New molecules for the treatment of rheumatoid arthritis

Novel small molecule therapeutics in rheumatoid arthritis

Victoria Kelly¹ and Mark Genovese¹

Abstract

A new wave of emerging therapies for the treatment of autoimmune and inflammatory diseases is under development. These therapies interrupt intracellular signalling through kinase inhibition. By interrupting one or more kinases it is possible to modulate the function of cellular structures such as surface receptors, signalling proteins and transcription of nuclear proteins and thus influence the behaviour of the cell types targeted. With these advances comes the significant potential to develop highly effective orally bioavailable therapeutics. The targets generating the greatest enthusiasm at this time for the treatment of autoimmune and inflammatory diseases include Janus-associated kinase, spleen tyrosine kinase, phosphodiesterase-4, Bruton’s tyrosine kinase and phosphatidylinositol-3 kinase. Ultimately human trials will help us understand the potential risks and benefits of these novel approaches across a number of diseases.

Key words: DMARDs, treatment, rheumatoid arthritis, small molecules, kinase, JAK, SYK, BTK, PI3K, PDE4.

Introduction

The field of rheumatology is by nature accustomed to and comfortable with the need for immunosuppression or modulation. For decades the field has used traditional small molecule DMARDs for the treatment of RA and many other inflammatory conditions. It has not always been clear how these individual agents have worked mechanistically; in some circumstances it remains poorly understood and in others it appears that there may be interruption of both extracellular and intracellular pathways. The 1990s ushered in the biologic DMARD era, allowing for the first time the effective use of protein-based therapeutics interrupting the two fundamental mechanisms for immunological communication, cytokines and cognate cell–cell interaction. These targeted approaches have taken advantage of our ability to target extracellular communication by blocking various receptors or ligands. The biologic classes such as TNF inhibition, IL-1 receptor antagonism, co-stimulatory blockade, B-cell depletion and IL-6 antagonism all represent advances in protein-based targeted therapeutics.

The 21st century has seen a resurgence in the development of small molecule therapeutics. This new wave of emerging therapies is based not on the blockade of extracellular communication, but on the interruption of intracellular signalling based on the inhibition of kinases. By interrupting one or more kinases it is possible to modulate the function of cellular structures such as surface receptors, signalling proteins and transcription of nuclear proteins, thus influencing the behaviour of the cell types targeted. With these advances comes significant potential to develop highly effective orally bioavailable therapeutics. While the field has advanced tremendously, it has not been without some notable failures in the attempt to generate new small molecule therapeutics. For much of the early 21st century many laboratories and clinical investigators toiled with the inhibition of p38 mitogen-activated protein kinase (p38). Unfortunately, despite appearing as a promising target in both in vitro assays and animal models, the promise of this targeted approach failed, as it became increasingly clear that despite selectivity and specificity for the intended target, there was inadequate clinical efficacy in the treatment of the human condition. Subsequent targets generating increasing enthusiasm for the treatment of autoimmune and
inflammatory diseases have included Janus-associated
kinases (JAKs), spleen tyrosine kinase (SYK), phospho-
diesterase-4 (PDE4), Bruton’s tyrosine kinase (BTK), phosphatidylinositol-3 kinase (PI3K) and others. Laboratory and clinical data have allowed these potential
targets to move into human study. Understanding how we
interrupt intracellular signalling based on the inhibition of
kinases starts with phosphorylation.

Phosphorylation, or the addition of a negatively charged
phosphate group from adenosine triphosphate to a cellular
protein, can induce significant conformational changes
in a protein’s structure and plays a major role in the acti-
vation and deactivation of many cellular enzymes [1].

While phosphorylation is generally initiated through an
extracellular signal, it is involved in almost all downstream
intracellular signalling pathways. Protein kinases are the
main enzymes responsible for phosphorylation of pro-
teins; in mammals, the majority of these protein kinases
fall into two major categories based on the amino acids
they phosphorylate: serine/threonine kinases and tyrosine
kinases [2]. Tyrosine kinases are activated primarily by
extracellular signals that control cell differentiation and
proliferation, otherwise known as growth factors. These
growth factors include hormones and cytokines, such as
IL-2, TNF or GM-CSF, that act as ligands for the associ-
ated tyrosine kinase receptor on the cell surface [3].

There are two ways in which tyrosine kinases transmit a
signal from an extracellular growth factor to the cell’s
interior: one is through membrane receptors with intrinsic
tyrosine kinase activity, also known as receptor protein
tyrosine kinases, and the other is through growth factor
receptors that have associated tyrosine kinases in the
cytosol, also known as non-receptor or cytoplasmic pro-
tein tyrosine kinases [4].

Within the family of non-receptor protein kinases, there
are 10 different subfamilies, including JAK, SYK and
tyrosine kinase expressed in hepatocellular carcinoma
(Table 1) [5]. Each of these families of non-receptor tyro-
mine kinases interact with associated cell surface recep-
tors, many of which are involved in the regulation and
proliferation of immune and haematopoietic cells. The
principal receptors that recruit and interact with non-receptor tyrosine kinases are type I and type II cyto-
kine receptors, the T-cell receptor, the B-cell receptor and
the high-affinity IgE receptor [4, 5]. Each of these receptor
subsets have a unique set of ligands that send signals into
the cell regarding growth, mitogenesis and cell–cell
interactions.

### JAK-STAT

JAKs are a family of non-receptor protein tyrosine kinases
that affect intracellular signalling through their association
with transcription factors known as STATs (signal trans-
ducer and activator of transcription), otherwise known as
the JAK–STAT pathway. JAKs are constitutively bound to
their associated receptors and are activated when the
corresponding cytokine or growth factor binds to its re-
ceptor. Activated JAKs phosphorylate the tyrosine resi-
dues on the receptor, causing a conformational change,
allowing the binding of a STAT protein to the SRC2 hom-
ology (SH2) domain. JAKs then phosphorylate the tyrosine
residues on the STAT proteins, allowing for dimerization of
the STATs, which then migrate into the cytoplasm and
translocate into the nucleus, allowing for transcription of
their target genes. [6] JAKs also autophosphorylate, but
the implication of this is still not well understood [7]
(Fig. 1).

Within the family of JAKs there are four members: JAK1,
JAK2, JAK3 and tyrosine kinase 2 (Tyk2). JAK1, JAK2 and
Tyk2 are fairly ubiquitously expressed in mammalian cells,
whereas JAK3 has a more limited repertoire, with expres-
sion primarily on haematopoietic cells [7]. Each of the
JAKs is associated with cytokine receptors and is acti-
vated when a cytokine binds to its cognate receptor.
JAK1 interacts with a wide variety of cytokines, through
the common γ chain receptor subunit, as well as the
gp130 subunit. Ligands for these receptors include IL-2,
-4, -7, -9, -15, -21 (γ chain), IL-6, -11, -27, -31 (gp130) and
INFα, β and γ [6, 7]. JAK2 also interacts with cytokines

### Table 1: Families of non-receptor protein tyrosine kinases

<table>
<thead>
<tr>
<th>ABL</th>
<th>ACK</th>
<th>CSK</th>
<th>FAK</th>
<th>FES</th>
<th>FRK</th>
<th>JAK</th>
<th>SRC-A</th>
<th>SRC-B</th>
<th>TEC</th>
<th>SYK</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABL1</td>
<td>ACK1</td>
<td>CSK</td>
<td>FAK</td>
<td>FER</td>
<td>BRK</td>
<td>JAK1</td>
<td>FGR</td>
<td>BLK</td>
<td>BMX</td>
<td>SYK</td>
</tr>
<tr>
<td>ARG</td>
<td>TNK1</td>
<td>MATK</td>
<td>PYK2</td>
<td>FES</td>
<td>SRMS</td>
<td>JAK3</td>
<td>SRC</td>
<td>LCK</td>
<td>BTK</td>
<td>ZAP-70</td>
</tr>
<tr>
<td>TYK2</td>
<td>YES1</td>
<td>LYN</td>
<td>TEC</td>
<td>TXK</td>
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</tbody>
</table>

ABL: Abelson murine leukaemia viral oncogene homologue; ACK: activated Cdc42 protein kinase; ARG: Abelson-related gene; BLK: B lymphocyte kinase; BMX: bone marrow kinase in chromosome 6; BRK: breast tumour kinase; CSK: C-terminal Src kinase; FAK: focal adhesion kinase; FES: feline sarcoma oncogene; FGR: Gardner-Rasheed feline sarcoma viral oncogene homologue; FRK: Fyn-related protein kinase; FYN: kinase associated with FYN gene; HCK: haemopoietic cell kinase; ITK: Lck-inducible T cell kinase; LCK: lymphocyte-specific protein tyrosine kinase; MATK: megakaryocyte associated tyrosine kinase; PYK2: proline rich tyrosine kinase 2; SRC: sarcoma; SRMS: src-related kinase lacking C-terminal regulatory tyrosine and N-terminal myristylation sites; TEC: tyrosine kinase expressed in hepatocellular carcinoma; TXK: kinase associated with the TXK gene; YES1: Yamaguchi sarcoma viral oncogene homologue 1. Reproduced with permission from Elsevier. This table was published in Signal Transduction (2nd edn) 2009; Ref [5], copyright Elsevier.
through the gp130 receptor family, and additionally with hormone receptors for erythropoietin, thrombopoietin, prolactin and growth hormone. JAK3 associates with the common \(\gamma\) chain cytokines, again in a limited population of cells. Tyk 2 is implicated primarily in signalling through the IL-12 receptor, and has a limited role in IFN\(\alpha\) and \(\beta\) signalling \[7\] (Table 2).

Regulation of the JAK–STAT pathway occurs through several mechanisms. One of the key mediators of this negative feedback is through the suppressor of cytokine signalling (SOCS) family of proteins. SOCS bind directly to activated JAKs and inhibit their catalytic activity.

Additional mechanisms of JAK regulation include inhibition by protein tyrosine phosphatases (PTPs) and through ubiquitylation, leading to enzyme degradation. STATs are similarly negatively regulated by PTPs, as well as by specific inhibitors known as protein inhibitors of activated STAT (PIAS) \[6\].

Much of what we know about JAKs comes from known human mutations and knockout mice. In humans, an autosomal recessive mutation resulting in deficiency of JAK3 gives the phenotype of severe combined immunodeficiency (SCID), with a complete absence of T and NK cells \[7\]. Constitutive activation of JAK2 and other
gain-of-function mutations in the JAK/STAT pathway have been implicated in myeloproliferative neoplasms, including essential thrombocytosis, polycythemia vera and myelofibrosis [8, 9]. In mouse models, JAK knockout mice display varying degrees of immunosuppression, from susceptibility to infections with Gram-negative bacteria and parasites (Tyk2), to SCID (JAK3), to full lethality with JAK1 and JAK2 knockouts [10-13]. Therapeutic targets in the JAK/STAT pathway, specifically JAK inhibitors, are currently under investigation for myeloproliferative neoplasms (JAK1 and JAK2), transplant rejection and autoimmune diseases, including RA [9, 14-16].

**SYK**

SYK is another cytoplasmic tyrosine kinase, and is a member of the ZAP70 (zeta-associated protein kinase of 70 kDa)/SYK family of non-receptor protein kinases. SYK is expressed on most hematopoietic cells, including B cells, immature T cells, macrophages, neutrophils and mast cells, and therefore plays a role in both the innate and adaptive immune response. It is also expressed on platelets and in some non-hematopoietic cells, including osteoclasts and lymphatic vessels [17, 18]. More recently, SYK has been discovered to play a role in IL-1b production through the NLRP3 (NOD-like receptor protein 3) inflammasome [18].

In immune cells, SYK signals through immunoreceptors, including B-cell receptors, pre-T-cell receptors and Fc receptors. Upon engagement of the antigen or antigen-presenting cell with an immunoreceptor, a member of the Src family of tyrosine kinases is recruited to the cytoplasmic membrane and phosphorylates a receptor-associated protein complex known as an immunoreceptor tyrosine-based-activation motif (ITAM). Once the ITAM is phosphorylated, it can then bind and activate SYK. Once it is bound, SYK autophosphorylates and then dissociates from the ITAM, with sustained kinase activated in the cytoplasm [17, 18]. Following the activation of SYK, several downstream events occur. In B cells, immature T cells, macrophages, neutrophils and mast cells, SYK participates in cytokine release and cell proliferation. In macrophages and mast cells, SYK is required for osteoclast differentiation and function [18, 19]. SYK has also been found to play a role in the regulation of the pro-inflammatory cytokine IL-12 in the context of Th1 cell differentiation [20].

**Table 2** JAK relationship with cytokine receptors

<table>
<thead>
<tr>
<th>JAK 1</th>
<th>JAK 2</th>
<th>JAK 3</th>
<th>TYK 2</th>
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</thead>
<tbody>
<tr>
<td>Type I and type II cytokine receptors</td>
<td>Common γ chain signals from IL-2 receptors</td>
<td>Common γ chain signals from IL-2 receptor receptor family</td>
<td>Type I IFNs γ/β</td>
</tr>
<tr>
<td>Common γ chain signals from IL-2 receptor family, IL-4 receptor family</td>
<td>Receptors for hormones</td>
<td>IL-2, -4, -7, -9, -15, -21</td>
<td>IL-12 receptor</td>
</tr>
<tr>
<td>IL-2, -4, -7, -9, -15, -21</td>
<td>erythropoietin</td>
<td>GM-CSF receptor family (IL-3 R, IL-5R GM-CSF-R)</td>
<td>B1 subunit (IL-12/IL-23)</td>
</tr>
<tr>
<td>gp130 receptor family IL-6, -11, -27, -31</td>
<td>thrombopoietin</td>
<td>gp130 receptor family IL-6, -11, -27, -31</td>
<td>GM-CSF receptor family (IL-3 R, IL-5R GM-CSF-R)</td>
</tr>
<tr>
<td>Type I IFNs (IFNα/β)</td>
<td>prolactin</td>
<td>Type I IFNs (IFNγ)</td>
<td>GM-CSF receptor family (IL-3 R, IL-5R GM-CSF-R)</td>
</tr>
<tr>
<td>Type II IFNs (IFNγ)</td>
<td>growth hormone</td>
<td>Type II IFNs (IFNγ)</td>
<td>IL-12 receptor</td>
</tr>
</tbody>
</table>

fibroblast-like synoviocytes [20]. In platelets, SYK is activated by three different cell surface receptors, and is involved in arterial thrombus formation through SLP76 [17, 19]. SYK is also involved in the differentiation of lymphatic vessels from the other blood vessels of the circulatory system [21].

Much of the current understanding of SYK function comes from in vitro studies and murine models. SYK-deficient mice demonstrate perinatal lethality, and an inability to produce mature B cells, arresting B-cell development in the pro-B- and pre-B-cell stage [22, 23]. In vitro, leukaemic cells from children with pro-B-cell leukaemia demonstrate markedly reduced levels of SYK [24]. Both of these models suggest that SYK is essential for normal B-cell maturation and viability. Recently Jakus et al. [25] have shown that mice who receive a bone marrow transplant with SYK-deficient stem cells are resistant to autoantibody-induced (K/BxN serum transfer) arthritis. Several SYK inhibitors are currently being investigated in clinical trials for human diseases, particularly RA [18].

**BTK**

BTK is another key intracellular kinase under investigation. It is a member of the Tec family of cytoplasmic protein tyrosine kinases. It is predominantly expressed in haematopoietic cells, including B cells, but also in cells of myeloid lineage such as macrophages, two cell lineages believed to be quite important in the treatment of inflammatory arthritis and other autoimmune diseases.

After an antigen engages the B-cell receptor, BTK is recruited to the cell membrane and phosphorylated by Src family members. Phosphorylated BTK recruits additional proteins to the plasma membrane. Subsequent phosphorylation and activation of phospholipase C-γ2 (PLC-γ2) affects calcium mobilization as well as resulting in potential effects on SYK and PI3K [26]. In addition to B-cell receptor signalling, BTK is also associated with macrophage signalling through the FcγRIII receptor and is involved in signalling downstream of the high-affinity receptor for IgE (FcεR) on basophils and mast cells [27] (Fig. 3).

Animal models of arthritis suggest that BTK inhibition can result in inhibition of B-cell receptor-dependent cell proliferation and a reduction of inflammatory cytokine production from myeloid cells (including TNF, IL-1 and IL-6) by preventing signalling through the FcγRIII receptor [28]. In humans, mutations in BTK can result in the development of X-linked agammaglobulinaemia (XLA), an immunodeficiency characterized by a defect in B-cell development and resulting in a significant absence of...

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**Fig. 2** Targeting SYK as a treatment for allergic and autoimmune disorders.

![Diagram of SYK signaling](https://example.com/syk_diagram.png)

Figure has been reproduced with permission from Informa Plc: Wong BR, Grossbard EB, Payan DG, Masuda ES. Targeting SYK as a treatment for allergic and autoimmune disorders. Expert Opin Investig Drugs 2004;13:743–62.
circulating B cells [29, 30]. Patients with XLA, also known as Bruton’s agammaglobulinaemia, are susceptible to recurrent infections but they can lead normal lives and are frequently treated with immunoglobulin.

BTK inhibition is being actively studied in lymphoma and other haematological malignancies. Based on the known target and the importance of these cell lines in autoimmunity, we are anticipating that we will see BTK inhibition explored in multiple human diseases, including RA, SLE, SS and possibly asthma.

**PI3K**

PI3Ks are a group of intracellular signalling molecules that phosphorylate the 3-hydroxyl group of the inositol ring of phosphatidylinositol lipid substrates. Their functions are diverse, affecting cell growth, survival, proliferation and metabolism in a variety of cell types. The PI3Ks can be divided into three classes based on their structure, regulation and lipid substrate specificity: class I, class II and class III. We will focus on the function of class I PI3Ks, as the role of class II and III PI3Ks in humans is not yet well understood [31, 32].

Class I PI3Ks consist of a regulatory subunit, p85, and a catalytic subunit, p110. Class I PI3Ks are present in all mammalian cell types, with a subset of p110 isoforms highly expressed in leucocytes. They are activated by G-protein-coupled receptors and tyrosine kinase receptors and act primarily through protein kinase B (Akt), with downstream effects through the mammalian target of the rapamycin pathway as well as other intracellular signalling pathways [31, 32].

In humans, class I PI3K gene mutations (PI3KCA) have been identified in many cancers and several non-selective class I PI3K inhibitors are in early stage clinical trials in oncology [33, 34]. However, there are potential toxicity issues with broad class I PI3K inhibition, and more selective inhibitors of class I PI3K p110 isoforms are being
studied in inflammatory diseases such as asthma, RA, SLE and certain haematological malignancies [35].

**Phosphodiesterase-4 inhibitors**

Phosphodiesterases are enzymes that hydrolyse the 3’ phosphate bond of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). By degrading these intracellular secondary messengers, PDEs regulate multiple downstream biologic processes. In mammals, PDEs are classified into 11 different subfamilies, PDE1–PDE11, based on their substrate specificity, tissue distribution, regulatory properties and amino acid sequence [36]. As each of the PDE families exhibit some degree of specificity, several PDE inhibitors have been developed, with widely varying clinical applications. As an example, PDE3 regulates cardiac and vascular smooth muscle contractility, as well as platelet aggregation through dual cAMP and cGMP specificity; pharmacological inhibitors of PDE3 include milrinone, which is used in heart failure, and cilostazol, used for intermittent claudication. PDE5 inhibitors, such as sildenafil, vardenafil and tadalafil, are cGMP specific and target vascular smooth muscle in the corpus cavernosum and pulmonary vasculature, with clinical applications in erectile dysfunction and pulmonary hypertension [36, 37].

PDE4 is a phosphodiesterase with specificity for cAMP, although it has more than 20 isoforms and is expressed in many different tissues, including in the brain, heart and lungs, as well as in inflammatory cells, including B cells, T cells, neutrophils, eosinophils, macrophages and mast cells [38, 39]. Several PDE4 inhibitors are in clinical trials for inflammatory diseases, including asthma, chronic obstructive pulmonary disease, inflammatory bowel disease, psoriasis and PsA [38]. PDE4 inhibitors have also shown therapeutic potential in mouse models of RA and in vitro suppression of TNF-α in human synovial cells [40]. Limitations in clinical trials have included off-target effects, including gastrointestinal events [38].

**Conclusion**

The field of rheumatology continues to change as we develop an increasing understanding of the diseases that we are treating and the pathways that are amenable to targeting. The new wave of emerging therapies in autoimmunity is based on the interruption of intracellular signalling through kinases. Through this process it is possible to modulate the function of cellular structures such as surface receptors, signalling proteins and transcription of nuclear proteins, thus influencing the behaviour of the cell types targeted. With these advances comes significant potential to develop highly effective orally bioavailable therapeutics. The targets generating increasing enthusiasm for the treatment of autoimmune and inflammatory diseases have included JAK, SYK, BTK, PI3K, PDE4 and others. Ultimately, however, it will take human trials to fully understand the potential risks and benefits of these approaches across a number of diseases.

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**Rheumatology key messages**

- Novel therapeutics are being developed in autoimmune diseases, particularly RA.
- Targeting of selective kinase inhibition appears efficacious for RA.
- Human studies will be necessary to understand the risk–benefit ratio of new treatment for RA.

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**References**