Abatacept ameliorates both glandular and extraglandular involvements in patients with Sjögren’s syndrome associated with rheumatoid arthritis: findings from an open-label, multicenter, 1-year, prospective study: the ROSE (Rheumatoid Arthritis with Orencia Trial Toward Sjögren’s Syndrome Endocrinopathy) and ROSE II trials

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**Running head:** Abatacept for glandular and extraglandular involvements in SS with RA
Abstract

Objective To clarify the efficacy and safety of abatacept for glandular and extraglandular involvements in Sjögren’s syndrome (SS) associated with rheumatoid arthritis (RA).

Patients and methods We performed an open-label, prospective, 1-year, observational multicenter study (ROSE and ROSE II trials) for SS with RA. The primary endpoint was the remission rate as measured by SDAI at 52 weeks after initiation of intravenous abatacept. The secondary endpoints included the changes in the Saxon’s test, Schirmer’s test, ESSDAI and ESSPRI. Adverse events and adherence rates during the study period were also analyzed.

Results 68 patients (36 in ROSE and 32 in ROSE II, all women) were enrolled in this study. The mean SDAI decreased significantly from 23.6±13.2 (±SD) at baseline to 9.9±9.5 at 52 weeks (P<0.05). Patients with SDAI remission increased from 0 (0 weeks) to 19 patients (27.9%) at 52 weeks. Saliva volume increased significantly from 2015.1±1695.4 (0 weeks) to 2311.3±1804.4 (24 weeks) mg/2 min (n=66, P<0.05). Tear volume increased significantly from 5.0±6.0 (0 weeks) to 5.6±6.3 (52 weeks) mm/5 min (n=52, P<0.05). Both ESSDAI and ESSPRI scores were significantly decreased at 12 weeks, and these responses were maintained up to 52 weeks. The rate of adherence to abatacept over the 52-week period was 83.8%. Twenty-two adverse events occurred in 15 patients, and 9 of these events were infections.

Conclusion Abatacept ameliorated both glandular and extraglandular involvements, as well as the systemic disease activities and patient-reported outcomes based on composite measures, in patients with SS associated with RA.

Introduction

Sjögren’s syndrome (SS) is an autoimmune disease characterized pathologically by lymphocytic infiltration into the exocrine glands (including the salivary and lacrimal glands) and clinically by dry mouth and dry eyes. SS is classified into primary SS, which is not associated with any other well-defined connective tissue disease (CTD), and secondary SS, which is associated with other well-defined CTD [1]. Importantly, 14.5% of patients with rheumatoid arthritis (RA) have been reported to have associated secondary SS [2].

With regard to the treatment for SS proposed by currently published European League Against Rheumatism (EULAR) recommendations [3] and clinical practice guideline for SS 2017 in Japan [4],
topical therapy and pharmacologic stimulation with muscarinic agonists against glandular involvements, as well as corticosteroids and immunosuppressants for extraglandular involvements such as in the joints, skin, lungs, kidneys, and nervous system based on disease activities have been recommended. Although some biologic agents including rituximab and abatacept have been suggested to improve active and intractable extraglandular manifestations of SS, the effectiveness of these drugs against glandular involvements and of the currently established composite measures such as EULAR Sjögren’s Syndrome Disease Activity Index (ESSDAI) and EULAR Sjögren’s Syndrome Patient Reported Index (ESSPRI) has not been confirmed [5]. For abatacept, 3 open-label prospective studies [6, 7, 8] revealed its effectiveness against glandular and extraglandular involvements of primary SS, as well as of the ESSDAI and ESSPRI. However, findings of 2 recently published randomized controlled trials (RCTs) [9, 10] showed that abatacept could not significantly improve the glandular manifestations, ESSDAI, and ESSPRI of primary SS as compared with the placebo, while some biologic markers including immunoglobulin, rheumatoid factor, and complement were significantly changed by abatacept when compared with the placebo.

On the other hand, it was reported that RA associated with SS showed higher RA disease activity [2], more progressive joint destruction [11], and more resistance against TNF inhibitors [12, 13] in comparison with isolated RA without SS. Thus, RA associated with SS seems to have a higher risk in terms of difficult to treat RA (D2T RA) [14, 15], which has been the focus of a recent research agenda by rheumatologists.

We previously revealed that intravenous abatacept could ameliorate SS manifestations including salivary and lacrimal secretions, in addition to RA manifestation among SS associated with RA in a previous open-label, prospective, 1-year, observational multicenter study named the ROSE (Rheumatoid Arthritis with Orencia Trial Toward Sjögren’s Syndrome Endocrinopathy) trial [16, 17]. A therapeutic study targeting SS associated with RA might be clinically important from the following 2 points of view: First, it could contribute to establishment of a new treatment strategy for SS. Second, it could provide new insight into the management of D2T RA mentioned above.

We performed an open-label, prospective, 1-year, observational, multicenter study (ROSE II trial) to confirm the efficacy and safety of intravenous abatacept against both SS and RA involvements in patients with SS associated with RA, targeting many more cases and centers in Japan. In this report, we present the combined results of the ROSE and ROSE II trials.
Patients and methods

Patients

Patients aged 20 years or older with a diagnosis of RA according to the American College of Rheumatology (ACR) 1987 [18] or ACR/EULAR 2010 [19] criteria and of SS according to the 1999 Japanese Ministry of Health criteria for the diagnosis of SS [20] and the 2002 revised version of the European criteria proposed by the American-European Consensus Group [1], who presented with sicca symptoms were eligible for this study. The 1999 Japanese Ministry of Health criteria for the diagnosis of SS include the presence of 2 or more of the following 4 clinicopathologic findings: (1) lymphocytic infiltration of the salivary or lacrimal glands, (2) dysfunction of the salivary glands, (3) keratoconjunctivitis sicca (KCS), (4) presence of anti-SS-A or SS-B antibodies [20]. The patients were followed up at the Departments of Rheumatology of 13 hospitals in Japan (University of Tsukuba Hospital, University of Occupational and Environmental Health Hospital, Nagasaki University Hospital, Hokkaido University Hospital, Kanazawa University Hospital, Keio University Hospital, Nihon University Hospital, Juntendo University Hospital, Saitama Medical University Hospital, Kyoto University Hospital, National Hospital Organization Osaka Minami Medical Center Hospital, Hyogo College of Medicine Hospital, and Kurashiki Medical Center Hospital). Approval for this study was obtained from the local ethics committee of each study site, and signed informed consent was obtained from each participant (approval number from the University of Tsukuba: H23-29, certification date of approval: 28 July, 2011 for the ROSE trial; H26-69 and 4 August, 2014 for the ROSE II trial). This study was registered at the University Hospital Medical Information Network (UMIN)-Clinical Trials Registry (CTR) (UMIN-ID: UMIN000005724 for the ROSE trial; UMIN000016273 for the ROSE II trial).

Patients with contraindications for abatacept (eg, hypersensitivity, severe infection, hepatitis B virus infection); aged older than 75 years or younger than 20 years; with leukopenia (leukocyte count ≤3000/mm$^3$); with severe liver or kidney disease; with severe hematologic disorders; negative for both anti-SS-A and SS-B antibodies and positive for anticientromere antibody were excluded from this study. Patients who were pregnant, nursing, or wanted to become pregnant and those being treated with palliative therapies for dryness including with cevimeline, anethole trithione, and pilocarpine within the last 4 weeks were also excluded. Patients who were considered unsuitable for this study by their attending physician were also excluded.
Medications

The dosing regimen approved for the treatment of RA was used in this study. The weight-adapted dose of abatacept (500 mg for patients weighing <60 kg, 750 mg for those weighing ≥60 kg) was administered intravenously at weeks 0, 2, and 4, every 4 weeks, over a period of 1 year. Other disease-modifying antirheumatic drugs (DMARDs), corticosteroids, and nonsteroidal antiinflammatory drugs (NSAIDs) were allowed to be used during the 1-year treatment period according to the clinical judgment of the attending physician.

Analysis of abatacept efficacy

The ROSE trial and ROSE II trial were each designed as an open-label, 1-year, prospective, observational study. For RA manifestations, the number of tender and swollen joints among 28 joints, the physicians’ global visual analog scale (VAS), the patients’ global VAS, the Simplified Disease Activity Index (SDAI), serum C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), rheumatoid factor (RF), and anticyclic citrullinated peptide (CCP) antibody were assessed at weeks 0 (baseline), 4, 12, 24, and 52. For SS manifestations, patients’ VAS for dry mouth, dry eye, and parotid pain; physicians’ VAS for dry mouth, KCS, and general condition; saliva volume measured using the Saxon’s test; tear volume measured using the Schirmer’s test; anti-SS-A/SS-B antibody; and serum IgG level were examined at weeks 0, 12, 24, and 52. Established composite measures for SS such as the ESSDAI [21, 22, 23, 24] and the ESSPRI [22, 23, 25] were also assessed in the ROSE II trial.

The primary endpoint was the percentage of patients who achieved clinical remission as assessed using the SDAI at 52 weeks. The secondary endpoints included changes in the SDAI, Saxon’s test, Schirmer’s test, IgG, ESSDAI, and ESSPRI.

Analysis of safety of abatacept

Adverse events (AEs) during the 52-week study period were analyzed at each visit. The types of AEs, onset, use of corticosteroids, treatment for AEs, hospital admission, cessation of abatacept use, association with abatacept, and outcome were recorded. We also recorded the rate of adherence to abatacept over the 52-week period and the causes of abatacept discontinuation.
Statistical analysis

Data were expressed as means±SDs. Differences between measures taken at baseline and those taken after treatment with abatacept were examined for significance using the Wilcoxon signed rank test. Differences between groups were examined using the Mann–Whitney U test for continuous variables. Categories of disease activity assessed using the SDAI and ESSDAI were examined by the Cochran-Armitage Test. Probability values less than 0.05 were considered to denote the presence of a significant difference. The rate of adherence to abatacept was analyzed using the Kaplan-Meier method. Deficits of data were compensated for by use of the last observation carried forward (LOCF) method.

Results

Clinicopathologic features at baseline

Sixty-eight patients (36 in ROSE and 32 in ROSE II, all women) were enrolled in this study. The baseline clinicopathologic features of the 68 patients are summarized in Table 1. The patients’ mean age was 56.1±12.5 years and their RA disease duration was 118.8±136.8 months. The disease in most of the patients were assessed as stage I or II and class 1 or 2 according to the Steinbrocker classification. The RA disease activity assessed using the SDAI was 23.6±13.2 (remission: 0 cases, low: 10 cases, moderate: 33 cases, high: 25 cases). The positivities of RF and anti-CCP antibody were 85.3% and 79.4%, respectively.

For SS manifestations, saliva volume assessed using the Saxon’s test was 2015.1±1695.4 mg/2 min (n = 66); tear volume assessed using the Schirmer’s test was 5.0±6.0 mm/5 min (n = 52); and Greenspan grading of the labial salivary gland (LSG) biopsy was grade 0 in 2 patients, grade 1 in 11 patients, grade 2 in 2 patients, grade 3 in 18 patients, grade 4 in 20 patients, and unknown in 13 patients. The positivities of anti-SS-A and anti-SS-B antibodies were 86.6% and 23.4%, respectively. 15 anti-SS-B antibody positive cases were all positive for anti-SS-A antibody. Thus, the present study did not include any patients with positive for isolated anti-SS-B antibody. In the ROSE II trial-enrolled patients, the ESSDAI was 9.5±4.6 (n = 32), and the ESSPRI was 5.7±2.0 (n = 32).

Twelve of the 68 patients (17.6%) were previously treated with biologics other than abatacept, whilst 56 patients were biologics-naïve. Forty-two of the patients (61.8%) were treated with concomitant methotrexate (mean dose: 9.4±3.5 mg/week), and 34 of the patients (50.0%) were treated with
Collectively, the enrolled patients had secondary SS with relatively preserved secretory functions and moderate systemic disease activity, in addition to moderately active long-standing RA.

**Effectiveness of abatacept against RA involvement**

The disease activity of RA assessed using the SDAI significantly decreased after treatment with abatacept, from 23.6±13.2 (0 weeks, baseline) to 9.9±9.5 (52 weeks) (P<0.05). Significant reduction in the SDAI relative to baseline (P<0.05) was noted at 4 weeks and was maintained over the 52-week period (Figure 1A). Patients with clinical remission, as assessed using the SDAI, increased from 0 patients at 0 weeks to 19 patients (27.9%) at 52 weeks (primary endpoint); patients with low disease activity also increased, from 10 patients (14.7%) at 0 weeks to 46 patients (67.6%) at 52 weeks (Figure 1B). In contrast, the number of patients with moderate or high disease activity, as assessed using the SDAI, decreased from 58 patients (85.3%) at 0 weeks to 22 patients (32.4%) at 52 weeks (Figure 1B). The number of patients with remission or low disease activity according to the SDAI was significantly increased (P<0.05), whereas those with moderate or high disease activity was significantly decreased (P<0.05), after 4 weeks as compared with baseline (Figure 1B). These observations confirmed the effectiveness of abatacept against RA manifestations in patients with SS with RA, which could become D2T RA.

**Effectiveness of abatacept against SS involvement**

Saliva volume as assessed using the Saxon’s test significantly increased after treatment with abatacept, from 2015.1±1695.4 mg/2 min at 0 weeks to 2219.1±1758.5 mg/2 min at 12 weeks and to 2311.3±1804.4 mg/2 min at 24 weeks (n = 66, P<0.05) (Figure 2A). However, saliva volume slightly decreased, to 2177.3±1739.9 mg/2 min at 52 weeks, resulting in no significant difference between the measurements at baseline and those at 52 weeks (Figure 2A). Importantly, in patients whose Greenspan LSG biopsy grading was grade 0, 1, or 2 (n = 16), saliva volume assessed using the Saxon’s test was significantly increased at 12 weeks, and the response was maintained up to 52 weeks as compared with baseline (P<0.05) (Figure 2B). On the other hand, in patients whose Greenspan LSG
biopsy grading was grade 3 or 4 (n = 37), saliva volume was also significantly increased at 12 and 24 weeks, but had returned at 52 weeks to the baseline level (P<0.05) (Figure 2B).

Tear volume measured using the Schirmer’s test significantly increased after treatment with abatacept, from 5.0±6.0 mm/5 min at 0 weeks to 5.9±7.1 mm/5 min at 24 weeks and to 5.6±6.3 mm/5 min at 52 weeks (n = 52, P<0.05) (Figure 2C).

Treatment with abatacept resulted in significant reductions in serum IgG levels, from 1727.7±529.5 mg/dL at 0 weeks to 1587.5±457.2 mg/dL at 12 weeks (n = 67, P<0.05), and this significant reduction in IgG was maintained over the 52-week period (Figure 2D). No patients showed hypogammaglobulinemia (<500 mg/dL) during the study period. Abatacept also significantly reduced serum RF levels from 195.6±316.8 IU/ml at 0 weeks to 151.5±232.6 IU/ml at 12 weeks, and 168.0±326.8 IU/ml at 24 weeks (n = 67, P<0.05), while serum RF levels significantly re-increased to 221.6±618.8 IU/ml at 52 weeks compared with baseline (n = 67, P<0.05) (Figure 2E).

In the ROSE II trial-enrolled 32 patients, the ESSDAI was significantly improved at 12 weeks, and the improvement was maintained up to 52 weeks as compared with baseline (P<0.05) (Figure 3A). The number of patients with high disease activity as assessed using the ESSDAI was significantly decreased at 12 weeks, whilst those with low disease activity was significantly increased, and the response was maintained over the 52-week period as compared with baseline (P<0.05) (Figure 3B). For each ESSDAI domain, disease activities in the constitutional, lymphadenopathy, glandular, articular, and biologic domains were reduced at 52 weeks as compared with baseline (Figure 3C). Moreover, the ESSPRI was also significantly improved at 12 weeks, and the improvement was maintained up to 52 weeks as compared with baseline in these 32 cases (P<0.05) (Figure 4A). Among the ESSPRI components, only the pain component was significantly improved at 12 weeks and was maintained up to 52 weeks as compared with baseline (P<0.05), whereas the fatigue did not significantly change, and the dryness was significantly and transiently improved at 12 weeks only (P<0.05) (Figure 4B, 4C, and 4D).

These results suggest the effectiveness of abatacept for secretory dysfunction, immunologic abnormalities, as well as for systemic disease activities and patient-reported outcomes (PRO), in patients with SS with RA.

Adherence to and safety of abatacept

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The rate of adherence to abatacept for 52 weeks was 83.8% (57/68) (Supplementary Figure 1). Eleven patients dropped out before completion of the 52-week study. The reasons for the dropout were inadequate effects in 5 patients, AEs in 3 patients, and other reasons (hospital transfer, economic reasons, and no visiting) in 3 patients.

Twenty-two AEs were recorded in 15 of the 68 patients (22.1%) over the 52-week period. Of those 22 AEs, 9 events (40.9%) were infections (2 urinary tract infections, 1 infectious cornea ulcer, 1 bronchitis, 1 herpes zoster, 1 sinusitis, 1 upper respiratory tract infection, 1 pharyngitis, 1 suspected cellulitis of the left lower leg). Abatacept was discontinued owing to AEs in 3 patients. Most AEs (86.4%, 19/22 events) fully recovered within the observation period (Table 2).

Discussion
In this multicenter, observational, and prospective study, we confirmed the effectiveness and safety of abatacept for patients with SS associated with RA, showing that both RA- and SS-related manifestations including established composite measures were significantly improved by 52 weeks’ treatment with intravenous abatacept. From these results, we have revealed the following four clinically important findings for the therapeutic strategy of SS associated with RA.

First, abatacept improved the RA disease activity of patients with SS associated with RA who had a higher risk for D2T RA [14, 15]. In comparison with isolated RA without SS, SS associated with RA could become D2T RA for several reasons, such as showing higher RA disease activity [2], more progressive joint destruction [11], and more resistance against TNF inhibitors [12, 13]. Regarding resistance against one of the TNF inhibitors, infliximab, among anti-SS-A antibody-positive RA, we previously proposed 3 possible mechanisms [12]: higher frequency of human antichimeric antibody (HACA), seroconversion of antinuclear antibody (ANA) positivity, and lower serum TGF-ß levels. Anti-SS-A antibody, which was detected in 33% to 74% of primary SS patients [26] but in 3% to 15% of RA patients [27], has 2 target antigens, Ro52kDa and Ro60kDa proteins [28]. Ro52kDa protein, also called tripartite motif-containing 21 (TRIM21), could function as an intracellular Fc receptor, resulting in activation of innate immune signaling as well as limiting of immune signaling via autophagy of activated dimeric IFN regulatory factor-3 (IRF3) [29]. Interestingly, a recent study showed that anti-Ro52 (TRIM21) antibody-positive patients with systemic lupus erythematosus (SLE) had enhanced differentiation of plasmablasts when compared with negative SLE patients and that anti-Ro52
(TRIM21) antibody might contribute to the pathogenesis of SLE and SS and B-cell differentiation via suppression of TRIM21 function [30]. Collectively, these observations suggest that RA associated with SS, which frequently has anti-SS-A (Ro52, TRIM21) antibody, could have enhanced B cells and plasmablast differentiation, causing higher HACA and ANA production after infliximab treatment. Abatacept reportedly reduced serum levels of rheumatoid factor (RF) and anticitrullinated peptide antibody (ACPA) in RA patients, and these reductions correlated with the clinical responses [31]. Thus, abatacept seems to be an appropriate therapeutic strategy for RA manifestation in SS associated with RA because it could inhibit B-cell activation and antibody production via suppression of T cells, which might contribute to resistance against TNF inhibitors, especially against infliximab.

Second, abatacept increased salivary and lacrimal secretion in patients with SS associated with RA. In contrast to our results, the results from 2 recently published RCTs showed that abatacept could not significantly improve glandular manifestations [9, 10]. One of the reasons why abatacept could improve the secretory function of SS associated with RA in the present study might be relatively preserved secretory salivary and lacrimal functions. Importantly, saliva volume was increased and was maintained over the 52-week period after initiation of abatacept in patients with mildly infiltrated LSG, whereas saliva volume returned at 52 weeks to baseline levels in patients with advanced infiltrated LSG. Moreover, a previous study showed that abatacept increased saliva secretion in primary SS patients, when adjusting for disease duration, indicating that treatment early in the disease course is more effective than at a later stage [6]. Thus, early intervention for SS, which has relatively preserved secretory function and mild infiltration and survival of glandular tissues in LSG, might be necessary to obtain and maintain the adequate secretory improvement by abatacept. For lacrimal glands involvements of SS, the histopathology of lacrimal glands has not been adopted by current 2016 ACR/EULAR classification criteria for primary SS [32, 33]. In the present study, we did not examine the pathological features of lacrimal glands. Thus, we could not analyze the association between the effect of abatacept on lacrimal secretion and the disease stage of SS in lacrimal glands such as the early disease of SS where lacrimal inflammation was active without fibrosis or the late disease with advanced fibrosis. However, we could speculate that intervention by abatacept might be more effective on lacrimal function in the early disease of SS than in the late disease, as same as salivary function. Regarding SS associated with RA, RA was reported to precede SS in the majority of patients [2]. Therefore, we have a chance to diagnose early SS among patients with RA who are followed up and
treated at rheumatology clinics. Unfortunately, we did not analyze whether SS preceded RA, or RA preceded SS in the enrolled cases. If RA patients develop secondary SS during RA treatment, abatacept might be an alternative therapy that could be beneficial for both RA and SS.

Third, in the present study we clarified, for the first time, that abatacept could improve the composite disease activity of SS assessed using the ESSDAI and the patient-reported outcomes based on the ESSPRI among patients with SS associated with RA. To date, many biologics including abatacept [9, 10], rituximab [34, 35, 36], infliximab [37], etanercept [38], tocilizumab [39], anti-ICOS ligand monoclonal antibody [40], ianalumab [41], and baminercept (lymphotoxin β receptor fusion protein) [42] have failed to obtain improvement in the ESSDAI and ESSPRI in current RCTs. Iscalimab (anti-CD40 monoclonal antibody) is the sole biologic that has been reported to significantly improve the ESSDAI when compared with placebo, in an RCT targeting primary SS [43]. Why is the effectiveness of biologics for SS manifestations limited? The following reasons have been proposed [44]. First, because of the chronicity and slowly progressive nature of the disease, the underlying pathogenic mechanisms are well established and the disease is advanced when biologics are initiated. Second, in most clinical trials, the observation time was limited to either 24 or 48 weeks, a relatively short period to record a significant improvement in clinical manifestations in a chronic and slowly progressive disease. Third, the diversity of phenotypes and endotypes of the disease may have also interfered with the outcomes and primary endpoints of many clinical trials conducted to assess biologic treatments in SS. Lastly, we still do not understand all the pathophysiologic mechanisms involved in different phases of the disease. SS associated with RA might be a distinct phenotype for which abatacept could modify the pathogenic process, and biologics could be initiated earlier for these patients than for patients with isolated SS without RA, as described above. Importantly, the previous immunohistochemical studies comparing the composition of lymphocytic infiltrates in LSG between RA patients with sicca symptoms and primary SS patients reported that milder lesions, together with increased prevalence of dendritic cells and lower prevalence of CD4+ cells in the RA-sicca subtype [45]. Therefore, we could suppose that abatacept might inhibit sialadenitis more successfully in patients with SS associated with RA than in primary SS, because of milder lesions in SS with RA. To confirm these speculations, RCTs of abatacept and other biologics targeted on not only primary SS but also secondary SS associated with RA would be needed.

Forth, abatacept was well tolerated among patients with SS associated with RA in the present study, showing that the rate of adherence to abatacept for 52 weeks was 83.8%. However, 22.1% of
enrolled patients experienced AEs of which 40.9% were infections. Although many infectious events were common and not specific for patients with SS associated with RA, one case developed infectious cornea ulcer which could be related with dry eye and KCS.

In conclusion, in patients with SS associated with RA, abatacept ameliorated both the glandular and the extraglandular involvements, as well as the systemic disease activities and patient-reported outcomes based on composite measures. To confirm the effectiveness of abatacept on SS and RA manifestations, RCTs especially targeting patients with SS associated with RA are needed.

References
1 Vitali C, Bombardieri S, Jonsson R, et al. Classification criteria for Sjögren’s syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. Ann Rheum Dis. 2002;61:554-558.
2 He J, Ding Y, Feng M, et al. Characteristics of Sjögren’s syndrome in rheumatoid arthritis. Rheumatology (Oxford). 2013;52:1084-1089.
3 Ramos-Casals M, Brito-Zerón P, Bombardieri S, et al. EULAR recommendations for the management of Sjögren's syndrome with topical and systemic therapies. Ann Rheum Dis. 2020;79:3-18.
4 Sumida T, Azuma N, Moriyama M, et al. Clinical practice guideline for Sjögren's syndrome 2017. Mod Rheumatol. 2018;28:383-408.
5 Brito-Zerón P, Retamozo S, Kostov B, et al. Efficacy and safety of topical and systemic medications: a systematic literature review informing the EULAR recommendations for the management of Sjögren's syndrome. RMD Open. 2019;5:e001064.
6 Adler S, Körner M, Förger F, et al. Evaluation of histological, serological and clinical changes in response to abatacept treatment of primary Sjögren's syndrome: A pilot study. Arthritis Care Res (Hoboken). 2013;65:1862-1868.
7 Meiners PM, Vissink A, Kroese FG, et al. Abatacept treatment reduces disease activity in early primary Sjögren's syndrome (open-label proof of concept ASAP study). Ann Rheum Dis. 2014;73:1393-1396.
8 Machado AC, Dos Santos LC, Fidelix T, et al. Effectiveness and safety of abatacept for the
treatment of patients with primary Sjögren's syndrome. Clin Rheumatol. 2020;39:243-248.

9 Jolien F van Nimwegen, Esther Mossel, Greetje S van Zuiden, et al. Abatacept treatment for patients with early active primary Sjögren's syndrome: a single-centre, randomised, double-blind, placebo-controlled, phase 3 trial (ASAP-III study). Lancet Rheumatol. 2020;2:E153-E163.

10 Baer AN, Gottenberg JE, St Clair EW, et al. Efficacy and safety of abatacept in active primary Sjögren's syndrome: results of a phase III, randomised, placebo-controlled trial. Ann Rheum Dis. 2021;80:339–348.

11 Brown LE, Frits ML, Iannaccone CK, Weinblatt ME, et al. Clinical characteristics of RA patients with secondary SS and association with joint damage. Rheumatology (Oxford). 2015;54:816-820.

12 Hagiwara S, Tsuboi H, Honda F, et al. Association of anti-Ro/SSA antibody with response to biologics in patients with rheumatoid arthritis. Mod Rheumatol. 2016;26:857-862.

13 Matsudaira R, Tamura N, Sekiya F, et al. Anti-Ro/SSA antibodies are an independent factor associated with an insufficient response to tumor necrosis factor inhibitors in patients with rheumatoid arthritis. J Rheumatol. 2011;38:2346-2354.

14 Nagy G, Roodenrijs NMT, Welsing PM, et al. EULAR definition of difficult-to-treat rheumatoid arthritis. Ann Rheum Dis. 2021;80:31-35.

15 Roodenrijs NMT, de Hair MJH, van der Goes MC, et al. Characteristics of difficult-to-treat rheumatoid arthritis: results of an international survey. Ann Rheum Dis. 2018;77:1705-1709.

16 Tsuboi H, Matsumoto I, Hagiwara S, et al. Efficacy and safety of abatacept for patients with Sjögren's syndrome associated with rheumatoid arthritis: rheumatoid arthritis with ocrenia trial toward Sjögren's syndrome Endocrinopathy (ROSE) trial-an open-label, one-year, prospective study-Interim analysis of 32 patients for 24 weeks. Mod Rheumatol. 2015;25:187-193.

17 Tsuboi H, Matsumoto I, Hagiwara S, et al. Effectiveness of abatacept for patients with Sjögren's syndrome associated with rheumatoid arthritis. An open label, multicenter, one-year, prospective study: ROSE (Rheumatoid Arthritis with Ocrenia Trial toward Sjögren's syndrome Endocrinopathy) trial. Mod Rheumatol. 2016;26:891-899.

18 Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum. 1988;31: 315-324.

19 Aletaha D, Neogi T, Silman AJ, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis
Rheum. 2010;62: 2569-2581.

20 Fujibayashi T, Sugai S, Miyasaka N, et al. Revised Japanese criteria for Sjögren’s syndrome (1999): availability and validity. Mod Rheumatol. 2004;14:425-434.

21 Seror R, Ravaud P, Bowman SJ, et al. EULAR Sjögren's Task Force. EULAR Sjogren's syndrome disease activity index: development of a consensus systemic disease activity index for primary Sjogren's syndrome. Ann Rheum Dis. 2010;69:1103-1109.

22 Seror R, Theander E, Brun JG, et al. EULAR Sjögren's Task Force. Validation of EULAR primary Sjögren's syndrome disease activity (ESSDAI) and patient indexes (ESSPRI). Ann Rheum Dis. 2015;74:859-866.

23 Seror R, Bootsma H, Saraux A, et al. on behalf of the EULAR Sjögren's Task Force. Defining disease activity states and clinically meaningful improvement in primary Sjögren's syndrome with EULAR primary Sjögren's syndrome disease activity (ESSDAI) and patient-reported indexes (ESSPRI). Ann Rheum Dis. 2016;75:382-389.

24 Seror R, Bowman SJ, Brito-Zeron P, et al. EULAR Sjögren's syndrome disease activity index (ESSDAI): a user guide. RMD Open. 2015;1:e000022.

25 Seror R, Ravaud P, Mariette X, et al. EULAR Sjögren's Task Force. EULAR Sjögren's Syndrome Patient Reported Index (ESSPRI): development of a consensus patient index for primary Sjögren's syndrome. Ann Rheum Dis. 2011;70:968-972.

26 Bournia VK, Vlachoyiannopoulos PG. Subgroups of Sjögren syndrome patients according to serological profiles. J Autoimmun. 2012;39:15-26.

27 Cavazzana I, Franceschini F, Quinzanini M, et al. Anti-Ro/SSA antibodies in rheumatoid arthritis: clinical and immunologic associations. Clin Exp Rheumatol. 2006;24:59-64.

28 Routtas JG, Tzioufas AG. Sjögren's syndrome--study of autoantigens and autoantibodies. Clin Rev Allergy Immunol. 2007;32:238-251.

29 Rhodes DA, Isenberg DA. TRIM21 and the Function of Antibodies inside Cells. Trends Immunol. 2017;38:916-926.

30 Kunishita Y, Yoshimi R, Kamiyama R, et al. TRIM21 Dysfunction Enhances Aberrant B-Cell Differentiation in Autoimmune Pathogenesis. Front Immunol. 2020;11:98.

31 Scarsi M, Paolini L, Ricotta D, et al. Abatacept reduces levels of switched memory B cells, autoantibodies, and immunoglobulins in patients with rheumatoid arthritis. J Rheumatol.
32 Shiboski CH, Shiboski SC, Seror R, et al. 2016 American College of Rheumatology/European League Against Rheumatism classification criteria for primary Sjögren's syndrome: A consensus and data-driven methodology involving three international patient cohorts. *Ann Rheum Dis.* 2017;76:9-16.

33 Shiboski CH, Shiboski SC, Seror R, et al. 2016 American College of Rheumatology/European League Against Rheumatism Classification Criteria for Primary Sjögren's Syndrome: A Consensus and Data-Driven Methodology Involving Three International Patient Cohorts. *Arthritis Rheumatol.* 2017;69:35-45.

34 Devauchelle-Pensec V, Mariette X, Jousse-Joulin S, et al. Treatment of primary Sjögren syndrome with rituximab: a randomized trial. *Ann Intern Med.* 2014;160:233-242.

35 Bowman SJ, Everett CC, O'Dwyer JL, et al. Randomized Controlled Trial of Rituximab and Cost-Effectiveness Analysis in Treating Fatigue and Oral Dryness in Primary Sjögren's Syndrome. *Arthritis Rheumatol.* 2017;69:1440-1450.

36 Fisher BA, Everett CC, Rout J, et al. Effect of rituximab on a salivary gland ultrasound score in primary Sjögren's syndrome: results of the TRACTISS randomised double-blind multicentre substudy. *Ann Rheum Dis.* 2018;77:412-416.

37 Mariette X, Ravaud P, Steinfeld S, et al. Inefficacy of infliximab in primary Sjögren's syndrome: results of the randomized, controlled Trial of Remicade in Primary Sjögren's Syndrome (TRIPSS). *Arthritis Rheum.* 2004;50:1270-1276.

38 Sankar V, Brennan MT, Kok MR, et al. Etanercept in Sjögren's syndrome: a twelve-week randomized, double-blind, placebo-controlled pilot clinical trial. *Arthritis Rheum.* 2004;50:2240-2245.

39 Felten R, Devauchelle-Pensec V, Seror R, et al. Interleukin 6 receptor inhibition in primary Sjögren syndrome: a multicentre double-blind randomised placebo-controlled trial. *Ann Rheum Dis.* 2020, Online ahead of print.

40 Mariette X, Bombardieri M, Alevizos I, et al. A Phase 2a Study of MEDI5872 (AMG557), a Fully Human Anti-ICOS Ligand Monoclonal Antibody in Patients with Primary Sjögren’s Syndrome [abstract]. *Arthritis Rheumatol.* 2019;71 (suppl 10).

41 Dörner T, Posch MG, Li Y, et al. Treatment of primary Sjögren's syndrome with ianalumab
(VAY736) targeting B cells by BAFF receptor blockade coupled with enhanced, 
antibody-dependent cellular cytotoxicity. *Ann Rheum Dis.* 2019;78:641-647.

42 St Clair EW, Baer AN, Wei C, *et al.* Clinical Efficacy and Safety of Baminercept, a Lymphotxin β 
Receptor Fusion Protein, in Primary Sjögren’s Syndrome: Results From a Phase II Randomized, 
Double-Blind, Placebo-Controlled Trial. *Arthritis Rheumatol.* 2018;70:1470-1480.

43 Benjamin A Fisher, Antonia Szanto, Wan-Fai Ng, *et al.* Assessment of the anti-CD40 antibody 
iscalimab in patients with primary SJÖGREN’S SYNDROME: a multicentre, randomised, double-blind, 
placebo-controlled, proof-of-concept study. *Lancet Rheumatol* 2020;2:e142–152.

44 Tzioufas AG, Goules AV. Limited efficacy of targeted treatments in SJÖGREN’S SYNDROME: why? *Clin 
Exp Rheumatol.* 2018;36 Suppl 112:27-28.

45 Fragoulis GE, Fragkioudaki S, Reilly JH, *et al.* Analysis of the cell populations composing the 
mononuclear cell infiltrates in the labial minor salivary glands from patients with rheumatoid 
arthritis and sicca syndrome. *J Autoimmun.* 2016;73:85-91.

**Conflict of interest**

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Authors’ contributions

All the authors contributed to the design of the study, collection of the data, and writing of the manuscript, and all agree to accept equal responsibility for the accuracy of this paper’s contents.

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Table 1. Baseline clinicopathologic features of the 68 enrolled patients

| Feature                                      | Value                |
|----------------------------------------------|----------------------|
| **Age, years**                               | 56.1±12.5            |
| **Sex, M / F**                               | 0 / 68               |
| **RA disease duration, mo**                  | 118.8±136.8          |
| **RA stage, I / II / III / IV**              | 19 / 28 / 7 / 14     |
| **RA functional class, 1 / 2 / 3 / 4**       | 21 / 40 / 6 / 1      |
| **SDAI (remission / low / moderate / high)** | 23.6±13.2 (0 / 10 / 33 / 25) |
| **IgG, mg/dL (n = 67)**                      | 1727.7±529.5         |
| **RF-positive (n = 68) RF-titer, IU/ml (n = 67)** | 58 (85.3%) 195.6±316.8 |
| **Anti-CCP antibody-positive (n = 63)**      | 50 (79.4%)           |
| **Anti-SS-A antibody-positive (n = 67)**     | 58 (86.6%)           |
| **Anti-SS-B antibodies (n = 64)**            | 15 (23.4%)           |
| **Organ involvement**                        |                      |
| Interstitial lung disease (n = 68)           | 7 (10.3%)            |
| Others (n = 68)                              | 13 (19.1%)           |
| **Saxon’s test, mg/2 min (n = 66)**          | 2015.1±1695.4        |
| **Schirmer’s test, mm/5 min (n = 52)**       | 5.0±6.0              |
| **Greenspan LSG grade, 0 / 1 / 2 / 3 / 4 / unknown** | 2 / 11 / 4 / 18 / 20 / 13 |
| **ESSDAI (n = 32) (low / moderate / high)**  | 9.5±4.6 (4 / 21 / 7) |
| **ESSPRI (n = 32)**                          | 5.7±2.0              |
| **Concomitant methotrexate (n = 68)**        | 42 (61.8%), mean dose: 9.4±3.5 mg/week |
| **Concomitant corticosteroid (n = 68)**       | 34 (50.0%), mean dose: 5.5±2.7 mg/day of equivalent PSL |
| **Previous biologics (n = 68)**              | 17.6% (bio-switch 12, bio-naïve 56) (including overlap) |
| IFX                                          | 7                    |
| ETN                                          | 6                    |
| ADA                                          | 2                    |
| TCZ                                          | 3                    |
| GLM | 2 |
|-----|---|
| CER | 1 |

Left column: n represents the number of patients tested.
*: assessed only in ROSE II trial-enrolled patients.
RA, rheumatoid arthritis; SDAI, Simplified Disease Activity Index; LSG, labial salivary gland; ESSDAI, EULAR Sjögren’s Syndrome Disease Activity Index; ESSPRI, EULAR Sjögren’s Syndrome Patient Reported Index; PSL, prednisolone; IFX, infliximab; ETN, etanercept; ADA, adalimumab; TCZ, tocilizumab; GLM, golimumab; CER, certolizumab.
Table 2. Adverse events in 68 cases over the 52-week period

| Study | Case | Adverse events                        | Onset, weeks | Treatment required | ABT administration | Outcome       |
|-------|------|---------------------------------------|--------------|--------------------|--------------------|---------------|
| ROSE  | 1    | Urinary tract infection               | 7            | +                  | Cessation          | Recovery      |
|       | 2    | Urticaria                             | 0            | +                  | Continuation       | Recovery      |
|       | 3    | Skin rash                             | 0            | +                  | Discontinuation    | Recovery      |
|       | 4    | Infectious cornea ulcer              | 24           | +                  | Continuation       | Recovery      |
|       | 5    | Compression fracture of lumbar spine  | 12           | +                  | Cessation          | Recovery      |
|       | 6    | Bronchitis                            | 4            |                    | Cessation          | *Unknown     |
|       | 7    | Herpes zoster                         | 4            | +                  | Continuation       | Recovery      |
|       | 8    | Vomit and diarrhea                    | 8            | +                  | Discontinuation    | No recovery   |
|       | 9    | Sinusitis                              | 36           | +                  | Discontinuation    | No recovery   |
|       | 10   | Upper respiratory tract infection     | 22           | +                  | Continuation       | Recovery      |
|       |      | Pharyngitis                           | 32           | +                  | Continuation       | Recovery      |
|       |      | Stomatitis                            | 48           | +                  | Continuation       | Recovery      |
| ROSE II| 1    | Endometriotic ovarian cyst            | 14           | +                  | Continuation       | Recovery      |
|       | 2    | Liver dysfunction                     | 8            | +                  | Continuation       | Recovery      |
|       |      | Nonalcoholic steatohepatitis          | 28           | +                  | Continuation       | No recovery   |
|       | 3    | Urinary tract infection               | 36           | +                  | Continuation       | Recovery      |
|       | 4    | Loss of consciousness                 | 37           | -                  | Discontinuation    | Recovery      |
|       | 5    | Fracture of right humerus             | 23           | +                  | Cessation          | Recovery      |
|       |      | Suspected cellulitis of left lower leg | 48         | +                  | Cessation          | Recovery      |
|       | 5    | Stomatitis                            | 3            | +                  | Continuation       | Recovery      |
|       |      | Ulcer of auricle                      | 49           | +                  | Cessation          | Recovery      |
|       |      | Fever                                 | 50           | +                  | Cessation          | Recovery      |

*: Unknown because of hospital transfer; ABT, abatacept; +, Yes; -, No.
Figure legends

Figure 1. Effects of abatacept on RA involvement

(A) Efficacy of abatacept treatment on the Simplified Disease Activity Index (SDAI) in 68 patients. Data deficits were compensated for by use of the last observation carried forward (LOCF) method. *P<0.05 vs 0 weeks (baseline), Wilcoxon signed-rank test. The numbers represent the mean SDAI at each time point.

(B) Efficacy of abatacept treatment on disease activity as assessed using the SDAI in 68 patients over the 52-week period. Data deficits were compensated for by use of the LOCF method. *P<0.05 vs 0 weeks (baseline), Cochran-Armitage test. The numbers represent the cases in each category.
Figure 2. Effects of abatacept on SS involvement

(A) Effects of abatacept treatment on saliva volume assessed using the Saxon’s test in 66 patients. Data deficits were compensated for by use of the LOCF method. *P<0.05 vs 0 weeks (baseline), Wilcoxon signed rank test. The numbers represent the mean Saxon’s test value at each time point.

(B) Comparison of effects of abatacept treatment on saliva volume assessed using the Saxon’s test between patients with Greenspan LSG biopsy grading of 0, 1, or 2 (n = 16) and those with grading of 3 or 4 (n = 37). Data deficits were compensated for by use of the LOCF method. *P<0.05 vs 0 weeks (baseline), Wilcoxon signed rank test. The numbers represent the mean Saxon’s test value at each time point.

(C) Effects of abatacept treatment on tear volume as assessed using the Schirmer’s test in 52 patients. Data deficits were compensated for by use of the LOCF method. *P<0.05 vs 0 weeks (baseline), Wilcoxon signed rank test. The numbers represent the mean Schirmer’s test value at each time point.

(D) Effects of abatacept treatment on serum IgG levels in 67 patients. Data deficits was compensated for by use of the LOCF method. *P<0.05 vs 0 weeks (baseline), Wilcoxon signed rank test. The numbers represent the mean serum IgG level at each time point.

(E) Effects of abatacept treatment on serum RF levels in 67 patients. Data deficits was compensated for by use of the LOCF method. *P<0.05 vs 0 weeks (baseline), Wilcoxon signed rank test. The numbers represent the mean serum RF level at each time point.
Figure 3. Effects of abatacept on ESSDAI in ROSE II trial-enrolled patients

(A) Effects of abatacept treatment on the European League Against Rheumatism (EULAR) Sjögren’s Syndrome Disease Activity Index (ESSDAI) in the 32 ROSE II trial-enrolled patients. Data deficits were compensated for by use of the LOCF method. *P<0.05 vs 0 weeks (baseline), Wilcoxon signed rank test. The numbers represent the mean ESSDAI value at each time point.

(B) Effects of abatacept treatment on disease activity as assessed using the ESSDAI (low: <5; moderate: 5-13; high: ≥14) in the 32 ROSE II trial-enrolled patients over the 52-week period. Data deficits were compensated for by use of the LOCF method. *P<0.05 vs 0 weeks (baseline), Cochran-Armitage test. The numbers represent the cases in each category.

(C) Effects of abatacept treatment on each ESSDAI domain in the 32 ROSE II trial-enrolled patients. Data deficits were compensated for by use of the LOCF method.
Figure 4. Effects of abatacept on ESSPRI in ROSE II trial-enrolled patients

(A) Effects of abatacept treatment on the EULAR Sjögren’s Syndrome Patient Reported Index (ESSPRI) in the 32 ROSE II trial-enrolled patients. Data deficits were compensated for by use of the LOCF method. *P<0.05 vs 0 weeks (baseline), Wilcoxon signed rank test. The numbers represent the mean ESSPRI value at each time point.

(B-D) Effects of abatacept treatment on each ESSPRI component including dryness (B), fatigue (C), and pain (D) in the 32 ROSE II trial-enrolled patients. Data deficits were compensated for by use of the LOCF method. *P<0.05 vs 0 weeks (baseline), Wilcoxon signed rank test. The numbers represent the mean ESSPRI components at each time point.
