Anti-Interleukin-6 Therapy in Rheumatoid Arthritis

Robert Woodrick, M.D., and Eric M. Ruderman, M.D.

Abstract

Recent advances in research have led to significant progress in unraveling the pathophysiology of rheumatoid arthritis (RA), including the cytokine-mediated signaling process. While therapies targeting one particular cytokine, tumor necrosis factor-alpha (TNF-α), have revolutionized the treatment of RA, other cytokines, including interleukin 6 (IL-6,) have been implicated in the disease process. In this review, we describe the research that ultimately led to large, randomized, controlled trials demonstrating the effectiveness of tocilizumab, a monoclonal antibody directed against the IL-6 receptor, as a potent new therapeutic agent in RA treatment. These data have shown this agent to be effective both in patients failing non-biologic DMARDs and those failing anti-TNF therapy, although the drug is currently approved for use only in the latter situation. Adverse events seen with tocilizumab therapy are also reviewed.

The answer to the question "Why target IL-6 for therapeutic intervention in rheumatoid arthritis?" lies in the search for improved treatments in rheumatoid arthritis (RA) populations that are inadequately responsive or non-responsive to current therapies. Over the past three decades, significant progress in our understanding of the pathophysiology of RA has identified the key role of cytokine signaling in this process, a finding that has led to potent new therapeutics. Several pro-inflammatory cytokines have been implicated in the RA disease process, including TNF-α, interleukin (IL)-1, and IL-6. Levels of all three cytokines have been shown to be elevated in the synovial fluid of patients with RA, compared to both controls and patients with other arthritides.[1-3] TNF-α inhibitors, and to a lesser extent IL-1 inhibitors, have revolutionized the treatment of RA; however, a substantial number of patients fail to respond adequately to treatment,[4-6] suggesting a role for inhibition of other pro-inflammatory signals, including IL-6. Studies have shown that IL-6 overproduction (i.e., detection in synovial fluid) correlates to increased RA disease activity.[7,8] Murine models indicate that IL-6 deficiency delays the onset and reduced the severity of collagen-induced arthritis[9]; blocking the IL-6 receptor leads to diminished joint disease and a decrease in anti-type II collagen antibodies in similar murine models.[10]

A chimeric antibody to the IL-6 receptor that blocks both soluble and membrane-bound IL-6 activity was found to inhibit collagen-induced arthritis in cynomolgus monkeys, paving the way for human trials targeting this pathway.[11,12] An initial study using in vitro human cell cultures found that the humanized monoclonal antibody called tocilizumab (previously known as MRA) decreased cell signaling via IL-6 in a dose-dependent manner.[13] Interestingly and importantly, other IL-6 family cytokines, including IL-11, leukemia inhibiting factor, and oncostatin M, were not affected. Given the promise of these pre-clinical data, human trials using tocilizumab to treat RA were initiated.

Early Tocilizumab Clinical Trials

The first clue to the efficacy of IL-6 inhibition in RA came from an early study in which 45 patients with active RA received a single, intravenous dose of 0.1, 1, 5, or 10 mg/kg of tocilizumab.[14] At 2 weeks, only the 5 mg/kg group had achieved the primary endpoint, an increased ACR20 response compared to placebo, although both the 5 mg/kg and the 10 mg/kg groups had a significantly increased ACR20 response rate at 6 and 8 weeks. C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) normalized after one

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dose in both the 5 mg/kg and 10 mg/kg treatment groups.

A follow-up multicenter, double blind, placebo-controlled trial was designed to assess the efficacy and safety of tocilizumab in patients with "established and active" RA, despite disease-modifying antirheumatic drugs (DMARD) therapy. Following DMARD washout, patients were randomized to placebo, 4- or 8-mg/kg tocilizumab.[15] The primary end point was the ACR20 response rate at 12 weeks. Tocilizumab produced a reduction in disease activity by this timepoint; 78% of the 8 mg/kg achieved at least an ACR20 response, compared with 57% ($p = 0.02$) and only 11% ($p < 0.001$) of the 4 mg/kg and placebo groups, respectively. The attrition rate in the placebo arm of this study was high. Of 53 patients assigned to placebo therapy, only 28 remained in the study at 12 weeks; 52/54 and 51/55 patients remained in the 4 mg/kg tocilizumab arm and 8mg/kg tocilizumab arms, respectively. Most of the withdrawals from the placebo arm were attributed to lack of efficacy, requiring additional DMARD therapy. Overall adverse events were distributed equally across all three groups. Serious adverse events occurred in five patients, including two in the placebo group. One patient receiving 4 mg/kg was hospitalized for leg infection due to a burn, but continued on tocilizumab. One patient receiving 8 mg/kg was hospitalized for allergic pneumonitis after three doses of tocilizumab. Another patient receiving 8 mg/kg developed hemophagocytosis syndrome after Epstein-Barr virus (EBV) reactivation and eventually died.

The first trial to evaluate inhibition of structural damage with tocilizumab was the SAMURAI (Study of Active Controlled Monotherapy Used for Rheumatoid Arthritis an IL-6 Inhibitor) study.[16] In this trial, 306 patients with active RA of less than 5 years duration were allocated to either 8 mg/kg tocilizumab infusions every 4 weeks or to "conventional DMARD" therapy (MTX with or without other DMARDs). DMARD and MTX doses could be adjusted according to disease activity. The mean dose of MTX was 7.1 mg/wk in those treated with MTX (a typical dose in Japan, where this study was performed). Radiographs were evaluated by two blinded radiologists at baseline, 28, and 52 weeks, using the van der Heijde modified Sharp method. At 52 weeks, there was significantly less change in the mean total Sharp (TSS), erosion, and joint-space narrowing scores in the tocilizumab-treated group, compared with the conventional DMARD group. DAS28 and health assessment questionnaire (HAQ) scores were significantly lower and the numbers of patients who achieved ACR20, 50, and 70 were significant higher in the tocilizumab group, compared with the DMARD group. It must be noted, however, that subjects and clinical assessors were unblended to treatment allocation in this study.

The first results from a European study of tocilizumab (CHARISMA, Chugai Humanized Anti-Human Recombinant Interleukin-6 Monoclonal Antibody) were published in 2006.[17] Patients with active RA, despite at least 6 months on methotrexate (MTX), were randomized to receive tocilizumab 2, 4, or 8 mg/kg every 4 weeks plus placebo or their existing dose of MTX. A seventh group received placebo infusions while continuing MTX. Both the 4 mg/kg and 8 mg/kg tocilizumab monotherapy groups produced greater ACR20 responses, compared to continued MTX monotherapy at 16 weeks, the primary end point of the study, although neither dose was superior to MTX for ACR50 and ACR70 responses. Combination therapy with MTX and tocilizumab also produced greater ACR20 responses than MTX monotherapy; however, only combination therapy with the 8 mg/kg tocilizumab plus MTX was statistically superior to MTX monotherapy for ACR50 and ACR70 responses. Remission rates were 34% with high dose (8 mg/kg) combination therapy, 17% with medium dose (4 mg/kg) combination therapy, and 8% among those receiving placebo plus MTX. Infections seen in this study included septicemia, osteomyelitis, and respiratory infection (most common). There were two non-life-threatening anaphylactic reactions (both in patients on monotherapy with tocilizumab, as were the 25 subjects with measurable anti-tocilizumab antibodies). Mean alanine aminotransferase (ALT) levels increased in a dose dependant manner with tocilizumab, and this was heightened in combination therapy with MTX. Five patients (all in the 8 mg/kg tocilizumab plus MTX group) showed marked (but reversible) increases in ALT (> 100 IU/L) and were withdrawn from the study. Lipid changes were seen and included elevated low density lipoprotein (LDL) and high density liproprotein (HDL) levels; however, the atherogenic index remained stable. A dose-dependent reduction of neutrophils was also seen in patients taking tocilizumab, although low neutrophil counts were not specifically associated with infection. Overall, this study established the dosing of 4 mg/kg or 8 mg/kg (every 4 weeks) of tocilizumab.

Phase III Experience with Tocilizumab

Building on the preliminary safety and dose-ranging data from the phase II trials, a series of phase III trials were designed to examine the safety and efficacy of tocilizumab in several distinct clinical situations. The OPTION study (Tocilizumab Pivotal Trial in Methotrexate Inadequate Responders) was a double-blind, randomized, placebo-controlled trial of 623 patients with moderate to severely active RA, who had not adequately responded to MTX.[18] Patients were required to have been taking MTX for more than 12 weeks with continued disease activity and were randomized to placebo infusions, 4 mg/kg or 8 mg/kg of tocilizumab every 4 weeks. Other DMARDs were discontinued; nonsteroidal antiinflammatory drugs (NSAIDs) and steroids (≤ 10 mg prednisone or equivalent) were kept at stable doses. The results showed that 120 patients (59%) in the 8 mg/kg group, 102 patients (48%) in the 4 mg/kg group, and 54 (26%) of those in the placebo group achieved ACR20 responses. More patients in the placebo and 4 mg/kg groups switched to the 8 mg/kg group (rescue therapy). By week 24, patients receiving tocilizumab had significantly better responses to all core set variables, including DAS28, HAQ,

Table 1 Major Clinical Trials with Tocilizumab in Rheumatoid Arthritis

Study	Number of Tocilizumab-Treated Patients	LFT Abnormalities	Hyperlipidemia	Neutropenia	Adverse Events	% Control Adverse Events	Hospitalizations, Serious Events, or Death
Nishimoto et al.[15]	164	12.8% (mostly grade I)	44%	15.6% (mostly grade I)	- 4 mg/kg: 59% - 8 mg/kg: 59% - Mostly colds, headache	56%	- One death: EBV reactivation - One hosp: allergic pneumonitis - One hosp: leg infection
CHARISMA[17]	179	127 total with ALT abnormality; 18 with greater than 100 IU/L	- No % or number: "moderate but reversible increase in mean HDL, TG, TC" - No increased cardiac deaths	- 40 pts, 13 with grade II/III - + dose related	Approx 50% with adverse reaction; 30 pts with 35 treatment-emergent reactions	Unclear	- Seven infections (between 4 mg/kg and 8 mg/kg groups), including osteomyelitis, septic arthritis, sepsis - Five cases of anaphylactic shock-hypersensitivity
OPTION[18]	- 4 mg/kg: 212 pts - 8 mg/kg: 206 pts	- ALT: 23 pts - AST: 3 pts	- Mean HDL, TC, TG elevated significantly above placebo - Some pts had an increase in arthrogenic ratio - No cardiac deaths	104 pts in combined treatment groups	- 4 mg/kg: 71% total AE - 8 mg/kg: 69% total AE - 26 (6%) serious reactions - 9/26 serious reactions were infection	- 63% total adverse reactions, 6% serious	
SAMURAI[16]	157	Unknown	- LDL elevation: 26% - TC elevation: 38% - TG elevation: 17% - No cardiac events reported	Unknown	- 89% total AE, with 18% considered serious - Most were mild to moderate	- 82% total AE in DMARD (control) group - 13% were serious	- 3 pts with PNA, 2 pts with URI, 2 pts with cellulitis, 1 pt with gastro, HSV, VZV - 2 pts with breast cancer, 1 pt with colon cancer - 3 pts with HTN after infusion, 2 pts with headache, 2 with injection site reaction

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RADIATE[19]	4 mg/kg + MTX: 163 pts 8 mg/kg + MTX 175 pts	- Common increases in ALT and AST - No pts developed MTX hepatotoxicity - ALT increase > 3xULN: 4 pts in the 4 mg/kg group; 4 pts in 8 mg/kg group; 1 pt in MTX group	- Mean TC increased in all groups - Significant TG increase seen in 2 pts in 4 mg/kg group - LDL/HDL increase > 30%: 4 mg/kg group, 19%; 8 mg/kg group, 22.2%; MTX only group, 10.1%	≥ one episode of neutropenia: 4 mg/kg group, 20.3%; 8 mg/kg group, 28% MTX only: < 1.0% Grade 4 neutropenia (requiring withdrawal): 4 mg/kg group, 1 pt; 8 mg/kg group, 4 pts	4 mg/kg total AE: 87.1% Serious AE: 7.4% 8 mg/kg total AE: 84.0% Serious AE: 6.3%	90.6% total AE Total serious AE: 11.3%	- 4 serious AE led to discontinuation of study: 4 mg/kg group, 1 pt with necrotizing PNA 8 mg/kg group, 1 pt with staphylococcal polyarthritis MTX group, urosepsis, osteomyelitis - No deaths - 1 control pt and 1 8 mg/kg pt withdrew due to infusion rxn - 1 pt in 4 mg/kg and 8 mg/kg groups had hepatic steatosis - 1 MI in control group
TOWARD[20]	- 8 mg/kg plus DMARD: 802 pts - DMARD group: 414 pts	- ALT increase under 3xULN - Tocilizumab group: 35.7% - DMARD only: 11.8% - Bilirubin increase under 3xULN: - 8 mg/kg plus DMARD: 8.9% - DMARD only: 0.7% - 4.1% tocilizumab group had ALT more than 3xULN	- Mean fasting lipid levels increased in tocilizumab group - Increase from < 240 mg/dl to > 240 mg/dl TC: - Tocilizumab group: 23% - DMARD group: 5.5% - Increase from < 160 mg/dl to > 160 mg/dl LDL - Tocilizumab group: 16% - DMARD group: 3% - TG levels increased in the tocilizumab group - Arthrogenic index was increased in the tocilizumab group	- Normal baseline PMN to low post-treatment PMN: - Tocilizumab group: 29% - DMARD group: 4% - No association of neutropenia with infection-related AE - 3.7% of pts in the tocilizumab group with grade III neutropenia	- Total AE 72.8% - Related serious AE: 2.9%[23] - Infection rate: 37.4% - GI disorders: 20.8% - Skin/subQ tissue disorders: 16.6%	-Total AE: 61.1% -Related serious AE:1.4%[6] - Infection rate: 14.7% - GI disorders: 14.7% - Skin/subQ tissue disorders: 7.0%	- Study withdrawals due to infection: 3 pts in tocilizumab group, 2 pts in DMARD only group - More infections, GI disorders, skin disorders were seen in the tocilizumab group - Deaths: tocilizumab group: 2 pts (bypass surgery complication, hemorrhagic stroke) - DMARD group: 2 pts (PNA, intestinal obstruction)

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AMBITION[21]	286 pts (8 mg/kg)	- ALT increase under 3xULN: - Tocilizumab group (8 mg/kg): 31.6% - MTX group: 30.3% - Bilirubin increase under 3x ULN: - Tocilizumab group: 7.6% MTX group: 0.7% - 1.0% tocilizumab pts and 2.5% MTX pts had ALT increase more than 3xULN	- Increase from < 240 mg/dl to > 240 mg/dl TC: - Tocilizumab group: 13.2% - MTX group: 0.4% - Increase from < 160 mg/dl to > 160 mg/dl LDL: - Tocilizumab group: 3.1% - MTX group: 0% - No cardiac events noted	- Normal baseline PMN to low post-treatment PMN: - Tocilizumab group: 40.2% - MTX group: 10.2% - 3.1% tocilizumab pts had grade III neutropenia; none in MTX group	- Total AE: 79.% - Serious AE: 3.8% - Related serious AE: 4 pts (1.4%) - Most common AE: infection - Higher skin/subQ infections with tocilizumab - 29.9% with GI disorder	- Total AE: 77.5% - Serious AE: 2.8% - Related serious AE: 4 pts (1.4%) - Most common AE: infection	- 5 pts in MTX group and 1 pt in Tocilizumab group withdrew, due to GI disorder - No pts in the tocilizumab group had fungal infection or TB Deaths: - Tocilizumab group: 3 pts (upper GI hemorrhage, myocardial ischemia, cardiorespiratory arrest with asthma) - MTX group: 1 pt (lung cancer) - Only GI hemorrhage was considered possibly related to tocilizumab

AE, adverse events; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DMARD, disease modifying anti-rheumatic drug; HDL, high density liproprotein; LDL: low density lipoprotein; MTX: methotrexate; PNA: pneumonia; pts: patients; TC: total cholesterol; TG: triglyceride; ULN: upper limit of normal; SubQ, subcutaneous.

pain VAS, global VAS, ESR and tender and swollen joint counts. Significantly more patients receiving tocilizumab also achieved ACR50 and ACR70 responses, compared to patients in the placebo group.

The RADIATE (Research on Actemra Determining Efficacy after Anti-TNF Failures) trial studied a combination of tocilizumab with MTX in RA patients refractory to TNF antagonist therapy.[19] The 499 patients enrolled in the study received 8 mg/kg or 4 mg/kg tocilizumab or placebo, plus MTX (10-25 mg/wk). Rescue therapy was allowed at 16 weeks; more patients in the placebo and 4 mg/kg tocilizumab groups required rescue than those in the 8 mg/kg tocilizumab group. The primary outcome was an ACR20 rate at 24 weeks, and both the 4-mg/kg and 8-mg/kg tocilizumab groups achieved significantly higher rates of response than the placebo group (50%, 30.4%, 10.1%, respectively). The 8 mg/kg tocilizumab group also had significantly higher ACR50 and ACR70 response rates at 24 weeks. Response in the tocilizumab groups was rapid (typically within 2 weeks). The number and type of TNF antagonists previously failed did not impact the ACR20 response rate. DAS28 remission rates were 30.1%, 7.6%, and 1.6% at 24 weeks for the 8 mg/kg, 4 mg/kg and placebo groups, respectively.

A fourth randomized, double-blind, placebo-controlled phase III trial (TOWARD, Tocilizumab in Combination with Traditional DMARD Therapy) evaluated 1220 patients with active disease, despite stable doses of DMARDs (the most common being MTX) randomized to receive monthly tocilizumab or placebo infusions.[20] At 24 weeks, the tocilizumab group had significantly improved ACR20 response rates and significantly improved DAS28 scores, as well as, significantly, rates of remission (also by DAS28). There was no difference in response rates in those patients taking more than two DMARDs. Levels of inflammatory markers (ESR, erythrocyte sedimentation rate; CRP, C-reactive protein) showed significant decreases in the tocilizumab group, compared to placebo. Improvements in function (measured with the HAQ) and fatigue (measured with FACIT-F) were also significantly greater in the patients in the tocilizumab group.

Finally, The AMBITION study (Actemra versus Methotrexate Double-Blind Investigative Trial in Monotherapy) assessed the efficacy of tocilizumab compared with methotrexate in a methotrexate-naïve population.[21] In this study, 673 RA patients with moderate to severe disease were randomized to receive tocilizumab 8 mg/kg every 4 weeks, MTX (titrated to 20 mg weekly, by week 8), or placebo for 8 wks followed by tocilizumab. At 24 weeks, tocilizumab was superior to MTX at achieving ACR20, ACR50, and ACR70 response rates. Improvement in HAQ scores with tocilizumab was also superior to MTX. At 24 weeks, patients in the tocilizumab-treated groups were five-times more likely to achieve DAS28 remission and four-times more likely to achieve at least a moderate EULAR response than in the MTX-treated group.

Adverse Reactions to Tocilizumab

Infections have been seen with tocilizumab therapy, although opportunistic infections have been uncommon. Rare incidences of gastrointestinal perforation have occurred. The most common patterns of laboratory abnormalities seen with tocilizumab therapy have included transaminase elevations, neutropenia, and hyperlipidemia. Despite the lipid abnormalities, clinical cardiovascular events have not been increased with tocilizumab therapy in the trials. In general, adverse events were somewhat more common with the 8 mg/kg dose of tocilizumab. Table 1 summarizes the adverse events seen in the large clinical trials of tocilizumab.

Conclusion

The experience with tocilizumab has confirmed that inhibition of the IL-6 pathway is an effective therapeutic strategy in RA. Based on the data from the clinical trial program, the U.S. Food and Drug Administration (FDA) has approved tocilizumab for use in patients with moderate to severely active RA with an inadequate response to DMARD therapy, including biologics. It may be used in combination with MTX or other DMARDs or as monotherapy. An initial dose of 4 mg/kg by IV infusion every 4 weeks is recommended (presumably because of the lower toxicity profile at this dose), with an increase to 8 mg/kg in the event of an inadequate clinical response, although there are no guidelines on the timing of this decision. Interestingly, the response to the 8 mg/kg dose in the TOWARD study in TNF inadequate responders, the situation where tocilizumab is likely to be used initially, was greater than the response to the 4 mg/kg dose. Infections are a concern, as with any immunologically active biologic therapy in RA, but do not appear to be more common than with existing biologics; post-marketing data may help identify whether these infections are more or less of an issue than with other therapies. Other common toxicities of tocilizumab, which will require monitoring, include elevations in lipids and transaminases and decreases in neutrophil counts. With the success of tocilizumab therapy, there is interest in other agents that might inhibit IL-6. Several antibodies to the IL-6 cytokine itself, rather than its receptor, are in development. This research is being pursued in the hope that their usage might avoid the hepatotoxicity and the lipid abnormalities, which have been postulated to result from antibody interaction with IL-6 receptors that are present on the surface of hepatocytes.

Disclosure Statement

None of the authors have a financial or proprietary interest in the subject matter or materials discussed, including, but not limited to, employment, consultancies, stock ownership, honoraria, and paid expert testimony.

References

1. Partsch G, Steiner G, Leeb BF, et al. Highly increased levels of tumor necrosis factor-alpha and other proinflammatory

cytokines in psoriatic arthritis synovial fluid. J Rheumatol. 1997 Mar;24(3):518-23.
2. Steiner G, Tohidast-Akrad M, Witzmann G, et al. Cytokine production by synovial T cells in rheumatoid arthritis. Rheumatology (Oxford). 1999 Mar;38(3):202-13.
3. Firestein GS, Alvaro-Gracia JM, Maki R. Quantitative analysis of cytokine gene expression in rheumatoid arthritis. J Immunol. 1990 May 1;144(9):3347-53.
4. Gomez-Reino JJ, Carmona L; BIOBADASER Group. Switching TNF antagonists in patients with chronic arthritis: an observational study of 488 patients over a four-year period. Arthritis Res Ther. 2006;8(1):R29. Epub 2006 Jan 6.
5. Hyrich KL, Lunt M, Watson KD, et al. British Society for Rheumatology Biologics Register. Outcomes after switching from one anti-tumor necrosis factor alpha agent to a second anti-tumor necrosis factor alpha agent in patients with rheumatoid arthritis: results from a large UK national cohort study. Arthritis Rheum. 56:13-20.
6. Nuki G, Bresnihan B, Bear MB, McCabe D; European Group Of Clinical Investigators. Long-term safety and maintenance of clinical improvement following treatment with anakinra (recombinant human interleukin-1 receptor antagonist) in patients with rheumatoid arthritis: extension phase of a randomized, double-blind, placebo-controlled trial. Arthritis Rheum. 2002 Nov;46(11):2838-46.
7. Sack U, Kinne RW, Marx T, et al. Interleukin-6 in synovial fluid is closely associated with chronic synovitis in rheumatoid arthritis. Rheumatol Int. 1993;13(2):45-51.
8. Madhok R, Crilly A, Watson J, Capell HA. Serum interleukin-6 levels in rheumatoid arthritis: correlation with clinical and laboratory indices of disease activity. Ann Rheum Dis. 1993 Mar;52(3):232-4.
9. Sasai M, Saeki Y, Ohshima S, et al. Delayed onset and reduced severity of collagen-induced arthritis in interleukin-6-deficient mice. Arthritis Rheum. 1999 Aug;42(8):1635-43.
10. Hata H, Sakaguchi N, Yoshitomi H, et al. Distinct contribution of IL-6, TNF-alpha, IL-1 and IL-10 to T cell-mediated spontaneous autoimmune arthritis in mice J Clin Invest. 2004 Aug;114(4):582-8.
11. Poli V, Maritano D. IL-6 Knockout mice. *In:* Fantuzzi G (ed): *Cytokine Knockouts*. Totowa, New Jersey: Humana Press, 2003, pp. 213-234.
12. Mihara M, Kotoh M, Nishimoto N, et al. Humanized antibody to human interleukin-6 receptor inhibits the development of collagen arthritis in cynomolgus monkeys. Clin Immunol. 2001 Mar;98(3):319-26.
13. Mihara M, Kasutani K, Okazaki M, et al. Tocilizumab inhibits signal transduction mediated by both mIL6-R and sIL-6R, but not by the receptors of other members of IL-6 cytokine family. Int Immunopharmacol. 2005 Nov;5(12):1731-40.
14. Choy EH, Isenberg DA, Garrood T, et al. Therapeutic benefit of blocking interleukin-6 activity with an anti-interleukin-6 receptor monoclonal antibody in rheumatoid arthritis. Arthritis Rheum. 2002 Dec;46(12):3143-50.
15. Nishimoto N, Yoshizaki K, Miyasaka N, et al. Treatment of rheumatoid arthritis with humanized anti-interleukin-6 receptor antibody. Arthritis Rheum. 2004 Jun;50(6):1761-9.
16. Nishimoto N, Hashimoto J, Miyasaka N, 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. nn Rheum Dis. 2007 Sep;66(9):1162-7; Epub 2007 May 7.
17. Maini RN, Taylor PC, Szechinski J, et al. Double-blind randomized controlled clinical control trial of the interleukin-6 receptor antagonist, tocilizumab, in European patients with rheumatoid arthritis who had an incomplete response to methotrexate. Arthritis Rheum. 2006 Sep;54(9):2817-29. Erratum in: Arthritis Rheum. 2008 Mar;58(3):887.
18. Smolen JS, Beaulieu A, Rubbert-Roth A, et al. Effect of interleukin-6 receptor inhibition with tocilizumab in patients with rheumatoid arthritis (OPTION study): a double-blind, placebo-controlled, randomized trial. Lancet. 2008 Mar 22;371(9617):987-97.
19. Emery P, Keystone E, Tony HP, et al. IL-6 receptor inhibition with tocilizumab improves treatment outcomes in patients with rheumatoid arthritis refractory to anti-tumor necrosis factor biologics: results from a 24-week multicentre randomized placebo-controlled trial. Ann Rheum Dis. 2008 Nov;67(11):1516-23; Epub 2008 Jul 14. Erratum in: Ann Rheum Dis. 2009 Feb;68(2):296.
20. Genovese MC, McKay JD, Nasonov EL, 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 Oct;58(10):2968-80.
21. Jones G, Sebba A, Gu J, et al. Comparison of tocilizumab monotherapy versus methotrexate monotherapy in patients with moderate to severe rheumatoid arthritis: the AMBITION study. Ann Rheum Dis. 2010 Jan;69(1):88-96.