Protein kinase small molecule inhibitors for rheumatoid arthritis: Medicinal chemistry/clinical perspectives

Charles J Malemud, David E Blumenthal

Charles J Malemud, David E Blumenthal, The Arthritis Research Laboratory, Department of Medicine, Division of Rheumatic Diseases, Case Western Reserve University, Cleveland, OH 44106-5076, United States
Charles J Malemud, David E Blumenthal, Department of Anatomy, Case Western Reserve University, Cleveland, OH 44106-5076, United States
Charles J Malemud, David E Blumenthal, School of Medicine, Division of Rheumatology, Veteran’s Administration Medical Center, Cleveland, OH 44106, United States

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Correspondence to: Charles J Malemud, PhD, Department of Medicine, Division of Rheumatic Diseases, The Arthritis Research Laboratory, Foley Medical Building, 2061 Cornell Road, Cleveland, OH 44106-5076, United States. cjm4@cwru.edu Telephone: +1-216-8447846 Fax: +1-216-8442288

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Abstract

Medicinal chemistry strategies have contributed to the development, experimental study of and clinical trials assessment of the first type of protein kinase small molecule inhibitor to target the Janus kinase/Signal Transducers and Activators of Transcription (JAK/STAT) signaling pathway. The orally administered small molecule inhibitor, tofacitinib, is the first drug to target the JAK/STAT pathway for entry into the armamentarium of the medical therapy of rheumatoid arthritis. The introduction of tofacitinib into general rheumatologic practice coupled with increasing understanding that additional cellular signal transduction pathways including the mitogen-activated protein kinase and phosphatidylinositol-3-kinase/Akt/mammalian target of rapamycin pathways as well as spleen tyrosine kinase also contribute to immune-mediated inflammatory in rheumatoid arthritis makes it likely that further development of orally administered protein kinase small molecule inhibitors for rheumatoid arthritis will occur in the near future.

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Key words: Clinical trials; Protein kinase; Signal transduction; Small molecule inhibitor; Rheumatoid arthritis

Core tip: Signal transduction is a regulator of gene expression in cells. Janus kinase/Signal Transducers and Activators of Transcription (JAK/STAT) signaling is activated by pro-inflammatory cytokines which contribute to immune-mediated inflammation in rheumatoid arthritis. Medicinal chemistry was employed to develop JAK small molecule inhibitors for determining their clinical efficacy in active rheumatoid arthritis patients. Tofacitinib, a JAK small molecule inhibitor, is now generally used to treat moderate to severe rheumatoid arthritis patients who have not adequately responded to disease-modifying anti-rheumatic drugs or various biologic agents. The clinical efficacy of JAK small molecule inhibitors provides the impetus for future drug discovery targeted at other signal transduction pathways in rheumatoid arthritis.

INTRODUCTION

Medical therapeutic intervention of rheumatoid arthritis...
(RA) was dramatically altered with the introduction of biologic drugs with monoclonal antibody or fusion protein structures into the armamentarium of disease-modifying anti-rheumatic drugs (DMARDs), which had previously included, non-steroidal anti-inflammatory drugs, immunosuppressive drugs, (e.g., glucocorticoids, methotrexate, sulfasalazine), anti-malarial agents (e.g., hydroxychloroquine), and modifiers of DNA synthesis (e.g., leflunomide or various combinations of these DMARDs. Among the biological drugs chosen for development for RA were those whose mechanism of action was attributed to their capacity to neutralize the downstream effects of the elevated levels of the pro-inflammatory cytokines in RA sera and synovial fluid, tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and IL-6, among other interleukins [11-13] as well as possessing activity towards the inhibition of proliferation and dysfunctional RA T-cells and B-cells [16-19].

However, the general requirement that the biologic drugs need to be employed in RA therapy for long periods of time has caused problems inherent in their chronic use, including, but not limited to, the elevated relative risk for developing cancers and infections, inadequate drug responses and drug refractoriness and death as well as the potential for antibodies to be produced that are directed against the monoclonal antibodies or fusion proteins themselves [24-27] thus neutralizing their effectiveness. These crucial considerations have resulted in the contention that there needs to be continual identification of novel therapeutic targets coupled to drug development for intervention in RA and autoimmune diseases in general [28-30].

**IDENTIFICATION OF PROTEIN KINASES AS POTENTIAL DRUG TARGETS FOR RA**

**The JAK/STAT pathway**

A central theme for considering which component of RA pathology should be targeted for novel drug development first involves identifying a pathway(s) that is involved in the aberrant cell and humoral-mediated immune response and inflammation which regulate abnormal survival of T-, B-cells, macrophages and synoviocytes as well as the loss of chondrocyte viability and vitality, all of which are characteristic elements of RA progression [29]. In that regard, the Janus kinase/Signal Transducers and Activators of Transcription (JAK/STAT) signaling pathway perfectly fits this viewpoint because JAK/STAT signaling has been shown to regulate so many of the diverse cellular functions critical to RA pathogenesis and progression, including, cell survival and proliferation, immune cell-fate determination and apoptosis [26,28,30,31]. There are 4 members of the JAK family, namely, JAK1, JAK2, JAK3 and TYK2 [32] and 7 STAT proteins, STAT1-4, STAT5A, STAT5B and STAT6 [33].

The elevated gene expression of several pro-inflammatory cytokines, including interferon-γ (INF-γ), IL-2, IL-6, IL-7, IL-17 receptor, IL-17, IL-15, IL-19, IL-21, IL-23 genes as well as other genes and transcription factors germane to RA pathology are all regulated by phosphorylated (i.e., activated) STAT proteins [33-37]. In addition, there are several STAT-target genes relevant to cell differentiation, survival, apoptosis and cytokine signaling (e.g., cyclin D1, e-Myc, Bcl-XL, Mcl-1, survivin, MKP-1, TNFRSF13b and SOCS-3), all of which play important roles in RA. For example, the complex interaction involving IL-7 and IL-7R appears to be critical for regulating the T-cell receptor-γ locus via phosphorylated STAT5 and histone acetylation. Thus, the findings reported by Hartgring et al [38] that RA synovial fluid contained elevated levels of IL-7R made the IL-7R gene an even more attractive target for SMI drug development, perhaps through the inhibition of STAT5 activation.

**Tofacitinib (CP-690,550)**

The development and FDA approval of the first small molecule inhibitor (SMI) of a protein kinase, for use in the therapy of moderate-to-severe active RA in which methotrexate did not work well, arose from a series of sequential optimization protocols involving pyrrolopyrimidine-based JAK3 inhibitors [39], which eventually resulted in the drug CP-690,550, now called tofacitinib [40]. The efficacy of this drug for RA was established in numerous RA clinical trials [20,41] (see below) and tofacitinib has now entered general rheumatology practice.

**Ruxolitinib (INCB018424)**

Ruxolitinib/INCB018424 now referred to as ruxolitinib is a JAK1 and JAK2 SMI. The results of studies conducted on normal volunteers [42] and RA patients [43] concluded that ruxolitinib was generally safe and well-tolerated and also exhibited acceptable oral bioavailability with dose-proportional systemic pharmacokinetics and pharmacodynamics with low oral dose clearance and a small volume of distribution. Additional results from that study showed that ruxolitinib inhibited the phosphorylation of STAT3 in whole blood that was correlated with the plasma levels of the drug. Additional clinical trials involving patients with mild-to-moderate psoriasis [44] or active RA [45] administered ruxolitinib have now been conducted. In the RA trial, Williams et al [46] showed that ruxolitinib achieved an American College of Rheumatology (ACR)-70 criteria in 33% of patients compared to 0% in the placebo arm. Pharmacokinetic analysis determined that ruxolitinib inhibited JAK1 and JAK2 and also reduced plasma levels of IL-6 and CD40, the latter a co-stimulatory protein found on antigen-presenting cells. Ruxolitinib was also a potent p-STAT3 SMI in *ex vivo* studies conducted on blood cells obtained from RA patients.

**Pre-clinical studies and development of JAK SMIs**

Clinical trials are presently being conducted with RA and psoriasis patients to determine the clinical efficacy of several JAK SMIs, including INCB020850 (specificity, JAK1
JAK2/V617F

JAK2/FLT3

i.e. JAK2/Src

α

September

Table 1  Janus kinase small molecule inhibitors in development

| SMI                  | JAK Specificity/other kinase inhibitory activity | Ref. |
|----------------------|-----------------------------------------------|------|
| SAR302503 (Fedratinib) | JAK2                                          | [46] |
| CEP701 (Lestaurtinib)  | JAK2                                          | [47] |
| SB1518 (Pacritinib)   | JAK2/FLT3                                      | [48] |
| XL-019                | JAK2                                          | [49] |
| LY274444              | JAK2/V617F                                    | [49,50]|
| AZD4180               | JAK2                                          | [51] |
| NS-108                | JAK2/Src                                      | [52] |
| BMS-911433            | JAK2                                          | [53] |

1FLT3: Fms-like tyrosine kinase 3; a receptor-type tyrosine-protein kinase; 2V617F: A point mutation in JAK2 (V617F) identified in the hematopoietic cells of patients with several chronic myeloproliferative disorders; 3Src: V-src avian sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog; JAK: Janus kinase; SMI: Small molecule inhibitor.

malignancies (Table 1) may be a more desirable result because JAK3 is known to be less involved in hematopoietic cell development than is JAK2[57].

THE MAPK, PI3K/AKT/MTOR AND SYK PATHWAYS

MAPK and PI3K/Akt/mTOR

Signal transduction pathways other than JAK/STAT which are relevant to RA are the mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3-kinase (PI3K) pathways which also involve MAPK and PI3K/Akt/mTOR signaling. Thus, it was not surprising that future drug development for RA has taken a page from those experimental interventions which particularly focus on inhibiting the proliferation of cancer cells. In that regard, insights gleaned from studies of MEK1/2, the upstream activator of extracellular signal-regulated kinase1/2 (ERK1/2) and mTOR activity[64,65] in mutant BRAF-metastatic melanoma[66,67] and other experimental models of malignancy may shed light on whether or not these molecules may be eventually applied to RA.

In that regard, the MEK1/2 SMI, AZD6244 (selumetinib) which used in combination with the mTORC1/mTORC2 SMI, AZD8055, showed significant anti-tumor activity in nude mouse xenograft models of human lung adenocarcinoma and colorectal carcinoma[68], whereas the MEK1/2 inhibitor, AZD6244, sensitized apoptosis-resistant NRAS-mutant lines of melanoma cells to undergo apoptosis. This was correlated with negative regulation of the Wnt/β-catenin signaling via ERK1/2 and increased levels of the downstream scaffolding protein, AXIN1[69]. Of note, a Phase 2 trial of selumetinib in patients with the BRAFV600E/K-mutated type of melanoma resulted in tumor regression in 3 of 5 patients with BRAF-mutated low p-Akt activity. However, no response was observed in the AZD6244 treatment group with high p-Akt activity. These results provide a rationale for the dual targeting of MEK1/2 and p-Akt, especially in those melanoma patients with documented high p-Akt activity. Although there was persuasive pre-clinical data supporting the targeting of p38 kinase-α in RA[70], the results from several clinical trials in which the efficacy of pamapimod was compared to methotrexate in RA patients was disappointing in favor of methotrexate. Thus it is unlikely that pamapimod will be further developed for treating RA[64-66], although the jury is still out, so to speak, regarding whether or not VX-702, another p38 kinase SMI should be further developed and assessed for clinical efficacy in RA patients[71].
SyK signaling

The clinical trial evidence is somewhat stronger, but not persuasive, for promoting the further development of the SyK inhibitor, fostamatinib (R-788)\[^{[69]}\], although in 3 RA clinical trials with this drug, the ACR20 response rate ranged from only 35%-38%\[^{[69,70]}\]. Moreover, in one of these clinical trials the ACR20 response in the fostamatinib (100 mg twice daily group) was 38%, compared to 35% in the placebo group after 3 mo and no significant differences were achieved in the ACR20, ACR50, or ACR70 response levels at that time.

Protein kinase C-θ

There is also increasing evidence for targeting protein kinase C-θ in RA\[^{[71]}\]. This is because protein kinase C-θ is known to play an integral role in regulating T-cell viability and cytoskeletal reorganization by regulating the activities of Vav, PI3K and Rac1 (guanyl-nucleotide exchange factor)\[^{[72,73]}\].

THE CLINICAL PERSPECTIVE

Data on the efficacy and safety of tofacitinib in RA was presented to the FDA in May 2012\[^{[74]}\]. In November 2012, tofacitinib was approved for use in the US for the treatment of adults with moderately to severe active RA with an inadequate response to, or intolerance to methotrexate. Assessment of the efficacy in RA clinical trials has become fairly standardized\[^{[75]}\] and the outcome measures used in the tofacitinib studies were similar to those used in previous clinical trials of biologic drugs for RA. The raw data included a measurement of the tender joint count and the swollen joint count by an examiner, the patient’s assessment of pain on a visual analog scale, the patient’s global assessment of disease activity on a visual analog scale, an examiner’s global assessment of disease activity on a visual analog scale, the patient’s assessment of physical function using the HAQ\[^{[76]}\], blood testing to determine erythrocyte sedimentation rate (ESR) or CRP, and radiographs of the hands and feet\[^{[70]}\]. In most RA studies the raw data is further “manipulated” to produce composite measures of drug efficacy. The ACR has defined the ACR20 response rate as a measure of efficacy in RA to be ≥ 20% improvement in tender joint count, ≥ 20% improvement in swollen joint count, and ≥ 20% improvement in 3 out of 5 of the following parameters: patient pain assessment, patient global assessment, physician global assessment, patient self-assessment of disability and blood acute phase reactant (ESR or CRP)\[^{[77]}\]. In the Phase 3 tofacitinib clinical trials, approximately 25%-30% of study patients achieved an ACR20 efficacy when placebo was added to their prior therapy with methotrexate or to another oral immunosuppressant. This was a result that was similar to that previously reported in clinical trials with biologic therapies for RA\[^{[74]}\]. In order to demonstrate efficacy that is less likely to be achieved by placebo alone, ACR50 and ACR70 data are also commonly reported, representing ≥ 50% and ≥ 70% improvement in the composite ACR score, respectively. Thus, the tender joint count of 28 joints, swollen joint count in 28 joints, serum ESR or CRP and the patient’s global assessment of disease activity can be entered into a formula to generate a DAS28-4 score ranging from 0 to 10\[^{[78]}\]. If the patient’s global assessment of disease activity is omitted, the resulting score is a DAS28-3. A DAS score of ≤ 2.6 is considered to represent clinical remission, although such a DAS28 score does not necessarily represent a cessation of all joint inflammation. However, DAS28 efficacy measurements are potentially relevant to clinicians, since the DAS28 can be used to track efficacy in clinical practice and a DAS28 ≤ 2.6 is often the therapeutic goal in treat-to-target clinical trials. Radiographs are also assessed to determine joint space narrowing and the presence of periarticular erosions, which are used to calculate a radiographic score. The method of Sharp as modified by Van der Heijde is commonly employed\[^{[79]}\]. This method generates a joint space narrowing score and an erosion score as secondary endpoints, which are combined to generate the primary endpoint, the total Sharp score\[^{[80]}\]. Although the publication of this type of radiographic data has become standard over the past 15 years, there are some methodological flaws in this analysis. When efficacy of a new pharmaceutical is assessed, the study population usually adds the new drug to a stable dose of an oral immunosuppressant such as methotrexate. The efficacy data of this population is compared to a group randomized to receive a stable dose of oral immunosuppressant plus placebo. As a result, both groups of subjects receive medication with potential efficacy in RA, and the rise in the modified Sharp/Van der Heijde score can be slow to rise, even in the placebo group. Therefore, to discern a meaningful difference between the new drug and placebo it may become necessary to choose study subjects with a high risk for the rapid accumulation of joint damage (for example, high serum levels of rheumatoid factor or anti-cyclic citrullinated peptide antibody), to continue to collect radiographic data for 1-2 years or more, or more often to enroll larger numbers of patients.

Ultrasound and Magnetic Resonance Imaging have been proposed as potential substitutes for radiography. However, issues of standardization, reproducibility, potential cost and correlation with other clinical outcome measures are still being worked out, but clinical trials employing these imaging techniques are beginning to appear in published reports.

The dose of tofacitinib used to treat RA in the US is 5 mg orally twice daily. The subjects enrolled in the 5 phase 3 clinical trials were those patients who had experienced an inadequate response to prior treatment with methotrexate, another oral immunosuppressant, or a TNF inhibitor\[^{[81]}\]. Most of the study subjects were given either tofacitinib or placebo under a double-blind study design while continuing a stable dose of methotrexate or other oral immunosuppressant. In one of these studies, subjects on a stable dose of methotrexate were given subcutaneous injections (either adalimumab or placebo) plus a pill (placebo pill to recipients of adalimumab, placebo or tofacitinib to recipients of placebo injections). A small
number of subjects were enrolled in a 3 mo study of tofacitinib vs placebo without therapy with another immunosuppressant, but there were ethical concerns about randomizing patients with active RA to a study arm in which they were to receive no treatment. In most RA trials the new drug is compared to an active immunosuppressant commonly used in RA (usually methotrexate). The outcome data demonstrated statistically significant efficacy for tofacitinib 5 mg twice daily vs placebo as determined by the following outcome measures: ACR20, ACR50, ACR70, DAS-4 (ESR) ≤ 2.6, DAS-4 (ESR) improving ≥ 1.2, and HAQ-Disability Index. When compared to 199 subjects receiving adalimumab, 40 mg by subcutaneous injection every 14 d, plus methotrexate plus placebo pills, tofacitinib plus placebo injection plus methotrexate was not inferior using the following outcome measures: ACR20, ACR50, ACR70, DAS-4(ESR) ≤ 2.6, DAS-4(ESR) improving ≥ 1.2, HAQ-Disability Index. One of these 5 studies also provided radiographic outcome data. Only 20% of the subjects receiving a stable dose of methotrexate plus placebo demonstrated worsening of the radiographic score at 1 year. In the tofacitinib 5 mg twice daily plus methotrexate treatment group there was a trend toward decreased progression of the total Sharp score, but the difference did not meet statistical significance at either 6 or 12 mo.

Tofacitinib was the first JAK SMI submitted to the FDA for approval in the treatment of RA. As a result the safety assessment was broad in scope, with data collected on mortality, total adverse effects, serious adverse effects, infections, malignancies other than non-melanoma skin cancer, cardiovascular events, and bowel perforations, with monitoring of cell counts, creatinine, liver enzymes, creatinine phosphokinase, and lipid levels in the blood. Data was available for the blinded placebo controlled phase of the study and also the unblinded long-term extension clinical trial. Treatment with tofacitinib was associated with drug dose-dependent neutropenia and lymphopenia, a rise in total HDL and LDL cholesterol, but without associated cardiovascular events, and a rise in serum creatinine. The increased LDL cholesterol improved after the addition of atorvastatin. Overall, the rates per 100 patient-years for all-cause mortality, serious infections, malignancy other than non-melanoma skin cancer, lymphoma, lung cancer, myocardial infarction and gastrointestinal perforation were similar to those reported in published clinical trials of biologic therapies for RA. However, the rate of Herpes zoster infection was higher in the subjects treated with tofacitinib than the infection rates for Herpes zoster reported in prior clinical trials of biologic drugs for RA. After review of the clinical trial data, tofacitinib was considered to be sufficiently safe and effective to be approved for use in the US for moderate to severe active RA not responsive to methotrexate. Of note, post-FDA approval monitoring of the long term safety of tofacitinib is ongoing.

Published RA treatment trials of other small molecule inhibitors have employed a study design similar to those used to assess the safety and efficacy of tofacitinib. As stated above, a phase 2 clinical trial of fostamatinib in RA has now been concluded. Outcome criteria included ACR20, ACR50, ACR70, and DAS28 as measures of efficacy. Imaging outcomes in that study were assessed by MRI. Patient-reported quality of life was assessed using the HAQ-Disability Index, multiple domains of the SF-36 questionnaire, and the Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-F) questionnaire. A 12 wk trial of pamapimod vs placebo added to a stable dose of methotrexate used ACR20, ACR50, ACR70, DAS28, change in mean serum CRP, HAQ-DI, SF-36, and FACIT-F as efficacy outcome measures.

Clinical trials of other small molecule inhibitors currently under development are likely to have a similar study design.

CONCLUSION

The development of SMIs of the JAK/STAT (including the newly developed JAK3 SMI, VX-509), MAPK, PI3K/Akt/mTOR and SyK signaling pathways has recently been the target of additional pre-clinical experimental arthritis studies and RA clinical trials assessment. The phase 3 clinical trial data for the JAK SMI, tofacitinib, illustrates the therapeutic potential of this class of SMI drug. For example, by comparison with the relative ease of storage and oral administration of these SMI drugs, the treatment of RA with biologic drugs such as the TNF blockade drugs, etanercept, adalimumab, golimumab, certolizumab, the T-cell co-stimulator inhibitor, abatacept, and the IL-6/IL-6R neutralizing monoclonal antibody, tocilizumab, requires that the medication be shipped by rapid delivery, stored at 2-8 °C, and maintained in a cool storage temperature during travel. Administration of these drugs also requires mastery of the correct injection technique, and safe and proper disposal of hypodermic needles. Therefore, if the efficacy and safety of protein kinase SMIs proves to be comparable to the injectable types of biologic drugs, many RA patients may prefer the convenience of an oral medication. However, the relatively short half-life of tofacitinib means that twice daily dosing will be necessary to achieve optimal clinical efficacy. This can be an advantage to the RA patient if the patient develops an infection such that the treating clinician may wish to reverse the immunosuppressive effect of the drug. Thus, at present, treatment with tofacitinib is a therapeutic option for moderate-to-severe RA where disease progression cannot be controlled with methotrexate.

Although SMIs have been primarily targeted to inhibit the activity of JAKs, specific members of the MAPK pathway (e.g., p38-α) and PI3K/Akt/mTOR signaling pathways were also shown to be relevant to the pathogenesis of immune-mediated inflammation associated with RA. Therefore, there are likely to be signaling components of the MAPK pathway, such as the upstream protein kinase, MEK1/2, whose activity is required for phosphorylation of ERK1/2 that may be targeted for further drug development. In addition, since one tar-
get of STAT activation is it’s potential to increase the expression of anti-inflammatory cytokines, such as, IL-4 and IL-10[25] and the signaling pathways these cytokines activate, it appears justified to consider developing SMIs that inhibit those protein kinases which can suppress the expression of anti-inflammatory cytokine genes.

However, as an example of the continuing SMI drug development for JAKs in RA, Baricitinib, (formerly known as LY3009104/INCBO28050) an inhibitor of JAK1 and JAK2 is presently under investigation in clinical trials in RA with the results from an open extension of the phase 2b trial having been recently reported[86] with additional studies entering the recruitment phase[96]. In the open-extension phase 2b trial, among all patients, the proportions of patients achieving ACR20, ACR50, ACR70, clinical disease activity index (CDAI) Remission, simplified disease activity index (SDAI) Remission, DAS28CRP ≤ 3.2, DAS28CRP < 2.6, DAS28ESR ≤ 3.2, DAS28CRP < 2.6 or the ACR/European League Against Rheumatism (EULAR) Boolean remission at the start of the open label extension (week 24) were similar or increased at week 52.

The ultimate place of protein kinase SMIs in RA therapy is not yet known. It is likely to be determined by the following conditions; more patient-years of follow-up to better understand the long-term efficacy and safety of this drug class as well as head-to-head safety and efficacy comparisons with conventional and biologic DMARDs already in use including cost issues relative to other RA treatment options.

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