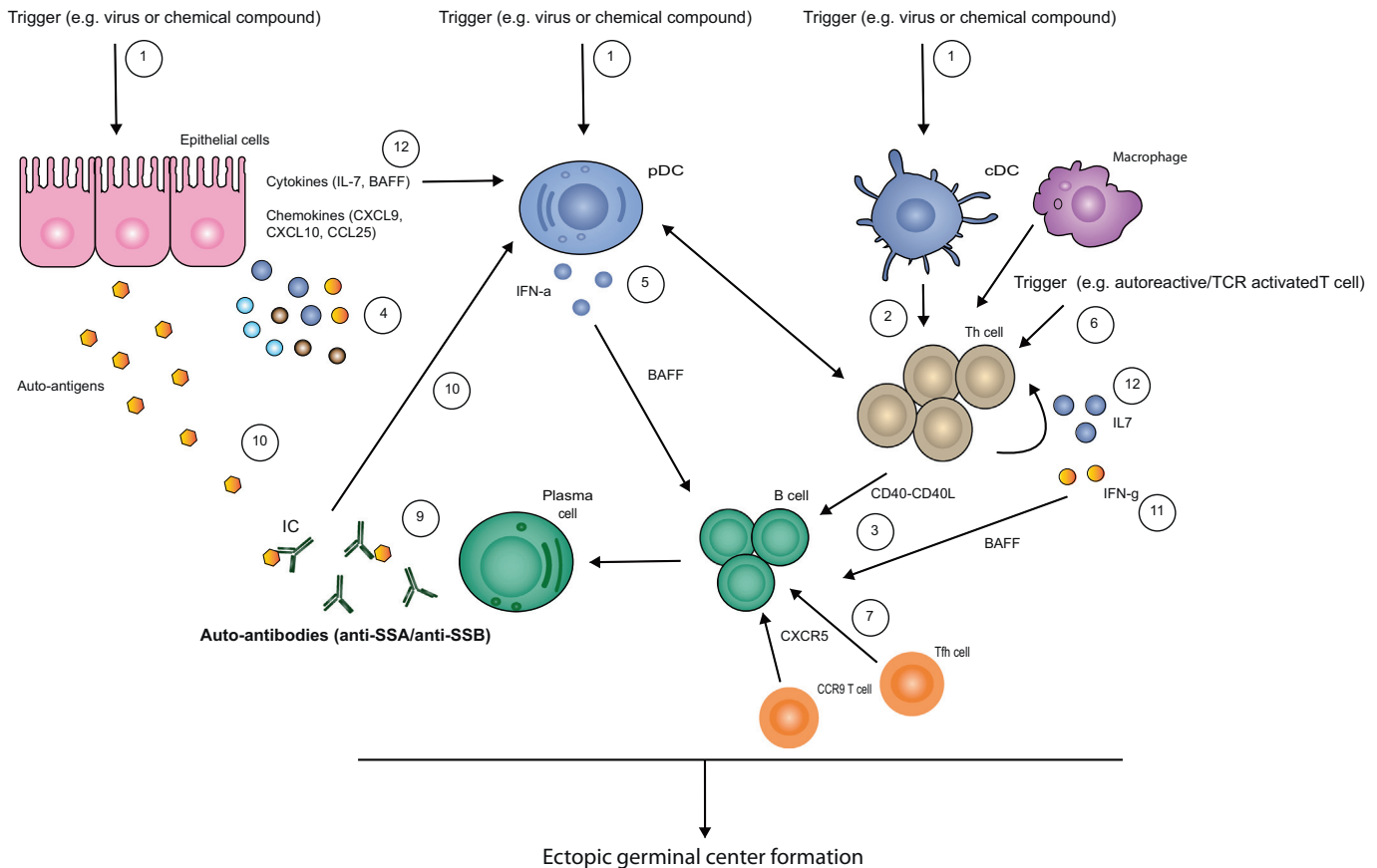


Fig. 1. The initiation and perpetuation of immunopathology of primary Sjögren's syndrome involves multiple pathways that additively/synergistically trigger inflammation. Initiation of the pathological process might occur in different ways. Environmental factors such as viruses or chemical compounds can trigger activation of epithelial cells, mDCs or pDCs (1), leading to activation of Th1 cells (2) associated with B-cell help (via IFN γ , CD40L, ICOSL upregulation) (3), production of chemokines to recruit inflammatory cells (4), contributing to the formation of lymphocytic aggregates. In addition, B-cell help comes from pDC by production of IFN- α associated with BAFF induction and by activation of Th cells (5). Inflammation can also be initiated by antigen-driven (TCR) activation of resident effector T-cells or newly recruited (via chemokines CXCL9 and 10) effector Th1 cells that have been educated by antigen-primed DCs in secondary lymphoid organs (6). B-cell differentiation is specifically promoted by CXCR5 Th cells and CCR9 Th cells (7) that are recruited to the inflamed tissue by specific chemokines CXCL13 and CCL25, respectively (4). The formation of (auto-)antibody producing plasma cells (9) subsequently leads to further activation of antigen presenting cells (DCs and macrophages) associated with further maturation/activation of these cells e.g. via intracellular TLR stimulation (via self or non-self RNA or DNA), resulting in a self-perpetuating loop (10). Environmental triggers, e.g. a virus, damaging epithelial cells could lead to release of non-self but also self-antigens, contributing to the chronicity of the inflammation (10). IFN- γ produced by Th1 cells and cytotoxic molecules like granzymes from CD8 T-cells finally can result in significant tissue damage in the glands (11). In approximately 25% of the patients lymphocytes are ultimately organized in germinal center like structures. Increased production of IL-7 and BAFF by epithelial cells and fibroblasts play key roles in these processes and have been associated with B-cell hyperactivity, extraglandular manifestations and lymphoma development (12).

Innate cells such as pDCs, cDCs, and macrophages are increased in LSG of pSS patients [22,29–31]. Upon activation of cDCs and pDCs both type I and type II interferon pathways are activated. In addition, activated conventional DCs produce IL-12, promoting activation of NK-cells and Th1 cells thereby further stimulating the production of IFN- γ , a type II IFN, which predominates in the salivary glands of pSS patients [26]. Both type I and II IFNs promote the production of B-cell activating factor (BAFF), an essential cytokine for maturation, proliferation and survival of B-cells and both BAFF and IFN type I and II play a critical role in (ectopic) germinal center formation [32,33].

Several pSS models support the important role of pDCs and cDCs in pSS immunopathology. ID3 is an inhibitory transcription factor suggested to be involved in inhibition of pDC development [34,35]. The ID3 knockout mouse spontaneously develop pSS-like symptoms [36]. Another model is the DC immunoreceptor (Dcir) knockout mouse model. Dcir is a type C lectin receptor with inhibitory function, mainly expressed in DCs. It negatively regulates DC expansion and therefore is important in maintenance of self-tolerance. Mice that are Dcir-deficient develop lymphocytic infiltrates in salivary glands accompanied by destruction of the tissue, anti-Ro/SSA and anti-La/SSB autoantibodies and increased numbers of T-cells with an activated phenotype and cDCs in lymph nodes when compared to normal mice [37].

In the LSG of pSS patients increased numbers of NK cells are found as compared to non-Sjögren sicca patients. These innate NK cells reside mainly outside the aggregates of lymphocytic cells (foci) that are typically found in the glands of pSS patients. Also, the number of NK cells outside the foci correlates with the focus score. Several findings implicate that NK cells seem to be involved in the pathogenesis of pSS [38]. Release of autoantigens as a result of epithelial cells undergoing apoptosis could possibly lead to activation of NK cells by engagement of apoptosis-induced molecules to their activating receptors. Upon this activation, NK cells subsequently activate DCs residing in the lymph nodes, leading to T-cells being primed and executing their effector functions in the peripheral tissues.

2.4. Acquired immunity in pSS

Infiltrates found in target organs are dominated by activated T-cells (predominantly CD4 but also CD8) and B-cells. The exact composition of the infiltrate varies with the severity of the lesion [30,39]. Apart from the huge body of evidence that shows B-cell hyperactivity and autoantibody production, numerous studies indicate that T-cells may play a pivotal role in the induction and perpetuation of glandular inflammation. T-cell intrinsic defects were shown to trigger pSS-like disease. In mice with T-cells lacking class IA phosphoinositide-3-kinase (1A PI3K), an enzyme involved in regulation of proliferation and survival of amongst others T-cells, infiltration and destruction of lacrimal glands develops and the pSS specific autoantibody anti-SS-A is produced [40]. Possibly, loss of central and/or peripheral tolerance plays a role, however, the process of thymic negative and positive selection in this model remains to be studied.

Cheng et al. [41] provide further evidence for an important role for aberrant T-cell regulation. In a mouse model with T-lymphocyte specific deletion of stromal interaction molecule (STIM) 1 and STIM2, key components that are critically involved in T-cell activation and function, the store-operated calcium entry (SOCE) and the cytokine production that is dependent on this process were heavily attenuated. This was accompanied by a decrease in number and function of regulatory T-cells. The mice spontaneously produced pSS-specific autoantibodies, developed inflammation and destruction of salivary glands, and loss of (stimulated) saliva production. In addition, they demonstrated that PBMCs from pSS patients show decreased levels of STIM1 and STIM2 and SOCE in T-cells. In addition, minor salivary gland biopsies of pSS patients with relatively high focus scores (>3) showed a marked reduction in STIM1 expression in the infiltrating cells compared to healthy controls and patients with a low focus score.

In addition, a role for auto-reactive CD4 T-cells was demonstrated by Arakaki et al. [42]. In their murine pSS model, adoptive transfer of autoreactive CD4 T-cells recognizing α -fodrin (previously shown to function as an autoantigen in the pathogenesis of pSS) induced significant cytotoxicity against salivary gland cells and the mice developed autoimmune lesions characteristic for pSS.

Also, cytokines such as IL-7 that are capable of inducing T-cell activation have been demonstrated to play a role in pSS. IL-7 is a potent pro-inflammatory cytokine primarily acting on T-cells expressing high levels of the IL7 receptor α -chain (IL-7R α), and it induces T-cell-dependent activation of B-cells, DCs and macrophages [43]. In the labial salivary gland (LSG) specimens of pSS patients, an increased number of IL-7 producing cells and IL7R-expressing cells was found as compared to patients with sicca symptoms without any sign of auto-immunity (nSS patients), and these increased numbers correlated with the lymphocytic focus score (LFS) and increased B-cell hyperactivity in the gland [44,45]. Moreover, levels of soluble IL-7 receptor (sIL-7R), considered a biomarker of IL-7 activity, are increased in pSS patients in serum and salivary gland tissue supernatant, and correlate to markers of inflammation [46]. Interestingly, IL-7 overexpression in mice plays a pivotal role in GC formation in lymphoid organs, but also in mucosa-associated tissue such as salivary glands [43].

Also Sjögren-like disease can be simulated by TLR3 triggering of epithelial cells inducing IL-7/T-cell-driven immune activation and Sjögren-like disease features in a mouse model of pSS [19]. NK-derived IFN- γ , CXCL9 and CXCL10 induced an influx of T-cells into the mouse salivary gland. At a later stage, IL-7 induced by poly I:C stimulation enhanced IFN- γ production by these T-cells, resulting in amplification of CXCL9 production and thereby ongoing T-cell infiltration.

Finally, CXCR5-expressing follicular helper T (T_{fh}) cells are cells specialized in providing B-cell help by promoting activation, differentiation of plasma cells and memory B-cells, and somatic hypermutation. Increased CXCL13 production in pSS patients by follicular dendritic cells (fDCs) and epithelial cells in the salivary gland is capable to attract T_{fh} cells into B-cell follicles via CXCR5. T_{fh} cells are crucial for the formation and maintenance of GCs or GC like structures and are increased in the glands and in peripheral blood of pSS patients [47]. They are characterized by expression of ICOS, PD-1, transcription factor Bcl-6 [48], and production of IL-21. IL21, produced by T_{fh} and a crucial cytokine for formation of GC-like structures, was demonstrated to be elevated in serum of pSS patients and IL21 expression within the salivary glands tended to increase with higher LFS [18,49]. pSS patients with elevated IL21 levels in the serum showed significantly higher systemic activity measured by ESSDAI [18].

Recently, a new subset of Th cells, the so-called CCR9-expressing T_{fh}-like effector cells as well as the chemokine that specifically attracts them, CCL25, were found increased in blood and salivary glands of pSS patients. CCR9 Th cells were found to express high levels of IL-7R α , and produce high levels of IFN- γ , IL-17, IL-21 and IL-4, typical of pathogenic effector Th cells. Like T_{fh} cells, CCR9 Th cells potently stimulated B-cells and increased proportions of ICOS-expressing CCR9 Th cells were found in the circulation and salivary glands of pSS patients [50]. Indicating their potential pathogenic capacity in pSS, in mice CCR9 Th cells induced inflammation and tissue destruction in mucosa-associated tissues, including salivary glands [51].

3. cDMARDs in pSS

As indicated above, cDMARDs have been shown effective in numerous clinical studies in generalized auto-immune diseases including RA, with detailed safety profiles. In addition, several of these drugs target several of the above-mentioned immunopathological pathways. In the following section, the cDMARDs that have been evaluated in relation to pSS (in vitro and animal models, case reports, clinical trials) will be discussed. Their mechanisms of action, components of the immune

system that are being targeted, and their efficacy in other auto-immune diseases and in pSS are reviewed.

3.1. Methotrexate

Methotrexate (MTX) at a low dose has proven to be clinically effective in RA. Its broad mechanisms of actions have not yet been fully elucidated, but MTX is best known for its ability to inhibit T and B-cell proliferation. It does so by multiple mechanisms including inhibition of *de novo* pyrimidine and purine synthesis, increase in adenosine levels [52] causing immunosuppression by inhibition of proinflammatory cytokines such as TNF- α , downregulation of adhesion molecules and chemokines resulting in a decreased influx of leukocytes into tissues and induction of anti-inflammatory cytokines such as IL-10 [53,54], increased apoptosis of memory T-cells via fas-dependent mechanisms [55] and inhibition of the JAK-STAT signaling pathway [56]. The achieved inhibition of pro-inflammatory T-cells leads to less activation of B-cells and subsequent antibody production.

Treatment with MTX in RA patients reduced the levels of IgA-rheumatoid factor (RF) and IgM-RF [57]. It also restored the imbalance between Th17/Treg cells in PBMCs of early RA patients in an *in vitro* system, and it reduced the levels of Th17 effector cells from early RA patients. MTX was shown to restore the regulatory T (Treg) cell function, that is known to be defective in RA patients [57–59].

IgG containing immune complexes, such as rheumatoid factor (RF), also present in a substantial proportion of pSS patients, potentially can activate monocytes [60]. Treatment-naïve RA patients, showing higher expression levels of Fc γ R of peripheral blood monocytes, compared to RA patients on DMARD therapy [61], showed downregulation of expression levels of activating Fc γ R on peripheral blood monocytes after treatment with MTX. An exclusive effect of MTX on these expression levels was demonstrated in an *in vitro* setting.

Overall, these results indicate that MTX is a very potent immune-suppressive drug with effects on multiple pathways of both acquired and innate immune cells.

MTX applied in low dose proved to have a favorable safety profile. Most frequent side effects are nausea, mucosal ulcers, mild bone marrow depression and dose-dependent hepatotoxicity reflected by mildly elevated levels of ALAT leading to cirrhosis in <1% of the cases. Frequency of side-effects can be lowered by administration of folic acid [62–64].

3.2. MTX in pSS

MTX has been poorly investigated in pSS. Only empirical use, described in case-reports, and one open study have provided some information about its efficacy in pSS. Its use (often in combination with corticosteroids) has been described in case reports concerning patients with pSS-associated myelopathy [65] and inclusion body myositis associated with pSS [66] demonstrating MTX to have little to moderate clinical effects.

Efficacy and safety of MTX was studied in an open pilot study with 17 pSS patients, receiving 0.2 mg/kg body weight once a week for one year [67]. Subjective parameters, consisting of dryness of eyes and mouth, ameliorated, according to the frequency of parotid gland enlargement, dry cough and purpura. In contrast, no improvement in objective measures of dryness of eyes and mouth (Schirmer's test, saliva flow rate) was found. IgG and ESR, elevated in 82% and 65% of the patients respectively at baseline, remained unchanged.

3.3. Sulfasalazine

Sulfasalazine (SSZ) is a combination of sulfapyridine (SP) and 5-aminosalicylic acid (5-ASA), linked by an azobond [68]. Alike MTX, SSZ increases extracellular adenosine, which has immunosuppressing properties as is described before, mainly targeting lymphocytes. Besides this, sulfasalazine inhibits nuclear factor kappa B (NF- κ B, a central pro-

inflammatory transcription factor), which is increased in pSS patients [16,69].

SSZ was shown to inhibit *in vitro* TNF- α production in monocyte-derived macrophages stimulated with LPS and was associated with induction of apoptosis of the macrophages. This apoptosis was also demonstrated *in vivo*, using a mouse model and seemed to be a result caspase 8-induced apoptosis and TNF inhibition [70].

Profound inhibiting effects of SSZ and of its metabolites SP and 5-ASA on IgG and IgM secretion by γ -cells were found in an *in vitro* system, in which stimulated B-cells from healthy individuals were treated with pharmacologically attainable concentrations SSZ [71].

A meta-analysis of 15 randomized controlled trials in RA patients (including 8 trials comparing SSZ to placebo) showed that in RA patients SSZ significantly decreases ESR, duration of morning stiffness, pain and swollen joints compared to placebo [72]. In current rheumatological practice SSZ is applied mainly in combination with other cDMARDs such as MTX, hydroxychloroquine (HCQ) and leflunomide (LEF).

Common side effects of sulfasalazine are headache, gastro-intestinal complaints, dizziness and rash. Also myelodepression can occur [73].

3.4. Sulfasalazine in pSS

The efficacy of SSZ in pSS-like disease was researched in New Zealand Black/New Zealand White (NZB/NZW) F1 hybrid mice, which show deviations in their salivary and lacrimal glands resembling those seen in pSS patients. SSZ treatment at 14–42 weeks or from 26 to 42 weeks of age did not decrease lymphocytic infiltration in the glands [74]. Other parameters such as IgG were not evaluated.

Clinical effects of SSZ have been poorly studied. Imai et al. [75] describe eleven pSS patients with hypergammaglobulinemia (>30 g/L) that were treated with 1000 mg SSZ/day. Statistically significantly decreased serum levels of IgG and IgA after 8 weeks of administration were observed. Side effects were mild and restricted to skin rash in 4 out of the 11 patients.

No randomized placebo-controlled clinical studies were performed investigating the effects of SSZ in patients with primary Sjögren's syndrome.

3.5. Azathioprine

Azathioprine (AZA) is a pro-drug that after several conversion steps yields its active metabolites that disturb the function of enzymes essential for replication and repair and cause DNA damage. Moreover, *de novo* purine synthesis is inhibited. Finally, the small GTP-ase Rac1 that regulates multiple signaling pathways that amongst other things rule cell proliferation [76], is inhibited, resulting in enhanced cell apoptosis in activated T-lymphocytes. AZA was shown to modulate CD28 induced Rac1 activation in T lymphocytes in both healthy individuals and patients with Crohn's disease, resulting in enhanced apoptosis of particularly memory, but also naive T-cells [77–81]. As stated before, endothelial cells are actively involved in the chronic inflammation process. To be able to migrate through the vascular wall into the tissues, leukocytes need to adhere to endothelial cells, upon which adhesion molecules such as VCAM-1 and ICAM-1 are induced. Rac1 is crucial for the formation of these adhesion molecules, and therefore inhibition of Rac1 could reduce downstream transcription of proinflammatory proteins [82]. Indeed, it was shown that TNF- α induced Rac1 activation in an endothelial cell line is inhibited by treatment with 6-MP and 6-T-GTP, metabolites of AZA. Also TNF- α induced NF- κ B activation was inhibited by 6-MP and 6-T-GTP. This was accompanied by a reduction of VCAM-1 expression and production of pro-inflammatory cytokines.

Taken together, azathioprine in pSS could be effective by its inhibition on T and B-cell lymphocytes. The safety profile of AZA is mainly researched in IBD patients. Most frequent side effects are myelotoxicity and hepatotoxicity and they typically occur in the first 4 months of treatment. Other side effects that can occur are gastro-intestinal

complaints, alopecia. AZA treatment is associated with a potential risk of developing lymphoma in patients with IBD, however the absolute risk remains small [83–85].

3.6. Azathioprine in pSS

The use of AZA in pSS is described in numerous case reports. Pragmatic use of AZA (often in combination with corticosteroids) is being applied in clinical practice for a variety of extraglandular manifestations, including pulmonary hypertension [86,87], interstitial cystitis [88], interstitial pneumonia/interstitial lung disease [89], interstitial nephritis [90] and myelopathy [91,92].

Yeoman et al. investigated the effect of AZA on lymphocytic focus score in submandibular and lacrimal glands, again in NZB/NZW mice. A decrease in the number of lymphocytic foci in the group of mice treated with AZA was observed. This accounted for both mice that were treated from the age of 14 weeks onwards, as for mice that started treatment at 26 weeks of age [93].

Clinical efficacy of AZA in pSS was studied in a double blind placebo controlled trial. Twenty-five patients with pSS were enrolled in this study (12 placebo, 13 AZA). Patients received treatment with 1 mg/kg bodyweight AZA during 6 months [94]. There was no significant beneficial effect of AZA on clinical parameters, serology or histological findings. Six patients (all receiving the active drugs) withdrew from the study because of side-effects.

In conclusion, no beneficial effect on common symptoms such as dryness, fatigue, myalgia and arthralgia could be demonstrated in this study. However, as described before, AZA is used in clinical practice as a corticosteroid sparing drug to treat serious extraglandular manifestations.

3.7. Cyclosporine

Cyclosporine A (CyA) is a cyclic polypeptide, consisting of 11 amino acids that exerts its effect by inactivating the serine-threonine phosphatase calcineurin. This results in inhibition of activity of Nuclear Factor of Activated T-cells (NFAT) which is essential for the expression of genes encoding amongst others IL-2, IFN- γ and granulocyte-macrophage colony-stimulating-factor (GM-CSF) [95,96]. This is substantially different from the upper mentioned cDMARDs that target both T and B-cells by affecting purine synthesis. CyA seems to more specifically target T-cells and T-cell-dependent responses.

In a recent study the *in vitro* effect of CyA on the activation of Th17 cells was assessed [97]. CyA decreased elevated levels of IL-17 and ROR- γ t mRNA levels in PBMCs of pSS patients with active disease but not in PBMCs of pSS patients with inactive disease and healthy individuals. Considering the proposed role of Th1 and Th17 cells in pSS [98,99], CyA could be an interesting treatment modality.

Peripheral T-cells co-stimulated with CD3 and CD28 and simultaneously were treated with CyA showed decreased IL-2 expression and thereby less proliferation by inhibition of nuclear translocation of NF-AT and NF- κ B p65/RelA [100].

(Reversible) nephrotoxicity is the most important side effect of CyA. Dose-related elevation of creatinine levels occurred in 48% of the patients in a study involving 154 RA patients treated with CyA. Other side effects that were frequently reported were new onset hypertension, gastro-intestinal complaints, headache and hypertrichosis [101].

3.8. Cyclosporine in pSS

Efficacy and safety of CyA in pSS patients *in vivo* were evaluated in a double blind, placebo-controlled trial with 20 pSS patients [102]. Ten patients were treated for six months with a CyA dosage of 5 mg/kg bodyweight, versus ten that received placebo. Significant clinical improvement in the CyA-treated group was restricted to xerostomia, this was not seen in the placebo group. All other subjective and objective parameters did not significantly change after treatment in both groups

(Table 1). Histological deterioration was observed in the placebo group, while the CyA treated group showed unaltered histological lesions after six months of treatment. This suggests that CyA retarded the natural course of the disease. Serum IgG levels were not evaluated.

Topical use of CyA has been investigated quite extensively. However, inclusion in most studies was not restricted to pSS patients but also patients with undefined dry eyes disease were incorporated. Sall et al. [103] reported on two identical randomized, double-blind vehicle-controlled trials investigating efficacy and safety of CyA cyclosporine A 0.05% and 0.1% ophthalmic emulsion compared to vehicle emulsion. A total of 877 patients were included, 30% being diagnosed with pSS based on presence of oral symptoms, ocular symptoms and a Schirmer test ≤ 5 mm as well as the presence of rheumatoid factor, antinuclear antibodies, anti-Ro/SSA or anti-La/SSB autoantibodies. Significantly greater improvements in both the 0.05% as in the 0.1% CyA group compared to the vehicle group were observed for the objective measures corneal staining and categorized Schirmer values, but not for tear break-up time. Subjective parameters blurred vision, use of lubricating eye drops and the physician's evaluation of global response to treatment showed a greater improvement for the group treated with 0.05% CyA emulsion compared to the group treated with vehicle. This study was followed by an open-label extended study [104] which enrolled 412 patients with dry eyes disease (% of patients diagnosed with pSS is unknown), all treated with CyA 0.1% ophthalmic emulsion during three extension periods of 12 months each in order to assess long-term safety. Topical CyA treatment proved to be safe. Mainly mild to moderate side effects were reported, only one was infectious of nature.

3.9. Leflunomide

Leflunomide (LEF) is an immune-suppressive drug, structurally not related to other immune-suppressants. It is an isoxazole derivate, which becomes active *in vivo* after conversion to its active form (A77 1726) by opening of the isoxazole ring. LEF is a known inhibitor of dihydro-orotate dehydrogenase (DHODH), the rate-limiting enzyme of *de novo* biosynthesis of pyrimidines, leading to inhibition of proliferation of B-cells and both naive and memory CD4 T-cells [105].

In B10.A mice that were immunized with a T-cell dependent and a T-cell independent antigen, LEF inhibited both T-cell dependent and T-cell independent B-cell antibody production, indicative of direct inhibition of B-cell responses [106]. This was due to the capacity of LEF to inhibit B-cell proliferation by blocking cell cycle transition from G1 to S phase and from entering G2/M phase. Thus, B-cell antibody production is reduced by LEF by blocking expansion of antibody-secreting cells.

A non-cell specific inhibitory effect of LEF on activation of the transcription factor NF- κ B was demonstrated *in vitro* [107]. Acute human T-cell leukemia cells (Jurkat cell line), pre-treated with LEF, were stimulated with different activators of NF- κ B. Activation of NF- κ B induced by these agents was remarkably reduced in the LEF pre-treated cells compared to the non-treated cells, indicating that LEF exerts its effect on a common step in the transduction pathway. The inhibition of NF- κ B activation was not restricted to Jurkat cells but was also demonstrated in different cell types (myeloid (U937), epithelial (HeLa) and glioma (H4)). By preventing translocation of NF- κ B to the nucleus LEF influences downstream (inflammation-associated) gene expression.

3.10. Leflunomide in pSS

Clinical effectiveness of LEF in patients with pSS was investigated by Van Woerkom et al. in an open label pilot study [108]. Fifteen patients diagnosed with early pSS and showing active disease were treated with LEF 20 mg once a day for 24 weeks. Modest clinical improvement was observed. After 24 weeks of treatment, patients reported less "general fatigue", a subscore from the Multidimensional Fatigue Inventory (MFI). Mean values of serum IgG, IgA and IgM levels decreased significantly from 8 weeks onwards compared to baseline measurement.

Also, a significant reduction in RF levels was seen after 24 weeks of treatment. There was a tendency towards increased mean values for the Schirmer's test. Repeated labial gland specimens in 5 of the 15 patients showed a decrease of 1 focus/4mm² in 4 of them. The remaining one patient exhibited an increase of 1 focus/4mm². Moreover, 3 patients with leucocytoclastic vasculitis prior to start of the study showed a remarkable improvement of their vasculitic purpura after treatment.

Side-effects were restricted to mild gastro-intestinal symptoms, alopecia, transient increase of liver enzymes (ALAT), aggravation of pre-existent hypertension, mild leucopenia and skin-lesions well responding to topical corticosteroid therapy.

Cytokine analysis of circulating cells of 13 of these LEF-treated patients was performed [109]. LEF suppressed TNF α and IL1 β production by PBMC from pSS patients after 24 weeks of treatment. In addition, serum concentrations of IFN-g, IL-2, TNF- α , IL-6 and IL-10 decreased after treatment. CD40L expression on CD4 T-cells also was suppressed by LEF treatment. Serum IgG, IgM, IgA levels and autoantibody production were significantly reduced. This could possibly be due to a direct effect of LEF on B-cell activity, but also due to reduced T-cell activation as can be deduced from the decreased levels of T-cell cytokines IFN-g and IL-2, the reduced expression of CD40L (costimulatory molecule enhancing B-cell maturation, important in formation of germinal centers) and B-cell activating cytokines IL-6 and IL-10.

Of the 13 LEF treated patients, 7 patients were considered clinical responders, based on 50% or more improvement of two out of three disease domains (ocular dryness, oral dryness and laboratory parameters). These responders, unlike the non-responders, showed a significant decrease of the production of T-cell associated cytokines IFN- γ and TNF- α in PBMCs upon stimulation with CD3/CD28, indicating that LEF-induced inhibition of T-cell activation contributes to disease improvement.

3.11. Hydroxychloroquine

Hydroxychloroquine (HCQ) is historically used as an anti-malarial and [110] several mechanisms have been proposed that possibly lead to immune inhibition by HCQ. By increasing pH within lysosomes, lysosomal degradation is inhibited and the process of autophagy, important for the synthesis of new macromolecules and creating a source of energy, is blocked, thereby creating a less optimal environment for cell proliferation [111]. Alteration of lysosome function in T-cells affects their capacity to degrade material that has been phagocytosed. The alteration in endocytic pH also affects cytokine production, and results in reduced production of e.g. IL-1, IL-6 and TNF [112]. Wallace et al. showed long-lasting suppressive effects of HCQ on IL-6 levels in patients with SLE [113].

HCQ mainly targets antigen-presenting cells such as dendritic cells and monocytes, concentrations of the drug are higher in these cells. Antigen presentation is decreased by HCQ, since MHC II molecules after synthesis are moved to the endocytic compartments. This mainly affects binding of low affinity self-peptides instead of antigenic pathogen derived peptides [114].

HCQ inhibits TLR mediated immune responses and subsequent induction of type I interferon and other pro-inflammatory cytokines. The mechanisms behind this inhibition are not yet fully elucidated. Two possible mechanisms have been proposed. First of all, the alteration of endosomal pH could lead to dysfunctioning of endosomal TLRs (non-competitive mechanism). Proper functioning of TLRs is pH dependent, requiring an acidic environment. By raising the endosomal pH, HCQ could possibly interfere with the functional transformation of the TLRs that is needed for their activation [112]. Also a competitive mechanism could be underlying inhibition by HCQ. Kuznik et al. showed that anti-malarials directly interact with nucleic acid TLR ligands in vitro. As a result of this interaction, structural alterations of the nucleic acid occur, preventing its binding to and subsequent activation of TLRs [115]. The capacity of HCQ to inhibit TLR mediated immune responses makes it a cDMARD with unique characteristics compared to other cDMARDs.

HCQ has a favorable safety profile. Common side effects of HCQ are gastro-intestinal complaints, rash and alopecia. A rare but serious adverse effect is retinopathy. The incidence of HCQ retinopathy is very low in the first 5 years of usage (approximately 0.3%), and increases slightly after this time period [116].

3.12. Hydroxychloroquine in pSS

Cholinesterase is an enzyme that degrades the parasympathic neurotransmitter acetylcholine (ACh), whose binding to cell surface muscarinic type-3 receptors is a critical step in the initiation of fluid secretion by salivary acinar cells. Elevated cholinesterase levels diminish production of saliva and thus salivary gland hypofunction. The effect of HCQ on cholinesterase was studied. Salivary cholinesterase levels were determined in 9 pSS patients and 9 healthy individuals. Cholinesterase activity was significantly elevated in the pSS patients and HCQ in clinically attainable levels decreased this cholinesterase activity in vitro. Additional studies have to be performed to confirm this result in vivo [117].

When compared to pSS patients not receiving any treatment, pSS patients treated by HCQ showed lower levels of MxA, which is a functional biomarker indicative of IFN type 1 activity [118].

Several clinical trials investigated the effect of HCQ in patients with pSS [119–123]. In 1993, Kruize et al. performed a double-blinded, placebo-controlled cross-over trial in 19 pSS patients with high disease activity parameters witnessed by high sIgG levels (mean 20.4 g/L), ESR (mean 32.5 mm/h) [119]. One group was treated with 400 mg HCQ once a day during 1 year, the second year they received placebo. The other patients had the same treatment, but in reverse order. No significant effect of HCQ on tear production or salivary scintigraphy was seen. Patients did not experience any difference during treatment with hydroxychloroquine of placebo. However, HCQ significantly reduced IgG and IgM levels and there was a trend towards a decrease in ESR.

Recently, the clinical effect of HCQ in pSS patients was investigated in a double-blind, placebo-controlled trial of 120 patients with low disease activity (ESSDAI median 2.5 and 2.0 for placebo group and treated group resp., sIgG mean 14.2 g/L and 14.5 g/L for placebo group and treated group resp) [120]. Patients were randomized and allocated to HCQ or placebo treatment until week 24. From week 24 to 48, all patients received HCQ. HCQ did not improve the most commonly present symptoms, being dryness of eyes and mouth, arthralgia/general pain and fatigue. ESR, IgM and IgG levels were reduced but these reductions did not reach significance.

4. Optimizing DMARD therapy for pSS

Data on cDMARDs from both experimental and clinical studies in multiple autoimmune diseases demonstrated unique and overlapping immunosuppressive properties. Several cDMARDs in relation to primary Sjögren's syndrome were reviewed in this literature study, showing that knowledge on effectivity of cDMARDs in pSS is quite limited. It is difficult to draw strong and definite conclusions about the potential efficacy of cDMARDs in pSS, for several reasons.

First of all, standardized outcome measures were lacking in the past. All studies performed to investigate the efficacy of the different cDMARDs used different outcome measures, resulting in results that are difficult to compare. Only recently the EULAR Sjögren's syndrome disease activity index (ESSDAI), a standardized measure for systemic disease activity in pSS patients was introduced [124], providing the opportunity to standardize studies and compare results of the different treatment options that are studied. In addition, other measures to indicate effects on disease activity or immune activation are often lacking. Immunoglobulin levels for example, an important hallmark of disease activity and immune activation in pSS, often are not evaluated in these studies.

In line with the lacking knowledge on efficacy of cDMARDs in pSS, little is known about the optimal dosing of these drugs in this disease.

Possibly other dosage regimes (higher or lower) should have been used in the studies that have been conducted so far. Usage of suboptimal study designs makes it hard to evaluate the efficacy of cDMARDs in pSS. Double-blind, placebo-controlled trials are lacking for most drugs and dosing-studies have not been performed. e.g. for MTX, only one open clinical study was performed, using a moderate dosage regimen (0.2 mg/kg/wk., corresponding to 14 mg/wk. for a patient with an average weight of 70 kg) [67]. Better effects might be reached using higher doses, however this will have to be tested. Of interest in this respect, in RA patients optimal disease inhibition was found at a dosage of 20 mg/wk. [125]. A minimal dose of 15 mg/week for oral administration and 12 mg/week for parental administration is recommended [126]. In view of its good safety profiles higher dosages of MTX or combinations with other cDMARDs or biologicals could be worthwhile to investigate. Likewise overdosing might occur. Recently our group observed that sub-optimal dosages of HCQ (10–30-fold lower than reported mean serum concentrations) extremely potently inhibit *in vitro* production of IgG and IgM levels by antigen/TLR stimulated PBMCs [127]. This implies that lower concentrations of HCQ than currently used in clinical practice might be sufficient to control B-cell hyperactivity and subsequently B-cell associated immunopathology such as lymphoma and extra glandular manifestations, thereby minimizing the risk on side effects.

In addition to the lack of knowledge on optimal dosing of cDMARDs in pSS, also safety profiles of the various drugs in patients with pSS are not investigated thoroughly. This is related to the fact that only a few (small) double-blind placebo-controlled trials are performed investigating efficacy of cDMARDs. AZA seems to have a relatively unfavorable safety profile in patients with pSS. Relevant myelotoxicity, hepatotoxicity and increased risk of lymphoma may hamper its suitability as a treatment modality for pSS. In a clinical study investigating AZA therapy in pSS, 6 out of 13 patients using the active drug withdrew because of side effects. LEF and HCQ, also investigated in a double-blind placebo controlled trial, proved to have favorable safety profiles. Common side effects of LEF are typically mild and include gastro-intestinal symptoms, alopecia, transient increase of liver enzymes (ALAT), aggravation of pre-existent hypertension, mild leucopenia and skin-lesions. For HCQ, common side effects are restricted to gastro-intestinal complaints, rash and alopecia. The risk on retinopathy is small especially in the first five years of usage.

Another drawback in comparing results of cDMARD treatments is lack of standardized inclusion criteria. Inclusion criteria between studies investigating cDMARDs were different; making comparison with recently conducted clinical trials difficult. For example this has resulted in selection of patient groups that were comprised of patients with rather stable disease and low to moderate activity at moment of enrollment. Logically, this can hugely influence the effect of the investigated treatment. For example, in a recent RCT [120] investigating HCQ in pSS, HCQ did not improve the most commonly present symptoms. ESR, IgM and IgG levels were reduced although not significant. It is important to realize that it was the goal of this study to investigate the clinical efficacy of hydroxychloroquine on symptoms that are widespread under pSS patients but are not indicative of current disease activity. As a result, mainly patients with low or mild disease activity were enrolled (ESSDAI median 2.5 and 2.0 for placebo group and treated group resp.). In this cohort, mean IgG, IgM and IgA levels at baseline were within the normal range, and thus not likely to decrease upon treatment with HCQ. Sub analyses using several clinical characteristics were performed, including a subdivision of patient groups with low or elevated IgG levels (≥ 12.6 g/L), observing no differences between HCQ and placebo groups. However, in our opinion, this cut-off point for defining hypergammaglobulinemia was set relatively low, since IgG levels below 16 g/L are considered normal. One could speculate that patients with higher levels of immunoglobulins might have shown significant reductions. In line with this assumption HCQ did significantly reduce serum Ig levels in patients that were included based on higher baseline serum Ig levels [119] (Fig. 2). As such, stratifying patients on the basis of

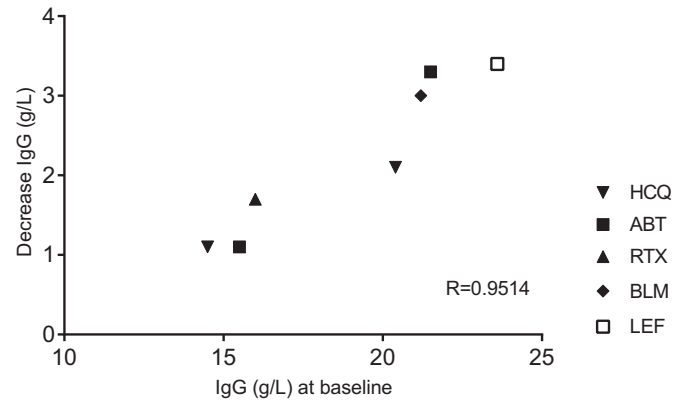


Fig. 2. Clinical trials of bDMARDs and cDMARDs demonstrate inhibition of B-cell hyperactivity. Inhibition of serum IgG levels after treatment with hydroxychloroquine (HCQ), abatacept (ABT), rituximab (RTX), belimumab (BLM) and leflunomide (LEF) are shown. Correlation (r) between IgG level at baseline and decrease of IgG level is indicated ($p = 0.001$). cDMARDs HCQ and LEF perform equal to biologicals ABT, RTX and BLM. References: HCQ [120] [119]; ABT [7] [5]; RTX [8]; BLM [10]; LEF [108].

these immunoglobulin values might have led to overlooking important effects of HCQ immune activation, which can have important implications for effects on long-term risks.

The before mentioned example indicates that no clear consensus exists on the clinical and immunological parameters that should lead to proper evaluation of efficacy of the different treatment options. In this respect it might be wise to target and evaluate different clinical outcomes separately. In particular since hallmarks of the disease such as dryness, fatigue, immune activation and clinical symptoms/disease activity as captured e.g. by Clinical ESSDAI (ClinESSDAI) are poorly correlating. Considering that dryness, often chosen as main outcome, has not been significantly influenced by any of the current systemic treatments, such an approach might open up ways for selective treatment modalities. In this respect, besides immunoglobulin levels, immunological outcomes of the different cDMARDs in pSS have been poorly studied. Nonetheless, if dryness is disregarded as main outcome, but for example immunoglobulin levels as a reflection of disease activity are evaluated, cDMARDs strikingly show equal results within patients with hypergammaglobulinemia compared to bDMARDs (Fig. 2). Given the association of B-cell hyperactivity with severe disease in particular on the long-term this is an important observation.

In addition to the above-mentioned drawbacks, biomarkers to predict response to therapy but also to monitor therapy efficacy are lacking. In this respect promising results were shown in a double-blind, placebo-controlled trial in SLE patients investigating efficacy of IFN- α receptor blocking monoclonal antibody Anifrolumab. This study showed a significantly greater response rate in patients with a high IFN-signature at baseline compared to IFN-signature negative patients. Considering the important role of IFNs in pSS pathology, in particular on B-cell hyperactivity and severe disease [128], the presence of an IFN-signature might hold promise in pSS patients as a biomarker predicting response to IFN-inhibiting therapy, possibly also cDMARDs like HCQ which potently targets the type I IFN response. In the light of the recent association between IFN- α signatures and immunoglobulin levels it is tempting to speculate that HCQ - as inhibitor of TLR signaling - is effective in diminishing long-term disease risk in those individuals with increased immunoglobulin levels. Biomarkers could also be used to monitor efficacy of therapy. An open-label study investigating efficacy of LEF in pSS patients [108] showed that LEF significantly inhibited T-cell produced IFN- γ only in the group of pSS patients that was classified as clinical responders, thereby validating the supposed inhibitory effect of LEF on T-cells secreting type II IFNs [109]. Possibly biomarkers like type I and II IFNs could serve as a biomarker to monitor therapy efficacy in pSS and provide tools to treat patients with tailored immunotherapy.

Numerous studies have shown synergism between multiple biological processes and different cell types. In pSS patients we recently showed synergism between IL-7- induced CD4 T-cell activation and TLR-7-induced B-cell activation, leading to enhanced Th cell cytokine and B-cell immunoglobulin production. The presence of antigen presenting cells (monocytes/macrophages in this case) was proven to be critical in this [134]. Considering the fact that potential additive pathways are upregulated in pSS patients (e.g. increased IL-7/IL7R [44] and TLR7 pathways [135]) it is anticipated that molecular and cellular synergism between the different key cell subsets in pSS will occur and might be of great importance. Hence it seems reasonable to conclude that targeting pathways affecting T-cell and B-cell activation, and at the same time also DC activation could be essential for successful treatment of pSS. Considering the different mechanisms of action of the different cDMARDs, combination therapy with two complementary cDMARDs might be worthwhile. MTX, SSZ, LEF and CyA, based on their shared immunomodulatory effects and favorable safety profiles, all are good candidates to be combined with a DMARD with a distinct mechanism of action, in order to target distinct immunopathological pathways in pSS pathology. The ability of HCQ to inhibit this and TLR-mediated immune

The combination of LEF, targeting mainly activated T-cells and to a lesser extent B-cells, and HCQ, targeting mainly B-cells and TLR-mediated processes, is a promising option that we recently tested *in vitro*. In a culture system mimicking pSS pathology by TCR/TLR9 stimulation of PBMCs of pSS patients, a combination of LEF and HCQ complementarily inhibited T and B-cell proliferation, production of IFN- α , CXCL13, IgG and IgM in clinically relevant concentrations. IFN- γ production persisted however additive inhibition was seen using a substantial concentration of HCQ in combination with a clinically relevant concentration of LEF [127]. These results indicate that a combination of LEF and HCQ inhibits the activity of the important players in pSS pathology, namely DCs, T and B-cells. Moreover, the positive clinical results of these separate cDMARDs in pSS are encouraging. It is striking how the clinical results of LEF and HCQ resemble those of the biologicals Rituximab, Abatacept and Belimumab (Table 1). When considering safety and clinical experience of these drugs in pSS treatment, a combination of LEF and HCQ seems a good therapeutic option and is currently being explored in a double blind placebo-controlled trial (EudraCT 2014-003140-12).

pSS is a multifactorial disease with an immunopathology that is the resultant of an interplay between tissue cells and both the innate and the acquired immune system. Several cDMARDs target many of the immunopathological pathways involved in pSS with unique mechanisms of action and therefore are potentially effective in inhibiting pSS immunopathology. Efficacy of cDMARDs in pSS is poorly studied and deserves proper comparison with bDMARDs by applying the same inclusion criteria and clinical outcome measures and by designing well-controlled clinical (dose finding) studies and employing biomarkers to stratify patients to optimize treatment efficacy. In this respect targeting multiple immunopathological pathways by combining complementary cDMARDs could be a promising strategy. The results of a clinical study in pSS investigating such a combination (leflunomide and hydroxychloroquine), with comparable inclusion and response criteria as used for bDMARDs, are underway. Clinical results of these cDMARDs separately show promising results on clinical parameters, in particular inhibition of B-cell hyperactivity, comparable to those of recently tested biologicals. Considering their harmonizing mechanisms of action, it is hypothesized that a combination will result in improved inhibition of disease activity and immune activation. If positive this might set the stage for combination trials of cDMARDs for treatment of pSS and might renew the interest in cDMARDs as therapeutic options for pSS, optimizing their efficacy.

Table 1
Clinical and immunological effects of different treatment options in pSS.

	Ref	ESSDAI	ESSPRI	LFS	Oral dryness (subjective)	Ocular dryness (subjective)	Saliva production	Schirmer	ESR	CRP	IgG
MTX	Skopouli et al. [63]	NI	NI	NI	Improved	Improved	No change	No change	No change	NI	No change
AZA	Price et al. [90] RCT	NI	NI	NI	No change	No change	NI	No change	No change	NI	No change
CYA	Drosos et al. [98] RCT	NI	NI	Stable in CyA group, worse in placebo group	Significant improvement compared to placebo	No change	No change	No change	NI	NI	NI
LEF	Van Woerkom et al. [104] Open study	NI	NI	Improved in 4 out of 5 patients	No change	No change	No change	Trend towards increase	No change	No change	Significant decrease

Table 1 (continued)

	Ref	ESSDAI	ESSPRI	LFS	Oral dryness (subjective)	Ocular dryness (subjective)	Saliva production	Schirmer	ESR	CRP	IgG
HCQ	Kruize et al. [115] RCT	NI	NI	NI	No change	No change	NI	No change	Trend towards decrease	NI	Significant decrease
	Gottenberg et al. [116]	No change	No change	NI	No change	No change	No change	No change	Significant decrease	NI	No change
ABT	Meiners et al. [5] Open study	Significant decrease	Significant decrease	NI	NI	NI	No change	No change	NI	NI	Significant decrease
	Adler et al. [7] Open study	NI	NI	Significant decrease	No change	No change	Significant increase(*)	NI	NI	NI	No change
BLM	Mariette et al. [10] Open study	Significant decrease	Significant decrease	NI	Significant improvement (dryness in general)	Significant improvement (dryness in general)	No change	No change	NI	NI	Significant decrease
RTX + pred	Devauchelle-Pensec [8] RCT	No change	NI	No change	No change	No change	No change	No change	No change	No change	Significant decrease
	Carubbi et al. [6] Prospective follow-up study (**)	Significant decrease	NI	Significant decrease	Significant improvement (dryness in general)	Significant improvement (dryness in general)	Significant increase	Significant increase	NI	NI	No change
	Dass et al. [130] RCT	NI	NI	NI	NI	NI	No change	No change	NI	NI	No change
	Meijer et al. [4] RCT	NI	NI	NI	Significant improvement	Significant improvement	Significant increase	No change	NI	NI	Significant decrease

ABT, abatacept; AZA, azathioprine; BLM, belimumab; CRP, C-reactive protein; CYA, cyclosporine A; ESR, erythrocyte sedimentation rate; HCQ, hydroxychloroquine; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; LEF, leflunomide; MTX, methotrexate; NI, no information; pred, prednisone; RTX, rituximab; SSZ, sulfasalazine.

* Only reached significance when corrected for disease duration.

** Results were compared to cDMARDs (HCQ, MTX or CYA): DMARD therapy also caused significant improvement in dryness and ESSDAI score.

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