Significance & Innovations

- A subcutaneous formulation of tocilizumab (TCZ) would greatly contribute to improving the quality of life in patients with rheumatoid arthritis (RA) because it would allow for a shorter administration time compared with an intravenous formulation and for home administration.
- Subcutaneous TCZ monotherapy demonstrated comparable efficacy and safety to intravenous TCZ monotherapy in patients with RA who have had an inadequate response to synthetic and/or biologic disease-modifying antirheumatic drugs.

Previously, patients with RA who did not respond to treatment, such as the 19th century French impressionist painter Pierre-Auguste Renoir, had limited alternatives available (8). Many treatment choices are now available that have proven clinical efficacy, including anti–tumor necrosis factor (anti-TNF) agents and TCZ. Most anti-TNF agents require concomitant methotrexate (MTX) for maximum efficacy, whereas TCZ has similar efficacy with and without MTX (9).

To optimize a patient's treatment, the efficacy, safety, and route of administration for each therapy should be considered along with a patient's disease status in order to achieve clinical, functional, and structural remission or the lowest disease activity state possible (10,11). Some patients prefer therapies with a biologic agent that can be administered by subcutaneous (SC) injection rather than IV formulations, and prefer to receive treatments at home (12–14). An SC formulation of TCZ (TCZ-SC) would provide an additional treatment option for patients with RA.

The efficacy and pharmacokinetics of TCZ-SC monotherapy were evaluated in an open-label, phase I/II study conducted in Japan at 3 doses (81 mg every 2 weeks, 162 mg every 2 weeks, and 162 mg weekly) over 6 months (15). To further expand on these results, the noninferiority, multicenter phase II study MUSASHI (Multi-Center Double-Blind Study of Tocilizumab Subcutaneous Injection in Patients Having Rheumatoid Arthritis to Verify Noninferiority Against Intravenous Infusion) was conducted to compare the efficacy and safety of TCZ-SC monotherapy 162 mg every 2 weeks and TCZ-IV monotherapy 8 mg/kg every 4 weeks in Japanese patients with RA with an inadequate response to synthetic and/or biologic DMARDs.

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PATIENTS AND METHODS

Patient population. Eligible patients were ages 20–75 years and had RA for ≥6 months, as diagnosed using the 1987 criteria of the American College of Rheumatology (ACR) for the classification of RA (16). Additional inclusion criteria were as follows: an inadequate response of ≥12 weeks to any synthetic DMARD (MTX, sulfasalazine, bucillamine, and leflunomide), biologic DMARD (infliximab, etanercept, and adalimumab), or immunosuppressant (e.g., tacrolimus); ≥8 tender joints (of 68 joints); ≥6 swollen joints (of 66 joints); and an erythrocyte sedimentation rate (ESR) ≥30 mm/hour or a C-reactive protein level ≥1.0 mg/dl.

Exclusion criteria included active tuberculosis, a history of serious allergies, and active hepatitis B or C. All candidates underwent tuberculin reaction or QuantiFERON testing. Patients testing positive for latent tuberculosis were enrolled if treatment with isoniazid was initiated 3 weeks prior to initial administration of TCZ and continued for 9 months. Patients with class IV Steinbrocker functional activity were excluded. Patients were also excluded if they had received previous treatment with TCZ; had received plasmapheresis, surgical procedures (except with locally and low invasive operations), or dose changes or added-in DMARDs or immunosuppressants within 4 weeks of TCZ treatment; had received oral glucocorticoids at a dosage of >10 mg/day of prednisolone or equivalent; or had a dose increase, new administration, or IV or intramuscular injections of glucocorticoids within 2 weeks of TCZ treatment.

Study design. MUSASHI was a 24-week, phase III, randomized, double-blind, double-dummy study in Japanese

patients with RA. The study protocol was approved by the Ministry of Health, Labour and Welfare of Japan and by the local ethical committees. All patients gave their written informed consent.

Patients were randomized 1:1 into 2 groups: 162 mg of TCZ-SC monotherapy every 2 weeks plus placebo TCZ-IV every 4 weeks or 8 mg/kg of TCZ-IV monotherapy every 4 weeks plus placebo TCZ-SC every 2 weeks. Throughout the study, DMARDs or immunosuppressants were not permitted. There was no washout period for synthetic DMARDs as long as treatment and dose were stable a minimum of 4 weeks prior to initial TCZ treatment. Concomitant use of low-dosage oral glucocorticoids (≤10 mg/day of prednisolone or equivalent without escalation from the baseline dosage) and 1 oral nonsteroidal antiinflammatory drug was permitted during the 24 weeks. Intraarticular injections of corticosteroids and hyaluronate preparations were avoided as much as possible.

Efficacy assessments. Efficacy assessments were conducted every 4 weeks. The primary end point was to demonstrate the noninferiority of TCZ-SC monotherapy to TCZ-IV monotherapy regarding the proportion of patients with 20% improvement in disease activity for ACR criteria (ACR20) responses at week 24 (17). Additional end points included ACR50 and ACR70 response rates, ACR/European League Against Rheumatism Boolean Index remission rates, Clinical Disease Activity Index (CDAI) remission rates, Disease Activity Score in 28 joints using the ESR (DAS28-ESR) remission rates, and a low disease activity rate at week 24. Mean changes in DAS28-ESR, CDAI score, and the proportion of patients who improved in the Japanese version of the Health Assessment Questionnaire (HAQ) by ≥0.3 units from baseline were assessed over time (18). For efficacy assessments, the per-protocol set (PPS) was used, excluding patients with protocol violations, early withdrawal, violations concerning concomitant medication use, or violations concerning the dose and administration. The last observation carried forward was used for any missing values. For patients receiving glucocorticoids or hyaluronic acid via intraarticular administration, any treated joints were treated as positive tender and swollen joints for that defined period.

Pharmacokinetics. Samples for pharmacokinetic analysis were collected at weeks 0, 2, 4, 8, 12, 16, 20, and 24. TCZ, which is not bound with the IL-6 receptor (free TCZ) in the serum, was determined by enzyme-linked immunosorbent assay (19). The lower limit of detection for free TCZ in serum was 0.1 μg/ml.

Safety and immunogenicity assessments. Safety and immunogenicity data were analyzed using the safety population, defined as all patients who received at least 1 dose of TCZ. Adverse events (AEs) and serious AEs were classified using the Medical Dictionary for Regulatory Activities, version 13.0. The number of patients with AEs and the total number of AEs were tabulated. Infusion and/or injection reactions were prespecified and classified as SC injection site reactions (ISRs; AEs at the site of SC injection), systemic reactions to SC injection (SIRs; AEs not at the site of SC injection within 24 hours of treatment), or IV infusion–related reactions (IRRs; AEs occurring within 24 hours of treatment). All AEs were graded as severe, moderate, or mild by physicians. Laboratory investigations were graded by Common Terminology Criteria for Adverse Events.

Blood samples for the anti-TCZ antibody screening assay were collected every 4 weeks. The anti-TCZ antibody screening assay was performed as previously described using a bridging enzyme-linked immunosorbent assay with an additional competitive displacement step as the confirmation assay (20).

Statistical analysis. The primary end point was analyzed using the PPS for the primary analysis and the modified intent-to-treat (ITT) population for the sensitivity analysis. The modified ITT population included all patients who received at least 1 dose of treatment. The noninferiority margin was set at 18%, as determined using the difference between the ACR20 results of SATORI (Study of Active Controlled Tocilizumab Monotherapy for Rheumatoid Arthritis Patients with an Inadequate Response to Methotrexate) (7); 18% was the more conservative criterion because it was less than one-third of the difference of the ACR20 response rate between the TCZ-IV monotherapy group and the control group in the SATORI study. Furthermore, it is less than half of the lower limit of the 95% confidence interval (95% CI) for the difference between the groups. The adjusted 95% CI for the difference between the ACR20 response rate in the TCZ-SC monotherapy and TCZ-IV monotherapy groups was calculated using the Mantel-Haenszel method, with patients stratified according to weight at enrollment (<60 or ≥60 kg) and previous use of anti-TNF agents. Noninferiority was demonstrated if the lower limit was not below the confidence limit for noninferiority (−18%). A sample size of 330 was calculated to provide 90% power to demonstrate the noninferiority of TCZ-SC monotherapy to TCZ-IV monotherapy. To determine the sample size, the ACR20 response rate was set to 70% because of the following assumptions: the ACR20 response rate at 24 weeks was 79.7% in the SATORI trial and the overall response rate potentially could be lower in the MUSASHI trial than in the SATORI trial because the patient population of inadequate anti-TNF responders was larger.

Simple logistic analysis was used to screen for potential predictive variables, including sex, age, weight (in kg, the fourth quartile versus the first to third quartiles), body mass index (BMI; in kg/m^2, the fourth quartile versus the first to third quartiles), disease duration, Steinbrocker class/stage, history of anti-TNF agents, rheumatoid factor, anti–cyclic citrullinated peptide antibody, glucocorticoid dose, number of previous DMARDs, DAS28-ESR, ACR core components, and IL-6 levels at baseline. Multiple logistic regression was used to identify the contributing baseline parameters to ACR20, ACR50, and ACR70 response rates in the TCZ-SC monotherapy group at week 24. The initial model contained the potential predictive variables and the predicting factor ($P \leq 0.05$) was identified in the final model by using a stepwise procedure.

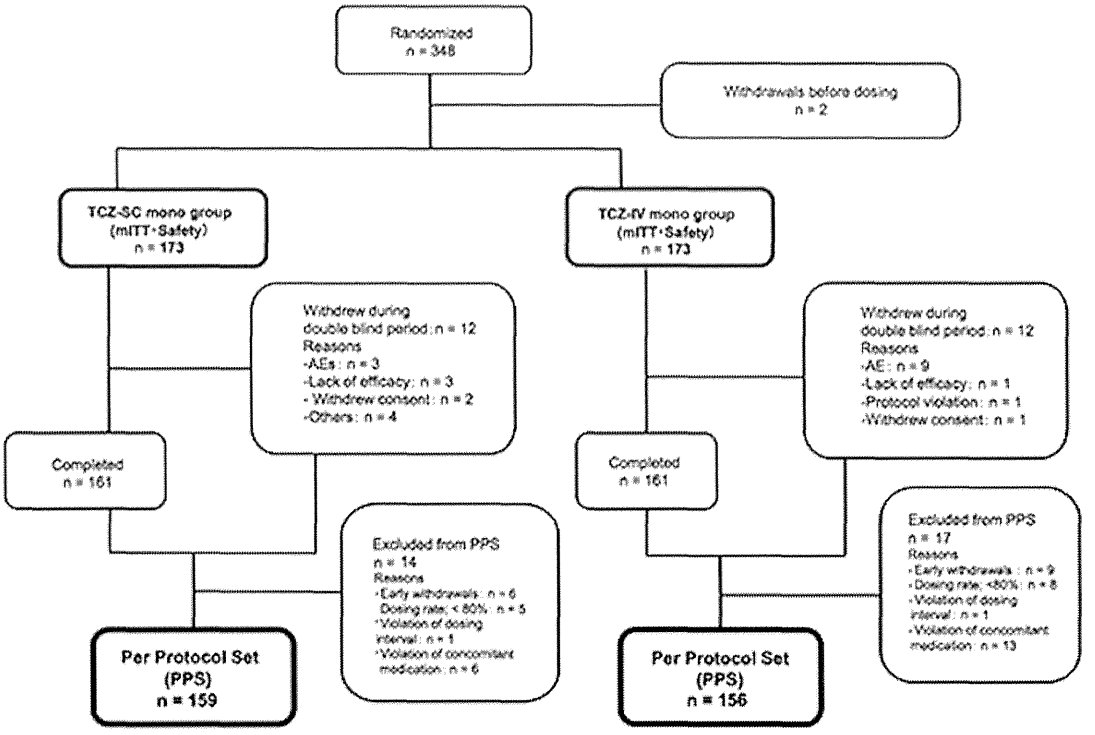


Figure 1. Patient disposition over 24 weeks (in the per-protocol set [PPS]). Two patients withdrew before treatment with tocilizumab (TCZ) was initiated. In the group receiving a subcutaneous injection of TCZ monotherapy (TCZ-SC mono), 3 patients withdrew because of adverse events (AEs), 3 patients withdrew because of a lack of efficacy, 2 patients withdrew consent, and 4 patients withdrew because of other reasons. In the group receiving an intravenous infusion of TCZ monotherapy (TCZ-IV mono), 9 patients withdrew because of AEs, 1 patient withdrew because of a lack of efficacy, 1 patient withdrew consent, and 1 patient withdrew because of a protocol violation. mITT = modified intent-to treat.

RESULTS

Patient disposition. A total of 348 patients were randomized (Figure 1). Two patients withdrew before treatment with TCZ and 346 patients were randomized into 2 groups; 173 patients in each group received the study drugs. Of these 173 patients, 161 (93.1%) completed the double-blind period in each group (Figure 1). In the PPS, 159 patients in the TCZ-SC monotherapy group and 156 patients in the TCZ-IV monotherapy group were eligible for analysis. The major reasons for patient exclusion from the PPS were receipt of <80% of the total dose, early withdrawal, and violations concerning concomitant medication use.

Baseline demographics and clinical characteristics. Patient demographics and clinical characteristics were similar between the TCZ-SC monotherapy and TCZ-IV monotherapy groups (Table 1). The patient population weighing ≥60 kg consisted of 23.3% in the TCZ-SC monotherapy group and 25.6% in the TCZ-IV monotherapy group. The percentages of patients who previously received anti-TNF agents were 18.9% in the TCZ-SC monotherapy group and 23.7% in the TCZ-IV monotherapy group (Table 1).

Clinical efficacy. The study met its primary end point of demonstrating the noninferiority of TCZ-SC monotherapy to TCZ-IV monotherapy. In the PPS, the ACR20 response rate at week 24 was achieved in 79.2% (95% CI 72.9, 85.5) of the TCZ-SC monotherapy patients and in 88.5% (95% CI 83.4, 93.5) of the TCZ-IV monotherapy patients (Figure 2A). The weighted difference between the groups was −9.4% (95% CI −17.6, −1.2), confirming the noninferiority of TCZ-SC monotherapy to TCZ-IV monotherapy. In the modified ITT population, the ACR20 response at week 24 was achieved in 79.2% (95% CI 73.1, 85.2) of the TCZ-SC monotherapy patients and in 86.0% (95% CI 80.9, 91.2) of the TCZ-IV monotherapy patients. The weighted difference between the groups was −7.0% (95% CI −15.0, 1.0), confirming the noninferiority of TCZ-SC monotherapy to TCZ-IV monotherapy in the sensitivity analysis. Another sensitivity analysis was conducted that was stratified according to disease duration and previous use of an anti-TNF agent. The weighted difference was −9.4% (95% CI −17.7, −1.1) and was consistent with the results of the PPS and modified ITT populations. ACR50 and ACR70 response rates at week 24 were also similar between the groups (Figure 2A).

The DAS28-ESR, CDAI, and Boolean Index remission rates at week 24 were 49.7%, 16.4%, and 15.7%, respectively, in the TCZ-SC monotherapy group. Conversely, the DAS28-ESR, CDAI, and Boolean Index remission rates at week 24 were 62.2%, 23.1%, and 16.0%, respectively, in the TCZ-IV monotherapy group (Figure 2B). A higher proportion of patients in the TCZ-IV monotherapy group (82.1% [95% CI 76.0, 88.1]) than in the TCZ-SC mono-

Table 1. Patient characteristics at baseline (per-protocol set)*

	TCZ-SC monotherapy (n = 159)	TCZ-IV monotherapy (n = 156)
Women, no. (%)	133 (83.6)	128 (82.1)
Age, years†	52.1 ± 12.6	51.8 ± 11.8
Body weight, median (min, max) kg†	53.0 (36.3, 83.3)	53.1 (37.5, 96.3)
Body weight, kg†	53.8 ± 8.7	54.4 ± 10.1
<60 kg, no. (%)	122 (76.7)	116 (74.4)
≥60 kg, no. (%)	37 (23.3)	40 (25.6)
Disease duration, years	7.3 ± 7.5	8.0 ± 7.3
Disease duration, median years	5.1	5.9
Steinbrocker functional class, no. (%)†		
I	25 (15.7)	20 (12.8)
II	112 (70.4)	118 (75.6)
III	22 (13.8)	18 (11.5)
Steinbrocker stage, no. (%)†		
I	20 (12.6)	8 (5.1)
II	53 (33.3)	60 (38.5)
III	47 (29.6)	42 (26.9)
IV	39 (24.5)	46 (29.5)
RF positive, no. (%)	126 (79.2)	131 (84.0)
ACPA antibodies, no. (%)	142 (89.3)	142 (91.0)
IL-6, pg/ml	39.1 ± 46.1	32.2 ± 42.8
SJC (in 66 joints)	14.3 ± 6.7	13.5 ± 6.8
TJC (in 68 joints)	18.1 ± 8.8	17.6 ± 9.4
Japanese HAQ score	1.18 ± 0.64	1.25 ± 0.65
Patient's pain assessment, mm	52.6 ± 23.1	58.4 ± 22.5
Patient's global assessment, mm	53.6 ± 24.9	59.7 ± 22.9
Physician's global assessment, mm	62.4 ± 20.0	61.3 ± 19.0
CRP, mg/dl	2.2 ± 2.3	2.1 ± 2.0
ESR, mm/hour	47.9 ± 24.4	48.8 ± 22.5
DAS28-ESR	6.1 ± 0.9	6.2 ± 0.9
CDAI score	34.2 ± 10.3	33.7 ± 10.8
Oral glucocorticoids administered, no. (%)	110 (69.2)	92 (59.0)
Dosage, mg/day‡	4.6 ± 2.3	4.7 ± 2.1
Previous MTX, no. (%)§	128 (80.5)	129 (82.7)
Dosage, mg/week§	8.2 ± 2.2	8.2 ± 2.3
Previous anti-TNF agents, no. (%)	30 (18.9)	37 (23.7)

* Values are the mean ± SD unless indicated otherwise. TCZ-SC = subcutaneous tocilizumab; TCZ-IV = intravenous tocilizumab; RF = rheumatoid factor; ACPA = anti–citrullinated protein antibody; IL-6 = interleukin-6; SJC = swollen joint count; TJC = tender joint count; HAQ = Health Assessment Questionnaire; CRP = C-reactive protein; ESR = erythrocyte sedimentation rate; DAS28-ESR = Disease Activity Score in 28 joints using the ESR; CDAI = Clinical Disease Activity Index; MTX = methotrexate; anti-TNF = anti–tumor necrosis factor.
† At randomization.
‡ Dosage is prednisolone or equivalent.
§ Patients who previously received MTX were analyzed within 4 weeks of initial TCZ treatment.

therapy group (65.4% [95% CI 58.0, 72.8]) achieved DAS28-ESR low disease activity at week 24. The mean change in DAS28-ESR and CDAI score decreased similarly over 24 weeks in both groups (Figures 2C and D). The proportions of patients who improved in physical function by ≥0.3 units (per the HAQ) from baseline between the TCZ-SC monotherapy and TCZ-IV monotherapy groups were 56.6% (95% CI 48.9, 64.3) and 67.9% (95% CI 60.6, 75.3), respectively, at week 24. The mean ± SD change in serum matrix metalloproteinase 3 (MMP-3) was similar in both groups (from 288.9 ± 204.7 ng/ml at baseline to 123.3 ± 89.9 ng/ml at week 24 in the TCZ-SC monotherapy group and from 290.0 ± 211.3 ng/ml at baseline to 101.7 ± 64.2 ng/ml at week 24 in the TCZ-IV monotherapy group).

To identify the background factors that influence efficacy, logistic regression analyses were applied to the ACR response rate. The result from stepwise regression, BMI in the fourth quartile (from 23.4 to 29.6 kg/m^2) at baseline, was detected as a significant variable for ACR20 response rate (63.4%; odds ratio [OR] 0.31 [95% CI 0.14, 0.70], P = 0.0048), ACR50 response rate (51.2%; OR 0.47 [95% CI 0.22, 0.98], P = 0.0444), and ACR70 response rate (24.4%; OR 0.39 [95% CI 0.17, 0.90], P = 0.0271).

Pharmacokinetics. The serum trough TCZ concentrations in the TCZ-SC monotherapy and TCZ-IV monotherapy groups were similar over time (Figure 3). More than 80% of patients maintained TCZ concentrations ≥1 μg/ml from week 4 onward in the TCZ-SC monotherapy group (Figure 3).

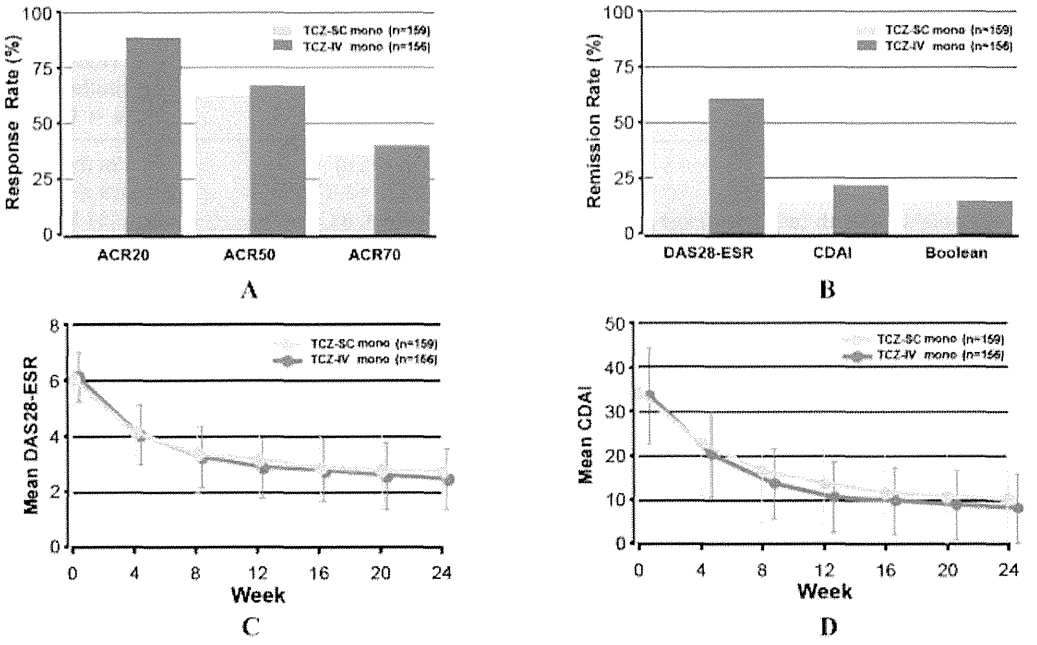


Figure 2. **A**, American College of Rheumatology (ACR) response rates of 20% (ACR20), 50% (ACR50), and 70% (ACR70) at week 24 (in the per-protocol set [PPS]) in patients receiving an intravenous infusion of tocilizumab monotherapy (TCZ-IV mono; n = 156) or a subcutaneous injection of tocilizumab monotherapy (TCZ-SC mono; n = 159). The ACR50 response rate in the TCZ-SC mono group was 63.5% (95% confidence interval [95% CI] 56.0, 71.0) and in the TCZ-IV mono group was 67.3% (95% CI 59.9, 74.7). The ACR70 response rate in the TCZ-SC mono group was 37.1% (95% CI 29.6, 44.6) and in the TCZ-IV mono group was 41.0% (95% CI 33.3, 48.7). The weighed differences of ACR50 and ACR70 response were −4.3% (95% CI −14.7, 6.0) and −3.8% (95% CI −14.5, 6.8), respectively. **B**, Disease Activity Score in 28 joints using the erythrocyte sedimentation rate (DAS28-ESR), Clinical Disease Activity Index (CDAI), and Boolean Index remission rates at week 24 (in the PPS). The rate of DAS28-ESR remission (<2.6) in the TCZ-SC mono group was 49.7% (95% CI 41.9, 57.5) and in the TCZ-IV mono group was 62.2% (95% CI 54.6, 69.8). The rate of CDAI remission (CDAI score ≤2.8) in the TCZ-SC mono group was 16.4% (95% CI 10.6, 22.1) and in the TCZ-IV mono group was 23.1% (95% CI 16.5, 29.7). The Boolean Index remission rate in the TCZ-SC mono group was 15.7% (95% CI 10.1, 21.4) and in the TCZ-IV mono group was 16.0% (95% CI 10.3, 21.8). **C**, DAS28-ESR over 24 weeks. The mean ± SD change in DAS28-ESR from baseline to week 24 in the TCZ-SC mono group was 6.1 ± 0.9 to 2.8 ± 1.4 and in the TCZ-IV mono group was 6.2 ± 0.9 to 2.5 ± 1.1. **D**, CDAI scores over 24 weeks. Error bars show the SD of the mean. The mean ± SD change in CDAI score from baseline to week 24 in the TCZ-SC mono group was 34.2 ± 10.3 to 10.3 ± 9.5 and in the TCZ-IV mono group was 33.7 ± 10.8 to 8.2 ± 7.8.

Safety. The safety profiles were comparable between the TCZ-SC monotherapy and TCZ-IV monotherapy groups, with the exception of ISRs, which occurred at a higher frequency in the TCZ-SC monotherapy group than in the TCZ-IV monotherapy group. Over 24 weeks, AEs occurred in 89.0% (154 of 173) and 90.8% (157 of 173) of patients, serious AEs occurred in 7.5% (13 of 173) and 5.8% (10 of 173) of patients, adverse drug reactions occurred in 83.2% (144 of 173) and 86.1% (149 of 173) of patients, and serious adverse drug reactions occurred in 3.5% (6 of 173) and 5.8% (10 of 173) of patients in the TCZ-SC monotherapy and TCZ-IV monotherapy groups, respectively. No deaths or malignancies were reported.

Infections were reported in 41.6% of the TCZ-SC monotherapy group and in 45.1% of the TCZ-IV monotherapy group. Nasopharyngitis was the most common event, occurring in 17.9% of the TCZ-SC monotherapy group and in 20.8% of the TCZ-IV monotherapy group. Serious infections (Table 2) occurred in 1.2% of patients in the TCZ-SC monotherapy group and in 2.9% of patients in the TCZ-IV monotherapy group.

ISRs occurred in 12.1% of patients (21 of 173) in the TCZ-SC monotherapy group and in 5.2% of patients (9 of 173) in the TCZ-IV monotherapy group (placebo injection). The most common event was injection site erythema (16 patients [9.2%] in the TCZ-SC monotherapy group and 5 patients [2.9%] in the TCZ-IV monotherapy group). Other ISRs included injection site hemorrhage, pruritus, hematoma, swelling, pain, and urticaria (see Supplementary Table 1, available in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/acr.22110/abstract). All ISRs were mild, and no cases resulted in withdrawal from the study.

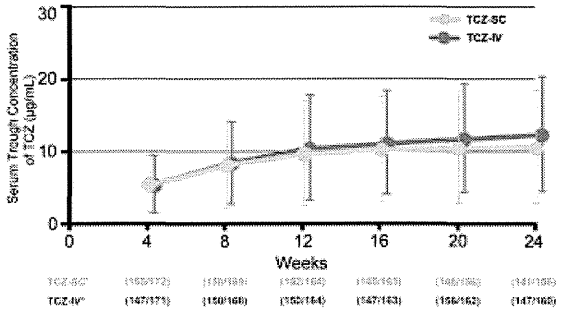
The incidence of SIRs from SC injection was 3.5% (6 of 173 patients) in the TCZ-SC monotherapy group, and the incidence of IV IRRs was 6.9% (12 of 173 patients) in the TCZ-IV monotherapy group. One patient (0.6%) in the TCZ-IV monotherapy group had an anaphylactic reaction after the second infusion (at week 4) and withdrew from

the study; this patient tested negative for anti-TCZ antibodies and recovered without sequelae. No patients in the TCZ-SC monotherapy group experienced serious hypersensitivity, including anaphylactic reactions.

The proportion of patients who experienced elevations in lipid levels and liver function tests during the blinded period was similar between the TCZ-SC monotherapy and TCZ-IV monotherapy groups (Table 3). The proportion of patients who experienced a grade 3 decrease in neutrophils (<1,000 to 500 cells/mm^3) was 2.9% (5 of 173 patients) in each group; 1 patient in the TCZ-SC monotherapy group withdrew. No grade 4 neutropenia (<500 cells/mm^3) was reported.

The incidence of elevated serum levels of Krebs von den Lungen-6 (KL-6) that exceeded the upper limit of normal (500 units/ml) and reached ≥1.5 times the baseline value was 3.8% in the TCZ-SC monotherapy group and 1.9% in the TCZ-IV monotherapy group. The incidence of elevated serum levels of pulmonary surfactant protein D (SP-D) that exceeded the upper limit of normal (110 ng/ml) and reached ≥1.5 times the baseline value was 6.9% in the TCZ-SC monotherapy group and 6.2% in the TCZ-IV monotherapy group. Patients who experienced increased levels of KL-6 and SP-D did not have any events of interstitial lung disease.

The proportion of patients who tested positive for anti-TCZ antibodies in the screening and confirmation assays was 3.5% (6 of 173) in the TCZ-SC monotherapy group and 0% in the TCZ-IV monotherapy group. Five of the 6 patients tested positive for anti-TCZ antibodies before week 12. No patients who developed anti-TCZ antibodies experienced ISRs, SIRs, or lack of efficacy after developing anti-TCZ antibodies.



Proportion of patients whose serum trough concentration of TCZ ≥ 1 μg/mL

Week	4	8	12	16	20	24
TCZ-SC mono (%)	80.2	85.8	87.2	85.7	91.7	85.9
TCZ-IV mono (%)	73.1	83.3	86.6	84.7	90.1	89.4

*Numbers of patients whose serum TCZ concentrations were above the limit of detection [> 0.1 μg/mL] and were collected at each point.

Figure 3. Mean serum trough tocilizumab (TCZ) concentrations over 24 weeks in patients receiving an intravenous infusion of TCZ monotherapy (TCZ-IV mono) or a subcutaneous injection of TCZ monotherapy (TCZ-SC mono). The table below the figure shows the proportion of patients in the TCZ-SC mono and TCZ-IV mono groups who had a serum trough TCZ concentration ≥1 μg/ml. At week 24, the mean ± SD serum trough TCZ concentration in the TCZ-SC mono group was 10.6 ± 7.8 μg/ml and in the TCZ-IV mono group was 12.4 ± 7.9 μg/ml.

Table 2. Summary of serious adverse events by patient*

SOC, preferred term	TCZ-SC monotherapy (n = 173)	TCZ-IV monotherapy (n = 173)
Infections and infestations		
Herpes zoster	–	2 (1.2)†
Pneumonia	–	2 (1.2)†
Cellulitis	1 (0.6)	1 (0.6)
Gastroenteritis	1 (0.6)	–
Gastrointestinal disorders		
Subileus	1 (0.6)†	–
Gastrointestinal hemorrhage	1 (0.6)	–
Ischemic colitis	–	1 (0.6)
Colonic polyp	1 (0.6)‡	–
Large intestine perforation	–	1 (0.6)
Vomiting	1 (0.6)†	–
Injury, poisoning, and procedural complications		
Spinal compression fracture	1 (0.6)‡	1 (0.6)†
Subdural hematoma	1 (0.6)†	–
Injury	1 (0.6)‡	–
Brain contusion	1 (0.6)†	–
Musculoskeletal and connective tissue disorders		
Synovitis	1 (0.6)‡	–
Spinal column stenosis	–	1 (0.6)†
Foot deformity	1 (0.6)‡	–
Respiratory, thoracic, and mediastinal disorders		
Pleurisy	–	1 (0.6)†
Chronic bronchitis	1 (0.6)‡	–
Asthma	1 (0.6)	–
Hepatobiliary disorders		
Hepatic function abnormal	–	1 (0.6)
Vascular disorders		
Hypertensive emergency	1 (0.6)†	–
Ear and labyrinth disorders		
Ménière disease	–	1 (0.6)
Nervous system disorders		
Intracranial hemorrhage	1 (0.6)†	
Metabolism and nutrition disorders		
Hyponatremia	1 (0.6)†	–
Immune system disorders		
Anaphylactic reaction	–	1 (0.6)
Benign, malignant, and unspecified neoplasms (including cysts and polyps)		
Neoplasm (benign)	1 (0.6)	–

* Values are the number (percentage). SOC = standard of care; TCZ-SC = subcutaneous tocilizumab; TCZ-IV = intravenous tocilizumab.
† Not related to the study drug. Occurred in the same patients, respectively.
‡ Not related to the study drug.

DISCUSSION

This noninferiority study was conducted to compare the efficacy of TCZ-SC monotherapy and TCZ-IV monotherapy in Japanese patients with RA who had inadequate responses to synthetic and/or biologic DMARDs. For the primary efficacy end point of ACR20 response rate at week

Table 3. Laboratory values*

	TCZ-SC monotherapy (n = 173)	TCZ-IV monotherapy (n = 173)
Shift in total cholesterol from baseline <200 mg/dl to worst value		
N	136	130
<200	39	37
200 to <240	65	58
≥240	32	35
Shift in HDL cholesterol from baseline <40 mg/dl to worst value		
N	29	14
<40	11	11
40 to <60	18	3
≥60	0	0
Shift in LDL cholesterol from baseline <100 mg/dl to worst value		
N	93	73
<100	17	17
100 to <130	51	44
130 to <160	24	8
160 to <190	1	4
≥190	0	0
Shift in ALT from normal at baseline to worst CTC grade		
N	164	165
Normal	124	124
Grade 1	35	32
Grade 2	4	7
Grade 3	1	2
Grade 4	0	0
Shift in AST from normal at baseline to worst CTC grade		
N	168	170
Normal	145	139
Grade 1	21	25
Grade 2	1	6
Grade 3	1	0
Grade 4	0	0
Shift in total bilirubin from normal at baseline to worst CTC grade		
N	173	172
Normal	149	154
Grade 1	21	13
Grade 2	3	5
Grade 3	0	0
Grade 4	0	0
Shift in neutrophils from normal at baseline to worst CTC grade		
N	170	172
Normal	130	125
Grade 1	19	20
Grade 2	16	22
Grade 3	5	5
Grade 4	0	0

* TCZ-SC = subcutaneous tocilizumab; TCZ-IV = intravenous tocilizumab; HDL = high-density lipoprotein; LDL = low-density lipoprotein; ALT = alanine aminotransferase; CTC = Common Terminology Criteria; AST = aspartate aminotransferase.

24, TCZ-SC monotherapy demonstrated noninferiority to TCZ-IV monotherapy in the PPS. The primary noninferiority analysis was made in the PPS, as recommended by the International Conference on Harmonisation E9 (21). To test the robustness of the noninferiority result, the results were validated by demonstrating the noninferiority of

TCZ-SC monotherapy to TCZ-IV monotherapy in the modified ITT population. From the results of secondary end points, the difference between TCZ-SC monotherapy and TCZ-IV monotherapy of ACR50 and ACR70 was smaller than ACR20. Furthermore, the mean change of the DAS28-ESR and CDAI score of TCZ-SC monotherapy was comparable to TCZ-IV monotherapy. These results support the noninferiority of TCZ-SC monotherapy to TCZ-IV monotherapy.

Two additional randomized, double-blind, phase III global studies (SUMMACTA and BREVACTA) evaluated TCZ-SC in combination with DMARDs in patients with RA from North America, Europe, South America, and Asia (other than Japan) (22,23). In the SUMMACTA study, TCZ-SC 162 mg every week was demonstrated to be noninferior to TCZ-IV 8 mg/kg every 4 weeks in combination with DMARDs using an ACR20 responder end point (noninferiority margin of 10%). The BREVACTA study demonstrated the superiority of TCZ-SC 162 mg every 2 weeks compared to placebo regarding the percentage of patients who achieved an ACR20 response at week 24. In both studies, the patients' mean body weight was 70–80 kg. In the MUSASHI study, TCZ-SC monotherapy dosing of every 2 weeks would be the most appropriate for Japanese patients with RA who have a lower body weight than patients in Western countries.

In Japan, the dose of TCZ-SC monotherapy of 162 mg every 2 weeks was selected from the previous phase I/II study with a mean body weight of 56 kg because it had a pharmacodynamic profile and TCZ trough concentration similar to those of the approved TCZ-IV dose of 8 mg/kg (15,24). In the current study, TCZ-SC monotherapy actually demonstrated TCZ trough concentrations comparable with those of TCZ-IV monotherapy despite a decrease in the given dose of TCZ in the TCZ-SC monotherapy group compared with the TCZ-IV monotherapy group if the weight is the same.

A previous TCZ-IV study reported that ≥ 1 μg/ml of serum TCZ was considered enough to suppress IL-6 signal transduction in the sera (19). In the current study, serum trough TCZ concentrations in the TCZ-SC monotherapy group were approximately equal to those in the TCZ-IV monotherapy group from week 4 onward, and most patients in both groups had TCZ concentrations ≥ 1 μg/ml. Prompt inhibition of IL-6 signaling by TCZ-SC monotherapy was also reflected in the time to improvement of disease activity, whereby the effectiveness of TCZ-SC monotherapy was approximately equal to that of TCZ-IV monotherapy from week 4 onward.

TCZ-SC monotherapy was administered as a fixed dose (162 mg), whereas the TCZ-IV monotherapy formulation was administered by body weight (8 mg/kg). In fact, trough TCZ concentrations tend to be lower in Japanese patients with a high body weight treated with TCZ-SC monotherapy (data not shown).

From the stepwise regression analyses, BMI in the fourth quartile at baseline was identified as a factor that contributed to low ACR response rates. However, more than half of patients in the fourth quartile of BMI achieved an ACR50 response. Therefore, it is unlikely that patients with high BMIs (23.4–29.6 kg/m^2) at baseline will have less response to therapy. With regard to the association between BMI and efficacy, further investigations are needed because the number of patients in the high BMI category was limited in this study. Previous use of anti-TNF agents was not identified as a factor that affected ACR response rates in the TCZ-SC monotherapy group. This suggests that the effect of TCZ-SC monotherapy on disease activity may be similar to that of TCZ-IV monotherapy in patients who have previously received anti-TNF agents.

Several studies have reported that TCZ as both monotherapy and in combination with DMARDs prevents joint destruction (4,6,9,23). The MMP-3 level in the TCZ-SC monotherapy group decreased at week 24 compared with baseline and was comparable with that in the TCZ-IV monotherapy group. Furthermore, the efficacy and serum TCZ trough concentrations were comparable between the TCZ-SC monotherapy and TCZ-IV monotherapy groups. These facts suggest that TCZ-SC monotherapy may also inhibit the progression of joint damage.

No new or unexpected safety issues were observed in this study. The safety profile of the TCZ-SC monotherapy group was similar to that of the TCZ-IV monotherapy group, except for ISRs. The incidence rate of ISRs was higher in the TCZ-SC monotherapy group than in the TCZ-IV monotherapy group. However, all events were mild and manageable. Although a direct comparison was difficult, the incidence of ISRs was not higher than that observed with other biologic agents that are administered by SC injection (10.4% with golimumab plus MTX and >30% with adalimumab monotherapy) (25,26). While the incidence rate of serious infection with TCZ-SC monotherapy was lower than with TCZ-IV monotherapy, there are not enough data to determine if this is a true difference. Additional data are being collected in the extension period. The serum levels of KL-6 and SP-D were reported to be elevated in patients with interstitial lung disease. The observed increase in serum KL-6 and SP-D levels was consistent with that in previous reports (27,28).

The number of patients who developed anti-TCZ antibodies was higher in the TCZ-SC monotherapy group than in the TCZ-IV monotherapy group. However, neither of these rates was numerically higher than the antidrug antibody rates reported for other biologic agents used to treat RA (29–32). None of the patients who tested positive for anti-TCZ antibodies experienced serious ISRs or hypersensitivity events, including anaphylaxis. The impact of anti-TCZ antibodies on efficacy was unclear because of the low number of patients who developed anti-TCZ antibodies. However, no patients who developed anti-TCZ antibodies experienced a lack of efficacy after developing anti-TCZ antibodies in this study.

The current study assessed the efficacy and safety of TCZ monotherapy without concomitant DMARDs. However, TCZ in combination with MTX was more commonly associated with elevated transaminases (9), and although the data on combination therapy with TCZ-SC are not yet available, the same effect is likely to be seen. Studies are currently ongoing to evaluate TCZ-SC in combination with DMARDs (22,23).

An SC formulation of TCZ would greatly shorten the administration time compared with the IV formulation

and would allow for home administration. Moreover, it would shorten the time and effort involved in the preparation of TCZ prior to administration and therefore would be more convenient for both patients with RA and health care professionals.

In summary, the noninferiority of TCZ-SC monotherapy to TCZ-IV monotherapy was confirmed. TCZ-SC monotherapy provided efficacy, safety, and serum trough concentrations of TCZ that were comparable with those of TCZ-IV monotherapy. The use of TCZ-SC monotherapy would provide an additional administration option for patients with RA.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Ogata had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Ogata.

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Review Article

Interleukin 6 and Rheumatoid Arthritis

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Interleukin-6 (IL-6) is a representative cytokine featuring pleiotropic activity and redundancy. A transient synthesis of IL-6 contributes to host defense against infectious agents and tissue injuries by inducing acute phase reactions and immunological and hematopoietic responses. However, uncontrolled persistent production of IL-6 may lead to the development of several immune-mediated diseases. Rheumatoid arthritis (RA) is a chronic disease with joint and systemic inflammation resulting from immunological abnormalities and it has been found that IL-6 plays a key role in the development of this disease. Clinical trials in various parts of the world of tocilizumab, a humanized anti-IL-6 receptor antibody, have proved its efficacy and tolerable safety either as monotherapy or in combination with disease-modifying antirheumatic drugs. As a result, it is currently used as a first-line biologic for the treatment of moderate-to-severe RA in more than 100 countries. Clarification of the mechanism(s) through which tocilizumab exerts its effect on RA and of the reason(s) why IL-6 is continuously produced in RA can be expected to lead to the best use of this agent for RA patients and aid in investigations into the pathogenesis of RA.

1. Introduction

Rheumatoid arthritis (RA) is characterized by synovial inflammation and hyperplasia, autoantibody production such as rheumatoid factor (RF) and anti-citrullinated protein antibody (ACPA), cartilage and bone destruction, and systemic features, including cardiovascular, pulmonary, psychological, and skeletal disorders [1]. Although its exact pathogenesis remains to be determined, a multistep progression is considered for the development of RA [1]. First, environment-gene interactions promote loss of tolerance to self-antigens that contain a citrulline residue generated by posttranslational modification. Second, the anticitrulline response is induced in T cells as well as B cells. Thereafter, localization of the inflammatory response occurs in the joint and synovitis is initiated and perpetuated by positive feedback loops and promotes systemic disorders. In this process, various cells and their products contribute to the development. For instance, as key molecules many cytokines including TNF-α, IL-1, IL-7, IL-15, IL-17A, IL-17F, IL-18, IL-21, IL-23, IL-32, and IL-33 are implicated in the pathogenesis of RA [1].

Before this century, the only drugs available for RA were nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and disease-modifying antirheumatic drugs (DMARDs) including gold, chloroxine, salazosulfapyridine, and methotrexate (MTX). However, these drugs were often not effective enough to completely suppress disease activity and joint destruction. The arrival of biological agents (biologics, biological DMARD) such as TNF inhibitors, abatacept, an inhibitor of T-cell costimulation, and rituximab, an agent leading to B-cell depletion induced a paradigm shift in the treatment of RA and Treat-to-Target (T2T) treatment proved to be successful for disease remission and protection against joint destruction [2].

Dysregulated persistent production of interleukin-6 (IL-6) also plays a key role in the development of the main characteristics of RA [3–5]. In response to the supposition

that IL-6 targeting could be a novel therapeutic strategy for RA, a humanized anti-IL-6 receptor monoclonal antibody (Ab), tocilizumab (TCZ), was developed. Subsequent clinical trials conducted all over the world have proved the efficacy and tolerable safety of TCZ and it is currently used as an innovative biologic for the treatment of RA in more than 100 countries. Moreover, TCZ was also approved for the treatment of systemic juvenile idiopathic arthritis in Japan, USA, EU, and India, and Castleman's disease in Japan and India, while recent various case reports or pilot studies of off-label use with TCZ suggest that it is widely applicable for the treatment of other immune-mediated diseases including vasculitis syndrome, adult-onset Still's disease, systemic lupus erythematosus, or others [4, 5]. In this paper, we present current evidence of the pathological role of IL-6 in the development of RA and the efficacy and safety profile of TCZ for RA and discuss future aspects of IL-6 targeting strategy for RA.

2. IL-6 and Signaling Pathway of IL-6

IL-6 is a glycoprotein with a molecular weight of 26 kDa and pleiotropic activity. It was first identified as B cell differentiation factor (BCDF) or B cell stimulatory factor 2 (BSF-2), which is a T-cell-derived soluble factor that induces the differentiation of activated B cells into Ab producing cells [6, 7]. Complementary DNA of IL-6 was successfully cloned by Hirano et al. in 1986 [8] and the resultant molecule was found to be identical to hybridoma growth factor (HGF), which derives its name from its promotion of growth of fusion cells with myeloma, to hepatocyte-stimulating factor (HSF) with its promotion of synthesis of acute phase proteins such as C-reactive protein (CRP), serum amyloid A (SAA), haptoglobin, fibrinogen, and hepcidin in hepatocytes, or to interferon (IFN)β2 due to its IFN anti-viral activity [9–11]. Subsequent studies also revealed that IL-6 performs multiple and essential functions in immune regulation, inflammation, and even oncogenesis and could be a key mediator for the development of many chronic inflammatory or autoimmune diseases including RA [12–14].

IL-6 triggers its signaling system through binding to an 80 kDa transmembrane IL-6 receptor (IL-6R) (Figure 1) [15, 16]. After binding to IL-6R, the complex consisting of IL-6 and transmembrane IL-6R associates with signal-transducing molecule gp130, resulting in the activation of downstream signaling events via Janus kinase (JAK) in target cells [17–20]. This activation is known as classic signaling pathway. Transmembrane IL-6R is expressed on only limited cells such as hepatocytes and some leukocytes, whereas gp130 is expressed on various cells. A soluble form of IL-6R (sIL-6R) lacking the cytoplasmic region exists in serum and has a similar affinity to IL-6 as transmembrane IL-6R. The complex of IL-6 and sIL-6R can also bind to gp130, leading to the activation of signaling cascade. This process is called trans-signaling. Accumulating evidence suggests that IL-6 trans-signaling is proinflammatory, whereas classic signaling is needed for regenerative or anti-inflammatory activities [21].

JAK is a member of the tyrosine kinase family, and its phosphorylation further induces the activation of signal transducer and activator of transcription (STAT) 3 and hyperphosphorylation of mitogen-activated protein kinases (MAPKs) [22]. The activation of the former is dependent on phosphorylation at tyrosine 759 (Y759) in gp130 and the latter requires phosphorylation on any residues of Y767, Y814, Y904, and Y915, which are all encountered in the YXXQ motif context. STAT3 then stimulates the expression of several genes leading to the induction of cell growth and differentiation [23–26]. MAPK also activates several transcription factors associated with acute phase protein synthesis and cell growth. Phosphorylation of a phosphoinositol-3 kinase (PI3K) by JAK results in activation of a third pathway by IL-6, which is the PI3K protein kinase B (PkB)/Akt pathway [27]. The activated Akt then phosphorylates several downstream targets to upregulate cellular survival [28].

3. Pathological Role of IL-6 in RA

RA is a chronic, progressive inflammatory disease of the joints and surrounding tissues accompanied by intense pain, if untreated, irreversible joint destruction, and systemic complications such as fatigue, anemia, and fever [1]. RA patients typically show immunological abnormalities leading to the production of autoantibodies such as RF and ACPA.

IL-6 has been shown to contribute to the production of autoantibodies by acting on plasmablasts [29]. Historically, IL-6 was originally identified as a helper T-cell-derived soluble factor that promoted immunoglobulin secretion by activated B cells [6, 7], while recent findings indicate that IL-6 also acts as regulator of CD4+ T cell differentiation and activation. IL-6 signaling has been found to control proliferation and resistance of resting T cells against apoptosis by promoting IL-2 production and STAT3 activation. In addition, IL-6 influences T cell effector functions by promoting Th2 cell differentiation through upregulation of nuclear factors of activated T cells (NFAT)c2 and c-maf, while it blocks IFN-γ-signaling and inhibits Th1 cell differentiation [30]. Moreover and more important, in the presence of transforming growth factor (TGF)-β, IL-6 is able to promote Th17 cell differentiation through STAT3-mediated upregulation of retinoid orphan receptor (ROR)γt, while it inhibits TGF-β-induced regulatory T cell (Treg) differentiation [31, 32]. IL-6 thus promotes predominance of Th17 over Treg in the effector CD4+ T cell subsets, which is thought to play a major role in the development of RA and various other immune-mediated diseases. In addition, IL-6 has been shown to promote T follicular helper cell development, which secretes IL-21, another B cell differentiation factor [33–35].

It has further been demonstrated that IL-6 is involved in local inflammation causing joint destruction by inducing endothelial cells to produce IL-8 and monocyte chemoattractant protein-1 (MCP-1) and to activate expression of adhesion molecules and recruit leukocytes to involved joints [36]. Synoviocytes can produce IL-6, while IL-6 can induce synoviocyte proliferation and osteoclast differentiation through receptor activator of NF-kappa B ligand (RANKL) expression

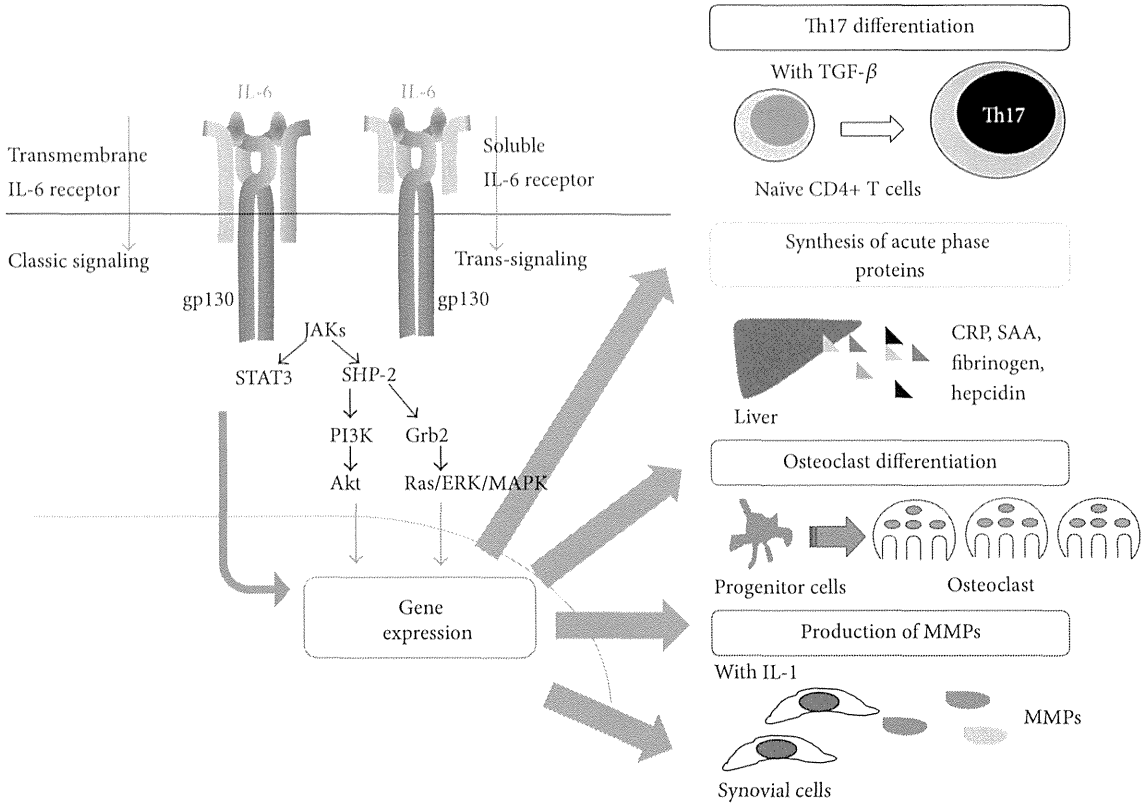

FIGURE 1: IL-6 exerts its pleiotropic activity by activation of gp130 through its binding to transmembrane or soluble IL-6 receptor. IL-6 initiates the IL-6 signaling pathway through binding to transmembrane or soluble IL-6 receptor. The resultant complex then induces homodimerization of gp130, which leads to activation of a signaling system. Transcriptional factors including STAT3 activate various gene expressions, resulting in cell differentiation or proliferation. JAKs: Janus kinases; STAT3: signal transducer and activator of transcription 3; SHP-2: SH2 domain-containing tyrosine phosphatase 2; PI3K: phosphoinositol-3 kinase; Grb2, growth factor receptor-bound protein 2; ERK: extracellular signal-regulated kinase; MAPK: mitogen activated protein kinase; Akt: protein kinase B; TGF-β: transforming growth factor beta; CRP: C-reactive protein; SAA: serum amyloid A; MMPs: matrix metalloproteinases.

[37, 38]. This stimulation by IL-6 is also associated with the development of osteoporosis and bone destruction. IL-6 and IL-1 synergistically enhance the production of matrix metalloproteinases (MMPs) from synovial cells, which may lead to cartilage and joint destruction [39]. Furthermore, enhanced angiogenesis and vascular permeability of synovial tissue are pathological features of RA resulting from the excess production of vascular endothelial growth factor (VEGF), which is also induced by IL-6 in synovial fibroblasts [40].

Systemic inflammatory signs and symptoms related to RA include fever, malaise, sleep disturbance, muscle weakness, and anemia, while laboratory findings observed in patients with RA are CRP elevation, hypercoagulability, and hypoalbuminemia. These are thought to be mostly mediated by IL-6 [5, 10, 11]. IL-6 induces hepcidin production, which blocks the action of iron transporter ferroportin 1 on gut and thus reduces serum iron and hemoglobin levels [41]. Moreover, RA patients often suffer from thrombocytosis, also mediated by IL-6, which promotes the differentiation of megakaryocytes into platelets [42].

These findings prove that IL-6 plays a key role in the induction of immunological abnormalities and in the development of joint and systemic inflammation of RA.

IL-6 was found to be elevated in serum as well as synovial fluid of patients with RA [43]. These levels correlated with disease activity of RA, while successful treatment with DMARDs or TNF inhibitors has been shown to reduce serum IL-6 concentrations [44–46]. Moreover, reduction in IL-6 levels during the first 12 months of treatment is reportedly a prognostic marker for better clinical outcome [47]. Recently, it was also shown that a decrease in serum IL-6 levels during TCZ treatment can be a predictive marker for maintenance of remission status [48]. These findings clearly point to the pathologic role of IL-6 in RA. However, it remains unknown what the exact mechanisms are through which IL-6 is continuously oversynthesized in RA and TCZ treatment leads to a reduction in intrinsic production of IL-6.

The pathological role of IL-6 in several animal models of RA was also documented. Collagen-induced arthritis (CIA) is the most well-known animal model of RA, in which injection of mice with type II collagen produces an immune response

directed at connective tissues. In the CIA model, activated T cells produce augmented amounts of both Th1 and Th17 cytokines, while deficiency of IL-6 activity through gene knockout suppresses Th17 cytokine production and clinical symptoms of arthritis [49, 50]. Similar results have been found for blockade of IL-6 signaling by using an anti-mouse IL-6R Ab [51, 52]. In this model, the proliferative response of B and T cells isolated from lymph nodes of anti-IL-6R-treated mice was significantly suppressed compared to controls. In addition, anti-IL-6R treatment led to amelioration of the histopathological features of arthritis including inflammatory synovitis and joint erosions. IL-6 gene deficiency and blockade of IL-6 activity also reduced severity of arthritis in other mouse models of RA, such as antigen-induced arthritis (AIA), an immune complex model of RA, and SKG mice which spontaneously develop autoimmune arthritis with ageing due to a spontaneous mutation in the zeta-chain-associated protein kinase-70 (ZAP-70) gene [53–57].

4. Development of Tocilizumab, a Humanized Anti-IL-6 Receptor Monoclonal Antibody

The findings described above led to the concept that IL-6 targeting might constitute a novel therapeutic strategy for RA. In response to this supposition, TCZ, a humanized anti-IL-6R monoclonal Ab of the IgG1 class, was developed [58]. TCZ blocks IL-6-mediated signal transduction through inhibition of IL-6 binding to transmembrane as well as soluble IL-6R. The first clinical evaluation of the efficacy of TCZ was conducted for the treatment of seven patients with Castleman's disease, a chronic inflammatory disease characterized by multiple lymph node swellings with massive infiltration of mature plasma cells [59]. Such patients present with severe inflammatory symptoms such as high fever, anemia, increased levels of acute-phase proteins, and hyper-γ-globulinemia. After TCZ administration, the fever promptly diminished, CRP levels became normalized, and hemoglobin levels increased. The efficacy of TCZ was next proved in a clinical trial using 28 patients with Castleman's disease [60], and this resulted in its approval as an orphan drug for the Japanese market in 2005.

The further development of TCZ entailed phase I and II clinical trials of TCZ for RA performed between 2002 and 2006 with favorable results [61–63]. The first trial was a randomized, double-blind, placebo controlled, dose-escalation trial in the UK [61]. Patients treated with 5 mg/kg or 10 mg/kg TCZ showed significant improvement by week 2. The next dosing determination trial was conducted in Japan. Patients were given a placebo or TCZ (4 or 8 mg/kg every 4 weeks) and 8 mg/kg TCZ resulted in the greatest improvement [62].

5. Efficacy of Tocilizumab in Phase III Clinical Trials and Actual as in Clinical Settings

Seven phase III randomized controlled trials (RCT) were conducted to evaluate the clinical efficacy of TCZ as either monotherapy or in combination with DMARDs including MTX (Table 1) [64–70].

5.1. Tocilizumab Combination Therapy. For further assessment of the efficacy of TCZ, RCTs of TCZ combination therapy were conducted. The OPTION trial was designed to evaluate the usefulness of TCZ (4 or 8 mg/kg every 4 weeks) in combination with MTX and the results demonstrated that this combination therapy was effective for and well tolerated by patients with active RA and an unsatisfactory response to MTX [64]. The TOWARD study compared the efficacy of TCZ (8 mg/kg every 4 weeks) plus DMARDs with that of DMARDs only for inadequate responders to DMARDs [65], and the RADIATE study compared the efficacy of TCZ (4 or 8 mg/kg every 4 weeks) plus MTX with that of MTX only for inadequate responders to TNF inhibitors [66]. Both studies showed evidence of a significant reduction of disease activity in the TCZ groups. The LITHE trial demonstrated that TCZ (4 or 8 mg/kg every 4 weeks) plus MTX had superior American College of Rheumatology (ACR20), 50 and 70 responses at 52 weeks compared with controls treated with placebo plus MTX [67].

5.2. Tocilizumab Monotherapy. The AMBITION trial was designed to compare the efficacy and safety of TCZ monotherapy with those of MTX monotherapy [68]. The results showed rapid improvement in RA disease activity and a favorable risk benefit profile for TCZ compared to MTX monotherapy. The SAMURAI study, which evaluated the efficacy of TCZ monotherapy for patients with an inadequate response to DMARDs, also showed a superior efficacy of TCZ compared to DMARDs [69]. Finally, the SATORI study investigated the efficacy of TCZ monotherapy for moderate-to-severe active RA patients with an inadequate response to low doses of MTX [70]. At week 24, the ACR20 response rate was 80.3% for the TCZ group and 25.0% for the MTX group.

In summary, TCZ as either monotherapy or in combination therapy with MTX or other DMARDs was highly efficacious for RA patients (Tables 1(a) and 1(b)).

5.3. Efficacy of TCZ in Protection of Radiographic Progression of Joints. In addition to clinical efficacy of TCZ in disease activity, TCZ showed beneficial effects in radiographic progression of joints (Table 1(c)). In the SUMURAI study, the TCZ group showed statistically significantly less radiographic change in the van der Heijde-modified Total Sharp Score (TSS) than the DMARD group at week 52 [69]. Moreover, the LITHE trial proved that at 52 week, the TCZ (either 4 mg/kg or 8 mg/kg) plus MTX group showed less progression of joint damage than the MTX group, as evaluated with the Genant-modified TSS (GmTSS) method [67].

5.4. Efficacy of TCZ in Phase IIIb/IV Trials and Clinical Practice. Following the seven phase III clinical trials, several phase IIIb/IV studies were conducted. The REACTION study performed in Japan showed that by 24-week treatment with TCZ, average disease activity score (DAS) 28 of 229 patients significantly decreased from 5.70 to 3.25 and a European League Against Rheumatism (EULAR) good response and DAS remission was achieved in 57.4% and 40.7% of the patients, respectively [71]. Moreover, at week 52, radiographic

Table 1: Randomized phase III controlled trials of tocilizumab.

(a) Clinical efficacy of tocilizumab (Tocilizumab combination therapy)

Study	Population	Week at evaluation	Treatment arms	Patient number	HAQ (% ≥MCID)	Response rates (%), OR (95% CI) ACR20	ACR50	ACR70	DAS28 < 2.6 remission rate (%), OR (95% CI)
TOWARD	DMARDs-IR	24 W	TCZ (8 mg/kg) + DMARDs	803	60****	61****	38****	21****	30****, 13.8
			DMARDs	413	34	25	9	3	3
RADIATE	Anti-TNF-IR	24 W	TCZ (4 mg/kg) + MTX	161	Δ − 0.3**	30***	17****	5	8, 4.3
			TCZ (8 mg/kg) + MTX	170	Δ − 0.4****	50***	29****	12****	30***, 21
			MTX	158	Δ − 0.1	10	4	1	2
OPTION	MTX-IR	24 W	TCZ (4 mg/kg) + MTX	214	Δ − 0.52*	48****, 2.6 (1.7–3.9)	31****, 3.8 (2.3–6.5)	12****, 7.0 (2.4–20.4)	13***, 18.8 (2.5–142)
			TCZ (8 mg/kg) + MTX	205	Δ − 0.55**	59****, 4.0 (2.6–6.1)	44****, 6.6 (3.9–11.2)	22****, 14.2 (5.0–40.4)	27****, 45 (6.1–332)
			MTX	204	Δ − 0.34	26	11	2	1
LITHE	MTX-IR	52 W	TCZ (4 mg/kg) + MTX	399	60	47*	29*	16*	30*, 4.92
			TCZ (8 mg/kg) + MTX	398	63*	56****	36****	20****	47****, 10.2
			MTX	393	53	25	10	4	8

(b) Tocilizumab monotherapy

Study	Population	Week at evaluation	Treatment arms	Patient number	HAQ (% ≥MCID)	Response rates (%), OR (95% CI) ACR20	ACR50	ACR70	DAS28 < 2.6 remission rate (%), OR (95% CI)
AMBITION	MTX, anti-TNF naïve	24 W	TCZ (8 mg/kg)	286	Δ − 0.7	70***	44**	28***	34$^{n.d.}$, 5.83 (3.27–10.4)
			MTX	284	Δ − 0.5	53	34	15	12
SAMURAI	DMARDs-IR	52 W	TCZ (8 mg/kg)	157	68***	78***	64***	44***	59***, 46.5
			DMARDs	145	40	34	13	6	3
SATORI	MTX-IR	24 W	TCZ (8 mg/kg)	61	67****	80***	49$^{n.d.}$	30$^{n.d.}$	43***, 37.0
			MTX	64	34	25	11	6	2

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

HAQ: health assessment questionnaire disability index; MCID: minimal clinical important difference; OR: odds ratio; CI: confidence interval; DMARDs: disease-modifying antirheumatic drugs; IR: inadequate response; TCZ: tocilizumab; TNF: tumor necrosis factor; MTX: methotrexate; n.d.: not described.

(c) Efficacy of tocilizumab in protection of radiographic progression of joints

Study	Radiographic assessment	Week at evaluation	Treatment arms	Proportion without progression TSS ≦ 0	Change in score (95% CI) Total score	Erosion score	JSN score
SAMURAI	van der Heijde-modified Sharp score	52 W	TCZ (8 mg/kg)	56**	2.3**, (1.5–3.2)	0.9***, (0.3–1.4)	1.5*, (0.9–2.1)
			DMARDs	39	6.1 (4.2–8.0)	3.2 (2.1–4.3)	2.9 (2.0–3.8)
LITHE	Genant-modified Sharp score	52 W	TCZ (4 mg/kg) + MTX	81****	0.34****	0.21*	0.13*
			TCZ (8 mg/kg) + MTX	84****	0.29****	0.17****	0.12**
			MTX	67	1.13	0.71	0.42

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

TSS: total Sharp score; CI: confidence interval; TCZ: tocilizumab; DMARDs: disease-modifying antirheumatic drugs; JSN: joint space narrowing; MTX: methotrexate.

nonprogression and functional remission were achieved in 62.8% and 26.4% of 232 patients, respectively [72]. Interestingly, progression of joint destruction was found to be similar with or without concomitant MTX, glucocorticoids, or previous use of TNF inhibitors. The ACT-RAY trial was performed to compare TCZ plus MTX with TCZ plus a placebo in a setting that closely resembled real-life clinical practice [73]. After 24 weeks, ACR20, 50, and ACR70 response rates were 71.5%, 45.5%, and 24.5%, respectively, for the TCZ plus MTX group and corresponding rate of 70.3%, 40.2%, and 25.4% for the TCZ monotherapy group. This study demonstrated that TCZ plus MTX combination therapy and TCZ monotherapy could both be expected to be effective in real-life clinical practice, and importantly, that TCZ plus MTX combination was not significantly superior to TCZ monotherapy (Table 2). These and other studies showed that TCZ treatment improved disease activity, joint destruction, and quality of life. Moreover, a recent trial comparing TCZ (8 mg/kg intravenously every 4 weeks) monotherapy with adalimumab (40 mg subcutaneously every 2 weeks) monotherapy (ADACTA trial) proved the clinical superiority of TCZ [74] (Table 2). TCZ as monotherapy can thus be considered to be more beneficial than other biologics [75]. However, a meta-analysis of systematic reviews of clinical trial data indicates that TCZ, TNF inhibitors, and abatacept have similar efficacy in combination with MTX [76].

5.5. Efficacy of Subcutaneous Injection of TCZ in Phase III Trials. Intravenous injection every 4 weeks of TCZ (4 or 8 mg/kg) is currently used for the treatment of moderate-to-severe active RA, but recent clinical trials (MUSASHI and SUMMACTA) demonstrated that subcutaneous administration of TCZ (162 mg) weekly or every 2 weeks showed efficacy and safety comparable to those of intravenous injection of TCZ (8 mg/kg every 4 weeks) [77, 78] (Table 2). The MUSASHI study was a double-blind, double-dummy, parallel-group, comparative phase III study to evaluate the efficacy and safety of subcutaneous (SC) versus intravenous (IV) TCZ monotherapy for patients with RA and an inadequate response to synthetic DMARDs and/or biologics. A total of 346 patients were randomized to receive TCZ-SC 162 mg every 2 weeks or TCZ-IV 8 mg/kg every 4 weeks. At week 24, ACR20 response was achieved in 79.2% of the TCZ-SC group and in 88.5% of the TCZ-IV group, showing that TCZ-SC was not inferior to TCZ-IV [77]. The incidences of all adverse events (AEs) and serious AEs were 89.0% and 7.5% for the TCZ-SC group and 90.8% and 16.4% for the TCZ-IV group, respectively, while serum trough TCZ concentrations were similar for the two groups during the test period. The SUMMACTA trial was a randomized, double-blind, parallel-group study to evaluate the safety and efficacy of TCZ-SC in comparison with TCZ-IV combined with DMARD for patients with moderate-to-severe RA. A total of 1,262 patients were randomly assigned to receive TCZ-SC 162 mg weekly or TCZ-IV 8 mg/kg every 4 weeks in combination with DMARD [78]. At week 24, 69.4% of the TCZ-SC-treated patients versus 73.4% of the TCZ-IV-treated patients attained an ACR20 response. Moreover, ACR50/70 responses, DAS28 improvement and the safety profiles were similar for the two groups.

6. Safety Profile of Tocilizumab

The comparison of AEs between the control population (4,199) and the TCZ-treated population (4,009) was reported in 2011 [79]. Overall AE and serious AE rates were 278.2/100 patient-year (PY) and 14.4/100 PY, respectively. These events included serious infections (4.7/100 PY), opportunistic infections (0.23/100 PY), gastrointestinal perforations (0.28/100 PY), malignancy (1.1/100 PY), myocardial infarction (0.25/100 PY), and stroke (0.19/100 PY). Short-term (28 weeks) safety of TCZ for 7,901 patients was monitored in a postmarketing surveillance in Japan [80]. The incidence of total AEs and serious AEs was 43.9% and 9.6%, respectively. Infection and infestation were the most frequent AEs (11.1%) and serious AEs (0.5%). Analysis of long-term safety showed that rates of serious AEs, serious infections, and cardiovascular events remained stable during continued exposure to TCZ in long-term clinical trials. Infection was identified as the most frequent serious AE. The most commonly reported infections in RCTs were pneumonia (0.9/100 PY) and skin or soft tissue infections (0.9/100 PY). These results lead to the conclusion that infections were the most frequent AEs but a meta-analysis comparing the safety profile of TCZ with that of other biologics including TNF inhibitors, anakinra (IL-1R antagonist), abatacept, and rituximab showed similar rates of infection [81]. In contrast to the finding for infections, no increase in the incidence of malignancy or reactivation of tuberculosis was seen in TCZ-treated RA patients [82]. Gastrointestinal perforation appeared to be an AE specific for TCZ with an incidence rate of 1.9/1,000 PY [83]. This rate fell between those of 3.9/1,000 PY for corticosteroids and 1.3/1,000 PY for TNF inhibitors listed in the United Health Care database. While it is not clear at present why IL-6 blockade induced perforation, most cases were complications of diverticulitis. IL-6 also affects metabolism. Increases in mean fasting levels of plasma lipids such as total cholesterol, low-density lipoprotein, triglycerides, and high-density lipoprotein were detected in 20–30% of patients treated with TCZ. These higher lipid levels resulting from TCZ treatment are perhaps mediated by the influence of TCZ on lipoprotein receptor expression, since it has been recently shown that overproduction of IL-6 lowers blood lipid levels via upregulation of the very-low-density lipoprotein (VLDL) receptor [84]. In spite of this elevation of lipids, an analysis combining the data of various clinical trials showed no apparent increase in cardiac events in a followup of up to 5 years [82].

7. Other IL-6 Inhibitors in Development

The success of the indication of TCZ for the treatment of RA clarified that IL-6 blockade was a therapeutic strategy for RA, so that other IL-6 inhibitors are now being

Table 2: Pivotal clinical trials of tocilizumab.

Study	Population	Week at evaluation	Treatment arms	Patient number	HAQ (% ≥MCID)	Response rates (%), OR (95% CI) ACR20	ACR50	ACR70	DAS28 remission rate (%), OR (95% CI)	Conclusion
ACT-RAY	MTX-IR	24 W	TCZ (8 mg/kg) + PBO	276	Δ − 0.5	70	40	25	35	No difference of efficacy between TCZ and TCZ + MTX
			TCZ (8 mg/kg) + MTX	277	Δ − 0.5	72	46	25	40, 5.6 (−2.4–13.7)	
ADACTA	MTX-IR	24 W	TCZ-IV (8 mg/kg/4 weeks)	163	Δ − 0.7	65** 2.0 (1.2–3.1)	47*** 2.4 (1.5–3.9)	33** 2.3 (1.3–3.8)	40**** 5.7 (3.1–10.3)	TCZ is superior to ADA as monotherapy
			ADA-SC (40 mg/2 weeks)	162	Δ − 0.5	49	28	18	11	
MUSASHI	MTX-IR	24 W	TCZ-IV (8 mg/kg/4 weeks)	173	68	89	67	41	62	Noninferiority of TCZ-SC to TCZ-IV
			TCZ-SC (162 mg/2 weeks)	173	57	79	63	37	50	
SUMMACTA	DMARDs-IR	24 W	TCZ-IV (8 mg/kg/4 weeks) + DMARD	631	67	73	48	27	36	Noninferiority of TCZ-SC to TCZ-IV
			TCZ-SC (162 mg/week) + DMARD	631	65	69	47	24	38	

** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

HAQ: health assessment questionnaire disability index; MCID: minimal clinical important difference; OR: odds ratio; CI: confidence interval; MTX: methotrexate; IR: inadequate response; TCZ: tocilizumab; PBO: placebo; IV: intravenous injection; ADA: adalimumab; SC: subcutaneous injection; DMARDs: disease-modifying antirheumatic drugs.

developed. These include fully human anti-IL-6R Ab (sarilumab/REGN88/SAR153191), anti-IL-6R nanobody (ALX-0061), anti-IL-6 Abs such as sirukumab (CNTO 136), BMS-945429 (ALD518), olokizumab (CDP6038), and MEDI5117, and soluble gp130-Fc fusion protein (FE301), which selectively inhibits trans-signaling but not classic signaling [5].

The favorable results of phase II, randomized, double-blind, placebo-controlled trials of sarilumab [85] and sirukumab [86] confirmed the effectiveness of IL-6 blockade strategy in RA. The phase II MOBILITY study evaluated efficacy and safety of subcutaneous injection of sarilumab, in which 306 RA patients were randomized to receive a 12-week administration of sarilumab 100 mg or 150 mg every week, 100 mg, 150 mg, or 200 mg every 2 weeks, or placebo added to stable MTX [85]. An ACR20 response was seen in 49.0% of the patients receiving the lowest sarilumab dose regime and in 72.0% of the patients receiving the highest dose regime, compared to 42.0% of those treated with placebo plus MTX. The types and incidence of AEs were consistent with those previously reported for TCZ. Sirukumab is a fully human monoclonal Ab to IL-6, and 151 RA patients were enrolled into a phase II trial [86]. The patients were randomized equally to receive subcutaneous injections of placebo every 2 weeks for weeks 0–10 and sirukumab 100 mg every 2 weeks for weeks 12–24, or sirukumab 25, 50, or 100 mg every 4 weeks, or 100 mg every 2 weeks for weeks 0–24. At week 12, more patients receiving sirukumab were in remission than those given the placebo according to Boolean- and simplified disease activity index (SDAI)-based ACR/EULAR criteria (2% versus 0% and 6% versus 3%). At week 24, high remission rates were attained with sirukumab at dose regimens ranging from 25 to 100 mg every 2–4 weeks, determined with ACR/EULAR or DAS28 (CRP) criteria. The types and incidence of AEs were consistent with those observed for TCZ.

8. Perspectives

In view of the outstanding clinical efficacy and tolerable safety of TCZ, TCZ is now recommended as one of first-line biologics for the treatment of active RA. However, several issues need to be clarified for realization of the optimal use of TCZ. First, an important issue is to clarify the mechanisms, which render IL-6 blockade efficacious for RA. Although it is clear that TCZ treatment led to improvements in markers related to systemic inflammation and bone and cartilage metabolisms [87–89], it remains to be determined whether the treatment can correct fundamental immunological abnormalities in RA [90]. As mentioned before, IL-6 has the capability of promoting autoantibody production and of causing imbalance between Th17 and Treg [31, 32]. Recent preliminary studies showed that TCZ treatment could rectify the imbalance in the peripheral blood CD4+ T cell population [91, 92]. Moreover, a 6-month treatment with TCZ led to a selective decrease in IL-21 production by memory/activated T cells in eight patients with RA [93]. Elevation of IL-21 has been detected in patients with RA [94] and is known to induce plasma cell differentiation and induce IgG4 production but the TCZ treatment resulted in a reduction in IgG4 subclass ACPA titer [35, 94]. These findings suggest that IL-6 blockade strategy may indeed correct immunological abnormalities in RA, but the findings of these studies have limited robustness due to the small sample size, so that further analyses will be required.

Second, the reason or reasons why IL-6 synthesis is continuously induced in RA remain to be clarified. One genetic polymorphism (−174) in the IL-6 gene promoter, which was found to affect IL-6 levels [95], did not appear to universally increase susceptibility to RA, but a recent meta-analysis showed that the −174 polymorphism might confer susceptibility to RA, at least in Europeans [96]. IL-6 can be produced by immune competent cells, fibroblasts, synoviocytes, endothelial cells, and many other cells in response to various stimuli [13]. The synthesis of IL-6 is strictly regulated by transcriptional and posttranscriptional mechanisms and a number of transcriptional factors, RNA binding proteins, and microRNAs have been shown to control IL-6 synthesis [97]. Moreover, it has been recently reported that newly found molecules such as Regnase-1 and Arid5a affect post-transcriptional regulation of IL-6 mRNA degradation [98–100]. Regnase-1 binds to the 3′ untranslated region of IL-6 mRNA and splits up IL-6 mRNA, whereas Arid5a binds to a similar region and stabilizes IL-6 mRNA. Moreover, some viral proteins or microRNAs reportedly activate the IL-6 gene and/or inhibit mRNA degradation [97]. It can therefore be anticipated that clarification of mechanisms by which dysregulated, persistent production of IL-6 is induced in RA will lead to an enhanced understanding of the pathogenesis of RA.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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