B-cell kinase inhibitors in rheumatoid arthritis

AD Chu1, BY Chang2

Abstract

Introduction

The pathogenesis of rheumatoid arthritis (RA) involves systemic dysregulation of T and B lymphocytes resulting in immune responses against self-antigens, and ultimately leading to downstream effects such as cartilage destruction, pannus formation, and bone erosion. Clinical evidence for the role of B cells in RA includes the effectiveness of rituximab, an antibody designed to deplete autoreactive B cells. A separate and newer approach to RA treatment involves interference with key intracellular signalling kinases present in B cells, notably spleen tyrosine kinase (SYK) and Bruton’s tyrosine kinase (BTK). Inhibition of SYK, a non-receptor tyrosine kinase predominantly expressed in hematopoietic cells that is involved in the activation of immune cells critical to RA, has proven effective in animal models of inflammation and inflammatory arthritis. BTK is a Tec-family kinase prominently expressed in B cells, with well-established function in B-cell receptor-mediated activation and survival. Ibrutinib, a first-in-class BTK inhibitor recently approved in mantle cell lymphoma and currently in clinical trials for B-cell malignancies, potently and dose-dependently reverses clinical arthritis, preventing cartilage and bone erosion in animal models of arthritis. Here we briefly review available data for SYK and BTK inhibitors in RA and other inflammatory diseases and outline their current clinical status.

Conclusion

With multiple small-molecule inhibitors being developed to treat chronic inflammation, the future appears promising for an increased variety of treatment options for patients with RA.

Introduction

Rheumatoid arthritis (RA) is a systemic disease characterized by circulating autoantibodies, synovial inflammation, pannus formation, and cartilage and bone destruction in affected joints. Initiation of the disease involves the systemic dysregulation of T and B lymphocytes, which results in immune responses directed against self-antigens.

During the chronic inflammatory phase of the disease, autoantibodies and immune complexes further activate a variety of cells such as neutrophils, monocytes/macrophages, dendritic cells, and mast cells that infiltrate the synovium and release proinflammatory cytokines and matrix metalloproteases, leading to cartilage destruction. Synovial hyperplasia leads to the formation of a pannus that invades the surrounding cartilage and bone, and inflammation enhances the activity of resident osteoclasts, leading to bone erosion1,2.

During the multi-step pathogenesis of RA, a host of intracellular signalling kinases are involved in initiation, inflammation, and bone resorption, including Janus kinase 1/3 (JAK1/3) in T cells and, in B cells, spleen tyrosine kinase (SYK) and Bruton’s tyrosine kinase (BTK). These mediate cell survival and the inflammatory response2. As such, RA has been a disease of interest for tyrosine kinase modulation, with small molecule JAK inhibitors leading the way in this approach to the clinical treatment of patients with RA. Modulation of the JAK/signal transducers and activators of transcription (STAT) pathway has proven to be effective in these patients, as evidenced by tofacitinib, which was approved in November 2012 for the treatment of patients with moderate-to-severe RA with an inadequate response to or intolerance of methotrexate. Tofacitinib primarily inhibits JAK1 and JAK3, and, to a lesser extent, JAK24. Multiple JAK inhibitors targeting different JAK homodimers are also being developed for patients with RA. The introduction of small molecule inhibition to RA treatment has led to clinical trials of other targeted therapies in various phases of development. This review focuses primarily on inhibitors of the B-cell kinases SYK and BTK that are currently in clinical development.

Discussion

The authors have referenced some of their own studies in this review. These referenced studies have been conducted in accordance with the Declaration of Helsinki (1964) and the protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed. All human subjects, in these referenced studies, gave informed consent to participate in these studies.

Clinical Significance of B Cells in RA

Ample clinical data support the role of B cells in the pathogenesis of RA. Rituximab, a monoclonal antibody targeting CD20 expressed on B cells, was the first B-cell-targeted therapy to gain approval for treatment of patients with RA. It was developed with the intention of depleting autoreactive B cells in order to reduce the production of pathogenic autoantibodies such as rheumatoid factor5. The efficacy of rituximab was established in patients who still had active disease despite being treated with methotrexate6,7 and in patients who were refractory or intolerant to anti–tumour necrosis factor (TNF) agents.

1 Clinical Sciences Department, Pharmacyclics Inc, Sunnyvale, California, USA
2 Research Department, Pharmacyclics Inc, Sunnyvale, California, USA

Corresponding author
Email: achu@phyc.com

Licensee OAPL (UK) 2013. Creative Commons Attribution License (CC-BY)

factor (TNF) therapy, B-cell depletion and improved clinical responses were demonstrated. Rituximab is approved for use in combination with methotrexate in adult patients with moderately to severely active RA who have experienced an inadequate response to one or more TNF therapies.

B-cell modulation for treatment of patients with RA has been attempted via B-lymphocyte stimulator (BLyS) and a proliferation-inducing ligand (APRIL), which play important functional roles in B-cell maturation, proliferation, and survival. Elevated BLyS and APRIL levels have been reported in mouse models of RA and in the serum and synovial fluid of patients with RA.

Figure 1: Role of inhibitors of BTK and SYK in the pathogenesis of rheumatoid arthritis.

Ataciept, a fully human, recombinant fusion protein of the transmembrane activator and CAML interactor (TACI) receptor and of human immunoglobulin G (IgG), binds to BLyS and APRIL to prevent binding to their respective receptors on B cells. Early-phase clinical trials with ataciept in patients with RA demonstrated decreases in Ig and autoantibody production, whereas its effect on inflammatory markers and clinical outcomes was less profound.

Tabalumab, an anti-BLyS human monoclonal antibody, was tested in phase 2 clinical trials for the treatment of patients with active RA and an inadequate response to methotrexate. In one 16-week study, significantly more patients in each of the tabalumab dosing groups met American College of Rheumatology 20% improvement criteria (achieved an ACR20 response) than in the placebo group. Observed ACR50 response rates in a longer study suggested that an initial response to treatment was not sustained to week 24. In another phase 2 clinical trial, ACR50 response rates at week 16 were not significantly different between tabalumab-treated patients with active RA with an inadequate response to TNF inhibitors and the placebo group. Subsequently, a phase 3 RA program was discontinued after an interim analysis of efficacy results.

The results of tabalumab and ataciept therapy for patients with active RA suggest that, while B cells may play a role in the perpetuation of the inflammatory response in RA as demonstrated by the studies with rituximab, only part of this response is related to the BLyS/APRIL pathway.

Mechanisms of Action for Kinase Inhibitors

SYK is a non-receptor tyrosine kinase predominantly expressed in hematopoietic cells. SYK binds to the phosphorylated immunoreceptor tyrosine-based activation motif (ITAM) of B-cell antigen receptor (BCR) and Fcγ receptor (FcγR), and plays a critical role in the signal transduction of early B-cell development and activation. It is also involved in FcγR and FcεR signaling of IgG and IgE immune complex-mediated activations and inflammations in macrophages and mast cells, respectively; Figure 1.

Therefore, SYK is a central player in the activation of the immune cells that are critical to the pathogenesis of RA. SYK is expressed differentially in RA synovia relative to osteoarthritis, and its inhibition suppresses both inflammation and bone erosions in animal models of RA. SYK inhibitors such as R406/R788 (Table 1) have been shown to be effective in inhibiting immune complex-mediated activations in animal models of inflammation (eg, reverse passive anaphylaxis [RPA] reactions, inflammatory arthritis, collagen-induced arthritis [CIA], and collagen antibody-induced arthritis [CAIA])

BTK is a Tec-family kinase that is specifically required for B-cell activation following engagement of the BCR. BTK is prominently expressed in B cells, and has a well-established function in BCR-mediated cell activation and survival. In the lymphoid lineage, expression of BTK is restricted to B cells and is not found in T cells. Functional null mutations of BTK in humans cause the inherited disease X-linked agammaglobulinemia (XLA), which is characterized by a lack of peripheral B cells and very low levels of serum IgG. In the mouse, point mutation or deletion of the BTK gene causes X-linked immunodeficiency (xid), with approximately 50% fewer conventional B2 B cells, absent B1 B cells.
### Table 1: BTK and SYK inhibitors.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Target(s)</th>
<th>Type</th>
<th>Activity</th>
<th>Mode(s) of Action</th>
<th>Clinical Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brutinib</td>
<td>BTK, ITK, BLK</td>
<td>I</td>
<td>Biochemical (BTK): Kᵢ 0.5 nM Cellular: Primary B-cell prolif. IC₅₀ 8 nM Animal model: murine therapeutic CIA ED₅₀ 3.125 mg/kg</td>
<td>Inhibits BCR and FcgR</td>
<td>3</td>
</tr>
<tr>
<td>RN486 (19)</td>
<td>BTK</td>
<td>R</td>
<td>Biochemical (BTK): Kᵢ 4 nM Cellular: Whole blood CD69 IC₅₀ 21 nM Animal model: murine prevention CIA ED₅₀ 3 mg/kg</td>
<td>Inhibits BCR and FcgR</td>
<td>N/A</td>
</tr>
<tr>
<td>CGI1746 (19)</td>
<td>BTK</td>
<td>R</td>
<td>Biochemical (BTK): Kᵢ 1.9 nM Cellular: Primary B-cell prolif. IC₅₀ 42 nM Animal model: murine prevention CIA ED₅₀ 200 mg/kg</td>
<td>Stabilizes inactive conformation, inhibits both BCR and FcgR</td>
<td>N/A</td>
</tr>
<tr>
<td>ONU-4059 (20)</td>
<td>BTK</td>
<td>R</td>
<td>Biochemical (BTK): IC₅₀ 2.2 nM Cellular: Primary B-cell activation: IC₅₀ 10 nM Animal model: 3, 10 mg/kg effective in murine CIA</td>
<td>Inhibits BCR and FcgR</td>
<td>1</td>
</tr>
<tr>
<td>“Compound 36” (21)</td>
<td>BTK, EGFR</td>
<td>I</td>
<td>Biochemical (BTK): IC₅₀ 1.9 nM Cellular: Primary B-cell prolif. IC₅₀ 3.4 nM Cellular: BTK pY 223 Ramos cell IC₅₀ 48 nM Animal model: 3, 10 mg/kg effective in murine CIA</td>
<td>Dose-dependent inhibition of TI-2 immune response (anti-NP IgM, IgG)</td>
<td>N/A</td>
</tr>
<tr>
<td>CC-292 (22)</td>
<td>BTK</td>
<td>I</td>
<td>Biochemical (BTK): IC₅₀ 5.9 nM Cellular: Primary B-cell prolif. IC₅₀ 3 nM Animal model: murine subtherapeutic CIA ED₅₀ 3 mg/kg</td>
<td>B-cell activation</td>
<td>1b</td>
</tr>
<tr>
<td>R406 (15)</td>
<td>SYK, JAK3, VEGFR</td>
<td>R</td>
<td>Biochemical (SYK): IC₅₀ 41 nM Cellular: Primary B-cell CD69: EC₅₀ 48 nM Animal model: rat therapeutic model: CIA ED₅₀ 20 mg/kg</td>
<td>Inhibits BCR, FceR, FcgR</td>
<td>Prodrug (R788, fostamatinib) phase 3</td>
</tr>
<tr>
<td>P505-15 (23)</td>
<td>SYK</td>
<td>R</td>
<td>Biochemical (SYK): IC₅₀ 6 nM Cellular: Primary B-cell CD69: EC₅₀ 280 nM Animal model: rat therapeutic CIA ED₅₀ 20 mg/kg</td>
<td>Inhibits BCR, FceR, FcgR</td>
<td>1</td>
</tr>
</tbody>
</table>

**BCR:** B-cell receptor; **BLK:** b-lymphoid tyrosine kinase; **BTK:** Bruton’s tyrosine kinase; **CIA:** collagen-induced arthritis; **ED₅₀:** median effective dose; **EGFR:** epidermal growth factor receptor; **FcR:** Fc receptor; **I:** irreversible; **IC₅₀:** inhibitory concentration of 50%; **Ig:** immunoglobulin; **ITK:** interleukin-2-inducible kinase; **JAK:** Janus kinase; **Kᵢ:** affinity constant; **NP:** nitrophenyl; **R:** reversible; **SYK:** spleen tyrosine kinase; **TI:** T-cell-independent; **VEGFR:** vascular endothelial growth factor receptor.

---

**SYK Inhibition in RA**

Fostamatinib (R788) was developed as an orally bioavailable small-molecule prodrug that is metabolized to R406, an active SYK inhibitor (Table 1). It has been tested in different populations of patients with RA with mixed results. Three phase 2 studies have reported results with fostamatinib treatment in patients with RA. Two of the studies examined the effect of fostamatinib therapy in patients with active RA who had an inadequate response to methotrexate. One of these was a 12-week, randomized, placebo-controlled study that enrolled 189 patients in a 3:1 ratio to receive

---

**Cells and reduced serum Ig levels**

Since RA is characterized by polyclonal B-cell activation that gives rise to B-cell expansion and the production of autoantibodies, BTK is a uniquely attractive target for selective B-cell inhibition in RA.

BTK is also expressed in specific types of myeloid cells that are known to be key players in the chronic inflammatory phase of RA, such as monocytes, macrophages, neutrophils, and mast cells. Loss of BTK in macrophages and myeloid cells has been linked to compromised inflammatory responses.

BTK is activated following FcyR/FcεR crosslinking by immune complexes in these cells. Lacking BTK, xid mice have reduced FcεR-dependent mast-cell degranulation and impaired macrophage function, including impaired TNF-α production. Accordingly, xid mice have demonstrated resistance to disease manifestations in CIA models, and BTK has been shown to be important for murine autoantibody production. The involvement of BTK not only in B cells but also in these myeloid cells provides further support for the potential role of BTK inhibition in treating RA.

---

**Competing interests:** None declared. Conflict of interests: None declared.

**Licensee OAPL (UK) 2013. Creative Commons Attribution License (CC-BY)**

**FOR CITATION PURPOSES:** Chu AD, Chang BY. B-cell kinase inhibitors in rheumatoid arthritis. OA Arthritis 2013 Oct 27;1(2):17.
Fostamatinib has also been tested in patients with active RA who did not respond to biologic agents. A 3-month, randomized, placebo-controlled, phase 2 study in a total of 219 patients with RA reported no significant differences achieved in the ACR20, ACR50, or ACR70 response levels at 3 months between patients receiving fostamatinib and those given placebo.

Contrary to the previous phase 2 studies, the primary endpoint of ACR20 response at month 3 was not significantly different between the fostamatinib group and the placebo group. The authors noted that this endpoint may have been compromised by the study’s difficult-to-treat patient population, as well as by differences in baseline disease activity and distribution of patients with refractory disease between the fostamatinib and placebo groups.

Additional phase 3 trials with fostamatinib include OSKIRA-2, which studied patients with RA who had inadequate responses to disease-modifying antirheumatic drugs (DMARDs), and OSKIRA-3, which studied patients with RA who had inadequate responses to methotrexate and a single TNF-α antagonist. Both of these studies demonstrated ACR20 response rates at 24 weeks that were significantly higher for the fostamatinib-treated groups than they were with placebo, but no further phase 3 development with fostamatinib in RA has occurred since the results of these trials.

**BTK Inhibition in RA**

Selective BTK inhibitors have been shown to block receptor signalling in human B cells, although BTK inhibitors are just emerging in clinical trials of patients with RA. Ibrutinib, a first-in-class BTK inhibitor that is approved for the treatment of mantle cell lymphoma in patients who have received at least one prior therapy, is currently being developed clinically to treat patients with various B-cell malignancies. It has also demonstrated therapeutic activity in animal models of RA.

In an RPA assay in which neutrophils, macrophages, and mast cells acted as the key effector cells independently of the complement system, ibrutinib potently inhibited acute vasculitis mediated by immune complexes and FcγR. Collectively, these studies established the dose-dependent inhibition of ibrutinib on immune complex mediated inflammation in vivo.

BTK inhibition by ibrutinib has also been shown to inhibit cytokine and chemokine release upon FcγR activation of monocytes and macrophages, as well as upon FcεR activation of mast cells. In both GIA and CAIA models, ibrutinib inhibited clinical inflammation and pannus formation and protected against cartilage and bone damage. Mechanistically, ibrutinib potently inhibited the release of FcγR-induced cytokines such as TNF-α, interleukin (IL)-6, IL-1β, and monocyte chemoattractant protein 1 (MCP-1) in monocytes, albeit less potently in macrophages. Additional benefit in the CIA model might have been derived from the inhibition of RANKL-induced osteoclastic differentiation, as suggested by findings that BTK and Tec are key downstream elements in RANK signalling.

Following ibrutinib treatment, Shinohara et al found a nearly complete inhibition of infiltrating cells (neutrophils and macrophages) in the synovial joints of mice. Cytokines and chemokines such as IL-1β, IL-6, TNF-α,
and MCP-1, and MCP-1 were also potently suppressed, consistent with in vitro results with primary monocytes and macrophages (Figure 1).

In combination with the in vitro studies with human primary cells and with preclinical data for other inhibitors of BTK (Table 1), these results suggest that BTK inhibition affects multiple cell types that contribute to the pathogenesis of RA, and that it is highly effective in models of several inflammatory diseases, including CIA, CAIA, and RPA. Inhibition of BTK by ibrutinib or other molecules thus constitutes a promising direction for therapeutic trials in diseases such as RA and other immune complex-mediated inflammatory diseases.

**Conclusion**

Biologic therapies such as rituximab that modulate the contribution of B cells to inflammatory pathways associated with RA are now used in clinical practice. Monoclonal antibodies and related biologics that are administered intravenously or subcutaneously have been the first targeted therapies for the treatment of patients with RA.

The regulatory approval of tofacitinib as a small-molecule inhibitor of the JAK-STAT pathway, however, has opened the path for the development of other targeted oral RA therapies.

Looking ahead, intracellularly targeted small molecules will likely rival the therapeutic space now inhabited by biologic therapies. Particularly, pathways that have proven efficacy in B-cell hematologic malignancies are viable candidates for modulation of RA, such as BTK signalling. Challenges involved in inhibiting intracellular targets include designing a therapeutic drug that is effective and durable over time while maintaining a safety profile that will limit off-target toxicities. With multiple small-molecule inhibitors being developed to treat chronic inflammation, the future appears promising for an increased variety of treatment options for patients with RA.

**References**


46. Dawes P, Dimic A, Genovese MC et al. Oskira-2: a phase III, multicenter, randomized, double-blind, placebo-


