2. Materials and methods

2.1. Materials

Tango™ CXCR7-bla U2OS cells, Tango™ CXCR4-bla U2OS cells and LiveBLAzer™ FRET B/G substrate were obtained from Invitrogen (Carlsbad, CA). pORF9-hCXCR7 expression vector was purchased from Invivogen (San Diego, CA). Human CXCL12/SDF-1α recombinant protein, anti-CXCR7 antibody (clone 11G8) and mouse IgG1 isotype control were obtained from R&D Systems, Inc. (Minneapolis, MN). Antibodies for Akt (rabbit polyclonal), pThr308 Akt (rabbit, clone 244F9) and β-actin (rabbit, clone 13E5) were obtained from Cell Signaling Technology Inc. (Beverly, MA). Rabbit polyclonal anti-CXCR4 antibody was purchased from Abcam plc. (Cambridge, UK).

2.2. Beta-lactamase reporter assay (Tango™)

The Tango™ U2OS cell lines (Invitrogen, Carlsbad, CA) were maintained as described [15]. When CXCR7 gene was transfected, the cells were plated at 3×10^5 cells/well in a 6-well-plate and incubated overnight at 37 °C with 5% CO_2. The cells were then transfected with 2 μg of receptor expression plasmids (treated with Ase1 and Ssp1 to cut the ampicillin resistant region beforehand) using FuGENE®6 Transfection Reagent (Promega Corporation, Madison, WI) as directed by the manufacturer's protocol. The details of β-lactamase reporter assay have been described elsewhere [16]. Specifically, the cell lines were exposed to the compound for 30 min prior to treatment with CXCL12 for 5 h at 37 °C with 5% CO_2. The fluorescence emission values at 460 nm and 535 nm were obtained using an Envision plate reader (PerkinElmer Inc., Waltham, MA).

2.3. In vitro tube formation assay

HUVECs were cultured for 4 days in endothelial basal medium (EBM-2) containing growth factors (EGM-2 bullet kit; Lonza, Basel, Switzerland). The medium was then changed to growth factor-free EBM-2 to remove angiogenesis-inducing activities. Growth factor-reduced Matrigel (BD Biosciences, San Jose, CA) was thawed at 4 °C over night, and 120 μl of Matrigel were added to each well of a 48-well plate and incubated for 30 min at 37 °C to polymerize. After 24 h-starvation, the HUVECs were incubated with or without the compound for 15 min and then cultured further on the Matrigel, with recombinant human CXCL12 (100 ng/ml, R&D Systems) added to the wells for 20 h. Cells were photographed using a BIOREVO BZ-9000 microscope equipped with a CCD camera (Keyence Corp., Osaka, Japan). The length of tube-like structures in the images was measured and the relative tube length was calculated as follows: the average length of the tubes per field with stimulation and/or inhibitor divided by the average length of the tubes without stimulation in each experiment.

2.4. Gene transfection

pORF9-hCXCR7 vector was transfected into HEK293FT cells by using FuGENE®6 Transfection Reagent (Promega Corporation) according to the manufacturer's instruction. After 2 days, cells were treated with CXCL12 or Compound 1 for following processes.

2.5. Quantitative RT-PCR

RNA was reverse transcribed using oligo-dT primers. Real time PCR was performed using KAPA SYBR® FAST qPCR Kits (Kapa Biosystems Inc., Woburn, MA). Gene-specific primers for human GAPDH, CXCR4 and CXCR7 were obtained from Takara Bio Inc. (Otsu, Japan).

2.6. Western blotting

Cells were lysed in ice-cold Cell Lysis Buffer (Cell Signaling Technology, Inc.) containing a cocktail of protease inhibitors (Nacalai Tesque Inc., Kyoto, Japan). Proteins were denatured by heating to 100 °C for 5 min in SDS sample buffer, loaded onto and separated by 4–20% gradient SDS polyacrylamide gels (Bio-Rad Laboratories Inc., Hercules, CA), and then transferred electronically to a polyvinylidene fluoride (PVDF) membrane. The membrane was blocked with Block Ace (DS Pharma Biomedical Co., Ltd., Osaka, Japan) for 1 h and then was incubated overnight with the following dilution of primary antibodies: polyclonal anti-Akt (1:1000), monoclonal anti-p-Akt (Thr308) (1:1000), polyclonal anti-CXCR4 (1:500) and monoclonal anti-β-actin (1:1000). The membranes were incubated with horseradish peroxidase-conjugated anti-rabbit secondary antibody at 1:5000 dilution for 1 h at room temperature, and after washes, visualized for immunoreactivity using an Enhanced Chemiluminescence (ECL) System (GE Healthcare UK Ltd., Amersham Place, UK).

3. Results

3.1. The compound specific for CXCR7 activated signal downstream of CXCR7

CXCR7 has only been identified as a chemokine receptor for CXCL12 relatively recently [6,7]and its biology is still largely unknown. We therefore tried to examine how its interaction with CXCL12 and possibly CXCR4 affects cellular events. Compound 1, and its analogs (Compound 2 and 3), are reported to be CXCR7-specific binding compounds, with potency in the low nanomolar range (WO2007/059108, Fig. 1A). To validate the biological activity of the generated compound, we performed SelectScreen® profiling in the Tango™ CXCR7-bla U2OS expression system (Invitrogen, San Diego, CA). These cells express CXCR7 modified to contain a TEV protease site that is linked to an integrated Gal4-VP16 transcription factor. Binding of CXCL12 to CXCR7 and consequent recruitment of β-arrestin leads to cleavage of Gal4-VP16 by the TEV protease tagged with β-arrestin, resulting in detectable β-lactamase activity. Compound 1 strongly induced β-arrestin recruitment to CXCR7 in this system in a dose dependent manner (Fig. 1B and Supplement 1). On the other hand, it failed to inhibit CXCL12-induced β-arrestin recruitment to CXCR7 and to recruit β-arrestin to CXCR4 (data not shown, Fig. 1B). As CXCL12 binding to CXCR7 was reported to activate Akt [17], the effect of Compound 1 on Akt phosphorylation was investigated. As a result, Compound 1 activated the phosphorylation of Akt in HEK293 cells (Fig. 1C). We therefore propose that the compound generated for targeting CXCR7 is a chemical agonist.

3.2. β-arrestin recruitment induced by CXCR7 agonists is required for the inhibition of angiogenesis

As blocking CXCL12 function has been reported to suppress angiogenesis [5], we performed tube formation assay on HUVECs to determine the effect of CXCR7 agonists. HUVECs were incubated with the compound for 15 min and then stimulated with CXCL12 for 20 h, after which tube lengths were measured. Compound 1 showed inhibitory effect on tube formation with high potency (IC50; 0.96 nM, Fig. 2A). Therefore, we suggest that the CXCR7 agonist suppresses CXCL12-induced angiogenesis. Since both CXCL12 and the CXCR7 agonist clearly recruited β-arrestin to CXCR7 (Fig. 1B and Suppl. Fig. S2), we next asked whether β-arrestin recruitment to CXCR7 is necessary for the inhibitory effect on CXCL12

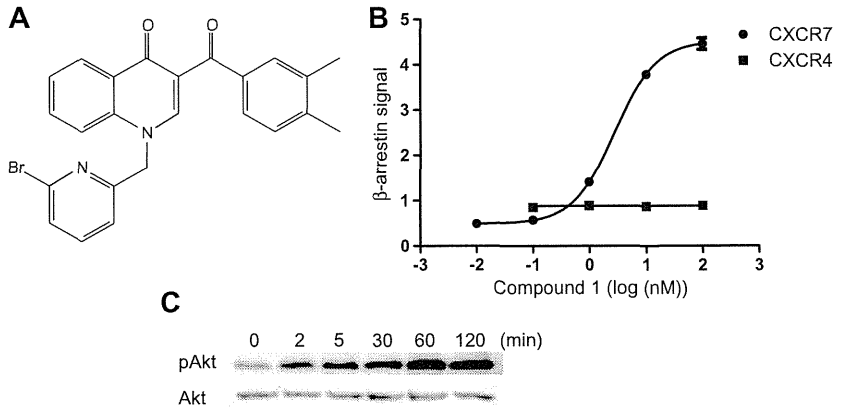

Fig. 1. Generation of Compound 1, an agonist that induces β-arrestin recruitment to CXCR7 and Akt activation. (A) The chemical structure of the CXCR7 agonist, Compound 1. Compound 1 was synthesized internally based on the published patent information from ChemoCentryx. (B) Compound 1 binds to CXCR7 and recruits β-arrestin to CXCR7. Tango™ CXCR7 (filled circles) and CXCR4 (filled squares) were exposed to increasing concentrations of Compound 1. $N = 3$, mean ± SEM. (C) Compound 1 activates Akt in HEK293 cells. HEK293 cells with overexpression of CXCR7 were exposed to Compound 1 for the indicated time and phosphorylated Akt was investigated with Western blotting.

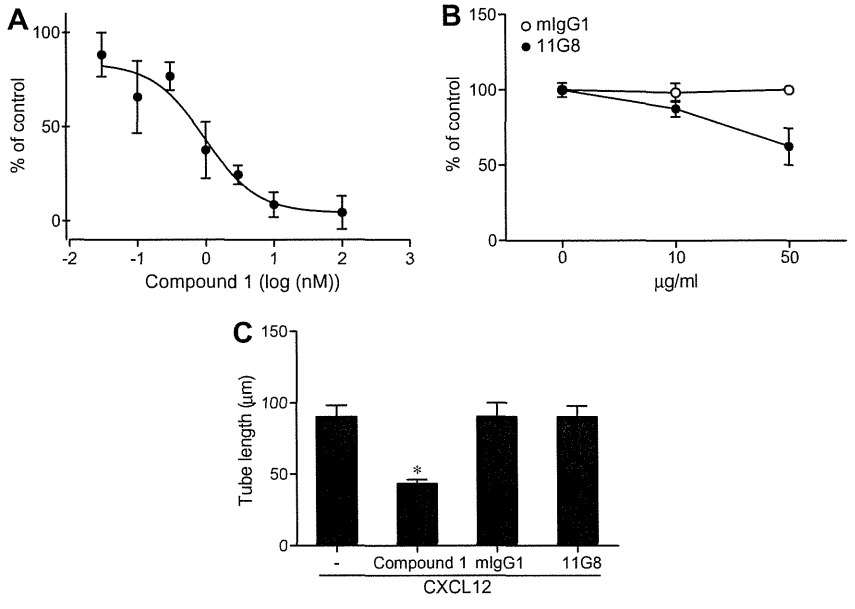

Fig. 2. CXCR7 agonist, but not antagonistic antibody, inhibited CXCL12 induced HUVEC tube formation. (A) Compound 1 inhibited CXCL12-induced angiogenesis. HUVECs were treated with increasing concentrations of Compound 1 for 15 min and seeded on the Matrigel with 100 ng/ml of CXCL12 for 20 h. Data were normalized as % of the tube length of HUVECs cultured without compound (CXCL12 only, 100%). The tube length of control (culture with PBS) was set to 0%. N = 6, mean ± SEM. (B) CXCR7 antibody blocks CXCL12-induced β-arrestin recruitment. The Tango™ CXCR7 cell line was exposed to 100 ng/ml of CXCL12 and anti-CXCR7 antibody or its isotype control (empty circles; mouse IgG, filled circles; anti-CXCR7, clone 11G8). Data are shown as % of control (CXCL12 only). N = 3, mean ± SEM. (C) CXCR7 antibody does not inhibit CXCL12-induced HUVEC tube formation. HUVECs were treated with anti-CXCR7 antibody (11G8) or its isotype control (mIgG1) and seeded on the Matrigel with 100 ng/ml of CXCL12 for 18 h and tube lengths were measured. $N = 6$, mean ± SEM. Compound 1 was used as a positive control. $^*P < 0.05$ compared to all the other groups.

signaling, or blocking the CXCL12 binding to CXCR7 is enough for the inhibitory effect. To clarify this question, anti-CXCR7 antibody was tested in the tube formation assay. The anti-CXCR7 antibody (clone 11G8), which has antagonistic activity in the CXCL12-induced β-arrestin recruitment to CXCR7 (Fig. 2B), showed no effect on the CXCL12-induced tube formation in HUVECs (Fig. 2C), demonstrating that the β-arrestin recruitment to CXCR7 is required for the inhibition of angiogenesis. Therefore, it is confirmed that β-arrestin recruitment to CXCR7 induced by the agonist is indispensable for the suppression of CXCL12-induced cellular events.

3.3. CXCR7 is a negative regulator of CXCR4

CXCL12 can bind not only CXCR7 but also CXCR4 and induce β-arrestin recruitment to CXCR4, whereas Compound 1 does not recruit β-arrestin to CXCR4 (Fig. 1B). These results prompted us to hypothesize that CXCR7 negatively regulates CXCR4, and CXCR7 agonists work indirectly as inhibitors of the CXCL12-CXCR4 signal relay. To determine whether CXCR7 suppresses signaling from CXCL12 binding to CXCR4, CXCR7 was overexpressed in CXCR4-bla U2OS cells to observe the effect on β-arrestin recruitment to CXCR4.

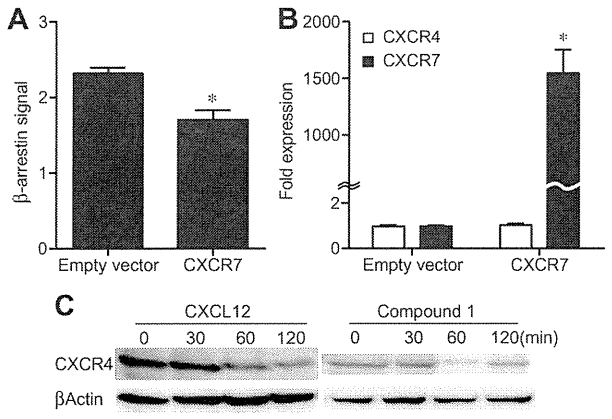

Fig. 3. Stimulation of CXCR7 reduces CXCR4 signaling. (A) The Tango™ CXCR4-bla cell line was transfected with CXCR7 or the empty vector and exposed to 100 ng/ml of CXCL12 for 5 h. $N = 6$, mean ± SEM. $^*P < 0.01$ vs. control (empty vector). (B) The mRNA expression level of CXCR4 and CXCR7 after CXCR7 gene transfection into Tango™ CXCR4-bla cells was measured by qRT-PCR. Empty columns show CXCR4 expression and filled columns show CXCR7 expression. Data are fold expression compared to the empty vector-transfected cells. $N = 6$, mean ± SEM. $^*P < 0.01$ vs. control (empty vector). (C) CXCR7over-expressed HEK293 cells were treated with CXCL12 or Compound 1 for the indicated time and CXCR4 expression was examined with Western blotting.

Upon CXCR7 overexpression, CXCL12-induced β-arrestin recruitment to CXCR4 was significantly reduced (Fig. 3A), implying that CXCR7 has an inhibiting effect on CXCR4 functions. When CXCR7 was overexpressed, the expression level of CXCR4 mRNA did not change in CXCR4-bla U2OS cells even when an extremely high level of mRNA expression of CXCR7 was induced (Fig. 3B). Since CXCR7 has been reported to be able to affect the expression of CXCR4, it is hypothesized that signal from CXCR7, but not just expression of CXCR7, affects the expression of CXCR4. Therefore the effect of Compound 1 on the expression of CXCR4 was examined. HEK293 cells were transfected with CXCR7-expression plasmid and treated with Compound 1 for 60 min. The protein expression level of CXCR4 was remarkably decreased by Compound 1 treatment and the similar effect was induced by CXCL12 (Fig. 3C). This result indicates that activation of CXCR7 reduces the protein amount of CXCR4.

4. Discussion

The current study provides evidence that CXCR7 agonism reduces the amount of CXCR4 protein and that inhibits CXCL12-induced cellular events. Since the finding that CXCR7 is a receptor for CXCL12 in 2005 [6], many studies on CXCR7 have been published. However, although expression on malignant cells and effects on angiogenesis have been proven, mechanisms of action for this receptor remained unclear. Chemical compounds originally developed by ChemoCentryx as "CXCR7 inhibitors" showed efficacy in models of tumor suppression and arthritis [5,7]. These studies suggest CXCR7 inhibitors may be an interesting intervention point for treating a variety of human diseases. Several consecutive studies, however, have revealed that CCX733, originally synthesized as a CXCR7 antagonist, or its derivatives recruit β-arrestin to CXCR7, suggesting that these compounds work as CXCR7 agonists. Furthermore, whereas CXCR4, another receptor for CXCL12, is expressed ubiquitously, CXCR7 is expressed on a limited number of cell types and the expression is transiently induced by certain stimuli like inflammation. Why the agonists of the transiently-upregulated receptor can reduce the effects of CXCL12 remains to be unclear. CCX733 is highly selective for CXCR7 and does not bind CXCR4, suggesting that a mechanism to indirectly inhibit the CXCL12-CXCR4 axis by CXCR7 agonists exists. In answer of this question, Naumann et al. reported that CXCR7 is a scavenger for CXCL12 and negatively regulates CXCL12 functions [12]. However, since CXCL12 might be released continuously under inflammatory conditions, only scavenging CXCR7 may not be enough to suppress the effect of CXCL12. We therefore hypothesized that there should be an additional mechanism negatively regulating CXCL12 function by CXCR7 agonists. It is documented that the expression of CXCR7 is upregulated by inflammatory cytokines such as IL-1β [5], and that CXCR7 forms heterodimers with CXCR4 and the interaction may regulate inter-receptor relationship [11]. Here we confirmed that CXCR7 itself modulates the function and amount of CXCR4.

CXCR7 does not couple with G-proteins, but interacts with β-arrestin as CXCR4 also does. Our results demonstrated that the CXCL12-induced β-arrestin recruitment to CXCR4 is inhibited by increasing CXCR7 expression, suggesting that CXCR7 affects signaling downstream of CXCR4. Others proved that β-arrestin is involved in receptor internalization [18,19], so it has been suggested that signaling through β-arrestin from activated CXCR7 plays important roles in the receptor recruitment. Indeed, our results showed that the increase of CXCR7 expression by transfection without any agonist stimulation did not influence the CXCR4 expression on mRNA level. CXCR7 agonists transduce the signal from CXCR7 and promote internalization of CXCR4, which forms heterodimers with CXCR7 [11]. It is reported that most of CXCR4 is degraded after internalization, whereas CXCR7 comes back to the cell surface [12]. CXCR4 contains a degradation motif (SSLKILSKGK) in the carboxyl terminus and ubiquitination on the lysine residues [20] triggers its degradation, whereas ubiquitination of CXCR7 is responsible for the correct trafficking of CXCR7 from and to the plasma membrane [21]. By overexpressing CXCR7 in HEK293 cells, which originally express CXCR7 at a low level, we observed that the CXCR7 agonists markedly reduced the protein expression of CXCR4. This observation leads us to propose that CXCR7 actively promotes CXCR4 degradation.

The effects of CXCL12-CXCR4 signal have been well studied, and CXCR4 antagonists are of high clinical interest in the context of mobilization of hematopoietic stem cells and cancer biology, as well as inflammatory diseases. However, CXCL12-CXCR4 signal is also critical for host homeostasis such as normal angiogenesis. As mentioned before, the expression level of CXCR7 is low under normal conditions, and it is up-regulated in tumor cells or under inflammatory conditions. Modulating the effect of CXCL12 by CXCR7 agonists could thus be a therapeutic option for treatment of CXCL12 involving diseases.

Competing interest statement

We have the following interest. Ayako Uto-Konomi, Julia Wirtz, Yayoi Sato, Ai Takano and Shinobu Suzuki are employed by Nippon Boehringer Ingelheim Co., Ltd. and Bryan McKibben is employed by Boehringer-Ingelheim Pharmaceutical, Inc. There are no patents, products in development or marketed products to declare. Other authors have declared that no competing interests exist.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.01.032.

References

[1] A. Muller, B. Homey, H. Soto, N. Ge, D. Catron, M.E. Buchanan, T. McClanahan, E. Murphy, W. Yuan, S.N. Wagner, J.L. Barrera, A. Mohar, E. Verastegui, A. Zlotnik, Involvement of chemokine receptors in breast cancer metastasis, Nature 410 (2001) 50–56.

[2] T. Kijima, G. Maulik, P.C. Ma, E.V. Tibaldi, R.E. Turner, B. Rollins, M. Sattler, B.E. Johnson, R. Salgia, Regulation of cellular proliferation, cytoskeletal function, and signal transduction through CXCR4 and c-Kit in small cell lung cancer cells, Cancer Res. 62 (2002) 6304–6311.

[3] T. Nanki, K. Hayashida, H.S. El-Gabalawy, S. Suson, K. Shi, H.J. Girschick, S. Yavuz, P.E. Lipsky, Stromal cell-derived factor-1-CXC chemokine receptor 4 interactions play a central role in CD4+ T cell accumulation in rheumatoid arthritis synovium, J. Immunol. 165 (2000) 6590–6598.

[4] F. Grassi, S. Cristino, S. Toneguzzi, A. Piacentini, A. Facchini, G. Lisignoli, CXCL12 chemokine up-regulates bone resorption and MMP-9 release by human osteoclasts: CXCL12 levels are increased in synovial and bone tissue of rheumatoid arthritis patients, J. Cell. Physiol. 199 (2004) 244–251.

[5] K. Watanabe, M.E. Penfold, A. Matsuda, N. Ohyanagi, K. Kaneko, Y. Miyabe, K. Matsumoto, T.J. Schall, N. Miyasaka, T. Nanki, Pathogenic role of CXCR7 in rheumatoid arthritis, Arthritis Rheum. 62 (2010) 3211–3220.

[6] K. Balabanian, B. Lagane, S. Infantino, K.Y. Chow, J. Harriague, B. Moepps, F. Arenzana-Seisdedos, M. Thelen, F. Bachelerie, The chemokine SDF-1/CXCL12 binds to and signals through the orphan receptor RDC1 in T lymphocytes, J. Biol. Chem. 280 (2005) 35760–35766.

[7] J.M. Burns, B.C. Summers, Y. Wang, A. Melikian, R. Berahovich, Z. Miao, M.E. Penfold, M.J. Sunshine, D.R. Littman, C.J. Kuo, K. Wei, B.E. McMaster, K. Wright, M.C. Howard, T.J. Schall, A novel chemokine receptor for SDF-1 and I-TAC involved in cell survival, cell adhesion, and tumor development, J. Exp. Med. 203 (2006) 2201–2213.

[8] I. Kalatskaya, Y.A. Berchiche, S. Gravel, B.J. Limberg, J.S. Rosenbaum, N. Heveker, AMD3100 is a CXCR7 ligand with allosteric agonist properties, Mol. Pharmacol. 75 (2009) 1240–1247.

[9] S. Rajagopal, J. Kim, S. Ahn, S. Craig, C.M. Lam, N.P. Gerard, C. Gerard, R.J. Lefkowitz, Beta-arrestin- but not G protein-mediated signaling by the "decoy" receptor CXCR7, Proc. Natl. Acad. Sci. USA 107 (2010) 628–632.

[10] T.N. Hartmann, V. Grabovsky, R. Pasvolsky, Z. Shulman, E.C. Buss, A. Spiegel, A. Nagler, T. Lapidot, M. Thelen, R. Alon, A crosstalk between intracellular CXCR7 and CXCR4 involved in rapid CXCL12-triggered integrin activation but not in chemokine-triggered motility of human T lymphocytes and CD34+ cells, J. Leukocyte Biol. 84 (2008) 1130–1140.

[11] A. Levoye, K. Balabanian, F. Baleux, F. Bachelerie, B. Lagane, CXCR7 heterodimerizes with CXCR4 and regulates CXCL12-mediated G protein signaling, Blood 113 (2009) 6085–6093.

[12] U. Naumann, E. Cameroni, M. Pruenster, H. Mahabaleshwar, E. Raz, H.G. Zerwes, A. Rot, M. Thelen, CXCR7 functions as a scavenger for CXCL12 and CXCL11, PLoS One 5 (2010) e9175.

[13] L. Cruz-Orengo, D.W. Holman, D. Dorsey, L. Zhou, P. Zhang, M. Wright, E.E. McCandless, J.R. Patel, G.D. Luker, D.R. Littman, J.H. Russell, R.S. Klein, CXCR7 influences leukocyte entry into the CNS parenchyma by controlling abluminal CXCL12 abundance during autoimmunity, J. Exp. Med. 208 (2011) 327–339.

[14] B.A. Zabel, Y. Wang, S. Lewen, R.D. Berahovich, M.E. Penfold, P. Zhang, J. Powers, B.C. Summers, Z. Miao, B. Zhao, A. Jalili, A. Janowska-Wieczorek, J.C. Jaen, T.J. Schall, Elucidation of CXCR7-mediated signaling events and inhibition of CXCR4-mediated tumor cell transendothelial migration by CXCR7 ligands, J. Immunol. 183 (2009) 3204–3211.

[15] B.J. Hanson, J. Wetter, M.R. Bercher, L. Kopp, M. Fuerstenau-Sharp, K.L. Vedvik, T. Zielinski, C. Doucette, P.J. Whitney, C. Revankar, A homogeneous fluorescent live-cell assay for measuring 7-transmembrane receptor activity and agonist functional selectivity through beta-arrestin recruitment, J. Biomol. Screen 14 (2009) 798–810.

[16] C. Doucette, K. Vedvik, E. Koepnick, A. Bergsma, B. Thomson, T.C. Turek-Etienne, Kappa opioid receptor screen with the Tango beta-arrestin recruitment technology and characterization of hits with second-messenger assays, J. Biomol. Screen 14 (2009) 381–394.

[17] R. Kumar, V. Tripathi, M. Ahmad, N. Nath, R.A. Mir, S.S. Chauhan, K. Luthra, CXCR7 mediated Gialpha independent activation of ERK and Akt promotes cell survival and chemotaxis in T cells, Cell. Immunol. 272 (2012) 230–241.

[18] P. Kumar, C.S. Lau, M. Mathur, P. Wang, K.A. DeFea, Differential effects of beta-arrestins on the internalization, desensitization and ERK1/2 activation downstream of protease activated receptor-2, Am. J. Physiol. Cell Physiol. 293 (2007) C346–C357.

[19] A. Lymperopoulos, Beta-arrestin biased agonism/antagonism at cardiovascular seven transmembrane-spanning receptors, Curr. Pharm. Des. 18 (2012) 192–198.

[20] A. Marchese, J.L. Benovic, Agonist-promoted ubiquitination of the G protein-coupled receptor CXCR4 mediates lysosomal sorting, J. Biol. Chem. 276 (2001) 45509–45512.

[21] M. Canals, D.J. Scholten, S. de Munnik, M.K. Han, M.J. Smit, R. Leurs, Ubiquitination of CXCR7 controls receptor trafficking, PLoS One 7 (2012) e34192.

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CASE REPORT

Expansion of range of joint motion following treatment of systemic sclerosis with tocilizumab

Yoshihito Shima · Naoki Hosen · Toru Hirano · Junsuke Arimitsu · Sumiyuki Nishida · Keisuke Hagihara · Masashi Narazaki · Atsushi Ogata · Toshio Tanaka · Tadamitsu Kishimoto · Atsushi Kumanogoh

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Abstract Systemic sclerosis (SSc) presents stiffness of extremities due to sclerosis of the tissue especially at fingers, hands, and forearms. Here we report the case of a patient with diffuse cutaneous SSc who was administered anti-interleukin-6 receptor antibody tocilizumab (TCZ). Skin condition of SSc is evaluated by pinching the skin according to the Rodnan skin score, but sometimes tissue atrophy results in overestimation of the condition. To understand how the extremities softened after initiation of TCZ, we observed mobility of extremities. Range of motion (ROM) of joints was measured every four months after initiation of TCZ. The patient presented not only reduction of Rodnan score but also amelioration of mobility of extremities. The Rodnan skin score reduced from 35 to 7 within sixteen months, and ROM of most joints except ankle was expanded.

Keywords Systemic sclerosis · Tocilizumab · Range of joint motion

Y. Shima (✉) · N. Hosen · T. Hirano · J. Arimitsu · S. Nishida · K. Hagihara · M. Narazaki · A. Ogata · A. Kumanogoh
Department of Respiratory Medicine, Allergy and Rheumatic Diseases, Osaka University Graduate School of Medicine, 2-2 Yamada-oka, Suita City, Osaka 565-0871, Japan
e-mail: ryanjin@imed3.med.osaka-u.ac.jp

T. Tanaka
Department of Clinical Application of Biologics, Osaka University Graduate School of Medicine, Osaka, Japan

T. Kishimoto
Laboratory of Immunoregulation, WPI Immunology Frontier Research Center, Osaka University, Osaka, Japan

Introduction

Systemic sclerosis (SSc) is a connective tissue disease that develops sclerotic changes in the skin and visceral organs. Patients present with stiffness of the limbs because of sclerosis in the skin and periarticular connective tissues. We present the case of a patient with SSc who showed improvement of joint motion after treatment with the anti-interleukin-6 (IL-6) receptor antibody tocilizumab (TCZ).

Although the etiology of SSc remains unclear, many factors have been proposed. IL-6 is a pleiotropic factor that plays a major role in inflammation; furthermore, it is a candidate factor that can reproduce the pathological conditions of SSc. Reportedly, culture supernatants of skin tissue or peripheral blood mononuclear cells from patients with SSc contain higher concentrations of IL-6 than those from normal controls [1, 2]. Elevation of serum IL-6 levels has also been reported, and these levels are reported to depend on skin score [3–5]. In addition, an anti-IL-6 antibody has been reported to suppress procollagen production by fibroblasts isolated from patients with SSc [6]. Given these facts, it is suggested that anti-IL-6 therapy may ameliorate the clinical symptoms of SSc. We have previously reported conventional therapy-resistant SSc cases that responded well to TCZ [7]. In our former study, two patients who were administered TCZ for six months showed a decrease in their modified Rodnan total skin (mRTS) scores, suggesting that their skin sclerosis could have been ameliorated by TCZ administration. However, the skin score in patients with SSc sometimes decreases spontaneously as a result of tissue atrophy. Therefore, it is necessary to examine not only the skin score but also the function of the extremities. In this case, we evaluated the range of motion (ROM) of joints before and after TCZ administration to investigate the effects of TCZ on mobility of extremities in a patient with SSc.

Published online: 01 March 2013

Case report

A 59-year-old woman noticed Raynaud's phenomenon and swellings in her fingers in 2004. This skin sclerosis developed from her fingers and expanded to her face and feet. She became aware of dyspnea on exertion, dysphagia, and stiffness of the hands, wrists, elbows, and shoulders. Although both anti-Scl-70 and anti-centromere antibodies were negative, she was diagnosed with SSc because skin biopsy revealed thick and tight collagen fiber bundles in the dermis. Antinuclear antibody was positive at a titer of 1:1280 with a speckled pattern. Closer examination revealed that anti-RNA polymerase III antibody was positive. Treatment was initiated with prednisolone at 10 mg/day, and cyclosporine was then added to this regimen, which was otherwise ineffective. In 2005, the patient developed subacute renal failure and hypertension; therefore, dialysis therapy was indicated. After temocapril at dosage of 4 mg/day and telmisartan at dosage of 80 mg/day were administered, her condition stabilized and hemodialysis was terminated. In 2007, the patient exhibited recurrent dyspnea on exertion and inadequate oral intake as a result of recurrent ileus with pneumatosis cystoides intestinalis. For a while, home parenteral nutrition was used; however, prolonged administration of antibiotics proved effective only to a limited extent. Though endoscopic examination showed normal esophageal mucosa, measurement of esophageal pressure indicated absence of peristaltic waves during swallowing. Chest computed tomography (CT) images detected no significant interstitial modification, however echocardiogram revealed pericardial effusion and elevated peak pressure gradient of tricuspid regurgitation (28 mmHg). Since right heart catheterization showed elevation of mean pulmonary artery pressure (25 mmHg), treatment with 125 mg/day bosentan was initiated. Her visceral organs became involved as described; furthermore, the skin sclerosis spread to her trunk. Her mRTS score was 35 in 2008. The patient had to use a wheelchair to move about, and she was unable to propel it by herself. Because her activities of daily living (ADL) were severely compromised because of skin sclerosis, we applied for a TCZ project which was supported by the National Institute of Biomedical Innovation (Ibaraki City, Osaka, Japan). After receiving informed consent by the patient and approval by the Ethics Committee of Osaka University Hospital, we initiated TCZ treatment. Laboratory data at TCZ initiation are presented in Table 1. The administration dosage and schedule of TCZ was 8 mg/kg every four weeks, which corresponds to the regimen used for rheumatoid arthritis. The following medications were administered concurrently: methylprednisolone (8 mg/day), telmisartan (40 mg/day), furosemide (80 mg/day), beraprost (120 μg/day), omeprazole (20 mg/day), cefdinir (300 mg/day), and bosentan (125 mg/day). ROM of the metacarpophalangeal joints of the hands as well as that of the wrist, elbow, shoulder, knee, and ankle joints was measured every 4 months using a goniometer.

ROM of the knee, wrist, and shoulder joints after TCZ initiation are shown in Fig. 1. ROM values, except for those in ankles, improved during the observation period. Skin sclerosis also improved over the course of treatment, and the patient's mRTS score decreased from 35 to 7. She could walk independently once again. In patients with SSc, problems concerning joint motion may result from sclerotic changes in the skin and subcutaneous tissue. In this case, the patient's knee, wrist, and shoulder joints, which were drastically affected, showed tendencies toward an inverse

Table 1 Laboratory data before TCZ therapy initiation

Blood cell count			Urine test			Biochemical data		
White blood cells	(3300–9400)	5660/μL	pH	(5.0–8.0)	5.0	Creatinine	(0.5–0.9)	2.09 mg/dL
Red blood cells	(390–510 × 10^4)	383 × 10^4/μL	Urine gravity	(1.005–1.030)	1.008	Aspartate aminotransferase	(<40)	19 IU/L
Hemoglobin	(12.0–15.0)	9.9 g/dL	Protein	(−)	−	Alanine aminotransferase	(<40)	11 IU/L
Hematocrit	(35.0–45.0)	30.6 %	Sugar	(−)	−	Gamma glutamyl transpeptidase	(8–51)	12 IU/L
Mean corpuscular volume	(84.0–98.0)	79.8 fL	Urobilinogen	(+/−)	+/−	Lactate dehydrogenase	(103–229)	241 IU/L
Mean corpuscular hemoglobin	(28.0–33.0)	25.8 pg	Bilirubin	(−)	−	Amylase	(44–153)	212 IU/L
Mean corpuscular hemoglobin concentration	(31.0–35.0)	32.3 %	Ketone	(−)	−	Creatinine kinase	(54–286)	11 IU/L
			Occult blood	(−)	−	Cholesterol	(150–220)	177 mg/dL
Platelet	(130–320 × 10^3)	187 × 10^3/μL				Albumin	(3.6–4.7)	3.5 g/dL
						C-reactive protein	(0.0–0.2)	0.13 mg/dL

Values in parentheses indicate normal limits at our hospital

relationship with the skin scores of the areas adjacent to the joints (Fig. 2a–c). In contrast, although the skin scores of the lower legs and dorsum of the feet improved, ROM of the ankle joint remained unchanged (Fig. 2d).

Discussion

This case report describes a patient with SSc who showed impaired mobility in addition to severe skin sclerosis. TCZ administration proved beneficial for the skin sclerosis, as described previously [7]. However, patients with SSc sometimes show improvements in their poor skin scores because of skin atrophy, considering that the Rodnan skin score is obtained by pinching the skin. In our patient, however, we observed an improvement in mobility of the limbs as well as the skin score. The patient regained the ability to walk independently, proving that the improved skin score not only represented the ease of pinching the skin but also functional improvement.

Several factors may contribute to the improvement in ROM during TCZ treatment in the present case. First, TCZ might act on arthritis. TCZ is a recognized medication for arthritis; therefore, it may improve joint movement through amelioration of joint inflammation. The patient, however, showed neither swelling nor tenderness of the joints before and during TCZ treatment. In addition, her C-reactive protein level was normal. Therefore, the observed improvement in ROM was probably not because of arthritis remission. Second, the ROM improvements might be the natural course of the disease or might indicate atrophic change. Four years passed between the onset of disease and initiation of TCZ treatment, and disability of limbs was worsening during this period. The fact that ROM improvement in the knee and shoulder joints was detected within the first 4 months of TCZ treatment gives an impression of the effect of this medicine. However, this possibility is remaining because there was no serial scoring data before TCZ treatment. Third, concomitant medicines might effect ROM improvement. Methylprednisolone and bosentan were used as concomitant medicines. Bosentan in

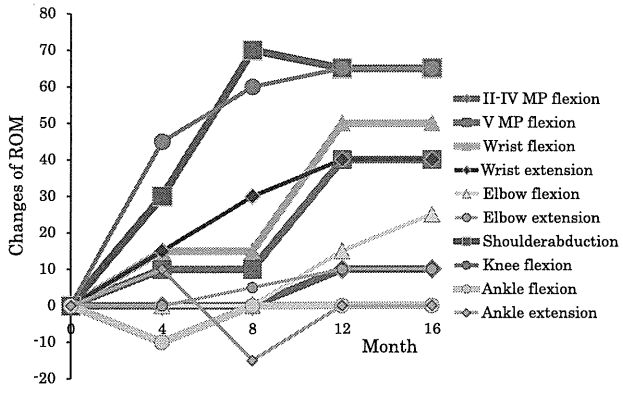

Fig. 1 Impact of tocilizumab (TCZ) on joint mobility in a patient with SSc. Joint range of motion (ROM) was initially set to zero, and data are expressed as the degree of improvement during the 16-month TCZ therapy. The *horizontal axis* indicates months after TCZ initiation. All ROM values, except for those in the ankles, improved considerably after 4 months and continued to improve until the end of study. The *unit* of angle is degrees

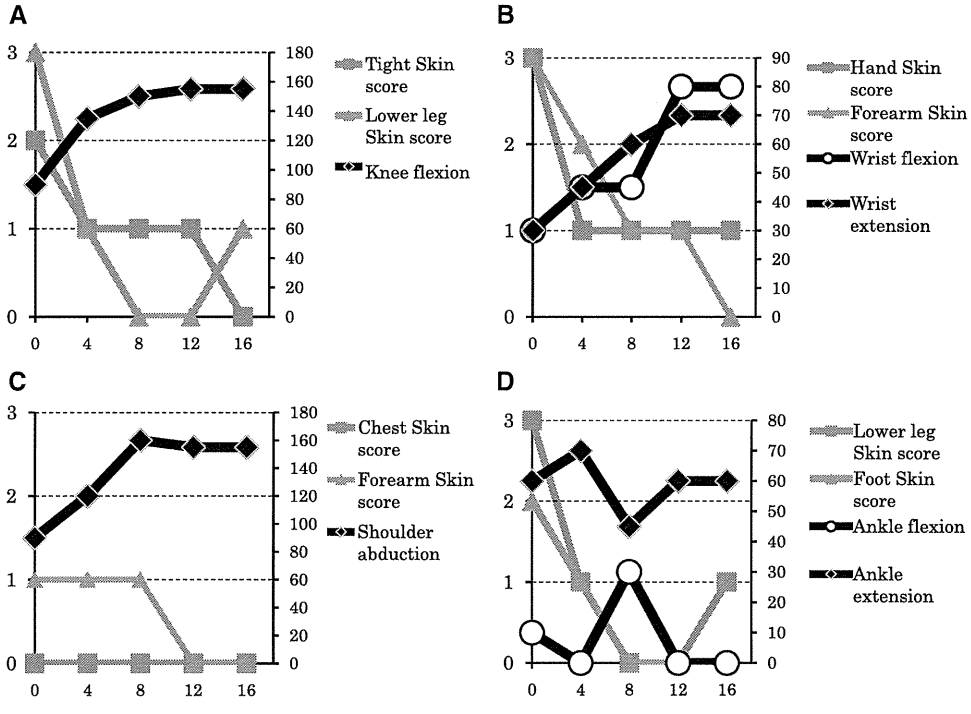

Fig. 2 Relationship between joint mobility and skin sclerosis in a SSc patient during TCZ treatment. The *left vertical axis* indicates the modified Rodnan total skin score, and the *right vertical axis* indicates ROM value. The *horizontal axis* indicates months after TCZ initiation. The skin scores for the knee (**a**), wrist (**b**), and shoulder (**c**) joints decreased as the ROM increased. In contrast, the ROM of the ankle joint did not improve, even though the skin scores of the lower leg and foot decreased to 0 after 8 months (**d**)

particular might contribute to reduction of skin score, because bosentan has protective efficacy for skin ulcer in SSc [8]. The possibility that bosentan acts to improve ROM might remain, but there is no report which presents an effect of bosentan on ROM improvement. The reason why the ROM of the ankle joints remained unchanged is unclear. There may be a relationship with long-term wheelchair use. This patient also had kidney, heart, and bowel involvement, and it is unclear how they were affected by TCZ administration. Although she has remained free from dialysis or home parenteral nutrition to date, she continues to require angiotensin receptor blockers, proton pump inhibitors, diuretics, and antibiotics. There is a possibility that the internal organ symptoms are being affected by these medications. TCZ administration, however, clearly resulted in improvement in the skin score for this case as well as former reported cases [7], and in this case, it was clear that the skin score decrement after TCZ initiation was not because the skin became easy to pinch but because the tissue was becoming soft and easy to move.

There is currently no standard pharmacological guideline for treatment of SSc, despite numerous clinical trials on steroids, antirheumatic drugs, and immunosuppressive agents. While an effective low-dose corticosteroid therapy with prednisolone has been proposed for early-phase diffuse cutaneous SSc [9], patients are at risk of developing sclerodermal renal crisis [10]. The effectiveness of penicillamine in SSc treatment remains controversial [11]. On the other hand, the European League Against Rheumatism recommends methotrexate for treatment of skin sclerosis in patients with early diffuse SSc [12], but an opposing view was also presented [13]. Other immunosuppressive agents such as cyclophosphamide, cyclosporine A, tacrolimus, and mycophenolate mofetil have been evaluated for treatment of SSc. Though the beneficial effects of one-year oral administration of cyclophosphamide on skin thickening have been reported [14], the long-term safety of this medicine has not been verified. There are no data which present late-occurring toxicities of cyclophosphamide in patients with SSc, but there are several reports which present oncogenicity after withdrawal of this medicine in patients with systemic lupus erythematosus and rheumatoid arthritis [15]. The usefulness of cyclosporine is also controversial because of the associated risk of sclerodermal renal crisis [16, 17]. The effectiveness of mycophenolate mofetil as an immunosuppressive agent in SSc treatment also remains inconclusive [18, 19], though a recent study presented beneficial effects in patients with recent-onset SSc [20]. Finally, several biologic agents are currently being evaluated for treatment of skin involvement in SSc, of which only rituximab has shown efficacy [21, 22]. Therefore, effective treatment for this disease is an ongoing challenge.

The effect of TCZ on skin sclerosis, pneumonitis, or the other symptoms in patients with SSc remains unclear, and further studies are required to verify this. The efficacy of TCZ for patients with SSc is currently being evaluated in an open-label trial in Japan (UMIN0000055550) and a double-blind trial in Europe and North America (NCT01532869); the results of these trials should provide further information on unresolved issues.

In this report, we described time-course changes of ROM observed in a patient with SSc during treatment with TCZ. The relation between TCZ treatment and ROM changes observed in the patient is currently unclear.

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Conflict of interest T. Kishimoto holds a patent for TCZ, and receives royalties for ACTEMRA®. A. Ogata received a consulting fee from Chugai Pharmaceutical Co. Ltd. for providing medical advice. Other authors have no conflict of interest to declare.

References

1. Gurram M, Pahwa S, Frieri M. Augmented interleukin-6 secretion in collagen-stimulated peripheral blood mononuclear cells from patients with systemic sclerosis. Ann Allergy. 1994;73:493–6.
2. Feghali CA, Bost KL, Boulware DW, Levy LS. Mechanisms of pathogenesis in scleroderma. I. Overproduction of interleukin 6 by fibroblasts cultured from affected skin sites of patients with scleroderma. J Rheumatol. 1992;19:1207–11.
3. Hasegawa M, Sato S, Fujimoto M, Ihn H, Kikuchi K, Takehara K. Serum levels of interleukin 6 (IL-6), oncostatin M, soluble IL-6 receptor, and soluble gp130 in patients with systemic sclerosis. J Rheumatol. 1998;25:308–13.
4. Sato S, Hasegawa M, Takehara K. Serum levels of interleukin-6 and interleukin-10 correlate with total skin thickness score in patients with systemic sclerosis. J Dermatol Sci. 2001;27:140–6.
5. Khan K, Xu S, Nihtyanova S, Derrett-Smith E, Abraham D, Denton CP, Ong VH. Clinical and pathological significance of interleukin 6 overexpression in systemic sclerosis. Ann Rheum Dis. 2012 [Epub ahead of print].
6. Kawaguchi Y, Hara M, Wright TM. Endogenous IL-1alpha from systemic sclerosis fibroblasts induces IL-6 and PDGF-A. J Clin Invest. 1999;103:1253–60.
7. Shima Y, Kuwahara Y, Murota H, Kitaba S, Kawai M, Hirano T, Arimitsu J, Narazaki M, Hagihara K, Ogata A, Katayama I, Kawase I, Kishimoto T, Tanaka T. The skin of patients with systemic sclerosis softened during the treatment with anti-IL-6 receptor antibody tocilizumab. Rheumatology (Oxford). 2010;49:2408–12.
8. Kuhn A, Haust M, Ruland V, Weber R, Verde P, Felder G, Ohmann C, Gensch K, Ruzicka T. Effect of bosentan on skin fibrosis in patients with systemic sclerosis: a prospective,

open-label, non-comparative trial. Rheumatology (Oxford). 2010;49:1336–45.
9. Takehara K. Treatment of early diffuse cutaneous systemic sclerosis patients in Japan by low-dose corticosteroids for skin involvement. Clin Exp Rheumatol. 2004;22:S87–9.
10. Steen VD, Medsger TA Jr. Case-control study of corticosteroids and other drugs that either precipitate or protect from the development of scleroderma renal crisis. Arthr Rheum. 1998;41: 1613–9.
11. Clements PJ, Furst DE, Wong WK, Mayes M, White B, Wigley F, Weisman MH, Barr W, et al. High-dose versus low-dose D-penicillamine in early diffuse systemic sclerosis: analysis of a two-year, double-blind, randomized, controlled clinical trial. Arthr Rheum. 1999;42:1194–203.
12. Kowal-Bielecka O, Landewé R, Avouac J, Chwiesko S, Miniati I, Czirjak L, Clements P, Denton C, et al. EULAR recommendations for the treatment of systemic sclerosis a report from the EULAR Scleroderma Trials and Research group (EUSTAR). Ann Rheum Dis. 2009;68:620–8.
13. Pope JE, Bellamy N, Seibold JR, Baron M, Ellman M, Carette S, Smith CD, Chalmers IM, Hong P, O'Hanlon D, Kaminska E, Markland J, Sibley J, Catoggio L, Furst DE. A randomized, controlled trial of methotrexate versus placebo in early diffuse scleroderma. Arthr Rheum. 2001;44:1351–8.
14. Tashkin DP, Elashoff R, Clements PJ, Goldin J, Roth MD, Furst DE, Arriola E, Silver R, et al. Cyclophosphamide versus placebo in scleroderma lung disease. N Engl J Med. 2006;354:2655–66.
15. Plotz PH, Klippel JH, Decker JL, Grauman D, Wolff B, Brown BC, Rutt G. Bladder complications in patients receiving cyclophosphamide for systemic lupus erythematosus or rheumatoid arthritis. Ann Intern Med. 1979;91:221–3.
16. Filaci G, Cutolo M, Scudeletti M, Castagneto C, Derchi L, Gianrossi R, Ropolo F, Zentilin P, Sulli A, Murdaca G, Ghio M, Indiveri F, Puppo F. Cyclosporin A and iloprost treatment of systemic sclerosis: clinical results and interleukin-6 serum changes after 12 months of therapy. Rheumatology (Oxford). 1999;38:992–6.
17. Denton CP, Sweny P, Abdulla A, Black CM. Acute renal failure occurring in scleroderma treated with cyclosporin A: a report of three cases. Br J Rheumatol. 1994;33:90–2.
18. Stratton RJ, Wilson H, Black CM. Pilot study of anti-thymocyte globulin plus mycophenolate mofetil in recent-onset diffuse scleroderma. Rheumatology (Oxford). 2001;40:84–8.
19. Nihtyanova SI, Brough GM, Black CM, Denton CP. Mycophenolate mofetil in diffuse cutaneous systemic sclerosis–a retrospective analysis. Rheumatology (Oxford). 2007;46:442–5.
20. Mendoza FA, Nagle SJ, Lee JB, Jimenez SA. A prospective observational study of mycophenolate mofetil treatment in progressive diffuse cutaneous systemic sclerosis of recent onset. J Rheumatol. 2012;39:1241–7.
21. Denton CP, Engelhart M, Tvede N, Wilson H, Khan K, Shiwen X, Carreira PE, Diaz Gonzalez F, Black CM, van den Hoogen FH. An open-label pilot study of infliximab therapy in diffuse cutaneous systemic sclerosis. Ann Rheum Dis. 2009;68:1433–9.
22. McGonagle D, Tan AL, Madden J, Rawstron AC, Rehman A, Emery P, Thomas S. Successful treatment of resistant scleroderma-associated interstitial lung disease with rituximab. Rheumatology (Oxford). 2008;47:552–3.

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ORIGINAL ARTICLE

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Phase III Study of the Efficacy and Safety of Subcutaneous Versus Intravenous Tocilizumab Monotherapy in Patients With Rheumatoid Arthritis

ATSUSHI OGATA,[1] KAZUHIDE TANIMURA,[2] TOYOHIKO SUGIMOTO,[3] HIROSHI INOUE,[4] YUKITOMO URATA,[5] TSUKASA MATSUBARA,[6] MASAKAZU KONDO,[7] YUKITAKA UEKI,[8] MITSUHIRO IWAHASHI,[9] SHIGETO TOHMA,[10] SHUJI OHTA,[11] YUKIHIKO SAEKI,[12] TOSHIO TANAKA,[1] AND THE MUSASHI STUDY INVESTIGATORS

Objective. **To evaluate the efficacious noninferiority of subcutaneous tocilizumab injection (TCZ-SC) monotherapy to intravenous TCZ infusion (TCZ-IV) monotherapy in Japanese patients with rheumatoid arthritis (RA) with an inadequate response to synthetic and/or biologic disease-modifying antirheumatic drugs (DMARDs).**

Methods. **This study had a double-blind, parallel-group, double-dummy, comparative phase III design. Patients were randomized to receive TCZ-SC 162 mg every 2 weeks or TCZ-IV 8 mg/kg every 4 weeks; no DMARDs were allowed during the study. The primary end point was to evaluate the noninferiority of TCZ-SC to TCZ-IV regarding the American College of Rheumatology criteria for 20% improvement in disease activity (ACR20) response rates at week 24 using an 18% noninferiority margin. Additional efficacy, safety, pharmacokinetic, and immunogenicity parameters were assessed.**

Results. **At week 24, ACR20 response was achieved in 79.2% (95% confidence interval [95% CI] 72.9, 85.5) of the TCZ-SC group and in 88.5% (95% CI 83.4, 93.5) of the TCZ-IV group; the weighted difference was −9.4% (95% CI −17.6, −1.2), confirming the noninferiority of TCZ-SC to TCZ-IV. Remission rates of the Disease Activity Score in 28 joints using the erythrocyte sedimentation rate and the Clinical Disease Activity Index at week 24 were 49.7% and 16.4% in the TCZ-SC group and 62.2% and 23.1% in the TCZ-IV group, respectively. Serum trough TCZ concentrations were similar between the groups over time. Incidences of all adverse events and serious adverse events were 89.0% and 7.5% in the TCZ-SC group and 90.8% and 5.8% in the TCZ-IV group, respectively. Anti-TCZ antibodies were detected in 3.5% of the TCZ-SC group; no serious hypersensitivity was reported in these patients.**

Conclusion. **TCZ-SC monotherapy demonstrated comparable efficacy and safety to TCZ-IV monotherapy. TCZ-SC could provide additional treatment options for patients with RA.**

INTRODUCTION

Tocilizumab (TCZ) is a humanized monoclonal antibody directed against the interleukin-6 (IL-6) receptor that is approved for the treatment of patients with rheumatoid arthritis (RA), polyarticular-course and systemic juvenile idiopathic arthritis, and Castleman's disease by intravenous (IV) administration. Multiple phase III trials of TCZ,

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[1]Atsushi Ogata, MD, PhD, Toshio Tanaka, MD, PhD: Osaka University, Osaka, Japan; [2]Kazuhide Tanimura, MD: Hokkaido Medical Center for Rheumatic Diseases, Hokkaido, Japan; [3]Toyohiko Sugimoto, MD: National Hospital Organization, Shimoshizu Hospital, Chiba, Japan; [4]Hiroshi Inoue, MD: Inoue Hospital, Gunma, Japan; [5]Yukitomo Urata, MD: Seihoku Central Hospital, United Municipalities of Tsugaru, Aomori, Japan; [6]Tsukasa Matsubara, MD: Matsubara Mayflower Hospital, Hyogo, Japan; [7]Masakazu Kondo, MD: Kondo Clinic of Rheumatology and Orthopedic Surgery, Fukuoka, Japan; [8]Yukitaka Ueki, MD: Sasebo Chuo Hospital, Nagasaki, Japan; [9]Mitsuhiro Iwahashi, MD: Higashihiroshima Memorial Hospital, Hiroshima, Japan; [10]Shigeto Tohma, MD: National Hospital Organization, Sagamihara Hospital, Kanagawa, Japan; [11]Shuji Ohta, MD: Taga General Hospital, Ibaraki, Japan; [12]Yukihiko Saeki, MD: National Hospital Organization, Osaka Minami Medical Center, Osaka, Japan.

AQ: 4 Dr. Ogata has received speaking fees (less than $10,000 each) from Abbott, BMS, Chugai, Mitsubishi-Tanabe, and Pfizer, and has received consulting fees (more than $10,000) from Chugai. Dr. Tanimura has received consulting fees, speaking fees, and/or honoraria (less than $10,000 each) from Abbott, BMS, Chugai, Eisai, and Mitsubishi-Tanabe.

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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.

in combination with synthetic disease-modifying antirheumatic drugs (DMARDs) or as monotherapy, demonstrated an improvement of clinical symptoms and prevention of joint destruction (1–7).

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.

Dr. Inoue has received consulting fees, speaking fees, and/or honoraria (less than $10,000 each) from BMS, Chugai, Eisai, Mitsubishi-Tanabe, and Takeda. Dr. Urata has received consulting fees, speaking fees, and/or honoraria (less than $10,000 each) from BMS, Chugai, Janssen, and Pfizer. Dr. Kondo has received consulting fees, speaking fees, and/or honoraria (less than $10,000 each) from Abbott, Chugai, Eisai, Mitsubishi-Tanabe, and UCB and (more than $10,000) from Pfizer. Dr. Ueki has received consulting fees, speaking fees, and/or honoraria (less than $10,000 each) from Abbott, BMS, Chugai, Eisai, Mitsubishi-Tanabe, and Pfizer. Dr. Iwahashi has received consulting fees, speaking fees, and/or honoraria (less than $10,000 each) from Abbott, Asahi Kasei, Janssen, Mitsubishi-Tanabe, Pfizer, and Santen and (more than $10,000 each) from BMS and Chugai. Dr. Tohma has received consulting fees, speaking fees, and/or honoraria (more than $10,000) from Chugai. Dr. Ohta has received consulting fees, speaking fees, and/or honoraria (less than $10,000 each) from Abbott, Astellas, BMS, Chugai, Eisai, Shionogi, and Takeda and (more than $10,000) from Mitsubishi-Tanabe. Dr. Saeki has received consulting fees, speaking fees, and/or honoraria (less than $10,000 each) from Abbott Japan, BMS, Chugai, Mitsubishi-Tanabe, Pfizer Japan, and Takeda. Dr. Tanaka has received consulting fees, speaking fees, and/or honoraria (less than $10,000 each) from Chugai and Mitsubishi-Tanabe.

Address correspondence to Atsushi Ogata, MD, PhD, Department of Respiratory Medicine, Allergy and Rheumatic Disease, Osaka University Graduate School of Medicine, 2-2, Yamadaoka, Suita, Osaka 565-0871, Japan. E-mail: ogata@imed3.med.osaka-u.ac.jp.

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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. AQ: 5

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 one 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 one 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.

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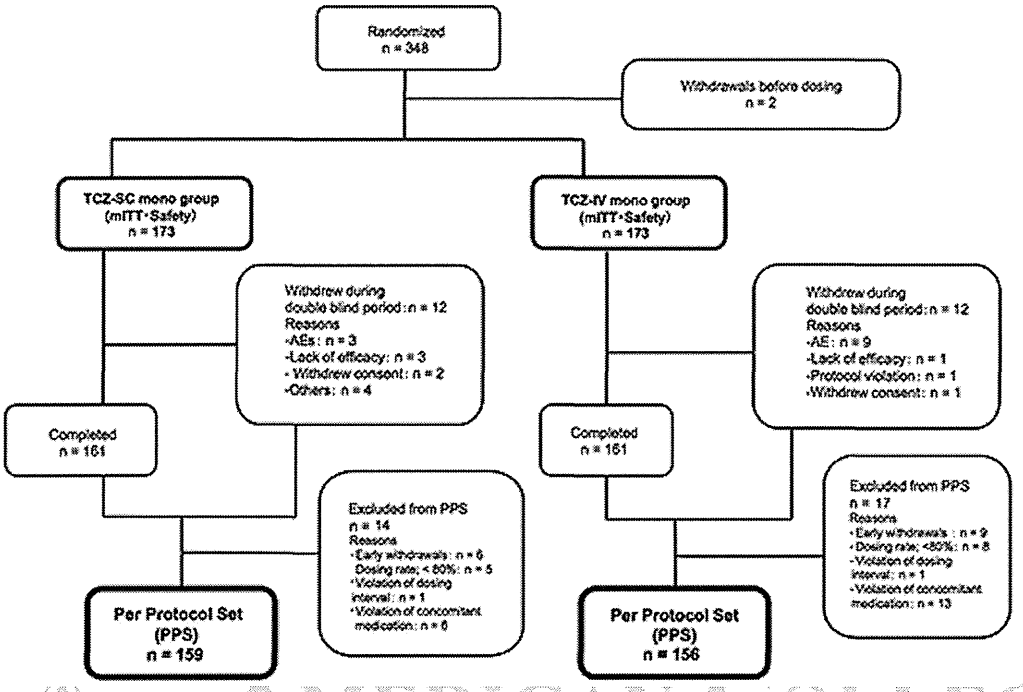

Figure 1. Patient disposition over 24 weeks (in the per-protocol set [PPS]). Two patients withdrew before treatment with tocilizumab (TCZ) was initiated. In the group receiving a subcutaneous injection of TCZ monotherapy (TCZ-SC mono), 3 patients withdrew because of adverse events (AEs), 3 patients withdrew 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

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. (%)	129 (88.4)	136 (90.7)
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 peptide 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.

(82.1% [95% CI 76.0, 88.1]) than in the TCZ-SC monotherapy 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

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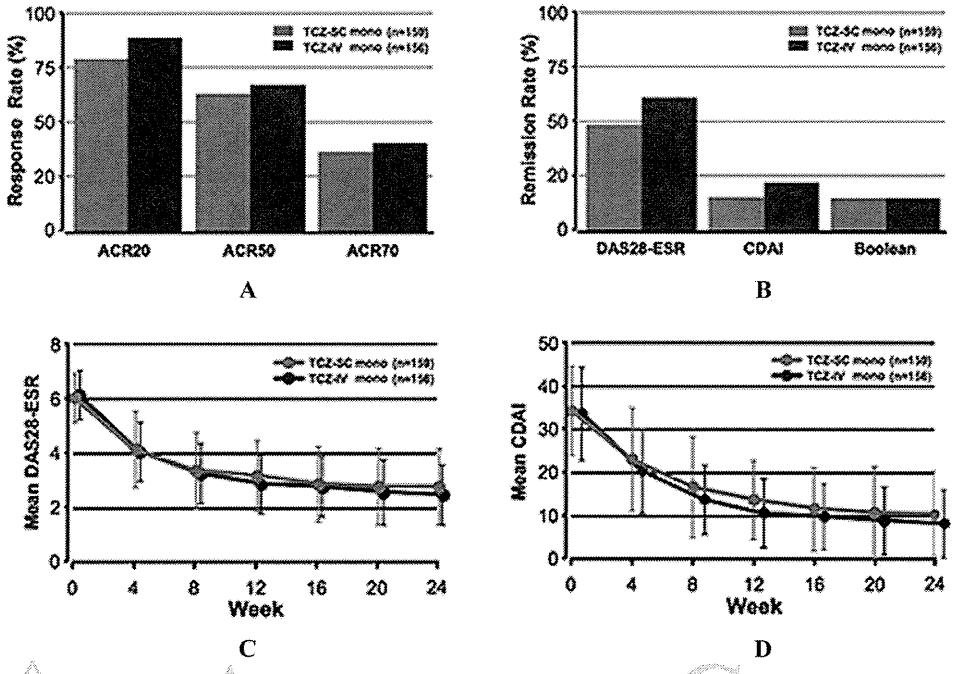


Figure 2. **A**, American College of Rheumatology (ACR) response rates of 20% (ACR20), 50% (ACR50), and 70% (ACR70) at week 24 (in the per-protocol set [PPS]) in patients receiving an intravenous infusion of tocilizumab monotherapy (TCZ-IV mono; n = 156) or a subcutaneous injection of tocilizumab monotherapy (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.

μg/ml from week 4 onward in the TCZ-SC monotherapy group (Figure 3).

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. T2

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.?????/abstract). All ISRs were mild, and no cases resulted in withdrawal from the study. AQ: 9

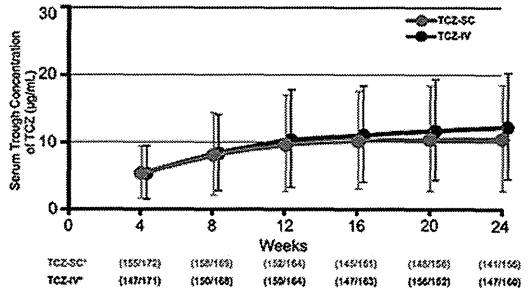
The incidence of SIRs from SC injection was 3.5% (6 of 173 patients) in the TCZ-SC monotherapy group, and the

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 T3 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

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.

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 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 for 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 of TCZ-SC monotherapy was lower than 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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Analysis and interpretation of data. Ogata.

ROLE OF THE STUDY SPONSOR

Chugai had no role in the study design, data collection, data analysis, or writing of the manuscript, as well as approval of the content of the submitted manuscript. Publication of this article was not contingent on the approval of Chugai. Genentech provided editorial support (performed by Denise Kenski, PhD, Ryan DeMasi, MD, and Angela Kam of Health Interactions, Atlanta, Georgia).

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REFERENCES

1. Smolen JS, Beaulieu A, Rubbert-Roth A, Ramos-Remus C, Rovensky J, Alecock E, et al. Effect of interleukin-6 receptor inhibition with tocilizumab in patients with rheumatoid arthritis (OPTION study): a double-blind, placebo-controlled, randomised trial. Lancet 2008;371:987–97.
2. Genovese MC, McKay JD, Nasonov EL, Mysler EF, da Silva NA, Alecock E, et al. Interleukin-6 receptor inhibition with tocilizumab reduces disease activity in rheumatoid arthritis with inadequate response to disease-modifying antirheumatic drugs: the Tocilizumab in Combination With Traditional Disease-Modifying Antirheumatic Drug Therapy Study. Arthritis Rheum 2008;58:2968–80.
3. Emery P, Keystone E, Tony HP, Cantagrel A, van Vollenhoven R, Sanchez A, et al. IL-6 receptor inhibition with tocilizumab improves treatment outcomes in patients with rheumatoid arthritis refractory to anti-tumour necrosis factor biologicals: results from a 24-week multicentre randomised placebo-controlled trial. Ann Rheum Dis 2008;67:1516–23.
4. Kremer JM, Blanco R, Brzosko M, Burgos-Vargas R, Halland AM, Vernon E, et al. Tocilizumab inhibits structural joint damage in rheumatoid arthritis patients with inadequate responses to methotrexate: results from the double-blind treatment phase of a randomized placebo-controlled trial of tocilizumab safety and prevention of structural joint damage at one year. Arthritis Rheum 2011;63:609–21.
5. Jones G, Sebba A, Gu J, Lowenstein MB, Calvo A, Gomez-Reino JJ, et al. Comparison of tocilizumab monotherapy versus methotrexate monotherapy in patients with moderate to severe rheumatoid arthritis: the AMBITION study. Ann Rheum Dis 2010;69:88–96.
6. Nishimoto N, Hashimoto J, Miyasaka N, Yamamoto K, Kawai S, Takeuchi T, et al. Study of active controlled monotherapy used for rheumatoid arthritis, an IL-6 inhibitor (SAMURAI): evidence of clinical and radiographic benefit from an x ray reader-blinded randomised controlled trial of tocilizumab. Ann Rheum Dis 2007;66:1162–7.
7. Nishimoto N, Miyasaka N, Yamamoto K, Kawai S, Takeuchi T, Azuma J, et al. Study of active controlled tocilizumab monotherapy for rheumatoid arthritis patients with an inadequate response to methotrexate (SATORI): significant reduction in disease activity and serum vascular endothelial growth factor by IL-6 receptor inhibition therapy. Mod Rheumatol 2009;19:12–9.
8. Boonen A, van de Rest J, Dequeker J, van der Linden S. How Renoir coped with rheumatoid arthritis. BMJ 1997;315:1704–8.
9. Dougados M, Kissel K, Sheeran T, Tak PP, Conaghan PG, Mola EM, et al. Adding tocilizumab or switching to tocilizumab monotherapy in methotrexate inadequate responders: 24-week symptomatic and structural results of a 2-year randomised controlled strategy trial in rheumatoid arthritis (ACT-RAY). Ann Rheum Dis 2013;72:43–50.
10. Singh JA, Furst DE, Bharat A, Curtis JR, Kavanaugh AF, Kremer JM, et al. 2012 update of the 2008 American College of Rheumatology recommendations for the use of disease-modifying antirheumatic drugs and biologic agents in the treat-

ment of rheumatoid arthritis. Arthritis Care Res (Hoboken) 2012;64:625–39.
11. Smolen JS, Landewe R, Breedveld FC, Dougados M, Emery P, Gaujoux-Viala C, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs. Ann Rheum Dis 2010;69:964–75.
12. Barton JL. Patient preferences and satisfaction in the treatment of rheumatoid arthritis with biologic therapy. Patient Prefer Adherence 2009;3:335–44.
13. Chilton F, Collett RA. Treatment choices, preferences and decision-making by patients with rheumatoid arthritis. Musculoskeletal Care 2008;6:1–14.
14. Williams EL, Edwards CJ. Patient preferences in choosing anti-TNF therapies-R1 [letter]. Rheumatology (Oxford) 2006; 45:1575–6.
15. Ohta S, Tsuru T, Terao K, Mogi S, Suzaki M, Nakashima H, et al. Optimal dose prediction by pharmacokinetic and biomarker response of subcutaneous tocilizumab treatment: a phase I/II study evaluating the safety, pharmacokinetics and clinical response in patients with rheumatoid arthritis [abstract]. Arthritis Rheum 2010;62 Suppl:S467–8.
16. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–24.
17. Felson DT, Anderson JJ, Boers M, Bombardier C, Furst D, Goldsmith C, et al. American College of Rheumatology preliminary definition of improvement in rheumatoid arthritis. Arthritis Rheum 1995;38:727–35.
18. Matsuda Y, Singh G, Yamanaka H, Tanaka E, Urano W, Taniguchi A, et al. Validation of a Japanese version of the Stanford Health Assessment Questionnaire in 3,763 patients with rheumatoid arthritis. Arthritis Rheum 2003;49:784–8.
19. Nishimoto N, Terao K, Mima T, Nakahara H, Takagi N, Kakehi T. Mechanisms and pathologic significances in increase in serum interleukin-6 (IL-6) and soluble IL-6 receptor after administration of an anti-IL-6 receptor antibody, tocilizumab, in patients with rheumatoid arthritis and Castleman's disease. Blood 2008;112:3959–64.
20. Stubenrauch K, Wessels U, Birnboeck H, Ramirez F, Jahreis A, Schleypen J. Subset analysis of patients experiencing clinical events of a potentially immunogenic nature in the pivotal clinical trials of tocilizumab for rheumatoid arthritis: evaluation of an antidrug antibody ELISA using clinical adverse event-driven immunogenicity testing. Clin Ther 2010;32: 1597–609.
21. International Conference on Harmonisation E9 Expert Working Group. ICH Harmonised Tripartite Guideline: statistical principles for clinical trials. Stat Med 1999;18:1905–42.
22. Burmester GR, Rubbert-Roth A, Cantagrel AG, Hall S, Leszczynski P, Feldman D, et al. A randomized, double-blind, parallel group study of the safety and efficacy of tocilizumab SC versus tocilizumab IV, in combination with traditional Dmards in patients with moderate to severe RA [abstract]. Arthritis Rheum 2012;64 Suppl:S1075.
23. Kivitz A, Olech E, Borofsky M, Zazueta BM, Navarro-Sarabia F, Rowell L, et al. A randomized, double-blind, parallel-group study of the safety and efficacy of tocilizumab subcutaneous versus placebo in combination with traditional DMARDs in patients with moderate to severe rheumatoid arthritis (BREVACTA). Presented at the 76th Annual Scientific Meeting of the American College of Rheumatology; 2012 November 9–14; Washington, DC.
24. Genentech, Inc. (a member of the Roche Group). Actemra prescribing information. South San Francisco: Genentech; 2010.
25. Tanaka Y, Harigai M, Takeuchi T, Yamanaka H, Ishiguro N, Yamamoto K, et al. Golimumab in combination with methotrexate in Japanese patients with active rheumatoid arthritis: results of the GO-FORTH study. Ann Rheum Dis 2012;71: 817–24.
26. Miyasaka N and the CHANGE Study Investigators. Clinical investigation in highly disease-affected rheumatoid arthritis patients in Japan with adalimumab applying standard and general evaluation: the CHANGE study. Mod Rheumatol 2008;18:252–62.
27. Takamura A, Hirata S, Nagasawa H, Kameda H, Seto Y, Atsumi T, et al. A retrospective study of serum KL-6 levels during treatment with biological disease-modifying antirheumatic drugs in rheumatoid arthritis patients: a report from the Ad Hoc Committee for Safety of Biological DMARDs of the Japan College of Rheumatology. Mod Rheumatol 2013;23: 297–303.
28. Harigai M, Takamura A, Atsumi T, Dohi M, Hirata S, Kameda H, et al. Elevation of KL-6 serum levels in clinical trials of tumor necrosis factor inhibitors in patients with rheumatoid arthritis: a report from the Japan College of Rheumatology Ad Hoc Committee for Safety of Biological DMARDs. Mod Rheumatol 2013;23:284–96.
29. Bartelds GM, Wijbrandts CA, Nurmohamed MT, Stapel S, Lems WF, Aarden L, et al. Clinical response to adalimumab: relationship to anti-adalimumab antibodies and serum adalimumab concentrations in rheumatoid arthritis. Ann Rheum Dis 2007;66:921–6.
30. Bartelds GM, Wijbrandts CA, Nurmohamed MT, Stapel S, Lems WF, Aarden L, et al. Anti-infliximab and anti-adalimumab antibodies in relation to response to adalimumab in infliximab switchers and anti-tumour necrosis factor naive patients: a cohort study. Ann Rheum Dis 2010;69:817–21.
31. Dore RK, Mathews S, Schechtman J, Surbeck W, Mandel D, Patel A, et al. The immunogenicity, safety, and efficacy of etanercept liquid administered once weekly in patients with rheumatoid arthritis. Clin Exp Rheumatol 2007;25:40–6.
32. Kaine J, Gladstein G, Strusberg I, Robles M, Louw I, Gujrathi S, et al. Evaluation of abatacept administered subcutaneously in adults with active rheumatoid arthritis: impact of withdrawal and reintroduction on immunogenicity, efficacy and safety (phase IIIb ALLOW study). Ann Rheum Dis 2012;71: 38–44.