CLINICAL INVESTIGATION

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

The medical therapy of rheumatoid arthritis (RA) was revolutionized more than a decade ago by the introduction of disease-modifying antirheumatic biological agents (DMARBDs) into the clinical practice of rheumatology. The development of DMARBDs for RA originally arose from positive results obtained in preclinical *in vitro* studies, as well as from the results of studies of the arthritis-ameliorating effects of experimental DMARBDs in experimentally induced inflammatory arthritis in mice, rats and rabbits. The results of these studies helped to clarify which of the many potential biological molecules involved in immune dysregulation and inflammation should be targeted for intervention in human RA. The results of these studies ultimately led to the full-scale commercial development of DMARBDs using this target-driven approach.

Among the targets chosen for drug development in RA were monoclonal antibodies or fusion proteins that neutralized receptor interactions or otherwise altered the biological activity of TNF-α [1–5], IL-1 [6–9], T-lymphocyte-derived CTLA-4 [10–13], IL-6 [14–17], B-lymphocyte-derived CD20/CD22 [18–20] and most

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recently, a proliferation-inducing ligand (APRIL) and B-lymphocyte stimulator, also known as B-cell activating factor, belonging to the TNF family [21–24]. APRIL can also be expressed as a cell surface fusion protein with TWEAK, termed TWE-PRIL [23].

Although many of these DMARBDs are now routinely employed in the therapy of RA, most are not recommended for use as first-line RA therapies or combined with more than one of the DMARBDs [25]. In RA clinical therapy DMARBDs are usually combined with, for example, a conventional disease-modifying antirheumatic drug (DMARD) such as methotrexate (MTX) [26–28]. At present, first-line RA therapy usually involves the use of only DMARDs, which can include, nonsteroidal anti-inflammatory drugs, glucocorticoids, sulphasalazine, antimalarial drugs and, of course, MTX as monotherapies [29,30] 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 [30] and/or glucocorticoids. Moreover, whilst there is relatively strong agreement that TNF-antagonists either employed as monotherapies, or in combination with MTX, reduce the progression of RA bone erosions [31–33], 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 cartilage destruction seen in RA [34,35].

Furthermore, development of drug refractoriness, inadequate drug responsiveness and, most significantly, serious adverse events (AEs), including infections, malignancies and death attributed to DMARBDs have been reported in RA patients who have been treated with these drugs for varying periods of time [33,36–38]. 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 [39].

History of development of JAK inhibitors in RA

The JAK family of proteins consists of three JAK isoforms, JAK1, JAK2 and JAK3 and TYK2 [40–45]. The differential stimulation of STAT proteins by each of the JAK proteins has been previously and extensively reviewed [46–48]. The functional role of JAK is basically to activate the STAT proteins [46,49–53]. Thus, the phosphorylation of STAT proteins by activated JAKs converts STATs into potent transcriptional factors that regulate the transcription activity of many of the cytokine genes for which DMARBDs have already been developed, including TNF-α and IL-6 as well as other STAT-target genes critical to cell survival, apoptosis and differentiation and cytokine signaling (e.g., cyclin D1, c-myc, Bcl-xL, Mcl-1, survivin, MKP-1, TNFRSF13b and SOCS-3). Additionally, the expression of several additional proinflammatory cytokines that appear to be critical to RA disease progression, such as IFN-γ, IL-7/IL-7R, IL-15, IL-19, IL-17, IL-21 and IL-23, as well as other transcription factors, are also regulated by STATs [44,45]; for example, the interaction between IL-7 and IL-7R was found to be critical for regulating the T-cell receptor-γ-locus by STAT5 and histone acetylase [45]. 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 [54].

With respect to IL-17, Nishihara *et al.* [55] posited that blockade of specific STAT protein activation via neutralizing the IL-6/gp130 pathway could be exploited to suppress the generation of T_h17 cells, which can also become T_h1 cells that produced IFN-γ [56]. In fact the activation of a specific STAT protein by IL-6 was later confirmed when it was shown that IL-6/sIL-6R only induced the activation of STAT3 [57].

For the sake of completeness, it should be noted that STATs can also be activated by several anti-inflammatory cytokines (e.g., IL-4, IL-10, IL-12, IL-13 and IFN-α) as well as other genes that are intimately involved in adaptive immune responses (e.g., TGF) [58,59]. Activation of specific STAT proteins by anti-inflammatory cytokines has also been reported. Thus, STAT6 and STAT4 activation was shown to result from the interaction of IL-4 and IL-12 with their respective receptors [58]. Therefore, in the current context it is appropriate to consider that proinflammatory as well as anti-inflammatory cytokine gene expression that is dysregulated in RA could be suppressed by inhibiting JAK activation [47,53].

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 synoviocyte survival and proliferation, immune-cell fate determination and apoptosis [17,45]. Although JAK inhibitors were originally formulated for preventing transplant rejection [60–62], the inhibition of JAK/STAT pathway activation was also considered to be of possible utility in RA patients because the JAK/STAT pathway was envisioned as a regulator of immune-mediated inflammation, and thus relevant to RA pathology [39].

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 immune-mediated inflammation [39,45]. For that reason, a library of compounds was produced by medicinal chemistry strategies, which were screened for their inhibitory activity against the catalytic domain of JAK3. This protocol resulted in the discovery of a series of pyrrolopyrimidine-based JAK3 inhibitors, among which CP-352,664 was further refined and developed [52]. Following that strategy synthetic analogues of 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 suppression of T-cell growth [63]. Additional evaluation to determine the effective use of these compounds included the use of another compound, namely PF-956,980 [64], which was also assessed for its preclinical efficacy and toxicity in a model of rodent inflammatory arthritis [64], in a model of rodent allograft rejection [64] and on the delayed hypersensitivity response in mice [65]. From these analyses where both efficacy and nontoxicity 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. The optimization of several series of JAK3 inhibitory compounds resulted in the identification of CP-690,550, now called tofacininib [66–68].

CP-690,550 at an approximate ED_{50} 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 (CIA) and in an adjuvant-induced arthritis model in the rat [67]. 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 dosage 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. Of note, CP-690,550 inhibited IL-4-dependent T_h2 differentiation *in vitro* [68]. Although T_h2 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 T_h1/T_h2 cells skewed to T_h1 [1,2]. Importantly, CP-690,550 also blocked the expression of the IL-23 receptor as well as the expression of the IL-17 cytokines IL17A and IL-17F. CP-690,550 also inhibited production of IL-22 by T_h1 cells in response to the treatment of T_h1 with exogenous IL-6 and IL-23 [68]. Finally, CP-690,550 blocked the activation of STAT1, induction of the T_h1-cell transcription factor, T-bet and the generation of T_h1-cells.

The efficacy of CP-690,550 in animal models of arthritis correlated with the suppression of STAT1-target genes as well as inhibition of JAK1/JAK3. Of note, Sewgobind *et al.* showed that CP-690,550 effectively inhibited T-cell effector function *in vitro* without altering the activity of $CD4^+$-regulatory T-cells [69]. Taken together, the results of these preclinical *in vitro* and *in vivo* studies strongly indicated that CP-690,550 was nontoxic and could be useful for the treatment of RA.

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

The initial clinical studies of CP-690,500 focused on its effect on renal toxicity and allograft rejection. Thus, Lawrendy *et al.* showed that CP-690,500 at a dosage of 15 mg twice-daily (b.i.d.) was well-tolerated in healthy volunteers in a placebo-controlled study with headache and nausea noted as the major clinically apparent side-effects [70]. However, CP-690,500 did not alter glomerular filtration rate, effective renal plasma flow or creatinine clearance, but the co-administration of CP-690,550 (30 mg b.i.d.) with mycophenolate mofetil resulted in 'over-immunosuppression' in *de novo* kidney allograft recipients, although CP-690,550 (15 mg b.i.d.) had an efficacy/safety profile that was comparable with a control group receiving tacrolimus [71].

AEs associated with CP-690,550 in clinical trials

Although CP-690,550 has shown more efficacy in treating transplant rejection and other autoimmune disorders in comparator studies with other immunosuppressant drugs, the inhibition of JAK1 and JAK2 by CP-690,550 was suggested as a likely cause of over-immunosupression. Although anemia has been associated with CP-690,550 therapy, this was a predictable clinical response based on the results of previous studies that showed that inhibition of JAK3 would be expected to alter red blood cell homeostasis [68].

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 [72] or in combination with MTX [73]. The results of these clinical trials revealed a significant clinical response, as measured by the American College of Rheumatology (ACR)20 criteria with only headache

and nausea noted as the major side-effects of monotherapy with CP-690,550. However, infection was more common in CP-690,550-treated RA patients 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 changes observed in liver enzyme 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 results of these Phase IIA and IIB trials with CP-690,550 in RA patients showed that CP-690,550 produced meaningful positive clinical responses.

The results of follow-up studies performed by Cohen *et al.* [74] have sustained the results previously reported with combination therapy of CP-690,550 and MTX [73]. In addition, Cohen's study concluded that dose-adjusting of CP-690,550 was not required to produce an optimal clinical response and that in RA patients CP-690,550/MTX combination therapy was safe and well-tolerated [74].

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 [75]. 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. [76].

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-naive. This study was also developed to determine the effect of CP-690,550 on several clinical assessment outcomes measurements, including, ACR20, ACR50, ACR70 responses and disease activity score-28 (DAS-28) scores over a 12-month period [201]. 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 [201].

Preclinical studies with CEP-33779

CEP-33779 is a 1,2,4-triazolo[1,5-a]pyridine derivative that was shown to be a highly selective, orally active inhibitor of JAK2 [101]. CEP-33779 was originally developed for intervention in immune-mediated organ rejection [201]. Following this development, CEP-33779 was later evaluated for its efficacy and toxicity in the CIA and collagen antibody-induced arthritis mouse model [77]. In this study, CEP-33779 reduced paw edema and the clinical score of arthritis severity in CIA and collagen antibody-induced arthritis as well as the local paw levels of IL-12, IFN-γ and TNF-α and the serum levels of IL-12 and IL-2. The reduced levels of these proinflammatory cytokines correlated with lower numbers of splenic-derived collagen II-specific T_h1 cells. The administration of CEP-33779 b.i.d. at dosages ranging from 10 to 100 mg/kg over a period of 4 to 8 weeks reduced cartilage matrix changes associated with murine arthritis, subchondral bone osteolysis, pannus formation and synovial inflammation. The improvements in synovial joint pathology correlated with reduced levels of activated STAT3 in the arthritic paws. There were no changes in body weight or anticollagen II antibodies associated with administration of CEP-33779. These preclinical results provide the impetus for the further assessment of the effects of CEP-33779 on biomarkers of human cellular and humoral immunity *in vitro*. It is anticipated that CEP-33779 will ultimately be tested in human RA clinical trials.

Clinical studies with INCB018424

INCB018424 (ruxolitinib) is an inhibitor of both JAK1 and JAK2 [78]. INCB018424 was originally designed to potentially be employed as an oral therapy for myelofibrosis, a form of the BCR-ABL-negative myeloproliferative neoplasm [79] as well as the treatment of psoriasis [78]. Shi *et al.* administered INCB018424 phosphate to a group of healthy volunteers where the drug was found to be generally safe and well-tolerated [80]. In that study, INCB018424 exhibited good oral bioavailability and dose-proportional systemic pharmacokinetics and pharmacodynamics with low oral dose clearance and a small volume of distribution. Studies on the whole blood of normal volunteers administered INCB018424 showed that inhibition of phosphorylated STAT3 had occurred, which correlated with INCB018424 plasma levels.

A series of clinical trials involving INCB018424 have also been conducted with patients having either mild-to-moderate psoriasis [202] or active RA [81]. A topical form of INCB018424 was employed in the psoriasis trial. In the short 28-day RA trial involving 12 patients, Williams *et al.* reported that 83% achieved the ACR20 response (placebo: 75%), 58% achieved ACR50 (placebo: 0%) and 33% achieved an ACR70 response (placebo: 0%) which correlated with lower DAS-28 scores [81]. The results of pharmacokinetic studies showed that INCB018424 inhibited JAK1 and JAK2 and reduced plasma IL-6 and CD40 levels. The results of *ex vivo* studies on blood cells from RA patients showed that INCB018424 inhibited IL-6-mediated STAT3 activation.

Preclinical & clinical studies with INCB028050

Ongoing studies have now shown that CP-690,550 is not JAK-isoform-specific at identical concentrations as was previously suspected [63], where CP-690,550 was shown to inhibit JAK1, JAK2 and JAK3 [82,83]. By contrast, INCB018424 was designed to be a relatively specific inhibitor of the JAK1/JAK2 isoforms [79] 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 [84,85]. In that regard, INCB028050 had no effect on Ba/F3-TEL-JAK3 cell proliferation [84] where 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 JAK3 [45].

Recent evidence also indicated that INCB028050 inhibited IL-6 and IL-23-mediated cell signaling at concentrations of <50 nM [85]. Furthermore, the efficacy, tolerability and amelioration of arthritis by orally administered INCB028050 was demonstrated in multiple murine models of arthritis where INCB028050 reduced the amount of T_h1- and T_h17-associated cytokines without altering biomarkers of humoral immunity or causing significant adverse effects. INCB028050 appeared to inhibit JAK1 (IC_{50}: 5.9 nM) and JAK2 (IC_{50}: 5.7 nM) equally. INCB028050 only exhibited moderate selectivity towards TYK2 (IC_{50}: 53 nM). INCB028050 had virtually no inhibitory activity against c-Met kinase (IC_{50}: $>10^4$ nM) and Chk2 kinase (IC_{50}: 10^3 nM).

There are two ongoing clinical trials in RA patients involving INCB020850 [203] or LY3009104 [204], the latter reportedly having JAK-inhibitory properties similar to INCB020850. NCT00902486 is a randomized, double-blind, placebo-controlled, dose-ranging, parallel-group study in active RA patients who have inadequately responded to DMARD therapy, including DMARBDs [203]. 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. NCT01185353 is a safety and efficacy study for 12 to 24 weeks of LY3009104 in patients with active RA on a background of MTX [204].

Preclinical studies with CYT387

CYT387 belongs to the phenylaminopyridine class of JAK inhibitors [86]. CYT387 was reported to block cellular activities dependent on JAK2 with an IC_{50} in the range of 100 to 500 nM and with limited cytotoxicity [87]. 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 hemopoietic cell lines [88]. 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 of human myeloma cell lines with IL-6 or IGF-1 [89]. 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 DMARBDs, the long-term effects of their use in this chronic disease remain unclear. Because several of the commonly employed DMARBDs possessing clinical efficacy for RA, 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

Table 1. Summary of the JAK inhibitors being developed for rheumatoid arthritis.

JAK inhibitor	JAK specificity	Clinical response(s)	Ref.
CP-690,550	Mostly JAK3	Positive ACR20 response ↓ pain, ↑ function, positive ACR20, ACR50 and ACR70 response	[73,75,76]
INCB018424	JAK1 and JAK2	Positive ACR50 and ACR70 response ↓ DAS-28, ↓ plasma IL-6 and CD40 levels	[81]
INCB028050	Mostly JAK1 and JAK2	Ongoing – no published data	[203]

ACR: American College of Rheumatology; DAS: Disease activity score.

Executive summary

History of development of JAK inhibitors in rheumatoid arthritis
- Targeted drug development has been applied to the treatment of rheumatoid arthritis (RA), including the development of drugs designed to block the action of TNF-α and IL-6 by inhibiting the activation of the JAK/STAT pathway.
- Novel small-molecule inhibitors have been developed to specifically inhibit the activity of JAK1, JAK2 and JAK3.

Development & preclinical studies of CP-690,550
- Novel JAK small-molecule inhibitor CP-690,550 ameliorated the severity of arthritis in animal models of RA.
- CP-690,550 suppressed the level of several biomarkers of immune-mediated inflammation such as IL-17 and IL-22 as well as inhibiting cell proliferation in well-defined *in vitro* test systems.

Preclinical studies with CEP-33779
- Novel JAK2 small-molecule inhibitor CEP-33779 ameliorated the severity of arthritis in animal models of RA.

RA clinical trials with CP-690,550
- JAK small-molecule inhibitor, CP-690,550, is being evaluated as a monotherapy or in combination with methotrexate.
- Preliminary results in RA clinical trials with CP-690,550 indicated that this JAK inhibitor was safe, well-tolerated and caused few serious adverse events.
- Orally administered JAK small-molecule inhibitor CP-690,550 produced a positive clinical response in RA patients as measured by the ACR20, ACR50 and ACR70 response criteria.

Clinical studies with INCB018424
- The JAK small-molecule inhibitor INCB018424 is being evaluated as a monotherapy or in combination with methotrexate in RA clinical trials.

Clinical studies with INCB028050
- The JAK small-molecule inhibitor INCB028050 is being evaluated in active RA patients who have an inadequate response to full therapy including disease-modifying antirheumatic biological agents.

Preclinical studies with CYT387
- The JAK2 inhibitor CYT387 inhibited IL-6-induced activation of STAT3, reduced phosphorylation of AKT and induced apoptosis in a myeloma cell line stimulated with IL-6 or IGF-1.

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 INCB020850 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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