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REVIEW ARTICLE

JAK inhibitor tofacitinib for treating rheumatoid arthritis: from basic to clinical

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Abstract Rheumatoid arthritis (RA) is a representative autoimmune disease characterized by chronic and destructive inflammatory synovitis. The multiple cytokines play pivotal roles in RA pathogenesis by inducing intracellular signaling, and members of the Janus kinase (JAK) family are essential for such signal transduction. An orally available JAK3 inhibitor, tofacitinib, has been applied for RA, with satisfactory effects and acceptable safety in multiple clinical examinations. From phase 2 dose-finding studies, tofacitinib 5 mg and 10 mg twice a day appear suitable for further evaluation. Subsequently, multiple phase 3 studies were carried out, and tofacitinib with or without methotrexate (MTX) is efficacious and has a manageable safety profile in active RA patients who are MTX naïve or show inadequate response to methotrexate (MTX-IR), disease-modifying antirheumatic drugs (DMARD)-IR, or tumor necrosis factor (TNF)-inhibitor-IR. The common adverse events were infections, such as nasopharyngitis; increases in cholesterol, transaminase, and creatinine; and decreases in neutrophil counts. Although the mode of action of tofacitinib remains unclear, we clarified that the inhibitory effects of tofacitinib could be mediated through suppression of interleukin (IL)-17 and interferon (IFN)-γ production and proliferation of $CD4^+$ T cells in the inflamed synovium. Taken together, an orally available kinase inhibitor tofacitinib targeting JAK-mediated signals would be expected to be a new option for RA treatment.

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Recent progress in treating rheumatoid arthritis

Rheumatoid arthritis (RA) is a representative autoimmune disease characterized by systemic, chronic, destructive inflammatory synovitis and multiple organ manifestations and causes severe disability and mortality rates. Conventional disease-modifying antirheumatic drugs (DMARDs), most commonly, methotrexate (MTX), remain the cornerstone of RA treatment. However, patients with active RA and an inadequate response to MTX (MTX-IR) are treated with biological agents targeting tumor necrosis factor (TNF) and interleukin (IL)-6, which play a pivotal role during the pathological processes of RA and that bring destructive inflammatory synovitis and multiple organ manifestations. The combinational application of TNF inhibitors and MTX has brought about a paradigm shift in RA management, and the RA treatment target has evolved to clinical, structural, and functional remission simply by reducing polyarthralgia [1–3].

On the other hand, orally available low molecular weight products targeting key molecules during the disease processes are attracting particular attention because they enter the cytoplasm and directly regulate intracellular signals, whereas high molecular weight biologics regulate intercellular signals by binding to cell-surface molecules or secreted protein. Among them, products targeting kinase proteins are emerging because multiple signaling kinases are involved in the pathological processes and can be designed to recognize particular conformations of target molecules, such as the relationships of a key and key hole, during the signaling cascade. For example, imatinib, which

Druker and others reported in 1996, is the derivative designed to antagonize an adenosine triphosphatase (ATP) binding site of the tyrosine kinase of BCR-ABL protein peculiar to chronic myelogenous leukemia and induces apoptosis of leukemic cells [4]. The multiple cytokines and cell-surface molecules play a pivotal role in RA pathogenesis, and binding of these molecules to their ligands on the cell surface induces various signals, including phosphorylation of kinase proteins. Thus, similar trials as those in leukemia using low molecular weight products targeting kinase proteins have been undertaken in RA treatment.

What is JAK, and why was JAK chosen as the target of treatment?

Five hundred and eighteen genes encoding kinase proteins have been identified from human genome-wide studies, and >99 % of them are serine/threonine kinases in physiological and ordinary condition. On the other hand, tyrosine kinase is the first intracellular signaling molecules to be phosphorylated following receptor binding in a cytokine response and is involved in fundamental functions such as cell proliferation, differentiation, and adhesion in various pathological processes, including inflammation and cancer. Therefore, many investigators have shed light on tyrosine kinases as the target of treatment of various diseases. More than 90 genes encoding tyrosine kinases have been identified from human genome-wide studies, and 14 tyrosine kinases are known to be involved in synovial membrane in patients with RA compared with patients with osteoarthritis [5]. Among them, members of the Janus kinase (JAK) family are essential for the signaling pathways of various cytokines and are implicated in RA pathogenesis of RA.

Kinase proteins of the JAK family are 120–140 kDa and possess two phosphorylation sites consisting of JAK1, JAK2, JAK3, and TYK2 (Fig. 1) [6–11]. After engagement of homodimeric or heterodimeric receptors, which are constitutively bound to JAKs, JAKs are activated by a conformational change in the receptor that allows trans- and/or autophosphorylation of the two bound JAKs. Several amino residues in JAKs phosphorylated by receptor ligation have been identified: an IL-2-induced phosphorylation site Y785 in JAK3, Y904 and Y939 in JAK3 as a positive regulator, Y813 in JAK2 by erythropoietin, and Y913 in JAK2 as a negative regulator [12–14]. These, in turn, phosphorylate cytokine receptors. Signal transducers and transcription activators (STAT) bind the phosphorylated receptor chains, which allow JAKs to phosphorylate STAT. Phosphorylated STAT forms dimers and/or changes its conformation and subsequently translocates into nucleus, where they regulate gene expression. Thus, JAK–STAT pathway regulates multiple immune functions. For instance, different STAT is involved in differential cytokine production from $CD4^+$ T-cell subsets: STAT1 and STAT4 mainly induce interferon (IFN)-γ from Th1, STAT6 induces IL-4 from Th2, STAT5 induces transforming growth factor (TGF)-β from regulatory T cells (Treg), and STAT3 induces IL-17 from Th17 [11].

JAKs are essential for the signaling pathways of various cytokines and are implicated in RA pathogenesis. Walker et al. reported that JAK3, STAT1, STAT4 and 6 were highly expressed in synovium in patients with RA, whereas the expression was scarce in synovium in normal volunteers and patients with osteoarthritis and spondyloarthropathy [15]. Among members of the JAK family, JAK3 was identified by O'Shea and colleagues who found that its expression is essentially limited to lymphocytes and constitutively binds to the common γ (γc) chain, which is a common receptor subunit for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 [11]. Therefore, the deficiency or dysfunction of JAK3 is synonymous with impairment in these γc cytokines, which impaired lymphocyte development and function and leads to immunodeficiency in mice. However, because of its limited expression on hematopoietic cells, the lack of JAK3 is not known to affect other organs, whereas deficiency of JAK1 or JAK2 results in fetal death [7–10]. Thus, numbers of tyrosine kinase inhibitors have recently been evaluated in clinical trials, and selective inhibition of JAK3 was considered a potential target in RA treatment without affecting other organ systems [15, 16].

What is tofacitinib action in RA?

Based on this background, an orally available JAK3 inhibitor, CP-690550, which is now designated tofacitinib, was developed with expectations to be a new immunosuppressant with few side effects. Tofacitinib was found by screening for inhibitors of in vitro JAK3 kinase activity from the Pfizer chemical library and extensive chemical modification by Changelian, O'Shea, and Borie and their colleagues [17]. Although tofacitinib was highly potent for JAK3 inhibition, it was 20- to 100-fold less potent for JAK2 and JAK1, respectively. Chrencik et al. [18] also reported that tofacitinib and CMP-6 (tetracyclic pyridone) is able to bind in adenosine triphosphatase (ATP)-binding cavities of JAK3 and TYK2 from their crystal structures like the relations of a key and the key hole. Karaman et al. [19] reported the interaction maps of 38 kinase inhibitors across a panel of 317 kinases and that among them tofacitinib most selectively interacts with the human kinome but that the specificity is not fully perfect and binds to other kinases. Further in vivo studies using graft-versus-host disease animal model proved immunosuppressive effects of tofacitinib with few side effects [20–22] and it also

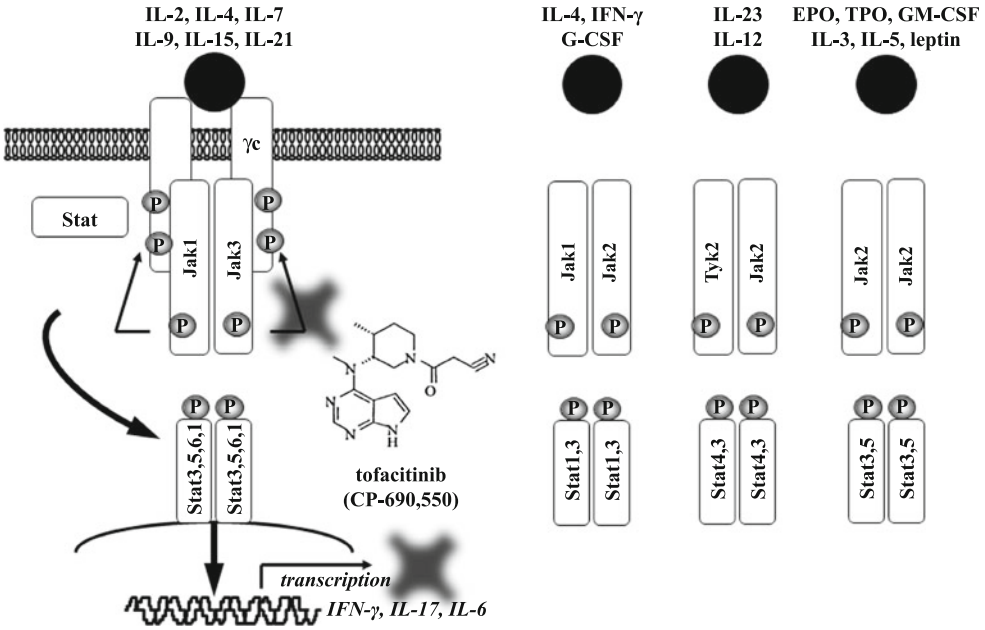

Fig. 1 The Janus kinase–signal transducer and activator of transcription (JAK–STAT) signaling pathway and mode of action of tofacitinib JAK3 expression is essentially limited to hematopoietic cells and constitutively binds to the common γ (γc) chain, which is a common receptor subunit for interleukin (IL)-2, IL-4, IL-7, IL-9, IL-15, and IL-21. Tofacitinib binds to JAK3 and could inhibit IL-17 and interferon (IFN)-γ production and $CD4^+$ T-cell proliferation

improved endpoints of both murine collagen-induced arthritis and rat adjuvant-induced arthritis [23, 24]. Thereafter, multiple clinical trials for RA were undertaken by tofacitinib with satisfactory effects and acceptable safety as described below.

Although the precise action of tofacitinib on JAK-STAT pathway in mice has been investigated, the exact mechanism of action in patients with RA remains unclear. Ghoreschi et al. [25] reported that tofacitinib potently inhibited JAK3 and JAK1 and to a lesser extent JAK2 with little effects on TYK2 and that it thereby inhibited signaling by IFN-γ, IL-6, and–to a lesser extent–IL-12 and IL-23, indicating that Th1 cell differentiation is therefore blocked, as is the generation of pathogenic Th17 cells. We also assessed the effect of tofacitinib on the proliferation of $CD4^+$ T cells isolated from synovium and peripheral blood in patients with active RA [26]. When $CD4^+$ T cells were stimulated with anti-CD3 and anti-CD28 antibodies, marked proliferation and production of IL-17 and IFN-γ was induced. However, the addition of tofacitinib to the culture inhibited IL-17 and IFN-γ transcription, as well as the proliferation in a dose-dependent manner (statistical difference starting at 10 nM), suggesting that tofacitinib exhibited its inhibitory effect by suppressing both proliferation and transcription. Tofacitinib also inhibited proliferation and cytokine production from $CD4^+$ T cells from peripheral blood in healthy individuals when stimulated with anti-CD3 and anti-CD28 antibodies in a similar manner as those from RA patients (unpublished data), indicating that tofacitinib mechanisms of action appears to be generalized in activated $CD4^+$ T cells from the different origin. Although the precise mechanism of its action requires further investigation, addition of IL-2 to $CD4^+$ T cells induced production of both IL-17 and IFN-γ, and its induction was inhibited by tofacitinib in a dose-dependent manner.

However, tofacitinib did not affect IL-6 and IL-8 production in vitro by $CD4^+$ T cells and, moreover, synovial fibroblasts and $CD14^+$ monocytes derived from synovium in patients with RA. These results support indications that JAK3 is limitedly expressed on lymphocytes. Meanwhile, 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. Our results suggest that tofacitinib mode of action appears to be restricted to proliferation and production of particular cytokines of $CD4^+$ T cells, presumably Th1 and Th17 cells, rather than $CD14^+$ monocytes and RA synovial fibroblasts (RASFs) in patients with RA at the early phase of treatment [26].

We next assessed in vivo effects of tofacitinib using SCID-Hu RAg mice, an RA animal model in which severe-combined immunodefficient (SCID) mice are implanted with synovium and cartilage from patients with RA. Tofacitinib was continuously given to the mice by the osmotic minipump [26], which decreased serum levels of human IL-6 and IL-8 in the mice. IL-17 and IFN-γ production are known to induce cytokine production from monocytes and fibroblasts, and IL-6 was known to be mainly derived from synovium macrophages and fibroblasts. These findings led us to speculate that tofacitinib specifically inhibited IL-17 and IFN-γ production by $CD4^+$ T cells (presumably Th1 and Th17 cells), which in turn

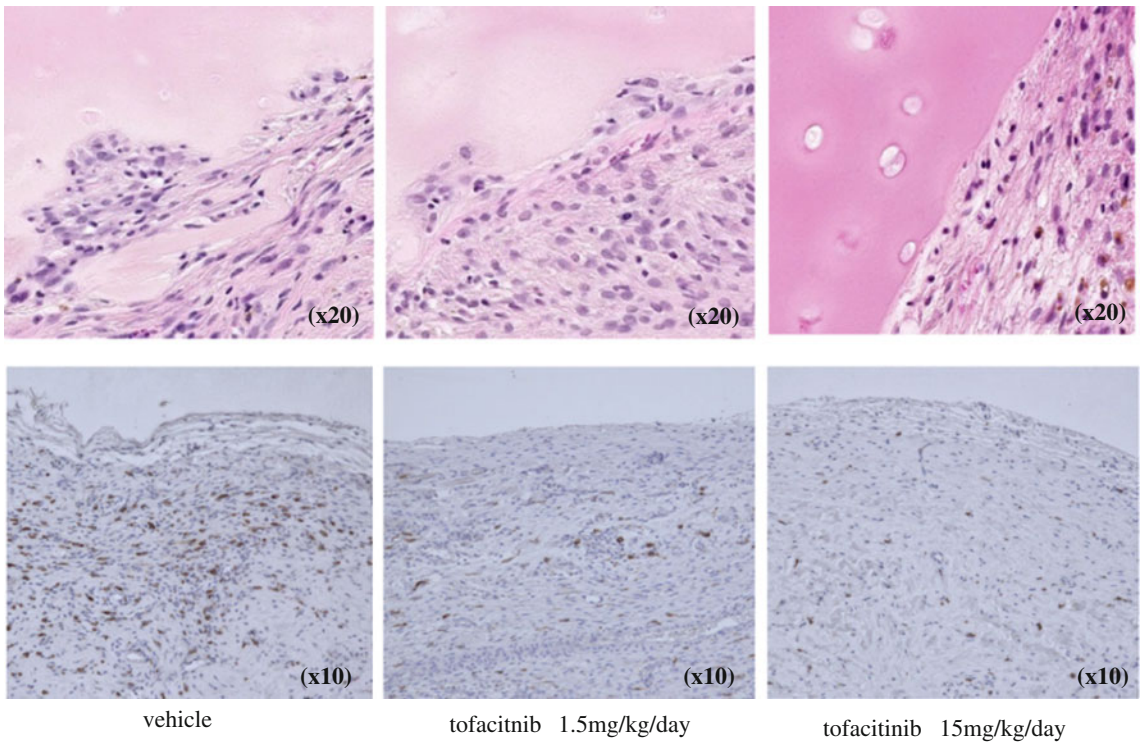

Fig. 2 Effects of tofacitinib on severe-combined immunodeficient (SCID)-Hu RAg mice—a rheumatoid arthritis (RA) animal model implanted with synovium and cartilage from patients with RA, and tofacitinib was continuously given to the mice by the osmotic minipump. The in vivo effects of tofacitinib were then assessed, *Upper column* shows the histological section stained with hematoxylin and eosin; *lower* column shows immunohistological findings using anti-interleukin (IL)-17 antibody

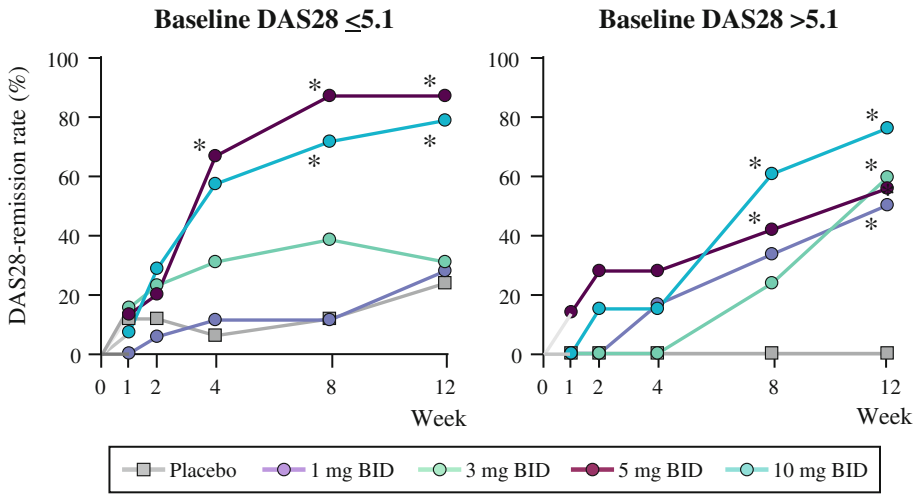

Fig. 3 Tofacitinib efficacy in patients with active rheumatoid arthritis (RA). A phase II double-blind study was carried out to investigate the efficacy and safety of orally available tofacitinib in 140 Japanese patients with active RA and inadequate response to methotrexate (MTX-IR). Patients were randomized to tofacitinib 1 mg, 3 mg, 5 mg, 10 mg, or placebo twice daily for 12 weeks. The percentage achievement of Disease Activity Score (DAS)28 remission for weeks 0–12 (nonresponder imputation) in patients whose baseline DAS28 was ≤5.1 or >5.1 are shown. $*P < 0.05$ versus placebo, no correlation for multiple comparisons

regulated synovitis by indirectly suppressing IL-6 and IL-8 from synovial fibroblasts and $CD14^+$ monocytes.

Next, 5 weeks after implantation implanted tissues were removed from SCID-HuRAg mice, embedded in paraffin, and stained with hematoxylin and eosin. By the histological evaluation, mice treated with vehicle alone showed prominent invasion of synovial tissue into the implanted cartilage. However, treatment with tofacitinib markedly

Table 1 Summary of clinical examination of tofacitinib for patients with rheumatoid arthritis (RA) carried out by Pfizer

Designation	Phase	Pretreatment	Concomitant therapy	No. patients	Tofacitinib dosage	Primary endpoint	Adverse events of tofacitinib	Ref. no.
A3921019	IIa	MTX or TNFi-IR	Mono	264	PL, 5, 15, 30 mg BID	5 mg, 70.5 %; 15 mg, 81.2 %, 30 mg, 76.8 %; PL, 29.2 % at week 6	Common AE; headache, nausea. Infection rate in 15 or 30 mg; 30.4 % (vs. 26.2 % in the placebo group). Increases in LDL and HDL cholesterol, creatinine level (0.04–0.06 mg/dl) in tofacitinib. No opportunistic infections or deaths	[27, 28]
A3921024	OLE	From 1019, 1025, 1035	Mono or with DMARD	3227	5, 10 mg BID	AE	441 (13.7 %) discontinued from OLE: 223 (6.9 %) due to AEs and 42 (1.3 %) due to insufficient response. 7747 TEAE in 2135 (66.2 %); infections and infestations (39.7 %), gastrointestinal disorders (18.8 %), musculoskeletal and connective tissue disorders (15.9 %), investigations (11.7 %). Decreased hemoglobin, 81 (2.5 %); raised aminotransferases, 1.7 % (ALT) and 1.1 % (AST); neutropenia, 16 (0.5 %); increase in creatinine, 393 (12.2 %). Serious AEs; 337 (10.9 %), 11.34/100 patients/year. SIE; 93 (2.9 %), 3.01/100 patients/year	[37]
A3921025	IIb	MTX-IR	With MTX	507	PL, 1, 3, 5, 10, 15 mg BID, 20 mg QD	$\geq$3 mg, 52.9 %; 5 mg, 50.7 %; 10 mg, 58.1 %, 15 mg BID, 56.0 %; 20 mg QD, 53.8 %; PL, 33.3 % at week 12	AE occurring in >10 % in any tofacitinib group; diarrhea, upper respiratory tract infection, urinary tract infection, arthralgia, headache. Sporadic increases in transaminases, increases in cholesterol and serum creatinine, and decreases in neutrophils and hemoglobin. SAEs: 21 (4.1 %)	[38]
A3921032 (ORAL Step)	III	TNFi-IR	With MTX	399	PL, 5, 10 mg BID	5 mg, 41.7 %; 10 mg, 48.1 %, PL, 24.4 % at Mo 6	AE at Mo 0–3 and Mo 3–6: 56.8, 53.4, 56.7 and NA, 42.9, 43.4 % in PL, 5 and 10 mg, respectively. Decreases in neutrophils, increases in LDL and HDL, in tofacitinib. SAE at Mo 0–3 and Mo 3–6: 4.5, 1.5, 1.4 and NA. 3.8, 4.5 % in PL, 5, 10 mg, respectively. 1 death due to pulmonary emboli in 10 mg. No SIE	[39]
A3921035	IIb	DMARD-IR	Mono (vs. ADA)	384	PL, 1, 3, 5, 10, 15 mg BID, ADA 40 mg SC (Q2W)	3 mg, 39.2 %; 5 mg, 59.2 %; 10 mg, 70.5 %, 15 mg, 71.9 %; ADA, 35.9 %; placebo, 22.0 % at week 12	AE; tofacitinib ($N = 272$) vs. placebo ($N = 59$), urinary tract infection (7.4 vs. 3.4 %), diarrhea (4.8 vs. 1.7 %), headache (4.8 vs. 1.7 %), bronchitis (4.4 vs. 1.7 %). Serious AEs; 2.9 % tofacitinib, severe anemia was most but no severe neutropenia	[40]
A3921039 (Japan)	IIb	MTX-IR	With MTX	140	Placebo, 1, 3, 5, 10 mg BID	1 mg. 64.3 %; 3 mg, 77.8 %; 5 mg, 96.3 %; 10 mg, 80.8 %, PL, 14.3 % at week 12	Nasopharyngitis ($n = 13$) and increased ALT ($n = 12$) and AST ($n = 9$). 5 severe AEs	[29]
A3921040 (Japan)	IIb	DMARD-IR	Mono	317	PL, 1, 3, 5, 10, 15 mg BID	1 mg. 37.7 %; 3 mg, 67.9 %; 5 mg, 73.1 %; 10 mg, 84.9 %, 15 mg, 90.7 %; PL, 15.4 % at week 12	Most common AEs; nasopharyngitis, hyperlipidemia, increased LDL, decreases in neutrophils. Common AEs; Increases in total, HDL, LDL cholesterol and small increases in serum creatinine. SAEs, 9 patients; no deaths in this study	[30]

Table 1 continued

Designation	Phase	Pretreatment	Concomitant therapy	No. patients	Tofacitinib dosage	Primary endpoint	Adverse events of tofacitinib	Ref. no.
A3921041	OLE	From 1039, 1040	Mono or with MTX	404	5 or 10 mg BID	AE	Most common AEs; infections such as nasopharyngitis (42.8 %) and herpes zoster (9.9 %). The most common AEs leading to discontinuation; herpes zoster (1.5 %), pneumonia (1.0 %), elevations in AST (0.5 %) and ALT (1.0 %)	[41]
A3921044 (ORAL Scan)	III	MTX-IR	With MTX	797	PL, 5, 10 mg BID	PL, 25.3 %; 5 mg, 51.5 %; 10 mg, 61.8 % at Mo 6	Most common AEs; infections. Common AEs; decreases in neutrophils, increases in LDL and HDL, and small increases in serum creatinine. SAEs were distributed across groups; 6 deaths (5 mg BID, 4; 10 mg BID, 1; PBO, 1)	[33]
A3921045 (ORAL Solo)	III	MTX-IR	Mono	610	PL, 5, 10 mg BID	PL, 26.7 %; 5 mg, 59.8 %; 10 mg, 65.7 % at Mo 3	In Mo 0-3, 330 patients (54.1 %) had 701 TEAE (54.9 % PBO, 51.0 % 5 mg, 56.7 % 10 mg) and 13 patients (2.1 %) discontinued due to these TEAE. In Mo 3–6, 244 pts (40.0 %) had 471 TEAE (36.1 % PBO → 5 mg, 39.3 % PBO → 10 mg, 39.9 % 5 mg, 41.2 % 10 mg) and 6 patients (1.0 %) discontinued due to these TEAEs. No opportunistic infections	[34]
A3921046 (ORAL Sync)	III	DMARD-IR	With DMARDs	792	PL, 5, 10 mg BID	PL, 31.2 %; 5 mg, 52.7 %; 10 mg, 58.5 % at Mo 6	AE at Mo 0–3 and Mo 3–6 were 61.0, 52.7, 54.5 % and 25.9, 38.4, 39.0 % in PL, 5 and 10 mg, respectively; 4 deaths and 4 opportunistic infections (1 herpes zoster disseminated, 1 cryptococcal pneumonia, 2 pulmonary tuberculosis)	[35]
A3921064 (ORAL Standard)	III	MTX-IR	With MTX (vs. ADA)	717	PL, 5, 10 mg, ADA 40 mg SC (Q2W)	PL, 28.3 %; 5 mg, 51.5 %; 10 mg, 52.6 %; ADA, 47.2 % at Mo 6	AE at Mo 0–3 and Mo 3–6; 47.2, 52.0, 46.8, 51.5 and 27.1, 32.8, 30.8, 33.3 % in PL, 5, 10 mg and ADA, respectively. Decreases in neutrophil in tofacitinib and ADA; increases in LDL and HDL, increases in creatinine in tofacitinib. SAE at Mo 0–3 and Mo 3–6 were 1.9, 5.9, 5.0, 2.5 and 3.4, 4.9, 3.5, 2.9 % in PL, 5, 10 mg and ADA, respectively. 2 deaths: sepsis (5 mg) and cardiac arrest (ADA), 2 with pulmonary tuberculosis (10 mg)	[36]

ADA adalimumab, *AE* adverse event, *BID* twive a day, *DMARD* disease-modifying antirheumatic drug, *HDL* high-density lipoprotein, *IR*: inadequately renonse, *LDL* low-density lipoprotein, *Mo* month, *mono* monotherapy, *MTX* methotrexate, *OLE* open-labeled extension study, *PL* placebo, *pt* patient, *Q2W* every 2 weeks, *QD* once a day, *SAE* severe adverse event, *SC* subcutaneous, *SIE* severe infectious event, *TEAE* treatment-emergent adverse event

inhibited this invasion, indicating that tofacitinib has the potential to inhibit progress in structural damage of joints in patients with RA (Fig. 2). Furthermore, addition of tofacitinib inhibited human IL-17 expression in synovial tissue. Taken together, tofacitinib directly suppressed IL-17 and IFN-γ production and $CD4^+$ T-cell proliferation, resulting in inhibition of IL-6 and IL-8 production by RASFs and $CD14^+$ cells and cartilage destruction. JAK in $CD4^+$ T cells, presumably Th1 and Th17 cells, plays a crucial role in rheumatoid synovitis.

Results of phase 2 clinical trials of tofacitinib in patients with RA

Based on this background, multiple clinical trials using orally available JAK inhibitor tofacitinib for patients with RA have been undertaken globally (Table 1). Kremer et al. first reported a phase 2 dose-ranging trial to investigate efficacy, safety, and tolerability of tofacitinib orally in 264 patients with active RA in whom MTX or TNF inhibitors caused an inadequate or toxic response [27, 28]. Patients

were randomized to placebo or 5, 15, or 30 mg of tofacitinib twice daily for 6 weeks and were followed up for an additional 6 weeks after treatment. The American College of Rheumatology 20 % improvement criteria (ACR20) response rate was 26.9 %. 70.5 %, 81.2 %, and 76.8 % in placebo and 5 mg, 15 mg, or 30 mg twice-daily groups, respectively, at 6 weeks. Thus, patients treated with tofacitinib in all treatment groups were satisfied with the primary efficacy end point: ACR20 response rate at 6 weeks. The most common adverse events (AEs) reported were headache and nausea. The infection rate in the 15-mg twice-daily group and the 30-mg twice-daily group was 30.4 % (26.2 % in placebo), and opportunistic infections or deaths were not observed.

A phase 2 double-blinded study was also carried out to investigate the efficacy and safety of tofacitinib in Japanese patients with active MTX-IR RA [29]. A total of 140 patients were randomized to tofacitinib 1 mg, 3 mg, 5 mg, and 10 mg, or placebo twice-daily groups in this 12-week study and remained on background MTX. ACR20 response rates at week 12, a primary endpoint, were significant for all tofacitinib treatment groups. The ACR20 response rate was 14.3 %, 64.3 %, 77.8 %, 96.3 %, and 80.8 % in the placebo and 1 mg, 3 mg, 5 mg, and 10 mg twice-daily groups, respectively, at 12 weeks. Significant improvements in ACR50, ACR70, and Health Assessment Questionnaire–Disability Index (HAQ-DI) were also obtained by the use of 5 mg or 10 mg. Furthermore, in patients with high Disease Activity Score (DAS28) at baseline (DAS28 >5.1), the greatest percentage of patients achieving DAS28 remission at week 12 was observed in the tofacitinib 10-mg twice-daily group (45.5 %). In patients with low to moderate disease activity at baseline (DAS28 ≤ 5.1), the tofacitinib 5-mg twice-daily group contained the greatest percentage of patients achieving DAS28 remission at week 12 (80.0 %) (Fig. 3).

A phase 2 double-blinded study of five doses of tofacitinib monotherapy was also done in Japanese patients with active RA and DMARD-IR [30]. A total of 317 patients were randomized to tofacitinib 1 mg, 3 mg, 5 mg, 10 mg, or 15 mg, or placebo twice daily in this 12-week study. ACR20 response rates at week 12, a primary endpoint, were 15.4 %, 37.7 %, 67.9 %, 73.1 %, 84.9 %, and 90.7 % in the placebo, 1 mg, 3 mg, 5 mg, 10 mg, and 15 mg twice-daily groups, respectively, at 12 weeks. Significant improvements in ACR50, ACR70, and HAQ-DI were also obtained by the use of 3–15 mg tofacitinib. The clinical trial data in Japan appears to be better than those in other, similar, global studies. Takeuchi et al. [31] indicated that the difference might depend on the lower body weight (means were about 55 kg and 72 kg in Japanese and global trials, respectively) and fewer number of tender joints (13.6–18.6 in Japanese vs. 24.1–27.1 in global trials), which might lead to higher response rates in Japanese trials. Fleischmann et al. reported that patients in the lowest quartile were more likely to achieve better response than patients in the highest quartile by the pooled analysis of five phase 3 studies with tofacitinib in patients with active RA. Baseline DAS28 [erythrocyte sedimentation rate (ESR)] was 5.87–6.42 in this Japanese phase 2 trial, which is the second lowest quartile in Fleischmann's category, and it might have brought about a better achievement to low clinical disease activity [32].

The most common AEs were nasopharyngitis, hyperlipidemia, and increased low-density lipoprotein (LDL); most were mild in severity in the Japanese trial [30]. Fifteen serious AEs (SAEs) were reported in nine patients. Nine treatment-related SAEs were observed in six patients. There were dose-dependent decreases in neutrophil counts, but there was no severe anemia. Dose-dependent increases in high-density-lipoprotein (HDL), LDL, and total cholesterol and small increases in serum creatinine were also observed.

Results of phase 3 clinical trials of tofacitinib in patients with RA

Subsequently, six phase 3 studies were performed to investigate the efficacy and safety of tofacitinib. A3921044 (ORAL Scan) study was carried out in patients with RA and MTX-IR using 5 or 10 mg tofacitinib twice a day or placebo in combination with MTX. Patients treated with tofacitinib with MTX were satisfied with ACR20 responses at 6 months compared with placebo. It is worth noting that significant improvement in 6-month changes of the modified total Sharp score (mTSS), erosion score, and joint-space-narrowing score was observed in patients treated with 10 mg tofacitinib compared with patients on placebo, indicating that tofacitinib has a potential to inhibit progress in joint destruction in patients with RA [32]. A3921045 (ORAL Solo) study was carried out in patients with RA and MTX-IR using 5 or 10 mg twice a day of tofacitinib monotherapy or placebo. Patients treated with tofacitinib alone reported significant clinical improvement compared with patients on placebo, suggesting that tofacitinib monotherapy is efficacious in MTX-IR patients [33]. A3921046 (ORAL Sync) study was done in patients with RA and DMARD-IR using 5 or 10 mg tofacitinib twice a day or placebo in the combination with DMARD. Patients treated with tofacitinib plus DMARD improved significantly compared with the placebo group, suggesting that a combination of tofacitinib with any DMARD is appropriate [34]. A3921064 (ORAL Standard) study was done in patients with RA and MTX-IR using 5 or 10 mg tofacitinib twice daily or placebo or subcutaneous injection

of adalimumab 40 mg every other week in combination with MTX. It is noteworthy that efficacy and safety were comparable between tofacitinib and adalimumab groups [35].

The most commonly reported AEs were infections, such as nasopharyngitis, dose-dependent increases in LDL, HDL, and total cholesterol, elevation of transaminase and serum creatinine, dose-dependent decreases in neutrophil counts, and anemia [27–30, 33–41]. The combination use of statins is required for patients who show increases in LDL or total cholesterol when treated with tofacitinib. Although the majority of AEs were tolerable and managed, opportunistic infections such as herpes zoster disseminated, pulmonary tuberculosis, cryptococcal pneumonia, and pneumocystis pneumonitis were reported. Careful pre- and postmarketing surveillance would be necessary, paying special attention to infections. Also, accumulating evidence regarding long-term safety would be warranted. Among 3,030 patients with RA from phase 3 and 3,227 from long-term extension (LTE) studies, there was no apparent increase in the rate of nonserious or serious infections over time. However, in LTE studies, the rate of serious infections with 10 mg twice daily of tofacitinib was approximately twice than that of 5 mg twice daily (4.9 vs. 2.3/100 patients-year), although that rate among tofacitinib 10 and 5 mg twice-daily groups was similar in phase 3 trials [42]. In our in vitro studies, proliferation of $CD4^+$ T cells in patients with RA stimulated with anti-CD3 and anti-CD28 antibodies was significantly reduced at week 52 after tofacitinib treatment compared with baseline, although no significant decrease in $CD4^+$ T-cell count was observed, indicating the possible relevance of impairment in T-cell responsiveness by tofacitinib to the serious infectious events [43].

Neutrophil changes and anemia may result from the pharmacology of tofacitinib which can inhibit signals by hematopoietic cytokines, such as erythropoietin and granulocyte–macrophage colony-stimulating factor, through JAK-2. It is interesting that some of the AEs observed here for tofacitinib, including decreases in neutrophils and increases in cholesterol and liver transaminases, were similar to those previously reported for tocilizumab, a humanized anti-IL-6 receptor antibody that blocks IL-6 signaling [44]. Results from the animal models of arthritis, showing decreased IL-6 levels in administration of tofacitinib-treated animals, and the dose-dependent increasing in blood lipids levels seen in clinical trials, suggest a possible inhibitory effect of tofacitinib on IL-6. Therefore, although tofacitinib has been reported to be specific to JAK3, it is now known that it functions as a pan-JAK inhibitor because the inhibition of JAK1 and JAK2 should be also taken into account.

Other than Pfizer, many companies are developing JAK3 inhibitors for treating RA. Recently, Vertex reported the results of a phase 2 double-blind study to investigate the efficacy and safety of an orally available JAK3 inhibitor VX-509 in patients with active RA and DMARD-IR [45]. Two hundred and four patients were randomized to VX-509 25-mg, 50-mg, 100-mg, or 150-mg monotherapy or placebo twice daily in this 12-week study. ACR20 response rates at week 12, a primary endpoint, were significant for ≥50 mg VX-509 treatment groups. The ACR20 response rate was 29 %, 39 %, 61 %, 65 %, and 66 % in the placebo group and 25-mg, 50-mg, 100-mg, and 150-mg twice daily groups, respectively, at 12 weeks. Significant improvements in ACR50, ACR70, DAS28, and %DAS28 remission were also obtained with 100 mg or 150 mg. Infections were the most common AE, occurring with similar frequency in placebo (17 %) and VX-509 groups (12–25 %). Serious AEs occurred in 4.9 % of VX-509 and 2.4 % of placebo groups, with serious infection in 3.1 % of VX-509 and none in placebo groups. Thus, the efficacy and safety profiles of VX-509 appear similar to those of tofacitinib, and the advanced phase of the clinical examination can be expected.

Taken together, in patients with active RA and MTX-naïve, MTX-IR, DMARD-IR, or TNF-inhibitor-IR, the orally available JAK inhibitor tofacitinib—as monotherapy or in combination with MTX—is efficacious and has a manageable safety profile. However, longer-duration studies and further accumulation of evidence of this novel JAK inhibitor, tofacitinib, in RA treatment would be prerequisite to clarify long-term safety and structural and functional efficacies. Also, improved knowledge of the underlying mechanisms of tofacitinib would contribute to better understanding the pathogenesis of RA and also for further application of the drug to other diseases.

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