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Basic science

Evaluating the therapeutic potential of tofacitinib in Sjögren's disease: a comprehensive clinical and immunological assessment

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Abstract

Objective: To evaluate the efficacy, safety and immunological effects of tofacitinib in patients with Sjögren's disease (SjD), focusing on its impact on disease activity and immune cell modulation.

Methods: Two independent cohort studies, one retrospective (Cohort I) and one prospective (Cohort II), were conducted to investigate the efficacy of oral tofacitinib treatment in patients diagnosed with SjD. All participants were evaluated for changes in disease activity and lab parameters. Circulating T cells were analysed, focusing on follicular helper T (Tfh) cells and peripheral helper T (Tph) cells.

Results: In cohort I, 112 patients treated with tofacitinib showed a significant improvement in the ESSDAI score [median (IQR), 8.00 (4.25, 15.75) *vs* 6.50 (2.25, 12.75), $P < 0.001$]. In cohort II, ten patients completed the 12-month treatment period. There was a significant reduction in ESSDAI scores at the sixth month compared with baseline ($P = 0.001$). In total, 80% (8/10) of patients achieved a decrease of at least one point or 15% in ESSPRI scores. A significant reduction in the proportion of Th17 cells was observed (mean ± SD, 14.84 ± 7.70 *vs* 7.74 ± 4.24, $P = 0.008$). A decrease in Tfh and Tph cells was also observed, along with decreased pSTAT-3 levels in CD4+ T cells and disease activity scores. No serious adverse events were observed in the two cohorts.

Conclusions: Tofacitinib effectively improves disease activity and immune regulation in SjD, and it is associated with suppressing Tfh and Tph cells, suggesting its potential as a treatment option.

Trial registration: ClinicalTrials.gov Identifier: NCT05087589.

Keywords: Sjogren's disease, tofacitinib, efficacy, safety, Tfh cells, p-STAT3.

Rheumatology key messages

- Tofacitinib is effective and safe for SjD, reducing ESSDAI scores significantly.
- Tofacitinib suppresses Tfh and Tph cells by inhibiting pSTAT-3, contributing to its immunomodulatory effects.

Introduction

Sjogren's disease (SjD) is a prevalent systemic autoimmune condition characterized by dry eyes and mouth. It is often accompanied by dysregulated T-cell functions, particularly the activity of Tfh cells, which play a pivotal role in B-cell differentiation and autoantibody production. These cells drive the aberrant immune response underlying SjD [1]. Presently, approved disease-modifying treatments that can effectively alter the progression of SjD remain lacking.

The Janus kinases-signal transducer and activator of transcription (JAK-STAT) pathways govern numerous crucial downstream immune functions, including cytokine, chemokine and interferon signalling, in particular type-I and type-II interferons (IFNs), interleukins (IL)-6, IL-7, IL-12 and IL-21,

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all of which are implicated in the pathogenesis of Sjogren's disease [2, 3]. Tofacitinib, a pan-JAK inhibitor, has emerged as a promising therapeutic agent, particularly in rheumatoid arthritis (RA) [4, 5]. It blocks JAK3 and JAK1 while exerting inhibitory effects on JAK2 and TYK2 [6]. Topical ophthalmic tofacitinib has been explored for treating dry eye in animal models and clinical trials, demonstrating notable enhancements in ocular surface inflammation, tear production and epithelial barrier function [7–9]. The efficacy of tofacitinib in suppressing the activation of CD4+ T cells has been demonstrated in both *in vitro* and *in vivo* studies, indicating its ability to modulate critical pathways involved in immune cell activation and signalling [10, 11]. However, despite these promising indications, the therapeutic effectiveness of tofacitinib in the specific context of SjD remains largely unknown.

Previously, we observed a correlation between Tfh and Tph cells, as well as the mean fluorescence intensity (MFI) of phosphorylated STAT3 (pSTAT3) and clinical disease scores such as ESSPRI and ESSDAI [12], implying that Tfh and Tph cells could serve as valuable immunological markers for assessing SjD disease activity (Supplementary Fig. S1, available at *Rheumatology* online). This finding underscores the potential utility of Tfh and Tph cells as immunological indices for monitoring the therapeutic effectiveness of tofacitinib and measuring the activity of SjD.

This paper reports the results of two complementary studies—a retrospective and a prospective investigation—aimed at evaluating the effects of oral tofacitinib treatment on individuals diagnosed with SjD. We examined changes in disease activity, laboratory parameters and effector Tfh and Tph cells. Consequently, our study not only assesses the impact of tofacitinib treatment on the clinical outcomes of SjD patients but also offers insights into its potential mechanism of action for treating autoimmune diseases.

Methods

A detailed materials and methods section is available in Supplementary Data S1, available at *Rheumatology* online.

Patients and procedures

The study was conducted in accordance with the principles of the Declaration of Helsinki and the International Conference on Harmonisation guidelines for good clinical practice. The protocol for this study was approved by the ethics committees of Peking University People's Hospital (Beijing, China). In cohort I, patients had to be aged 18–75 years, fulfill the 2002 American and European Consensus Group (AECG) criteria for SjD, and be validated according to the 2016 American College of Rheumatology/European League Against Rheumatism (ACR-EULAR) classification criteria for SjD [13]. At the time of inclusion, patients had to exhibit at least one of the following characteristics: systemic complications (defined as the presence of polysynovitis, vasculitis, autoimmune cytopenia, or cutaneous, renal, pulmonary or neurological involvement) or significant symptoms (defined as dryness, limb pain, fatigue and mental fatigue). The following concomitant treatments were allowed if kept at a stable dose for at least four weeks (28 days) at the time of screening: non-steroidal anti-inflammatory drugs, immunosuppressive/immunomodulatory or antimalarial agents, secretagogue therapy or tear substitutes. Some patients received a combination therapy with immunosuppressive agents and steroids. No change in immunosuppressive/immunomodulatory drugs was allowed during the study period, but glucocorticoid dose could be reduced if indicated. Patients with a history of malignancy or underlying cardiac, pulmonary, metabolic, renal or gastrointestinal conditions, chronic or latent infectious diseases, immune deficiency and previously treated with biological agents were excluded (Fig. 1). A total of 112 Chinese patients were included in this study, recruited from the Department of Rheumatology, Peking University People's Hospital, spanning from March 2020 to September 2023. Patients were treated with tofacitinib 5 mg twice daily for six months.

In cohort II, we recruited 10 Chinese patients who met the 2002 AECG classification criteria for SjD, which was further validated by the 2016 ACR-EULAR classification criteria for SjD. These patients exhibited moderate or high disease activity, as defined by an ESSDAI score of ≥ 5 [14, 15]. Before enrolment and throughout the study period, patients were required to maintain a stable dose of antimalarials, prednisone or its equivalent, cholinergic stimulants and topical ciclosporin for a minimum of 4 weeks. The maximum allowed daily dose for hydroxychloroquine was 400 mg and for prednisone it was 10 mg. Exclusion criteria comprised concurrent tumors, infections, other autoimmune diseases and associated use of other immunosuppressants or biological agents. The online supplementary text provides additional details regarding the inclusion and exclusion criteria. The trial is registered at https://clinicaltrials.gov/ (NCT05087589).

A designated individual collected the patients' clinical data at each visit and documented it in a standardized database. Patients with SjD received an oral dose of tofacitinib 5 mg twice daily for six months, followed by continuation of the current treatment and a 6-month follow-up period. Study visits were scheduled at months 0, 1, 3, 6, 9 and 12. Disease activity scores, including ESSPRI and ESSDAI, were assessed. At each visit, laboratory parameters such as ESR, immune globulin, gamma globulin, complements and complete blood count were evaluated. Minimal clinically significant improvement (MCII) in ESSDAI was defined as an improvement of at least three points.

In contrast, MCII in ESSPRI was defined as a decrease of at least one point or 15%. Flow cytometry detected Th17, Tfh cells and other immune cells at each visit. Fasting venous blood samples were collected in the morning for laboratory examinations. Safety assessments were conducted at each study visit throughout the entire study duration. The incidence and severity of all adverse events were recorded and classified according to the Medical Dictionary for Regulatory Activities (v.14.0).

JAK inhibitor-tofacitinib

Tofacitinib, sourced from Sichuan Kelun Pharmaceutical Research Institute, was dissolved in DMSO for *in vitro* applications.

Cell isolation and treatment

Peripheral blood mononuclear cells (PBMCs) were extracted from blood samples obtained from SjD patients as described [16] and prepared for flow cytometry or Western blotting analysis.

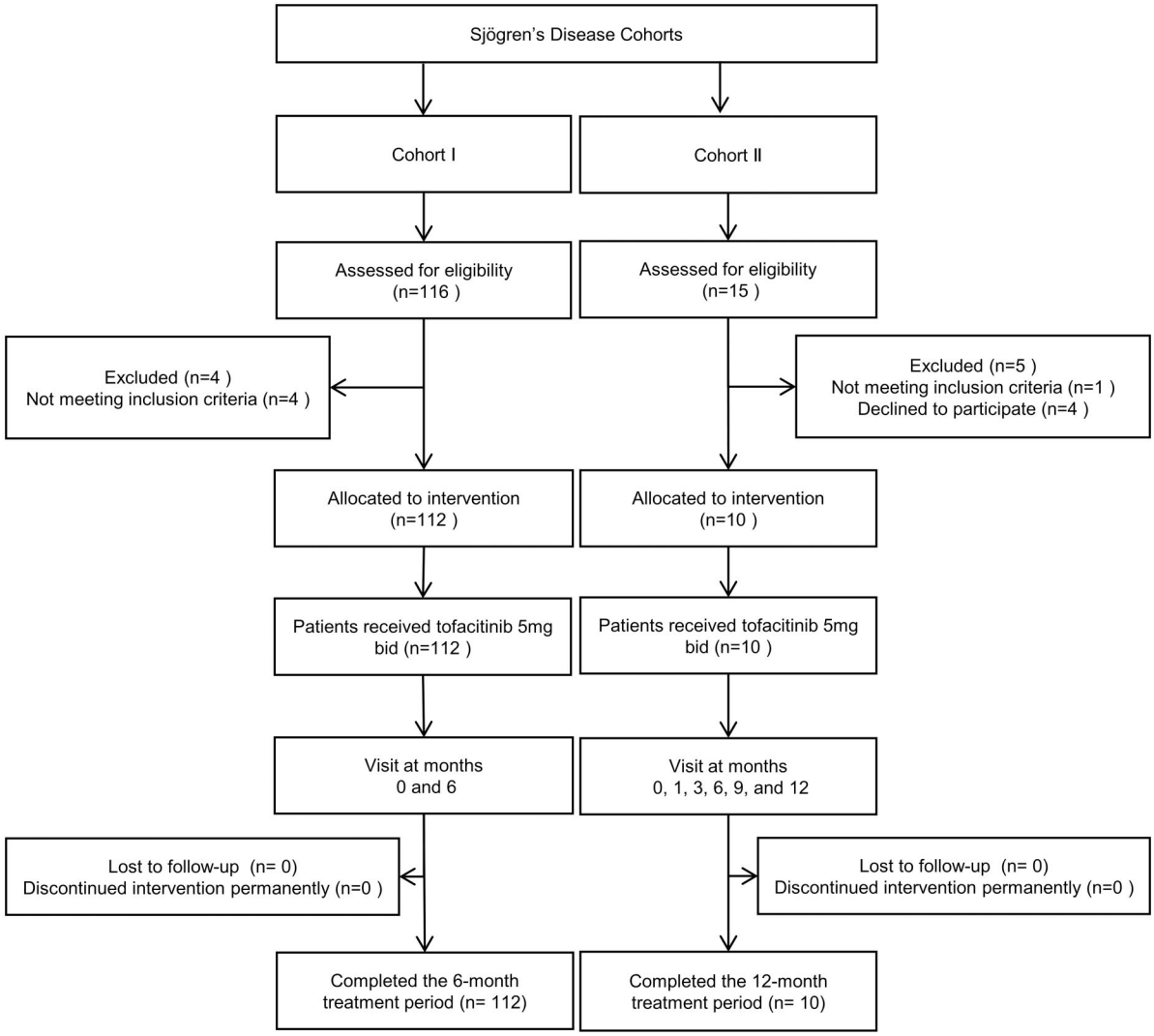

Figure 1. Study design. All patients were treated with tofacitinib (5 mg, twice daily). Symptoms, signs, laboratory results, side effects and lymphocyte subsets were documented at each visit in two cohorts. Bid, two times per day

Flow cytometry

To assess the response of immune cells and STAT phosphorylation to tofacitinib *in vivo* and *in vitro*, PBMCs were stained with a panel of fluorescence-conjugated monoclonal antibodies as before [16–18]. A comprehensive list of all antibodies employed in the study is provided in Supplementary Table S1, available at *Rheumatology* online.

Western blot analysis

We conducted Western blot analysis to evaluate the expression and phosphorylation status of STAT3 and STAT5 in the *in vitro* tofacitinib suppression experiment. As previously described, proteins were detected in blots using the antibodies listed in Supplementary Table S2, available at *Rheumatology* online [19].

Outcomes

Evidence shows that tofacitinib reduces T-cell activation and Th1 and Th17 polarization *in vitro* [20, 21]. The trial's primary end point was the alteration in the percentage of Th17 cells within total $CD4^+$ T cells at month 6. Secondary endpoints included enhancements in the ESSPRI and the safety and tolerability profile of tofacitinib. For patient-reported outcomes, a response was defined as an improvement of at least one point or 15% in the ESSPRI. Safety assessment encompassed monitoring the incidence of reported and observed adverse events, including infections, tumors, abnormal neutrophil and lymphocyte counts, anaemia and drug-induced liver and kidney damage.

Sample size calculations

As previously described, we concluded that our prospective study needed a minimum of nine participants [22–24].

Statistical analysis

Data was analysed using SPSS Statistics 24.0 software (SPSS Inc., Armonk, NY, USA). Figures were generated using GraphPad Prism (version 8). Continuous variables were analysed using Student's *t* test, the non-parametric Mann–Whitney *U* test, and the Kruskal–Wallis test, while categorical variables were assessed using Fisher's exact test. Correlation analysis was performed using the non-parametric Spearman correlation coefficient. HRs and 95% CIs were

calculated using univariate Cox proportional hazards models and further adjusted for potential confounders. All data were filled in. All tests were two-tailed with a 95% confidence interval, and *P*-values ≤0.05 (*$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$) were considered statistically significant.

Results

Efficacy of tofacitinib therapy in SjD patients from cohort I

In cohort I, the clinical characteristics of these SjD patients are presented in Supplementary Table S3, available at *Rheumatology* online. Cutaneous involvement includes erythema multiforme [38.46% (5/13)], maculopapular rash [38.46% (5/13)] or urticarial rash (23.08% [3/13]). Treatment with tofacitinib resulted in a significant improvement in the ESSDAI score [median (IQR), 8.00 (4.25, 15.75) *vs* 6.50 (2.25, 12.75), $P < 0.001$]. Noteworthy reductions were observed in the levels of immunoglobulin G (IgG) [median (IQR), 14.35 (11.62,17.75) g/l *vs* 13.65 (11.27, 16.94) g/l, $P = 0.021$], erythrocyte sedimentation rate (ESR) [median (IQR), 20.00 (11.00, 44.00) mm/h *vs* 17.00 (8.50, 32.50) mm/h, $P = 0.023$], and C-reactive protein (CRP) [median (IQR), 1.20 (0.40, 6.70) mg/l *vs* 0.40 (0.40, 1.95) mg/l, $P = 0.003$], alongside increased levels of complement C4 [median (IQR), 0.21 (0.16, 0.28) g/l *vs* 0.24 (0.19, 0.32) g/l, $P = 0.007$] at the sixth month of treatment (Table 1). No significant alterations were noted in routine laboratory parameters such as white blood cell count, haemoglobin and platelet count throughout the treatment duration.

Clinical efficacy was categorized into responders and non-responders based on the MCII achievement in ESSDAI, which is defined as an improvement of at least three points. Among the cohort, 36 patients (32.14%) exhibited a response to tofacitinib therapy, manifesting higher frequencies of arthritis [22 (61.11%) *vs* 29 (38.16%), $P = 0.023$], cutaneous manifestations [8 (22.22%) *vs* 5 (6.85%), $P = 0.036$], and hyperglobulinemia [19 (52.78%) *vs* 21 (27.63%), $P = 0.012$] compared with non-responders [76 (67.86%)]. Additionally, responders presented significantly elevated baseline ESR and CRP levels ($P < 0.001$, $P = 0.013$, respectively, Supplementary Table S4, available at *Rheumatology* online). There were no significant differences in the median initial oral glucocorticoids (GC) dose or the proportion of patients with different GC doses between responders and non-responders (Supplementary Fig. S2A, available at *Rheumatology* online). A higher proportion of responders were using prednisone (dose ≥15 mg/day), although this difference did not reach statistical significance (Supplementary Fig. S2B, Supplementary Table S5, available at *Rheumatology* online). The doses of GCs were significantly reduced in both groups at 6 months compared with baseline ($P < 0.05$, Supplementary Fig. S2A, available at *Rheumatology* online).

Patients were divided into three groups according to the cut-off value of ESSDAI scores at baseline: low disease activity [28 patients (25%), ESSDAI score <5], moderate disease

Table 1. Response of SjD patients to tofacitinib treatment in cohort | ($n = 112$)

Variable	Month 0	Month 6
ESSDAI, median (IQR)	**8.00 (4.25, 15.75)**	**6.50 (2.25, 12.75)*****
Routine laboratory tests		
IgG titre, g/L, median (IQR)	**14.35 (11.62, 17.75)**	**13.65 (11.27, 16.94)***
IgA titre, g/L, median (IQR)	3.31 (2.29, 4.42)	3.12 (2.00, 3.95)
IgM titre, g/L, median (IQR)	1.06 (0.73, 1.75)	1.02 (0.68, 0.38)
C3, g/L, median (IQR)	0.99 ± 0.24	1.03 ± 0.28
C4, g/L, median (IQR)	**0.21 (0.16, 0.28)**	**0.24 (0.19, 0.32)****
ESR, mm/h, median (IQR)	**20.00 (11.00, 44.00)**	**17.00 (8.50, 32.50)***
CRP, mg/L, median (IQR)	**1.20 (0.40, 6.70)**	**0.40 (0.40, 1.95)****
WBC, × 10^9/L, median (IQR)	5.40 (4.14, 7.53)	4.84 (3.94, 7.02)
lymphocyte count, × 10^9/L, median (IQR)	1.40 (1.00, 1.95)	1.36 (1.02, 1.84)
Neutral cell count, × 10^9/L, median (IQR)	3.37 (2.12, 4.83)	3.07 (2.14, 4.70)
Hb, g/L, median (IQR)	122 (110, 133)	125 (118, 134)
PLT, × 10^9/L, median (IQR)	226 (180, 270)	223 (176, 276)
ALT, U/L, median (IQR)	18.00 (12.00, 25.20)	16.00 (13.00, 24.00)
AST, U/L, median (IQR)	**20.50 (17.80, 26.00)**	**24.00 (20.00, 30.00)*****
GGT, U/L, median (IQR)	18.00 (14.00, 34.00)	19.50 (15.00, 34.50)
Cr, umol/L, median (IQR)	63.00 (54.25, 73.00)	65.00 (58.00, 73.00)
Immune cells		
Total T cells, %, median (IQR)	**74.00 (70.50, 82.50)**	**74.00 (70.00, 79.00)***
CD4+T cells, %, median (IQR)	40.65 (32.02, 46.88)	36.80 (31.40, 43.40)
pTfh cells, %, median (IQR)	2.80 (1.65, 3.95)	2.15 (1.70, 3.28)
Foxp3+Treg cells, %, mean ± SD	8.37 ± 2.67	7.00 ± 2.55
CD4+TNF-α+Th1 cells, %, mean ± SD	**48.18 ± 13.01**	**39.52 ± 14.54***
CD4+IFN-γ+Th1 cells, median (IQR)	**17.80 (14.60, 23.40)**	**15.40 (11.70, 19.80)***
CD4+IL-2+Th1 cells, %, mean ± SD	44.46 ± 13.60	41.54 ± 10.78
CD4+IL-4+Th2 cells, mean ± SD	1.55 (1.06, 2.12)	1.93 (1.60, 2.07)
CD4+IL-17A+Th17 cells, mean ± SD	**1.40 (0.87, 1.72)**	**1.00 (0.80, 1.45)***
B cells, %, mean ± SD	11.00 ± 8.08	10.58 ± 6.76
NK cells, mean ± SD	11.45 (6.72, 16.80)	8.90 (5.30, 14.90)

Data are presented as median (IQR), mean ± SD or *n* (%). Asterisks and boldface highlight significant outcomes (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).
ALT: alanine transaminase; AST: aspartate transaminase; C3: complement 3; C4: complement 4; CRP: C-reactive protein; Cr: creatinine; ESR: erythrocyte sedimentation rate; ESSDAI: EULAR SS Disease Activity Index; GGT: gamma-glutamyltransferase; Tfh: follicular helper T cells.

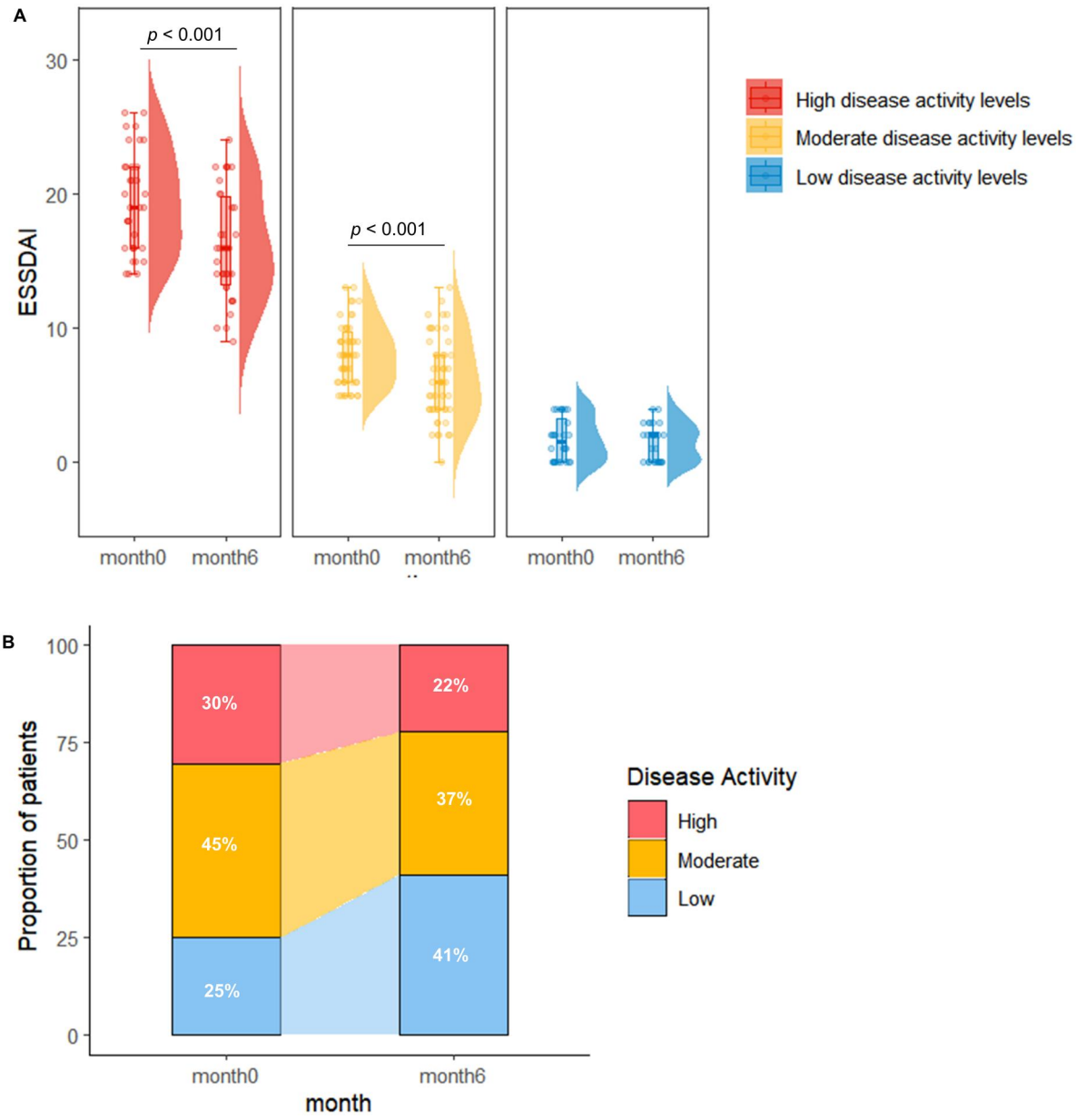

Figure 2. Evolution of disease activity in each cluster of cohort I ($n = 112$). (**A**) Changes of ESSDAI scores in different groups after 6-month tofacitinib treatment. (**B**) Distribution of disease activity [proportion of patients with low (<5), moderate (5–13) or high (>13) ESSDAI disease activity] at baseline and month 6. Data are presented as median (IQR), mean ± SD or *n* (%)

activity [50 patients (44.64%), ESSDAI score between 5 and 13], and high disease activity [34 patients (30.36%), ESSDAI score ≥14].

ESSDAI score showed a significant decrease from baseline to 6 months in both the moderate-disease-activity group [median (IQR), 8.00 (6.00–10.00) *vs* 6.00 (4.00–8.25), $P < 0.001$] and high-disease-activity group (mean ± SD, 19.41 ± 3.75 *vs* 16.15 ± 4.11, $P < 0.001$). However, there were no significant differences in ESSDAI scores at month 6 for patients in the low disease activity group (Fig. 2A). In the ESSDAI responder analysis at month 6, the proportion of the high disease activity group reached 50% (17/34), the moderate disease activity group reached 38% (19/50) and no responders were in the low disease activity group. Changes in disease activity levels over the 6-month follow-up period are shown in Fig. 2B, indicating systemic disease activity levels improved after receiving tofacitinib ($P = 0.036$).

Concomitant medications and their impact on the clinical efficacy of tofacitinib therapy in cohort I

A stepwise logistic regression analysis was conducted to investigate the impact of concomitant medications on the clinical efficacy of tofacitinib therapy in SjD patients (Supplementary Table S5, available at *Rheumatology* online). Patients were stratified into response ($n = 36$) and non-response ($n = 76$) groups based on their use of associated medications. Results indicated a higher prevalence of responders receiving medications such as hydroxychloroquine, leflunomide, methotrexate, azathioprine, mycophenolate

mofetil, low-dose interleukin-2, salazosulfapyridine and prednisone (dose ≥15 mg/d). Interestingly, fewer responders received low doses of prednisone or therapies like cyclophosphamide compared with non-responders.

Univariate analysis identified factors associated with therapy response including hydroxychloroquine therapy (OR = 1.90, 95% CI 0.83–4.33, *P*-value=0.129), leflunomide therapy (OR = 5.13, 95% CI 1.72–15.33, *P*-value=0.003), methotrexate therapy (OR = 4.49, 95% CI 1.48–13.58, *P*-value=0.008) and cyclophosphamide therapy (OR = 0.13, 95% CI 0.16–1.00, *P*-value=0.05). Multivariable analysis highlighted hydroxychloroquine and cyclophosphamide as significant factors associated with clinical response at month 6 (OR = 3.03, 95% CI 1.28–7.21, *P*-value = 0.012, and OR = 0.08, 95% CI 0.01–0.61, *P*-value = 0.016, respectively).

Immunological analysis of tofacitinib therapy in SjD patients from cohort I

The immunological analysis revealed notable reductions in peripheral blood Th1 [median (IQR), 17.80% (14.60%–23.40%) compared with 15.40% (11.70%–19.80%), $P = 0.044$] and Th17 cells [median (IQR), 1.40% (0.87%–1.72%) *vs* 1.00% (0.80%–1.45%), $P = 0.044$] at month six compared with that at baseline (Table 1). Comparing response and non-response groups, responders exhibited higher baseline levels of pTfh cells [median (IQR), 2.50% (1.55%–3.52%) *vs* 1.70% (1.30%–2.10), $P = 0.041$], Th1 cells (mean ± SD, 44.68 ± 13.46% *vs* 34.16 ± 12.51%, $P = 0.025$), and Th17 cells [median (IQR), 1.12% (0.79%–1.93%) *vs* 0.86% (0.64%–1.02%), $P = 0.038$], indicating distinct immunological changes associated with treatment efficacy (Supplementary Table S4, available at *Rheumatology* online).

Clinical efficacy of cohort II

In cohort II, we recruited 10 Chinese patients (Supplementary Table S6, available at *Rheumatology* online) with SjD from 20 November 2021 to 1 January 2023 (Fig. 1). Ten patients completed the 12-month treatment period. A clinical trial was conducted to explore further the clinical and immune effects of tofacitinib on patients with SjD. Following tofacitinib treatment, there was a significant reduction in ESSDAI scores at the 6th, 9th, and 12th months compared with baseline (Fig. 3A, $P = 0.001$, $P < 0.001$, $P < 0.001$, respectively), indicating reduced disease activity. ESSPRI scores decreased by the 12th month (Fig. 3B, $P = 0.015$). By the end of the six months, 90% (9/10) of the participants achieved MCII in ESSDAI, and this percentage increased to 100% (10/10) at the 12-month follow-up (Fig. 3C). Additionally, at the end of 6 months, 80% (8/10) of patients achieved MCII in ESSPRI, which increased to 90% (9/10) by 12 months (Fig. 3D). Notably, patients with arthritis displayed significant improvement at the 12th month compared with baseline ($P = 0.011$). Other manifestations, such as fever, weight loss, lymphadenopathy, glandular swelling, arthritis, skin rash, interstitial lung disease and myositis, also exhibited improvement (Supplementary Table S7, available at *Rheumatology* online). At the same time, after 12 months of treatment, the doses of glucocorticoids (GCs) were gradually reduced compared with the baseline doses (Supplementary Fig. S3E, available at *Rheumatology* online).

Laboratory parameters of cohort II

Cohort II demonstrated a consistent decline in IgA, IgG, γG and ESR levels at different time points. There was a significant decrease in IgA levels (Supplementary Fig. S3A, available at *Rheumatology* online), IgG levels (Supplementary Fig. S3B, available at *Rheumatology* online), gamma globulin (γG) levels (Supplementary Fig. S3C, available at *Rheumatology* online) and ESR (Supplementary Fig. S3D, available at *Rheumatology* online) at follow-up time points. Improvements were also noted in IgM, complement C3 and C4 levels, although no statistical differences were observed.

Immunological responses following tofacitinib treatment in patients with SjD of cohort II

This study also investigated the immunological impact of tofacitinib on SjD in cohort II. A decrease in the proportion of Tfh, Tph, Th17 cells and $CD19^+$B cells post-treatment was observed, with statistical significance ($P < 0.05$) (Fig. 4A–C, Supplementary Fig. S4C, available at *Rheumatology* online). While there was a decline in the proportion of Th1 and Treg cells, the reduction was not statistically significant (Supplementary Fig. S4A and B, available at *Rheumatology* online).

Further exploration of the downstream STAT proteins influenced by tofacitinib revealed a significant decrease in pSTAT-5 levels in $CD4^+$ T cells over time, highlighting the targeted impact of tofacitinib on the JAK-STAT pathway. Although reductions in pSTAT-3 levels were noted, they did not reach statistical significance (Supplementary Fig. S4D and E, available at *Rheumatology* online). Notably, a significant correlation was observed between circulating Tfh cells, Tph cells and pSTAT3 MFI in CD4+ T cells, underscoring the intricate relationship between specific STAT proteins and immune cell modulation in SjD (Tfh: r = 0.5733, $P < 0.001$; Tph: $r = 0.2620$, $P = 0.043$).

The effects of tofacitinib on immune cells were also investigated *in vitro*

Isolated PBMCs from 10 active SjD patients of cohort II were treated with one μM tofacitinib and DMSO as a control for 72 h. Significant decreases were observed in the proportions of Th17, Tfh and Tph cells when isolated PBMCs were treated with tofacitinib (Fig. 5A, $P = 0.012$, $P = 0.004$, $P = 0.001$, respectively). Reductions were also noted in Treg and B cells, indicating a broad immunomodulatory effect (Supplementary Fig. S5A, available at *Rheumatology* online). While Th1 cells exhibited a decreasing trend, the change was not statistically significant (Supplementary Fig. S5A, available at *Rheumatology* online). Furthermore, strong correlations were found between Tph and Tfh cells, as well as between circulating Tfh and Tph cells with B cells (Fig. 5C, $r = 0.8120$, $P < 0.0001$; Fig. 5D, Tfh: $r = 0.6491$, $P = 0.0020$; Fig. 5E, $r = 0.5980$, $P = 0.0054$).

To explore the potential downstream molecules of JAK1 and JAK3, changes in pSTAT3/STAT3 and pSTAT5/STAT5 ratios were evaluated. Reductions were observed in the ratios of pSTAT3/STAT3 and pSTAT5/STAT5, indicating tofacitinib's modulation of these pathways potentially influences lymphocyte regulation (Fig. 5B, Supplementary Fig. S5B, available at *Rheumatology* online, $P = 0.005$, $P = 0.004$, respectively). A notable positive correlation was found between

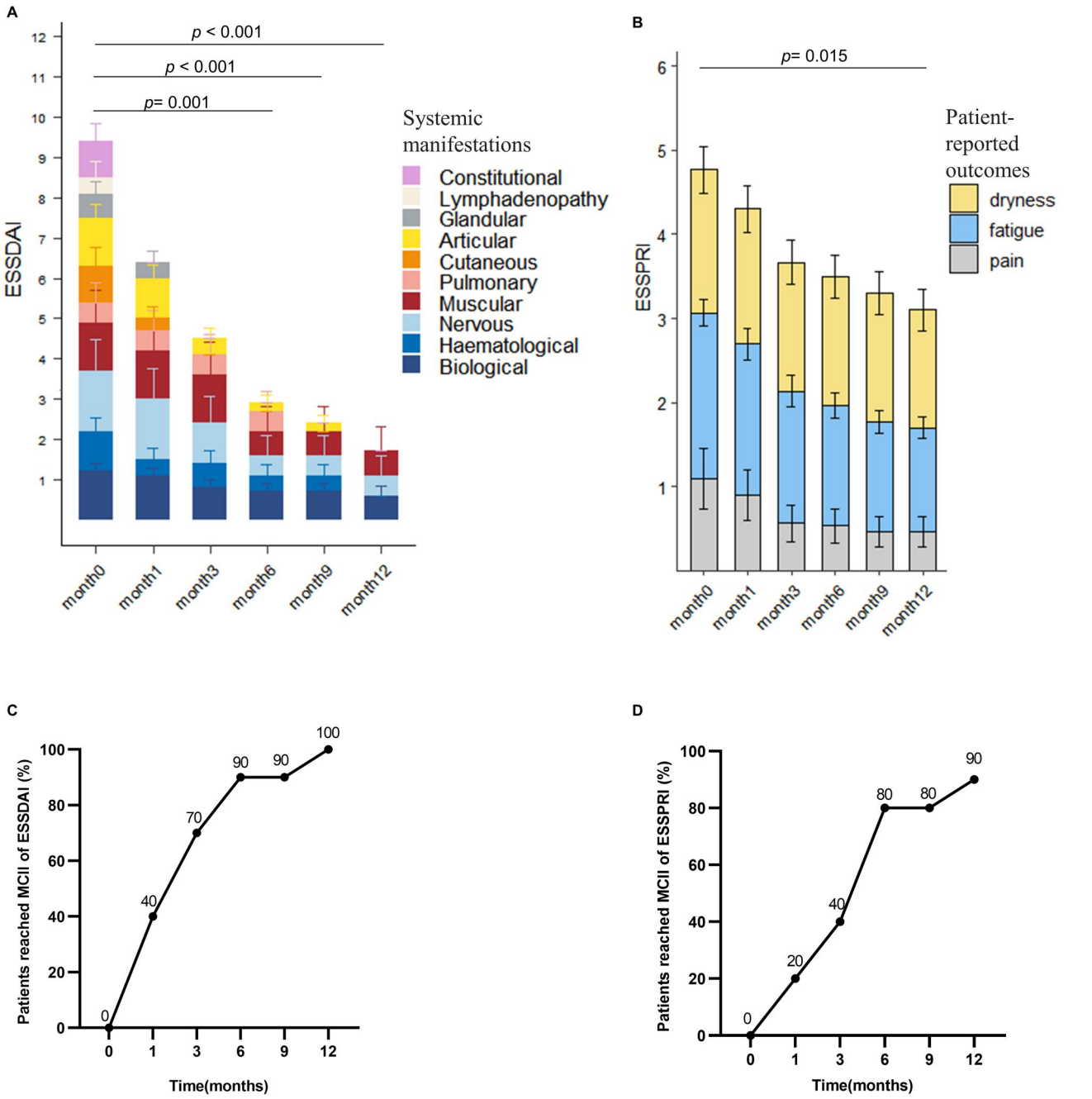

Figure 3. Clinical response to tofacitinib treatment in cohort II. Changes of ESSDAI (**A**) and ESSPRI (**B**) scores of SjD patients after the treatment of tofacitinib. After 6 months of treatment, 90% of patients achieved MCII of ESSDAI (**C**) and 80% achieved MCII of ESSPRI (**D**). Data are presented as median (IQR). ESSPRI: EULAR primary SS Patient Reported Index; ESSDAI: EULAR primary SS Disease Activity Index

the pSTAT3/STAT3 ratio and the proportions of Tfh and Tph cells in CD4+ cells, suggesting pSTAT3 plays a pivotal role in tofacitinib's mechanism of action, particularly in suppressing Tfh and Tph cell activity *in vitro* (Fig. 5F, $r = 0.6902$, $P = 0.0008$; Fig. 5G, $r = 0.5609$, $P = 0.0101$, respectively).

Safety

In two cohorts, the safety profile of tofacitinib revealed that most adverse events (AEs) were mild to moderate, with upper respiratory infections being the most common. Importantly, no serious AEs were reported. Notably, there were no instances of herpes zoster reactivation, BK viremia or venous thromboembolic events, highlighting the tolerability of tofacitinib in patients with SjD (Supplementary Tables S8 and S9, available at *Rheumatology* online).

Discussion

In cohort I, we discovered that tofacitinib effectively alleviated the symptoms of SjD, particularly in patients with arthritis, cutaneous manifestations and hyperglobulinemia. We also noted a significant reduction in IgG, ESR, CRP and C4 levels. The proportion of ESSDAI responders and the degree of improvement in ESSDAI were higher in the high disease

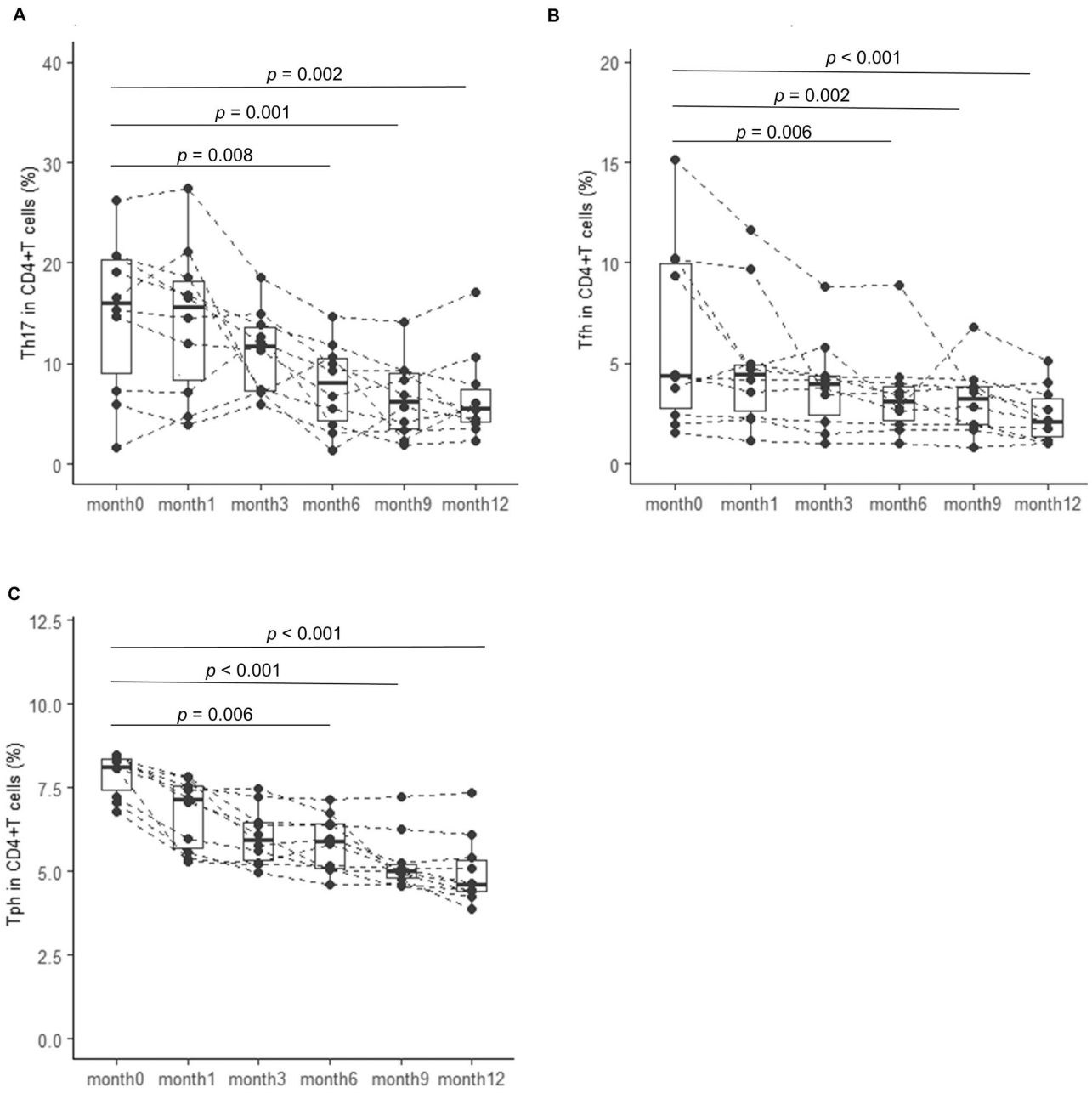

Figure 4. Tofacitinib modulates CD4+ T-cell differentiation in the peripheral blood of SjD patients in cohort II. The percentage of Th17 (**A**), Tfh (**B**) and Tph (**C**) in CD4+T cells dramatically decreased after the treatment of tofacitinib. Data are presented as median (IQR), mean ± SD

activity group than in the moderate and low disease activity groups.

In cohort II, tofacitinib was administered at a dosage of 5 mg twice daily to patients with active SjD. By the sixth month, there were clinically meaningful and statistically significant reductions in ESSPRI scores from baseline. Additionally, patients receiving tofacitinib experienced substantial decreases in ESSDAI scores at month 6, consistent with the findings from our retrospective study. The higher proportion of patients responding to ESSDAI in the prospective phase 2 trial at six months than in the retrospective study may be related to the higher ESSDAI scores of patients. Moreover, levels of IgG significantly decreased by month 6, and the efficacy of tofacitinib remained sustained up to month 12.

In univariate analysis, the combined use of prednisone (dose ≥15 mg/d), hydroxychloroquine, antimetabolites (including leflunomide, methotrexate, azathioprine, mycophenolate mofetil) and low-dose interleukin-2 was associated with increased clinical efficacy of tofacitinib at six months [25]. Our study also revealed that antimalarial drugs, such as HCQ, provided safe and favourable therapeutic effects in SjD patients treated with tofacitinib. The combination of glucocorticoids plays an essential role in the treatment of Sjögren's disease patients receiving tofacitinib, possibly due to their potent anti-inflammatory effects and their ability to control active systemic disease [26, 27]. The efficacy of low-dose IL-2 therapy may be attributed to its ability to induce substantial and dose-dependent increases in the proportions and absolute numbers of regulatory T cells (Treg), thus partially restoring

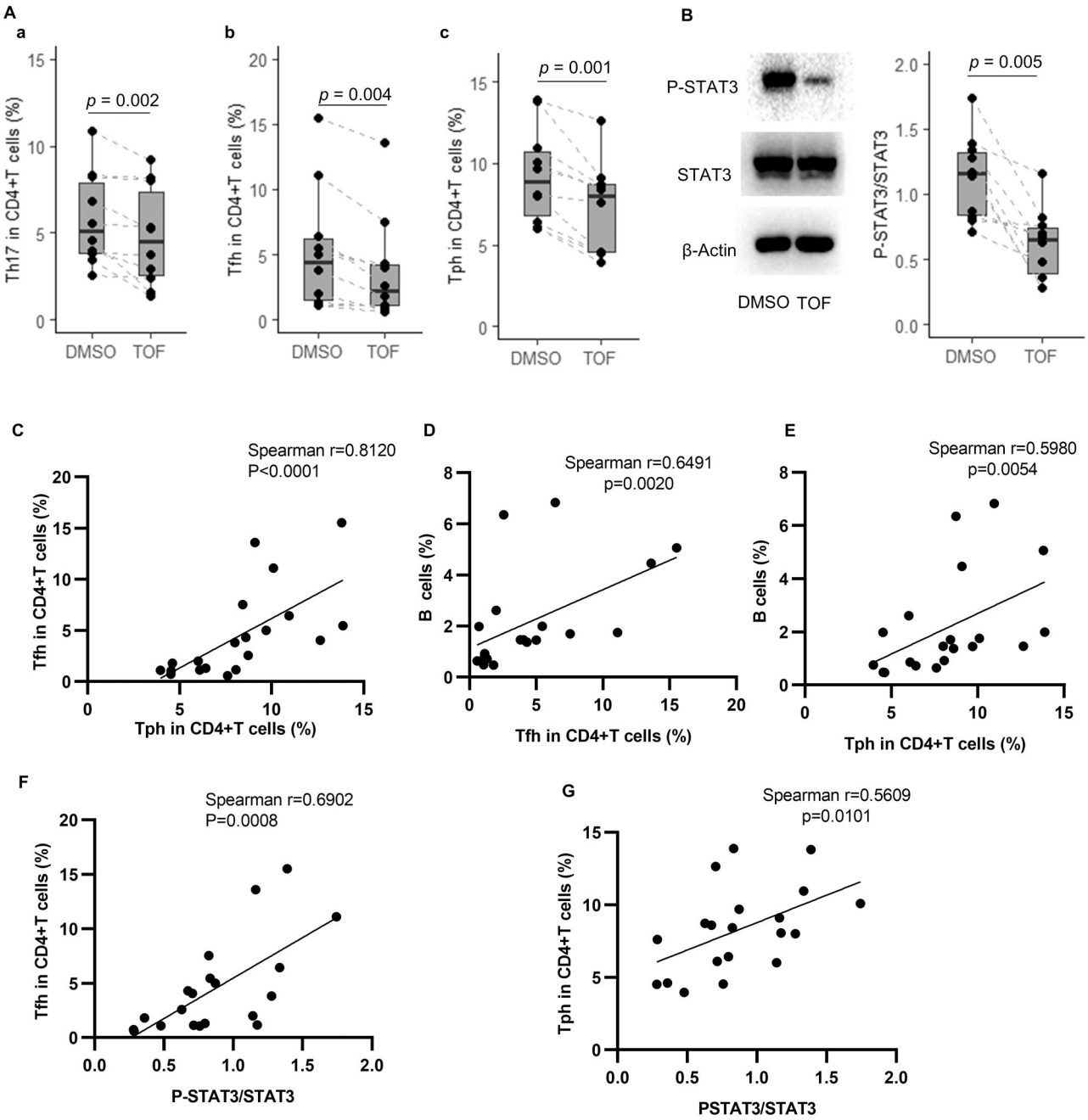

Figure 5. Effect of tofacitinib on human cells of untreated patients with Sjogren's disease *in vitro*. PBMCs from active SjD patients were incubated with DMSO or one μM tofacitinib for 72 h. (**A**) Changes of percentage of Th17 (a), Tfh (b), Tph (c) in CD4+T cells. (**B**) The expression of STAT3 and P-STAT3 were detected in PBMCs by Western blot. (**C**) The correlation between change of percentage of Tfh and Tph in CD4+T cells *in vitro*. (**D**) The correlation between change of percentage of B cells and Tfh iCD4+T cells *in vitro*. (**E**) The correlation between change of percentage of B cells and Tph in CD4+T cells *in vitro*. (**F**) The correlation between change of percentage of Tph in CD4+T cells and pSTAT3/STAT3 ratios *in vitro*. (**G**) The correlation between change of percentage of Tph in CD4+T cells and pSTAT3/STAT3 ratios *in vitro*. Data are presented as median (IQR), mean ± SD

the homeostatic balance between Treg and effector T cells (Teff). However, the combination of alkylating agents (including cyclophosphamide) and calcineurin inhibitors (CNIs) (including ciclosporin and tacrolimus) was associated with reduced clinical efficacy of tofacitinib at six months. The negative association between combined CNIs and clinical efficacy may be due to the immunosuppressive nature of CNIs, which inhibit T-cell activation through the inhibition of the calcium/calmodulin-dependent phosphatase calcineurin [28], potentially reducing the effectiveness of tofacitinib. However, further research is needed to elucidate the underlying mechanisms.

Treatment with tofacitinib had several side effects, including increased liver enzyme levels, slight rises in serum creatinine, and reductions in various blood cell counts, such as white blood cells, neutrophils, lymphocytes and platelets. Additionally, hair loss was observed in patients with SjD treated with tofacitinib despite JAK inhibitors being considered a treatment option for alopecia areata [29–31]. No serious adverse events led to treatment withdrawal, but four

(3.57%) of 112 patients discontinued for non-serious adverse events: one (0.89%) for headache; one (0.89%) for creatinine slightly elevation; one (0.89%) for increased blood pressure; one (0.89%) for chest distress. These adverse effects were consistent with findings reported in other studies involving tofacitinib at 5 mg or 10 mg doses taken twice daily [32–34]. Throughout the study period, no deaths, malignancies (including non-melanoma skin cancer), major adverse cardiovascular events, thromboembolic events, gastrointestinal perforations, drug-induced liver injuries, opportunistic infections or cases of interstitial lung disease were reported.

The Th17 pathway plays a crucial role in disease pathogenesis and the formation of germinal centres in SjD [35]. Recent studies have demonstrated that Tph and Tfh cells may contribute to B cell hyperactivity in SjD and are associated with clinical and laboratory parameters such as ESSDAI, IgG, ESR levels and anti-SSA antibodies [36, 37]. This clinical trial met the primary end point: Th17 cells showed a significant decrease after tofacitinib treatment at the month six time point. Additionally, decreases in Tfh, Tph and B cells were observed *in vitro* and *in vivo* after tofacitinib administration. These findings suggest that inhibiting T-cell activation and potentially reducing B-cell activity could be a promising therapeutic strategy for SjD [36–38].

In our research, we have substantiated that tofacitinib treatment effectively reduces levels of p-STAT3 and p-STAT5 in immune cells of SjD patients [39], demonstrating efficacy in controlled laboratory conditions and real-world clinical settings. Furthermore, we have observed a notable positive correlation between p-STAT3 expression and the frequency of circulating Tph cells across both environments. Despite recent studies suggesting a potential new role for STAT3 and STAT5 in the differentiation of Th17 cells [40], our prospective phase 2 trial did not uncover significant correlations between the phosphorylation of STAT3 or STAT5 in $CD4^+$ T cells and Th17 cells.

Moreover, our results reveal that tofacitinib therapy influences the equilibrium between Teff and Treg. Given the critical role of STAT5 activation in mediating the immunosuppressive functions of Tregs [41], the observed initial decline followed by a subsequent rise in Treg proportions during tofacitinib treatment can be elucidated as a consequence of the shifting balance among various subsets of CD4+ T cells.

Limitations

However, it is important to note several limitations in our study. Firstly, it was not a double-blind, placebo-controlled trial, which is a superior design for clinical research. Although each patient served as their control in this study design, any improvements observed in an open-label study must be interpreted cautiously. This is because it does not eliminate the potential impact of natural remission on the disease process, nor does it account for the inherent heterogeneity of SjD, the subjectivity of many disease measures or the possibility of observer bias [42, 43]. Although we imposed restrictions on concomitant medications at the time of screening and during the study period in two cohorts, we cannot completely rule out the potential impact of concomitant medications on the efficacy of tofacitinib treatment for SjD. Therefore, larger, randomized, placebo-controlled clinical trials are needed to further assess the efficacy and safety of tofacitinib therapy for SjD. Secondly, the study's sample size was relatively small, and the *post hoc* nature of the analysis may restrict the generalizability of the clinical profiling of tofacitinib treatment response, and caution should be exercised when interpreting the findings. This exploratory analysis warrants further confirmatory studies with larger patient cohorts to validate the results and assess which clinical profiles in SjD patient populations are most strongly associated with treatment response. Thirdly, the study primarily focused on short-term outcomes, necessitating longer-term follow-up to assess sustained efficacy and safety.

Conclusion

This study represents the inaugural assessment of tofacitinib's efficacy and safety in SjD. Our findings indicate that tofacitinib demonstrates effectiveness and favorable tolerability among patients with active SjD.

In summary, the data gleaned from our investigation imply that tofacitinib's therapeutic impact on SjD may stem from its ability to regulate Tfh and Tph cells, achieved through the suppression of pSTAT3. These mechanisms may contribute to the potential induction of disease remission.

Supplementary material

Supplementary material is available at *Rheumatology* online.

Data availability

See Supplementary Data Sharing Statement.

Contribution statement

J.H. had full access to all of the study's data and is responsible for its integrity and accuracy. Concept and design: J.H., D.Y., L.Sh., La.S. Acquisition, analysis or interpretation of data: Q.L., Y.Z., X.X., B.H., R.F. Drafting of the manuscript: J.H., A.J., J.L.A, Y.W., N.W. Critical manuscript revision for important intellectual content: J.H., X.Z., Y.L., D.Y., L.Sh., La.S., Q.L., Y.Z. Statistical analysis: Q.L., Y.Z., X.X., B.H., R.F., Y.W., N.W., Li.S., L.Sh., La.S. Obtained funding: J.H., X.Z., Y.L., L.Sh., La.S. Administrative, technical or material support: X.Z., A.J., J.L.A. Supervision: J.H., X.Z., Y.L., L.S., D.Y., L.Sh., La.S., A.J., J.L.A.

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