Inhibitors of JAK for the treatment of rheumatoid arthritis: rationale and clinical data

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The JAK/STAT signal transduction pathway is primarily involved in regulating STAT-target gene transcription. Many of the STAT-target genes are those responsible for the synthesis of proinflammatory cytokines, proteins that regulate apoptosis and/or cell survival and genes that control determination of cell fate. The aberrant over production of proinflammatory cytokines, the imbalance between cell survival and apoptosis skewed towards survival and the abnormal proliferation of cells of the immune system and synoviocytes are several hallmark characteristics of the pathophysiology of human rheumatoid arthritis (RA). Several of the disease-modifying antirheumatic biological drugs, including TNF-α antagonists and IL-6-receptor neutralizing monoclonal antibodies retard the clinical and radiographic progression of RA and also inhibit JAK/STAT pathway activation. The long-term goal of developing JAK-specific small-molecule inhibitors through medicinal chemistry strategies may ultimately be to reduce the dependency on the use of biologics by directly inhibiting activation of JAK/STAT signaling. A few of these small-molecule inhibitors have been proven to have efficacy by ameliorating the severity of arthritis in rodent models of inflammatory arthritis. Several of these small-molecule JAK inhibitors are now being evaluated in human RA clinical trials where the preliminary evidence indicates that JAK inhibitors are safe and well-tolerated and produce positive RA clinical responses, as measured by the American College of Rheumatology response criteria.

Keywords: clinical trials • disease-modifying antirheumatic biological drugs • experimentally induced arthritis • JAK • rheumatoid arthritis • small-molecule inhibitors • STAT
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Therapeutic Perspective

Recently, a proliferation-inducing ligand (APRIL) and B-lymphocyte stimulator, also known as B-cell activating factor belonging to the TNF family (BAFF). APRIL can also be expressed as a cell surface fusion protein with TWEAK, termed TWE-PRIL [3]. Although many of these DMAPRs are now routinely tested in patients with RA, most are not recommended for use as first-line RA therapies or combined with more than one of the DMAPRs [4]. In RA clinical therapy DMAPRs are usually combined with conventional disease-modifying antirheumatic drug (DMARD) such as methotrexate (MTX) [46–48]. At present, first-line RA therapy usually involves the use of only DMARDs, which can include, nonsteroidal anti-inflammatory drugs, glucocorticoids, sulfasalazine, antimarial drugs and, of course, MTX as monotherapies [36,50] or in various combinations and dosages. Of note, some anti-TNF antagonists have also been approved for a first-line therapy indication, but MTX is generally used as the first-line treatment for mild to moderately severe RA patients who become unresponsive to therapy with nonsteroidal anti-inflammatory drugs [36] and/or glucocorticoids. Moreover, whilst there is reliably strong agreement that TNF-antagonists either employed as monotherapies, or in combination with MTX, fail to control the progression of RA bone erosion [36,45,51], controversy still exists regarding the extent to which early and aggressive RA therapy with anti-TNF biologics alone employed for treatment in moderate-to-severe RA patients curtail the progression of the cascade destruction seen in RA patients [36,45].

Furthermore, development of drug refractoriness, inadequate drug responsiveness and, most significantly, serious adverse events (AEs), including infections, malignancies and death attributed to DMAPRs have been reported in RA patients who have been treated with these drugs for varying periods of time [36,50–54]. These important issues have led many to contend that there must be a continuous development of novel therapeutic targets for clinical intervention in RA and other rheumatic diseases [36].

History of development of JAK inhibitors in RA

The JAK family of proteins consists of three JAK isoforms, JAK1, JAK2 and JAK3 and TYK2 [46–48]. The differential stimulation of STAT proteins by each of the JAKs has been previously and extensively reviewed [46–48]. The functional role of JAK is basically to activate the STAT proteins [46–48]. Thus, the phosphorylation of STAT proteins by activated JAKs converts STATs into potent transcription factors that regulate the transcription activity of many of the cytokine genes for which DMAPRs have already been developed, including TNFα and IL-6 as well as other STAT-target genes critical to cell survival, apoptosis and differentiation (for a review see [36,46–48]). (e.g., c-jun, Dc, c-myc, Bcl-xL, Mcl-1, survivin, MKP-1, TNFFRSF1B and SOCS-3). Additionally, the expression of several additional proinflammatory cytokines that are critical to RA pathogenesis, such as IFNγ, IL-7, IL-7R, IL-15, IL-19, IL-21, IL-22 and IL-23, as well as other transcription factors, are also regulated by STATs [46–48]. For example, the interplay between IL-6 and IL-23 was found to be critical for regulating the T-cell receptor-γ locus by STAT5 and histone acetylation [46]. Importantly, Hartgring et al. found elevated levels of IL-7R in the synovial fluid of RA patients as well as in the synovial fluid of patients with undifferentiated arthritis [46,48]. With respect to IL-17, Nishihara et al. [46,48] posited that blockade of specific STAT protein activation via neutralizing the IL-6/STAT3 pathway could be exploited to suppress the generation of T cells, which can become Th1 cells that produced IFNγ [46,48]. In fact the activation of a specific STAT protein by IL-6 was later confirmed when it was shown that IL-6/MJL-6R only induced the activation of STAT3 [46,48]. For the sake of completeness, it should be noted that STATs can also be activated by several anti-inflammatory cytokines involved in the regulation of RA bone erosion. For example, IFNγ as well as other genes that are intimately involved in adaptive immune responses (e.g., TGF) [46,48]. Activation of specific STAT proteins by anti-inflammatory cytokines has also been reported. Thus, STAT1 and STAT4 activation was shown to result from the interaction of IL-4 and IL-12 with their respective receptors [46]. Therefore, in the current context it is appropriate to consider that proinflammatory as well as adaptive immunological cytokine gene expression that is dysregulated in RA could be suppressed by inhibiting JAK activation [46,48]. Furthermore, JAK/STAT pathway signaling also appears to regulate many other cellular processes that are integral to RA pathogenesis and disease progression, including aberrant immune-cell and synovocyte survival and proliferation, immune-cell fate determination and apoptosis [46,48]. Although JAK inhibitors were originally formulated for preventing transplant rejection [46–48], the inhibition of JAK/STAT pathway activation was also considered to be of possible utility in RA patients because the JAK/STAT pathway was enriched as a regulator of immune-mediated inflammation, and thus relevant to RA pathology [46,48].

Development & preclinical studies of CP-690,550

JAKs were identified as potential targets for intervention in RA because previous studies had shown that these protein kinases were intimately involved in the initiation and progression of RA [46–48]. For that reason, a library of compounds was produced by medicinal chemistry strategies, which were screened for their inhibitory activity against the JAKs domain of JAK3. This protocol resulted in the discovery of a series of pyrolonimidine-based JAK3 inhibitors, among which CP-352,664 was further refined and developed [46]. Following that, a series of preclinical studies showed that CP-352,664 were analyzed for their pan-JAK activity inhibitory and JAK-specificity using an in vitro IL-2-induced T-cell blast proliferation assay where CP-352,664 showed potent inhibition of T-cell growth [46]. Additional evaluation to determine the effective use of these compounds included the use of another compound, namely PF-956,980 [46,48], which was also assessed for its preclinical efficacy and toxicity in a model of rodent inflammatory arthritis [36], in a model of rodent allograft rejection [46,48] and on the delayed hypersensitivity response in mice [36]. From these analyses where both efficacy and non-toxicity were demonstrated, the JAK-specificity of PF-956,980 was shown to be mainly towards JAK3, although inhibition of the more ubiquitous JAK1 and JAK2 was also indicated from these results [46,48]. The most promising series of JAK3 inhibitory compounds resulted in the identification of CP-690,550, now called tofacitinib [46–48]. CP-690,550 at an approximate ED50 dose of 1.5 mg/kg/day corresponding to a serum level of 5.8 ng/ml resulted in the amelioration of the severity of arthritis in murine collagen-induced arthritis [36] and in an adjuvant-induced arthritis model in the rat [36]. A reduction in the severity of arthritis was characterized by a lower level of inflammatory cell influx into the affected joints and a histological assessment revealed a significant reduction in joint damage. In fact, CP-690,550 employed at a dose of 15 mg/kg/day showed no histological evidence of arthritis.

More recently, several biomarkers of innate and adaptive immunity and the inflammatory response associated with human RA have been shown to be altered by CP-690,550 [36,48]. Of note, CP-690,550 inhibited IL-4-dependent T cell differentiation in vitro [46]. Although T cells are nonpathogenic in rodent CIA the inhibitory effect of CP-690,550 may have a negative impact on human RA disease progression because RA is characterized, in part, by an imbalance of Th1/Th2 cells skewed to Th2 [36,48]. Importantly, CP-690,550 also showed potent suppression of IL-23 receptor as well as the expression of the IL-17 cytokines IL17A and IL17F. CP-690,550 also inhibited production of IL-22 by Th1 cells in response to the treatment of T cells with exogenous IL-6 and IL-23 [46]. Finally, RA clinical trials revealed the clinical expression of CP-690,550, inhibition of the T1+ cell transcription factor, T-bet and the generation of Th1 cells.

The efficacy of CP-690,550 in animal models of arthritis and in RA patients treated with orally administered CP-690,550 alone [46–48] or in combination with MTX [46,48]. The results of these preclinical trials revealed the clinical expression of CP-690,550 as a novel oral agent for the treatment of RA.

AEs associated with CP-690,550 in clinical trials

Clinical studies with CP-690,550 in normal volunteers & in kidney transplant recipients

The initial clinical studies of CP-690,550 focused on its effect on renal toxicity and allograft rejection. Thus, Lawrenz et al. showed that CP-690,550, at a dosage of 15 mg twice daily (b.d.) was well-tolerated in healthy volunteers in a placebo-controlled study with headache and nausea noted as the major clinically apparent side-effects [46,48]. However, CP-690,550 did not alter glomerular filtration rate, renal function and any proteinuria or albuminuria. In addition, the co-administration of CP-690,550 (30 mg b.d.) with mycophenolate mofetil resulted in ‘over-immunosuppression’ in de novo kidney allograft recipients, although CP-690,550 (15 mg b.d.) had an efficacy/safety profile that was comparable with a control group receiving tacrolimus [36].

RA clinical trials with CP-690,550

Riese et al. summarized the results of several dose-ranging Phase IIA and IIB clinical trials in RA patients treated with orally administered CP-690,550 alone [46–48] or in combination with MTX [46,48]. The results of these studies strongly indicated that CP-690,550 was nontoxic and could be useful for the treatment of RA.
and nausea noted as the major side-effects of mono-therapy with CP-690,550. However, infection was noted in 4 patients (CP-690,550 treated RA patients as compared with RA patients in the placebo arm of these clinical trials). Of note, a decrease in hemoglobin and white blood cells were seen, as were small increases in serum creatinine. Elevated transaminase levels as well as elevated low-density lipoprotein and high-density lipoprotein were also observed when CP-690,550 was co-administered with MTX. Interestingly, the change in low-density lipoprotein levels and in biomarkers of cholesterol synthesis that were seen in RA patients treated with CP-690,550 were similar to those changes in biomarkers of liver metabolism that were also elevated in RA clinical trials involving the anti-IL-6 receptor monoclonal antibody tocilizumab, the latter also resulting in suppression of JAK/STAT pathway activation [14–16,45]. However, when taken together, the latter also resulting in suppression of JAK/STAT pathway activation [14–16,45]. However, when taken together, the latter also resulting in suppression of JAK/STAT pathway activation. IL-6-receptor monoclonal antibody tocilizumab, the latter also resulting in suppression of JAK/STAT pathway activation [14–16,45]. However, when taken together, the latter also resulting in suppression of JAK/STAT pathway activation. IL-6-receptor monoclonal antibody tocilizumab, the latter also resulting in suppression of JAK/STAT pathway activation. IL-6-receptor monoclonal antibody tocilizumab, the latter also resulting in suppression of JAK/STAT pathway activation. IL-6-receptor monoclonal antibody tocilizumab, the latter also resulting in suppression of JAK/STAT pathway activation. IL-6-receptor monoclonal antibody tocilizumab, the latter also resulting in suppression of JAK/STAT pathway activation. IL-6-receptor monoclonal antibody tocilizumab, the latter also resulting in suppression of JAK/STAT pathway activation. IL-6-receptor monoclonal antibody tocilizumab, the latter also resulting in suppression of JAK/STAT pathway activation.

The results of follow-up studies performed by Cohen et al. [16] have supported the results previously reported with combination therapy of CP-690,550 and MTX [30]. In addition, Cohen's study concluded that dose-adjusting of CP-690,550 was not required to maintain clinical response as compared with RA patients CP-690,550/MTX combination therapy was safe and well-tolerated [25].

Most recently, monotherapy with CP-690,550 also proved to be effective in improving the pain, function and overall health in RA patients over a 6-week clinical trial period [71]. Furthermore, CP-690,550 and MTX orally administered to a group of Japanese RA patients who had previously exhibited an inadequate response to MTX alone produced meaningful ACR20, ACR50 and ACR70 responses after 12 weeks at CP690,550 concentrations ranging from 3 to 10 mg b.i.d. [73]. Finally, a clinical trial was designed to evaluate whether monotherapy with CP-690,550 suppressed joint damage using magnetic resonance imaging and longitudinal radiographs of RA patients with moderate-to-severe disease who were MTX naïve. This study was also developed to determine the effect of CP-690,550 on several clinical assessment outcomes, including, ACR20, ACR50, ACR70 responses and disease activity score (DAS-28) scores over a 12-month period [103]. Thus, the results of NCT01164579 is likely to reveal the extent to which monotherapy with CP-690,550 can suppress the progression of joint damage in RA patients [103].

Preclinical studies with CP-33779

**Clinical studies with INCB018424**

INCB018424 (ruxolitinib) is an inhibitor of both JAK1 and JAK2 [49]. INCB018424 was originally designed to be a JAK inhibitor with similar properties called CEP-33779 was designed to be a selective inhibitor of JAK1 and JAK2 isosforms and JAK3, and a JAK inhibitor with similar properties called INCB028050 was developed to determine the extent to which INCB028050 could be employed as a differential inhibitor of JAK1, JAK2 and JAK3 [49]. In that regard, INCB028050 had no effect on Ba/F3-TEL-JAK3 kinome activation [49] and proliferation of Ba/F3-TEL-JAK3 is dependent on the activity of JAK3. Thus, it is likely that INCB028050 does not inhibit JAK3. This finding is particularly critical in the development of JAK1-specific inhibitors because JAK1 can interact with JAK2 and JAK3.

INCB028050 was shown to inhibit IL-6 and IL-23-mediated cell signaling at concentrations of >50 nM [49]. Furthermore, the efficacy, tolerability and amelioration of arthritis by orally administered INCB028050 was demonstrated in multiple murine models of arthritis where INCB028050 reduced the mortality of T1- and T17-associated cytokines without altering biomarkers of humoral immunity or causing significant adverse effects. INCB028050 appeared to inhibit JAK1 (IC50: 5.9 nM) and JAK2 (IC50: 5.7 nM) equally. INCB028050 also inhibited selective memory T cells [49]. INCB028050 had virtually no inhibitory activity against c-Met kinase (IC50: >104 nM) and Chk2 kinase (IC50: 104 nM).

There are two ongoing clinical trials in RA patients involving INCB028050 [50] or LY3009104 [50,51], the latter reportedly having JAK-inhibitory properties similar to INCB028050. CTEP0902486 is a randomized, double-blind, placebo-controlled, dose-ranging parallel-group study in active RA patients who have inadequately responded to DMARD therapy, including DMARDs [52]. The study will be performed over a 6-month period with the primary end point being the ACR20 response criteria at 3 months. Safety, tolerability and AEs will also be monitored. NCT0185535 is a safety efficacy study for 12 to 24 weeks of LY3009104 in patients with active RA on a background of MTX [52].

**Preclinical studies with CYT387**

CYT387 belongs to the phenylaminopyridine class of JAK inhibitors [53]. CYT387 was reported to block cellular activities dependent on JAK2 with an IC50 in the range of 100 to 500 nM and with limited cytotoxicity [53]. The results of recent studies also indicated that CYT387 blocked JAK1, JAK2 and TYK2 in the low nanomolar concentration range while causing growth suppression and inducing apoptosis in JAK2-dependent hematopoietic cell lines [50]. Most recently, Monaghan et al. showed that CYT387 inhibited IL-6-induced activation of STAT3, reduced the phosphorylation of AKT and induced apoptosis following stimulation with osteoclastogenic factor in human RA clinical trials [50]. At the time that this review was submitted there were no active RA clinical trials employing CYT387 listed in the ClinicalTrials.gov database.

**Conclusion**

Although the successful treatment of RA was significantly advanced by the development of DMARDs, the long-term effects of their use in this chronic disease remain unclear. Because several of the commonly employed DMARDs possessing cellular activities dependent on JAK2 including those that target soluble TNF or membrane-bound TNF receptors and those that interfere with IL-6/IL-6 receptor interaction, also inhibit JAK/STAT signaling, it was conjectured that small molecule inhibitors that directly inactivate specific JAK isoforms would also reduce not only the clinical symptoms of RA, but also suppress the upregulation of many of the

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**Table 3. Summary of the JAK inhibitors being developed for rheumatoid arthritis.**

<table>
<thead>
<tr>
<th>JAK inhibitor</th>
<th>JAK specificity</th>
<th>Clinical response(s)</th>
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<tbody>
<tr>
<td>CP-690,550</td>
<td>Mostly JAK1</td>
<td>Positive ACR20 response + pain, function, positive ACR20, ACR50 and ACR70 response</td>
</tr>
<tr>
<td>INCB018424 JAK1 and JAK2</td>
<td>Positive ACR50 and ACR70 response + DAS-28, + plasma IL-6 and CD40 levels</td>
<td></td>
</tr>
<tr>
<td>INCB028050 Mostly JAK1 and JAK2</td>
<td>Ongoing – no published data</td>
<td></td>
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ACR: American College of Rheumatology; DAS: Disease activity score.
Proinflammatory cytokines that are critical in driving RA disease progression.

Future perspective
The commercial development of various JAK-specific inhibitors was spurred on by their success in ameliorating the severity of arthritis in rodent models of human RA. Several of these JAK inhibitors, including CP-690,550, INCB018424 and CP-690,550, are now being evaluated in RA clinical trials (Table 1). The preliminary results from these clinical trials have indicated that JAK inhibitors with or without concomitant use of MTX improve RA clinical responses as measured by the ACR20, ACR50 and ACR70 criteria.

Financial & competing interest disclosure
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