# Differences in the strength of inhibition of interleukin-6 signalling by subcutaneous sarilumab and tocilizumab in rheumatoid arthritis patients

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## Abstract

### Objective

*To assess differences in the strength of inhibition of IL-6/STAT3 signalling induced by subcutaneously (sc) administered tocilizumab (TCZ) and sarilumab (SAR).*

### Methods

*Data were collected on patients with rheumatoid arthritis (RA) who achieved low disease activity (Clinical Disease Activity Index [CDAI]≤10) following treatment with weekly or bi-weekly administration of 162 mg sc of TCZ (TCZ qw group, n=8; TCZ q2w group, n=8), bi-weekly doses of 200 mg sc of SAR (SAR q2w group, n=7), or MTX (n=8) as a control. The clinical characteristics of each group were collected, and the serum concentrations of IL-6 and soluble IL-6 receptor (sIL-6R) were measured using ELISA. Whole blood samples from each group were stimulated with 100 ng/ml of IL-6. The proportion of phosphorylated (p)STAT3-positive CD4+ T cells was measured using phosflow cytometric analysis.*

### Results

*The proportion of pSTAT3-positive CD4+ T cells following stimulation with 100 ng/ml of recombinant human IL-6 was significantly different among the groups (median 1.8% [0.9–3.0] vs. 7.7% [2.9–8.0] vs. 12.5% [11.4–16.6] vs. 71.5% [68.0-78.5] for the TCZ qw, SAR q2w, TCZ q2w, and MTX control groups, respectively; p<0.01 for all comparisons).*

### Conclusion

*SAR 200 mg q2w showed significantly stronger inhibition of IL-6/STAT3 signalling than TCZ sc q2w but weaker inhibition than TCZ sc qw. The results of this study may be useful for adjusting the IL-6 blockade treatment for patients with RA.*

**Key words**

sarilumab, tocilizumab, IL-6, rheumatoid arthritis

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*Competing interests: see page 1455.*

## Introduction

Interleukin-6 (IL-6) plays an important role in the pathophysiology of rheumatoid arthritis (RA) (1). The IL-6 signalling cascade is initiated after IL-6 binds its receptor, leading it to form a complex. This complex subsequently binds to gp130 and induces phosphorylation of JAK kinase. This in turn induces phosphorylation of signal transducer and activator of transcription (STAT) 3 (pSTAT3), which mediates further signal transduction (2). Tocilizumab (TCZ) and sarilumab (SAR) are humanised monoclonal antibodies which combine with membrane-bound and soluble forms of the human IL-6 receptor (mIL-6R and sIL-6R) to inhibit IL-6/STAT3 signalling. Meanwhile, several characteristics such as forms of antibodies, dosage, and affinity to IL-6 receptors, are different (3). In addition, since levels of IL-6 and sIL-6R in serum and synovial fluid differ significantly, the strength of IL-6 signal inhibition required among individual patients with RA is thought to vary (4). In our previous study, we developed a highly sensitive bioassay to measure the strength of TCZ-induced inhibition of IL-6/pSTAT3 signalling (5). We successfully showed that there was a significant difference in inhibition of IL-6/pSTAT3 signalling in patients with RA treated with an adjusted dose frequency of intravenous TCZ and those treated with methotrexate (MTX)(5).

A common SAR dosage regimen for patients with RA is subcutaneous (sc) administration of 200 mg once quaque 2 weeks (q2w) (6). The recommended initial dose of TCZ is 162 mg q2w sc, and an increase in dose frequency up to TCZ 162 mg sc weekly (qw) is recommended if clinical response is inadequate, in Japan (7) and America (https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/125276s114lbl.pdf). In Europe, the standard regimen is TCZ 162mg sc qw (https://www.ema.europa.eu/en/documents/product-information/roactemra-epar-product-information_en.pdf) and considers dose frequency decrease when the response is good.

This study aimed to evaluate the strength of IL-6 signalling inhibition with subcutaneous TCZ and SAR by measuring pSTAT3-positive CD4$^+$ T cells from patients with RA.

## Methods

*Patients and data collection*

To avoid the influence of RA disease activity, we collected data on patients with RA who achieved low disease activity (Clinical Disease Activity Index [CDAI] ≤10) following treatment with weekly or bi-weekly administration of 162 mg sc of TCZ (TCZ qw, n=8; TCZ q2w group, n=8), bi-weekly doses of 200 mg sc of SAR (SAR q2w group, n=7), or MTX without biologics or JAK inhibitors (n=8) as a control. Patients who received TCZ and SAR had all blood samples collected on the same days as drug administration, before IL-6 blockade treatment. In addition, all blood samples were collected more than 6 months after the date on which stable dose frequency was initiated for each medication. The clinical characteristics of each group were collected, and the concentrations of serum IL-6 and soluble IL-6 receptor (sIL-6R) were measured using ELISA.

This study was approved by the ethics committee of our institution (Ethics Committee of Keio University School of Medicine, approval number: 20100080). Written informed consent was obtained from all patients. The investigation was conducted according to the principles of the Declaration of Helsinki.

*Detection of pSTAT3-positive CD4$^+$ T cells*

The protocol used to determine the proportion of pSTAT3-positive CD4$^+$ T cells was the same as that described in our previous report (4). A 100-μL volume of whole blood sample taken from each participant was stimulated with each concentration of recombinant human (rh) IL-6 (0, 0.1, 1, 10, and 100 ng/ml) for 15 minutes each at 37°C. The proportion of pSTAT3-positive CD4$^+$ T cells was measured using phosflow cytometric analysis, according to the manufacturer's protocol (BD Phosflow, Franklin Lakes, NJ, USA). Briefly, after stimulation, 1 ml of Lyse/Fix Buffer (BD Phosflow) was added

**Table I.** Clinical and serological characteristics of sc TCZ and SAR-administered patients and the MTX control group.

| | TCZ qw (n=8) | SAR q2w (n=7) | TCZ q2w (n=8) | MTX (n=8) | *p*-value |
|---|---|---|---|---|---|
| Age (y/o) | 66 (47-81) | 56 (53-76) | 61 (52-71) | 66 (59-78) | 0.80 |
| Female (n, %) | 8/8 (100%) | 7/7 (100%) | 8/8 (100%) | 6/8 (75%) | 0.12 |
| RA duration (m) | 134 (46-190) | 103 (18-204) | 134 (85-241) | 48 (21-147) | 0.29 |
| Duration of TCZ/SAR use (m) | 12 (10-13) | 8 (7-10) | 12 (8-18) | - | 0.91 |
| RF positive (n, %) | 7/8 (88%) | 7/7 (100%) | 8/8 (100%) | 5/8 (63%) | 0.09 |
| Anti-CCP positive (n, %) | 7/8 (88%) | 6/7 (86%) | 7/8 (88%) | 4/8 (50%) | 0.24 |
| Body weight (kg) | 54.2 (44.4-58.2) | 52.0 (43.7-70.0) | 53.0 (48.3-58.6) | 55.5 (46.9-64.0) | 0.95 |
| BMI (kg/m$^2$) | 20.3 (18.8-22.9) | 21.6 (19.6-26.9) | 22.8 (19.0-24.5) | 22.7 (21.1-24.4) | 0.66 |
| MTX use (n, %) | 3/8 (38%) | 3/7 (43%) | 3/8 (38%) | 8/8 (100%) | ***0.03*** |
| MTX dose (mg/week) | 0 (0-8) | 0 (0-6) | 0 (0-8) | 10 (4-12) | ***0.03*** |
| PSL use (n, %) | 1/8 (13%) | 2/7 (29%) | 0/8 (0%) | 1/8 (13%) | 0.43 |
| PSL dose (mg/day) | 0 (0-2) | 0 (0-1) | 0 (0-0) | 0 (0-0) | 0.10 |
| CRP (mg/dl) | 0.01 (0.00-0.01) | 0.01 (0.00-0.02) | 0.01 (0.00-0.06) | 0.13 (0.03-0.25) | ***0.01*** |
| ESR (mm/hr) | 3 (2-7) | 5 (2-9) | 6 (2-20) | 19 (5-19) | ***0.02*** |
| IL-6 (pg/ml) | 65.4 (7.6-117.4) | 37.7 (6.8-81.3) | 16.8 (7.0-25.5) | 0.9 (0.6-2.4) | ***<0.01*** |
| sIL-6R (ng/ml) | 201.4 (170.8-234.0) | 181.9 (162.1-227.7) | 175.7 (161.4-223.4) | 30.1 (20.8-35.2) | ***<0.01*** |
| DAS-ESR | 1.86 (0.89-2.59) | 1.67 (1.04-1.99) | 1.36 (0.69-2.96) | 2.4 (1.9-3.1) | 0.11 |
| CDAI | 6 (1.6-8.1) | 2 (0.4-6.8) | 1.8 (0.3-5.5) | 2.7 (0.6-4.5) | 0.30 |
| HAQ-DI | 0.375 (0.0-1.75) | 0.375 (0.0-1.125) | 0.125 (0.0-1.00) | 0.375 (0.0-0.75) | 0.82 |

Values are expressed as median (Q1-Q3). TCZ: tocilizumab; SAR: sarilumab; CCP: cyclic citrullinated peptide; BMI: Body Mass Index; MTX: methotrexate; PSL: prednisolone; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; IL-6: interleukin-6; sIL-6R: soluble interleukin-6 receptor; DAS: disease activity score; CDAI: Clinical Disease Activity Index; HAQ-DI: Health Assessment Questionnaire Disability Index.

to the blood samples for erythrocyte lysis and fixation to preserve levels of pSTAT3. After the fixation, cells were subsequently permeabilised with 1 ml of Perm buffer III (BD Phosflow), then washed and stained for 60 minutes at room temperature in darkened conditions with the following monoclonal antibodies: anti-CD3-PerCP-Cy5.5 (Biolegend, San Diego, CA, USA), anti-CD4-phycoerythrin (PE)-Cy7 (BD Pharmingen, Franklin Lakes, NJ, USA), anti-pSTAT3 (pY705)-AF488 (BD Phosflow), and anti-mouse immunoglobulin G isotype-matched controls (all from BD Biosciences). Stained cells were additionally washed once with 3 ml of Wash Buffer (BD Phosflow) and analysed using flow cytometry (MACS Quant Analyzer; Miltenyi Biotec, Bergisch Gladbach, Germany).

*Statistical analysis*

Descriptive values are expressed as median (Q1-Q3). Results from three or more groups were compared using the Kruskal-Wallis test and chi-squared test. Comparisons between two groups were conducted using the Wilcoxon test and Fisher's exact test. *p*-values <0.05 were regarded as significant. All statistical analyses were performed using JMP software 16.0.0 (SAS Institute, Cary, NC, USA).

## Results

*Characteristics of patients administered TCZ, SAR and MTX*

The clinical and serological characteristics of patients administered TCZ, SAR and MTX are summarised in Table I. There were no significant differences in age, sex, RA duration, body weight, RF and ACPA positivity, or RA activity among the patient groups. However, the dose of MTX used was higher in the MTX group than the TCZ and SAR groups, because some of the patients in the TCZ and SAR-administered groups did not use MTX at the time of recruitment. While CDAI was comparable between the groups, CRP and ESR levels were slightly but significantly high in the MTX control group, which is attributed to differences in the modes of action of the medications. Similarly, levels of IL-6 and sIL-6R were significantly higher in the TCZ and SAR-administered groups than the MTX control group, reflecting differences in the drugs' IL-6 signalling blockade. CRP, ESR, IL-6, sIL-6R levels were not significantly different between the TCZ and SAR-administered groups.

*Proportion of pSTAT3-positive CD4$^+$ T cells in each treatment group*

Following stimulation of whole blood with 100 ng/ml of rhIL-6, the proportion of pSTAT3-positive CD4$^+$ T cells was significantly different among the treatment groups (median 1.8% [0.9–3.0] *vs*. 7.7% [2.9–8.0] *vs*. 12.5% [11.4–16.6] *vs*. 71.5% [68.0–78.5] in the TCZ qw, SAR q2w, TCZ q2w, and MTX control group, respectively; $p<0.01$ for all comparisons; Fig. 1). The proportion of pSTAT3-positive CD4$^+$ T cells that resulted from stimulation of whole blood with each concentration of rhIL-6 (0, 0.1, 1, 10, and 100 ng/ml) is summarised in Figure 2.

## Discussion

SAR 200 mg q2w showed significantly stronger inhibition of IL-6/STAT3 signalling than TCZ sc q2w but weaker inhibition than TCZ sc qw. In our previous study, we assessed the influence of serum IL-6, soluble and membrane IL-6 receptor, and gp130 on CD4$^+$ T cells and found that while these parameters are affected by IL-6 blockade, they have no effect on the level of pSTAT3 in our assay (5). Levels of pSTAT3 in CD4$^+$ T cells stimulated by exogenous IL-6 reflect the strength of TCZ and SAR-induced IL-6 inhibition. However, a limitation of this method is that it does not directly indicate the strength of inhibition of IL-6-induced inflammation in the synovial environment, nor does it directly reflect each drug's clini-

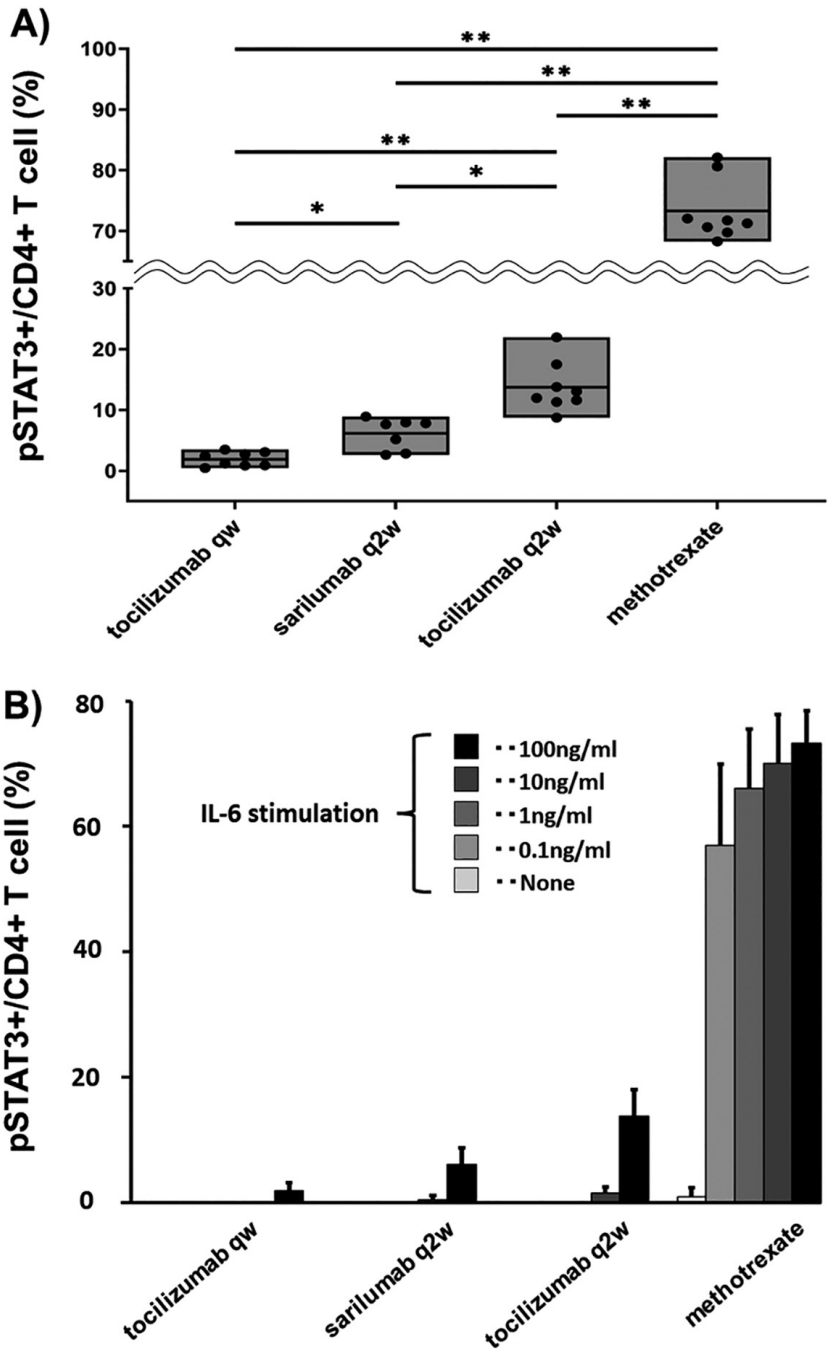


**Fig. 1. A**: Proportion of pSTAT3-positive CD4+ T cells in blood samples following stimulation with 100 ng/ml of IL-6. * $p<0.01$; ** $p<0.001$.
**B**: Proportion of pSTAT3-positive CD4+ T cells in blood samples following stimulation with IL-6 of 0, 0.1, 1, 10, and 100 ng/ml.
IL-6: interleukin-6; pSTAT3: phosphorylated STAT3.

cal efficacy. Our study demonstrated that the strength of inhibition of IL-6/pSTAT3 signalling differed among the drug regimens, all of which clinically lower disease activity in each group of patients with RA. This suggests that it is necessary to optimise inhibition of IL-6 signalling to each individual RA patient to lower disease activity. In addition, to see the previous report referring to the machine learning technology to predict the response to SAR (8), our result might be useful to consider the most suitable patient profile for treatment with SAR and TCZ when added as one of the variables in such systems. The results of this study are supported by similar reports from other groups. One study that simulated the IL-6 receptor occupancy rate based on blood samples acquired from patients who were administered a subcutaneous injection of TCZ and SAR showed that IL-6 receptor occupancy in the SAR sc q2w group was significantly higher than that in the TCZ sc q2w group, but lower than that in the TCZ sc qw group (9). The authors suggested that the higher affinity of SAR than TCZ for the IL-6 receptor may have influenced the receptor occupancy rate. Meanwhile, the SUMMACTA, BREVACTA, and MONARCH trials examined the change in CRP level after administration of SAR sc q2w, TCZ sc qw, and TCZ sc q2w. In these studies, RA patients had a comparable average CRP concentration of approximately 20 g/L at the initiation of IL-6 blockade treatment. Consistent with our results, the trials found that SAR administration led to intermediate average CRP concentrations between those observed for TCZ qw and TCZ q2w (6, 9, 10).

Several limitations of this study warrant mention. First, the main limitation of the study is the small number of patients in each group. Second, it is not clear whether the observed differences in strength of IL-6 signal inhibition are due to differences between the drugs' affinity for the IL-6 receptor or the amount of IL-6 blocker molecule in blood samples. Third, we could not examine the relationship between the proportion of pSTAT3-positive CD4+ T cells and the SAR trough level because of the lack of research kit to examine SAR concentration. Although, our previous report showed that the trough TCZ concentration is strongly and inversely correlated with the proportion of pSTAT3-positive CD4+ T cells, suggesting that the amount of free IL-6 blocker molecule and the concentration of TCZ or SAR may be important (5). Taken together with the preceding report (11), TCZ and SAR could be a potential candidate for therapeutic drug monitoring. The limitations notwithstanding, our findings suggest that there is a difference in strength of IL-6 signal blockade by TCZ and SAR.

The current study demonstrated that the strength of inhibition of IL-6/pSTAT3

signalling differed among drugs and administration regimens. The results of this study will be useful for adjusting the IL-6 blockade treatment for patients with RA.

**Key messages**

- SAR 200mg sc q2w showed significantly stronger IL-6/STAT3 signalling inhibition than TCZ 162mg sc q2w.
- SAR 200mg sc q2w showed significantly weaker IL-6/STAT3 signalling inhibition than TCZ 162mg sc qw.
- This is the first report to directly demonstrate differences in the strength of IL-6/STAT3 signalling inhibition between subcutaneously injected TCZ and SAR.

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**Competing interests**

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