Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by synovial inflammation and joint destruction. However, the combined use of synthetic disease-modifying anti-rheumatic drug (DMARD) such as methotrexate and a biological DMARD targeting tumour necrosis factor (TNF) has revolutionized treatment of RA. Clinical remission is a realistic target to treat and the maintenance of remission has produced significant improvements in structural and function outcomes. However, biological DMARDs are limited to intravenous or subcutaneous uses and orally available small but strong products have been developed. The multiple cytokines and cell surface molecules bind to receptors, resulting in the activation of various signalling, including phosphorylation of kinase proteins. Among multiple kinases, Janus kinase (JAK) plays pivotal roles in the pathological processes of RA. Tofacitinib, a small product targeting JAK, inhibits phosphorylation of JAK1 and JAK3, subsequent Stat1 and expression of Stat1-inducible genes, which contribute to efficient propagation of its anti-inflammatory effects for the treatment of RA. The primary targets of tofacitinib are dendritic cells, CD4+ T cells such as Th1 and Th17 and activated B cells which leads to multi-cytokine targeting. Six global phase 3 studies revealed that oral administration of 5 or 10 mg tofacitinib was significantly effective than placebo with or without methotrexate in active RA patients with methotrexate-naïve, inadequately responsive to methotrexate or TNF-inhibitors. Therapeutic efficacy of tofacitinib was observed in a short term after administration and was as strong as adalimumab, a TNF-inhibitor. The most commonly observed adverse events were related to infection, hematologic, hepatic and renal disorders and association of tofacitinib with carcinogenicity and infections remains debated. Further investigation on post-marketing survey would help us understand the positioning of this drug.

Keywords: DMARD/JAK inhibitor/remission/rheumatoid arthritis.

Abbreviations: CIA, collagen-induced arthritis; DMARD, disease-modifying anti-rheumatic drug; IFN, interferon; IL, interleukin; IRF, IFN regulatory factor; JAK, Janus kinase; LPS, lipopolysaccharide; MHC, major histocompatibility complex; RA, rheumatoid arthritis; SCID, severe combined immunodeficiency; STAT, signal transducers and activators of transcription; TNF, tumour necrosis factor.

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized with chronic inflammatory synovitis, progressive joint destruction and multiple organ manifestations that causes severe disability and mortality. Auto-reactive T cells and inflammatory cytokines such as tumour necrosis factor (TNF) play a pivotal role in the pathological processes of RA through the accumulation of immune cells and the self-perpetuation of inflammation. These cells also induce the production of matrix metalloproteinase as well as the maturation and activation of osteoclasts, which results in destruction of cartilage and bone (1–3). Because progress in a joint damage derived from synovial inflammation is apparent in the early stage of the disease, it is required to treat patients at a stage when the evolution of joint destruction can still be prevented.

The combined use of methotrexate, a synthetic disease-modifying anti-rheumatic drug (DMARD), and biologic DMARD targeting TNF, interleukin (IL)-6 and T cells has revolutionized treatment of RA. Clinical remission has recently become an achievable goal in many patients and rapid and appropriate induction of remission is prerequisite to halt joint destruction. Actually it has proven that early therapeutic intervention using synthetic DMARD and biological DMARD not only improves clinical outcomes but also reduces the occurrence of joint damage and disability (Fig. 1) (1–5). Furthermore, not only the maintenance of clinical remission but also inhibition of functional disability and structural changes has become a long-term outcome of the treatment using biological DMARD and methotrexate.

However, biological DMARDs are limited to intravenous or subcutaneous uses because of their size, 90,000–150,000 Dalton. Orally available low molecular weight products, targeting key molecules during the disease processes, therefore, currently attract particular attention because they enter into cytoplasm and directly regulate intracellular signals. Among them, products targeting kinase proteins have been emerging because multiple signalling kinases are involved in the pathological processes of RA. We here review recent progress in the development of kinase inhibitors for
The treatment of RA, shedding light upon a small molecule inhibitor of the Janus kinase (JAK).

The Development of JAK Inhibitors

The multiple cytokines and cell surface functioning antigens bind to receptors, resulting in the activation of various signalling, including phosphorylation of kinase proteins. Five hundred and eighteen genes encoding kinase proteins have been identified from human genome-wide studies. Among them, the tyrosine kinase is phosphorylated following cytokine-receptor binding and is involved in multiple cellular functions during pathological processes of various inflammatory diseases such as RA. Therefore, tyrosine kinases have been emerging as the target for the treatment of these diseases. More than 90 genes encoding tyrosine kinases have been identified from human genome-wide studies and nine receptor tyrosine kinases and five non-receptor tyrosine kinases are known to be involved in synovial inflammation in patients with RA, compared with those with osteoarthritis (6).

Among them, members of JAK family are essential for the signalling pathways of multiple cytokines, growth factors and hormones and are implicated in the pathogenesis of RA. These molecules consist of homodimer or heterodimer of JAK1, JAK2, JAK3 and TYK2 (Fig. 2). After the engagement of cytokines receptors constitutively bound to JAK, JAK is activated by a conformational change and phosphorylated. These in turn phosphorylate the cytokine receptors, resulting in phosphorylation of the signal transducers and activators of transcription (STAT) that subsequently translocate into the nucleus, where they regulate gene expression. Reflecting the involvement of multiple inflammatory cytokines in the pathological processes of RA, both JAKs and STATs were increased in the synovium from RA patients compared with osteoarthritis patients and the expression of JAK-STAT was diminished following treatment with synthetic DMARDs (7).

Among members of a JAK family, the expression of JAK3 is limited to lymphocytes and constitutively binds to the common γ-chain for IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21 (8). Therefore, the deficiency or dysfunction of JAK3 is synonymous with impairment in these cytokines that impaired lymphocyte development and function and leads to severe combined immunodeficiency (SCID) in both human and mouse. However, because the expression of JAK3 was known to be limited on haematopoietic cells, the lack of JAK3 was supposed to marginally affect other organs, whereas the deficiency of JAK1 or JAK2 results in fetal death. Based on these backgrounds, selective inhibition of JAK3 was considered as a potential target for the treatment of RA without affecting other organ systems (9–11). Tofacitinib was found by screening for inhibitors of in vitro JAK3 kinase activity from the Pfizer chemical library and extensive chemical modification by the company (12, 13).

In Vitro Mode of Action of a JAK Inhibitor

Tofacitinib is an orally available compound that binds to the ATP binding pocket of JAK3 and its molecular weight is 504.49 Da with nitrile citrate. However, recent kinome binding maps have shown that tofacitinib inhibits not only JAK3 but also JAK2 and is also able to bind to the ATP pocket in both JAK1 and JAK2 (14, 15). Furthermore, both activation of JAK1/JAK2 by IL-6 and JAK1/JAK3 by IL-15 and subsequent phosphorylation of relevant STAT proteins were efficiently inhibited by tofacitinib in animal models. Ghoreschi et al. (16) also reported that tofacitinib potentially inhibited signalling by interferon-γ (IFN-γ) and IL-6 and to a lesser extent IL-12 and IL-23. Therefore, tofacitinib is currently categorized as a pan-JAK inhibitor preferentially inhibiting...
JAK1 and JAK3 and, to a lesser extent, JAK2 with minimum effect on TYK2.

We assessed the effects of tofacitinib on the proliferation of CD4+ T cells purified from synovium in patients with active RA and those purified from and peripheral blood in RA patients or healthy individuals (Fig. 3) (17). When CD4+ T cells were stimulated with anti-CD3 and anti-CD28 antibodies, marked proliferation and production of various cytokines were induced. However, the addition of tofacitinib to the culture inhibited the transcription of IL-17 and IFN-γ, but not IL-6, IL-8 and TNF-α, as well as the proliferation in a dose-dependent manner. Also, addition of IL-2 to CD4+ T cells induced production of both IL-17 and IFN-γ, and the induction was also inhibited by tofacitinib in a dose dependent manner, whereas tofacitinib did not affect IL-6 and IL-8 production by CD4+ T cells. Furthermore, although tofacitinib did not affect synovial fibroblasts and CD14+ monocytes derived from synovium in patients with RA, conditioned medium from CD4+ T cells cultured with tofacitinib inhibited IL-6 production from synovial fibroblasts and IL-8 production from CD14+ monocytes, indicating the indirect effect of tofacitinib on monocytes and fibroblasts in synovium in patients with RA. These results supports that the primary targets of tofacitinib appear limited on lymphocytes.

On the other hand, we also clarified that tofacitinib inhibited antigen-presentation capacity of dendritic cells by inhibiting type I IFN-mediated signalling and subsequently reducing expression of costimulatory molecules such as CD80 and CD86 (18). Dendritic cells possess antigen-specific T-cell activation abilities that are accompanied by the induction of major histocompatibility complex (MHC) and co-stimulation molecules such as CD80 and CD86. We have reported that dendritic cells express JAK1, JAK2 and JAK3, but dendritic cells derived from a JAK3-deficient mouse overproduced IL-10 and exhibited anti-inflammatory activity. Moreover, the number of dendritic cells and the expression of JAK on synovial dendritic cells increased in the synovium in patients with active RA, indicating the involvement of JAK on dendritic cells in the pathological processes (7). We found that tofacitinib decreased the expression of CD80 and CD86 on lipopolysaccharide (LPS)-stimulated human dendritic cells in vitro, whereas it did not change expression of MHC class II antigen. The expression of CD80 and CD86 on dendritic cells was enhanced by type-I IFN stimulation, but the LPS-induced CD80/CD86 expression was inhibited by an anti-type-I IFN receptor antibody. Furthermore, tofacitinib suppressed the production of type-I IFN and the activation of IFN regulatory factor (IRF)-7, a transcription factor involved in CD80/CD86 and type-I IFN expression. Moreover, tofacitinib decreased the T-cell stimulatory capability of dendritic cells. Thus, we found that dendritic cells are potent targets for tofacitinib; tofacitinib suppressed production and stimulation loop of a type-I IFN through JAK1/JAK3, decreased CD80/CD86 expression and suppressed T-cell stimulatory capacity, which leads to its immunomodulatory effects (18).

We also clarified that tofacitinib inhibited differentiation and antibody production of B cells (19). B cells initiate and perpetuate autoimmune disease processes. IL-4 and IL-21 produced by follicular helper T cells are required for B cell activation, germinal center formation, immunoglobulin class switching and plasma cell differentiation. Purified human CD19+ B cells were fully activated with crosslinking of B cell receptor, sCD40L and cytokines. Tofacitinib abrogated
AICDA, BCL6, XBP-1 expression and IgG production in the activated B cells to the basal levels. Tofacitinib also inhibited IL-6 gene expression and protein production in the activated B cells, whereas it did not change the production of IL-10 from B cells. These results suggest that tofacitinib suppresses B cell activation, differentiation and class switching, whereas it maintains B cell regulatory function. Taken together, the primary targets of tofacitinib appear dendritic cells, CD4+ T cells and activated B cells that leads to multicytokine targeting beyond simply a JAK3 inhibitor.

**In Vivo Mode of Action of a JAK Inhibitor**

*In vivo* efficacies of tofacitinib were initially demonstrated by its prevention of transplant rejection in a murine heterotopic heart transplantation model and in a non-human primate renal transplant model with prolongation of graft survival by tofacitinib monotherapy. Number of T lymphocytes in the periphery did not change by tofacitinib, although CD8+ T cells and NK cells tended to decrease (12). Additional studies with rodent aorta transplantation model and non-human primate kidney transplantation model treated with tofacitinib also showed significantly enhanced graft survival (20, 21).

The effect of tofacitinib on arthritis animal model *in vivo* has been assessed with murine collagen-induced arthritis (CIA), rat adjuvant-induced arthritis and K/BxN serum transfer arthritis (21). Administration of tofacitinib at the time of disease onset significantly reduced inflammatory cell influx and joint damage. When tofacitinib was administered into an established murine CIA, arthritis and inflammation were rapidly ameliorated through the inhibition of the JAK1 and JAK3 signalling pathways and the suppression of Stat1-dependent gene expression in the joint. Furthermore, tofacitinib reduced acute response to LPS *in vivo*, a model known to be dependent on IFN-γ and STAT1 (16). These results indicate that tofacitinib efficiently inhibits JAK1 and JAK3, subsequent STAT1 and expression of Stat1-inducible genes, which contribute to efficient propagation of the anti-inflammatory effect of tofacitinib.

We also assessed the *in vivo* effects of tofacitinib using the SCID-HuRAg mice, an RA animal model utilizing SCID mice implanted with synovium and cartilage from patients with RA and tofacitinib was continuously given to the mice by the osmotic minipump (17). Treatment of SCID-HuRAg mice with tofacitinib suppressed synovial inflammation, cartilage destruction and the production of human IL-6, IL-8 and matrix metalloproteinase-3 from implanted synovium. Tofacitinib also directly suppressed the production of IL-17 and IFN-γ and the proliferation of CD4+ T cells, resulting in inhibition of IL-6 and IL-8 production by synovial fibroblasts and CD14+ cells as well as cartilage destruction. Thus, in CD4+ T cells, Th1 and Th17 cells, JAK plays a crucial role in RA synovitis.

**The Efficacy and Safety of Tofacitinib on the Treatment of RA**

Based on these backgrounds, multiple clinical trials using an orally available JAK inhibitor tofacitinib in patients with RA have been globally undertaken. Subsequently to multiple phase 2 trials, 6 phase 3 studies were performed to investigate the efficacy and safety of tofacitinib (22–28). Briefly, oral
administration of 5 or 10 mg twice a day of tofacitinib was significantly effective than placebo with or without methotrexate in RA patients with methotrexate-naïve, inadequately responsive to methotrexate or inadequately responsive to TNF-inhibitors. The efficacy occurred rapidly and strongly. It is noteworthy there was not significant difference between tofacitinib and adalimumab, a representative TNF-inhibitor. Also, it is worth noting that significant improvement in 6 months-changes of modified total Sharp score, bone erosion score and joint space narrowing score, was observed in patients treated with 10 mg of tofacitinib, compared with placebo, indicating that tofacitinib has a potential to inhibit progress in joint destruction in patients with RA. Thus, tofacitinib was approved in the USA in 2012 and Japan in 2013 and subsequently it is now approved in many countries including Switzerland, except for the European Union. In Japan, oral administration of 5 mg twice a day of tofacitinib is used in RA patients with inadequate response to at least one synthetic DMARD such as methotrexate.

The most commonly reported adverse events were infections such as nasopharyngitis, increases in total cholesterol, elevation of transaminase and serum creatinine, decreases in neutrophil counts and anaemia (22–28). Although the majority of the adverse events have been tolerable and managed, opportunistic infections such as herpes zoster disseminated, pulmonary tuberculosis, cryptococcal pneumonia and pneumocystis pneumonia were reported. In our in vitro studies, statistical analysis revealed that proliferation of CD4+ T cells in patients with RA stimulated with anti-CD3 and anti-CD28 antibodies was significantly reduced at Week 52 after the tofacitinib treatment, compared with that at the baseline. However, no significant decrease in CD4+ T cell count was observed. Also, receiver operating characteristic analysis identified a CD8+ T cell count <21/µl at baseline as a significant predictor of clinically significant infectious adverse events. These results indicate the possible relevance of the impairment in T cell responsiveness by tofacitinib to the serious infectious events (29). There are also safety concerns regarding malignancy because tofacitinib inhibits the signalling by IL-2 and IL-15 that may reduce differentiation and activation of NK cells. Currently, careful post-marketing surveillances would be required to pay special attention on infections and malignancies and the accumulation of evidence regarding long-term safety would be warranted.

Conclusion

Tofacitinib targets the JAK that plays pivotal roles in the beginning of the intracellular cytokine signalling pathway. Six phase 3 studies revealed that oral administration of 5 or 10 mg tofacitinib was significantly effective than placebo with or without methotrexate in active RA patients with methotrexate-naïve, inadequately responsive to methotrexate or TNF-inhibitors. Therapeutic efficacy of tofacitinib was observed in a short term after administration in patients and was as strong as adalimumab, a TNF-inhibitor. The most commonly observed adverse events are related to infection, hematologic, hepatic and renal disorders and the association of tofacitinib with carcinogenicity is under debate.

According to the launch of tofacitinib, multiple low molecular weight products targeting JAK are emerging for the development. The JAK3 inhibitors decernotinib and peficitinib showed strong and rapid efficacy and similar adverse events to tofacitinib in their phase 2 trials. Phase 2 clinical trials were over regarding baricitinib targeting JAK1/2 and filgotinib targeting JAK1 and similar efficacy were reported (30). Thus, orally available small products targeting specific kinase could represent a valuable addition to the current DMARDs and biological DMARDs and these kinase inhibitors such as ‘Jakibs’ would take in the therapeutic armamentarium in RA and multiple autoimmune diseases (31). The development of tofacitinib is an example to encourage the translation from bench to bedside, shedding light upon basic research regarding intracellular signalling mechanisms and their relevance to pathological processes, but safety concerns of tofacitinib also have brought about the significance of translational research from bedside to bench.

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Conflict of Interest

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