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Kinase inhibitors in the treatment of immune-mediated disease

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Abstract

Protein kinases are fundamental components of diverse signaling pathways, including immune cells. Their essential functions have made them effective therapeutic targets. Initially, the expectation was that a high degree of selectivity would be critical; however, with time, the use of "multikinase" inhibitors has expanded. Moreover, the spectrum of diseases in which kinase inhibitors are used has also expanded to include not only malignancies but also immune-mediated diseases. At present, thirteen kinase inhibitors have been approved in the United States, all for oncologic indications. However, there are a growing number of molecules, including several Janus kinase inhibitors, that are being tested in clinical trials for autoimmune diseases such as rheumatoid arthritis, psoriasis and inflammatory bowel diseases. It appears likely that this new class of immunomodulatory drugs will have a major impact on the treatment of immune-mediated diseases in the near future.

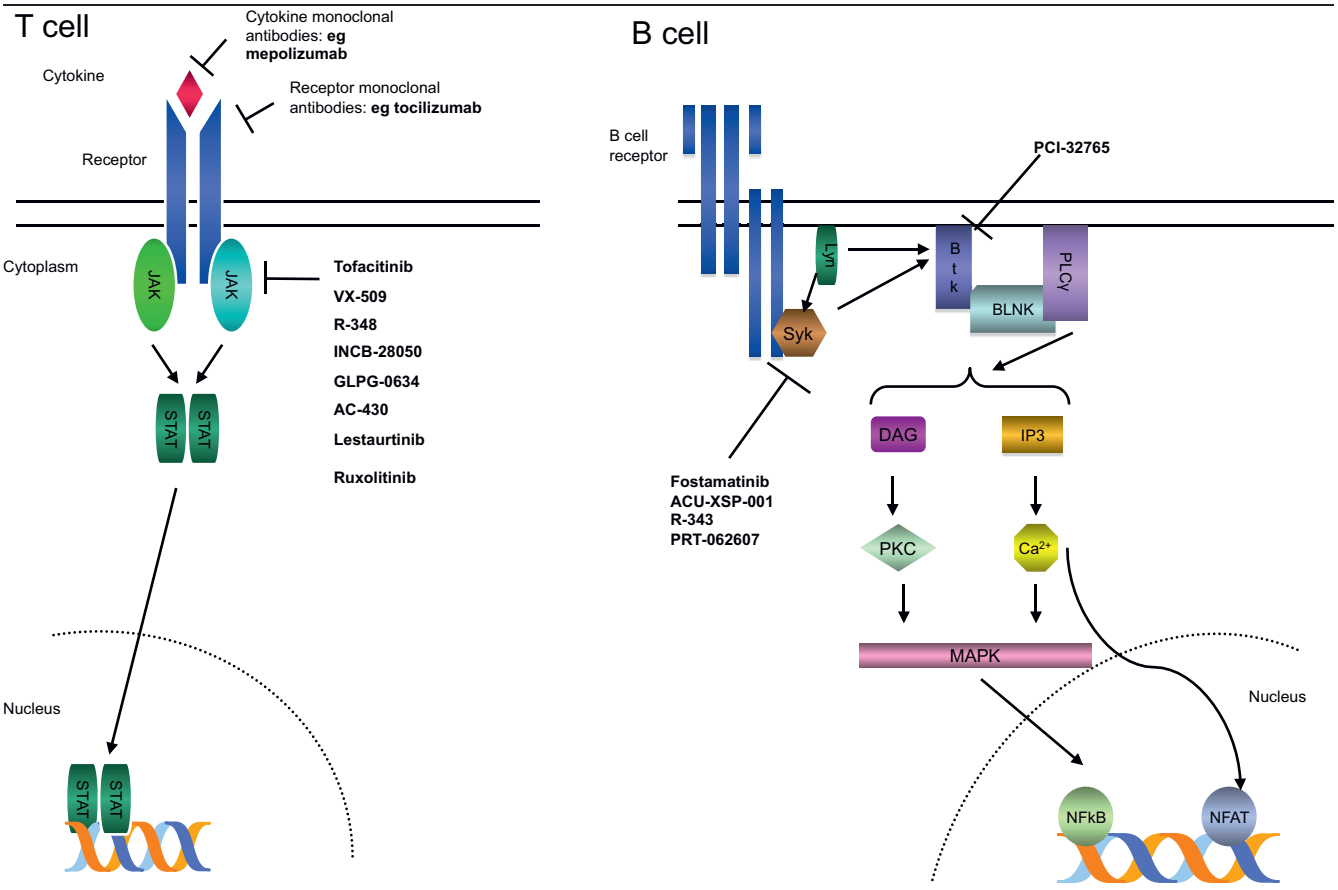
Introduction

Work over the last 20 years has firmly established that reversible protein phosphorylation is a fundamental mechanism of cell signaling. Protein kinases, also termed phosphotransferases, are the enzymes that catalyze the transfer of the γ phosphate of a purine nucleotide triphosphate (i.e. ATP and GTP) to the hydroxyl groups of their protein substrates. Importantly, many of the major classes of receptors that trigger immune cell activation are linked to protein phosphorylation and physically associate with kinases. In fact, the first event in T cell receptor (TCR), B cell receptor (BCR), NK (natural killer) and Fc receptor signaling is phosphorylation of receptor subunits on tyrosine residues. Likewise, cytokine receptors, especially Type I/II cytokine receptors, signal directly by activating kinases, which phosphorylate receptor subunits and thereby initiate signaling. This has led to the idea that blocking kinases may be an effective way to block immune cell activation and, in turn, treat autoimmune disease. Multiple kinase inhibitors are now in clinical trials for rheumatoid arthritis, inflammatory bowel disease, psoriasis and other diseases. This is surely an area that will expand in the next few years, so it is appropriate to briefly review some of the key issues.

Kinases: the first step in immune cell signaling

There are 518 kinases in the human genome, divided into eight major groups. The first step in signaling by multi-chain immune recognition receptors, which include the TCR, BCR, Fc receptors and others, is tyrosine phosphorylation of the receptor itself and associated adapter molecules like LAT (linker for activation of T cells). This is mediated initially by Src family protein tyrosine kinases, followed by kinases such as Syk (spleen tyrosine kinase) or Zap-70, Tec family PTKs and later by serine-threonine kinases, such as mitogen activated protein kinases (MAPKs) and protein kinase C (PKC) family (see Figure 1). Initial protein phosphorylation ultimately links membrane events to calcium modulation, cytoskeletal rearrangement, gene transcription and other canonical features of lymphocyte action. Cytokines that use Type I and II cytokine receptors signal via the activation of receptor-associated Janus kinases (Jaks).

Figure 1. Proximal signaling pathways upon stimulation of immune receptors in B and T cells



Type I and II cytokine receptors associate with Janus kinases (Jaks). Cytokine binding activates Jaks, which then phosphorylate cytokine receptors allowing STAT (signal transducer and activator of transcription) DNA-binding proteins to attach to receptors and become phosphorylated. STAT activation leads to their dimerization and translocation to the nucleus where they regulate gene expression. Targets along the signal transduction pathway, including specific kinase inhibitors, are shown (left). In B cells, antigen ligation leads to activation of three main protein tyrosine kinases (PTKs) — the Src-family kinases Lyn, Syk and the TEC-family kinase Btk. Syk phosphorylates adaptor protein BLNK and, along with Btk, activates PLC γ. Activation of PLC γ leads to the release of intracellular Ca^{2+} and activation of protein kinase C (PKC), which activate mitogen-activated protein kinases (MAPKs). The MAPK cascade activates transcription factors nuclear factor-κB (NF-κB) and nuclear factor of activated T cells (NFAT), allowing gene regulation (right). Abbreviations: BLNK: B cell linker protein; MAPK, mitogen-activated protein kinase; NFAT, nuclear factor of activated T cells; NF-κB, nuclear factor-κB; PLC γ, Phospholipase γ; PIP3K, phosphatidylinositol triphosphate kinase; PKC, protein kinase C; STAT, signal transducer and activator of transcription; Syk: Spleen tyrosine kinase; DAG, diacylglycerol; IP3, inositol 1,4,5-triphosphate.

Other cytokines, such as stem cell factor or transforming growth factor family cytokines, bind to receptors with intrinsic tyrosine or serine-threonine kinase properties respectively. Receptors for cytokines such as interleukin (IL)-1 and tumor necrosis factor (TNF) are not themselves directly associated with kinases, but they too link to downstream kinase cascades.

Where it all started

Since all protein kinases bind ATP, the prospect of developing a therapeutically useful kinase inhibitor seemed daunting, since many enzymes use ATP as a substrate, and the structure of protein kinases is highly conserved. Nonetheless, it is now appreciated that kinase inhibitors have become one of the most successful new categories of drugs. The story begins with the Abl tyrosine kinase. BCR-Abl is a fusion protein that results from a chromosomal translocation (Philadelphia chromosome) in patients with chronic myeloid leukemia (CML) and this kinase seemed to represent an ideal target, despite the caveats of targeting protein kinases [1]. In fact, the inhibitor imatinib has revolutionized the treatment of CML with relatively modest side effects [2]. Imatinib was subsequently shown to inhibit several unrelated tyrosine kinases [3,4]. In fact, these actions led to imatinib being used successfully in other

malignancies, such as gastrointestinal stromal tumors, and the hypereosinophilic syndrome [3,4], by inhibiting kinases Kit [5] and PDGFR [6]. The success of imatinib and the epidermal growth factor receptor inhibitors erlotinib and gefitinib led to the problem of tumors developing drug resistance associated with mutations in the targeted kinase [7]. This led to the development of new multikinase inhibitors such as dasatinib and sunitinib, which are also now FDA approved [8,9]. Currently, there are several small molecule kinase inhibitors in routine clinical use, all of which are FDA approved for oncologic indications (see Table 1).

Targeting cytokine signaling by inhibiting Janus kinases

The role of cytokines in mediating an immune response has made them attractive targets for immunomodulatory drug development. Consequently, monoclonal antibodies against cytokines (e.g. mepolizumab against IL-5) and cytokine receptors (e.g. tocilizumab against IL-6 receptor), as well as recombinant receptors, have been used successfully in the clinic [10]. Of note, a large subset of cytokines (roughly 60), which bind type I/II cytokine receptors and includes many interleukins, interferons, colony stimulating factors and other cytokines [11], has a shared mechanism of signal transduction. The Type I/II cytokine receptors bind Jaks, which are essential for signaling [12-14].

The importance *in vivo* of Jaks was first established by the identification of patients with immunodeficiency and *JAK3* mutations and by knockout mice. Mutation of *JAK3* results in a severe combined immunodeficiency (SCID), characterized by an almost complete absence of T cells and NK cells, with defective B cells [15-18]. In contrast with other Jaks, JAK3 is primarily expressed in hematopoietically derived cells, where it is associated with the IL-2 receptor common γ chain (cγc) and mediates signaling by IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21, cytokines that are critical for the development and maturation of T cells [19]. The profound, but selective, phenotype associated with JAK3 deficiency led to the suggestion that targeting Jaks might be a strategy for the development of a new class of immunomodulatory drugs [20].

JAK inhibitors in the clinic

Tofacitinib

Tofacitinib, formerly designated CP-690,550, was one of the first JAK inhibitors to enter the clinic. It inhibits JAK3 and JAK1 and, to a lesser extent, JAK2, but has little effect

Table 1. US FDA-approved kinase inhibitors

Agent	Targets for therapeutic activity	US FDA-approved indication
Direct kinase inhibitors by competing for the ATP-binding pocket		
Imatinib	BCR–ABL, PDGFR and KIT	CML and GIST
Dasatinib	BCR–ABL	CML
Nilotinib	BCR–ABL	CML
Gefitinib	EGFR	Non-small cell lung cancer
Erlotinib	EGFR	Non-small cell lung cancer and pancreatic cancer
Lapatinib	EGFR and ERBB2	Breast cancer
Sunitinib	VEGFR2, PDGFR and KIT	Renal cell carcinoma, GIST, pancreatic cancer
Sorafenib	VEGFR2 and PDGFR	Renal cell carcinoma and hepatocellular carcinoma
Pazopanib	VEGFR2, PDGFR and KIT	Renal cell carcinoma
Crizotinib	ALK/c-MET	Non-small cell lung cancer
Vemurafenib	BRAF	Melanoma
Vandetanib	VEGFR-2, EGFR, RET and ErbB-1	Medullary thyroid cancer
Ruxolitinib	JAK1/JAK2	Myelofibrosis
Indirect kinase inhibitors by binding to FK506 binding protein 12 (FKBP12)		
Sirolimus	mTOR	Solid organ and bone marrow transplantation
Everolimus	mTOR	Renal cell carcinoma, Subependymal Giant Cell Astrocytoma (SEGA) associated with Tuberous Sclerosis (TS) and Progressive Neuroendocrine Tumors of Pancreatic Origin (PNET)
Temsirolimus	mTOR	Renal cell carcinoma
Monoclonal antibodies binding to receptor tyrosine kinases		
Trastuzumab	ERBB2	Breast cancer
Cetuximab	EGFR	Colorectal cancer, and squamous carcinoma of head and neck
Panitumumab	EGFR	Colorectal cancer
Bevacizumab	VEGF	Colorectal cancer, non-small cell lung cancer, breast cancers, glioblastoma and renal cell carcinoma

Abbreviations: CML, chronic myeloid leukemia; EGFR, epidermal growth factor receptor; FDA, Food and Drug Administration; GIST, gastrointestinal stromal tumor; PDGFR, platelet-derived growth factor receptor; VEGFR2, vascular endothelial growth factor receptor 2.

on TYK2 [13,21]. Consequently, tofacitinib potently inhibits cγc cytokines but also blocks IFN-γ, IL-6 and, to a lesser extent, IL-12 and IL-23. Functionally, tofacitinib affects both innate and adaptive immune responses by inhibiting pathogenic Th17 cells and Th1 and Th2 cell differentiation [13]. In Phase II trials in rheumatoid arthritis, tofacitinib as monotherapy met the American College of Rheumatology 20% improvement (ACR20) criteria in 61-70% of patients at doses between 5 and 15 mg twice daily [22]. These results were replicated in phase III trials at doses 5 and 10 mg twice daily [23]. In combination with methotrexate, tofacitinib met its primary endpoint (pre-agreed measure of success) in a highly active disease group [24,25]. Additionally, tofacitinib significantly reduced progression of structural damage compared with placebo in patients with active rheumatoid arthritis on methotrexate [26]. Tofacitinib was also found to be beneficial in patients with rheumatoid arthritis who were refractory to biologics [27]. Tofacitinib is also under clinical investigation for psoriasis, inflammatory bowel disease and prevention of transplant rejection.

The major adverse effects of tofacitinib include increased incidence of infections and increased low density lipoprotein levels; however, the incidence of infection with opportunistic organisms appears to be limited [28]. The former is perhaps expected given the roles of diverse cytokines in host defense. The latter is likely related to inhibition of IL-6 signaling [29]. Anemia and neutropenia were also reported [30], presumably related to JAK2 inhibition and interference with cytokines, such as erythropoietin and colony stimulating factors [31]. Little reduction in $CD4^+$ T cells has been seen, but significant reduction in NK cells and $CD8^+$ T cells does occur, with an as yet undetermined infection risk [32,33]. Thus, the major adverse effects of tofacitinib appear to be consequences of blocking cytokine signaling as one might expect, and seemingly not related to off-target effects. The balance of efficacy and safety of tofacitinib compared to standard of care therapy will need to be ascertained in clinical trials and, if approved, ultimately in the routine clinical use of these drugs.

VX-509

VX-509 is another inhibitor designed to selectively inhibit Jak3. A phase IIa study has just been completed and, like tofacitinib, use of VX-509 was also associated with a dose-dependent increase in clinical response in rheumatoid arthritis.

GLPG0634

The results of a Phase II trial of a selective Jak1 inhibitor GLPG0634 have also been released, and it too is efficacious and causes no unexpected adverse advents.

Ruxolitinib

As gene targeting of either *Jak1* or *Jak2* in mice was embryonically lethal, it was thought that pharmacological inhibition might be problematic. However, the discovery that *JAK2* gain-of-function mutations underlie polycythemia vera and myelofibrosis provided the impetus to purposely target JAK2. This led to the development of the drug, ruxolitinib, which blocks JAK1 and JAK2. In a phase II study, patients receiving ruxolitinib for myelofibrosis showed significant clinical improvement. Despite the drug's ability to block both JAK1 and JAK2, it was well tolerated [34]. In addition, efficacy was seen in patients that did not exhibit *JAK2* mutations, suggesting that the drug might be affecting kinases other than JAK2. This drug was recently approved by the FDA for treatment of myelofibrosis.

As cγc cytokines employ both JAK1 and JAK3 for signaling, ruxolitinib and tofacitinib will block many of the same cytokines. It is therefore of interest to note that, in a phase II study in rheumatoid arthritis, ruxolinitib had efficacy that was not dissimilar from tofacitinib [35].

INCB028050

Another selective JAK1 and JAK2 inhibitor, INCB028050, showed dose-dependent efficacy in active rheumatoid arthritis patients refractory to disease-modifying drugs and biologics, with the most frequent side effects being headache, upper respiratory infections and diarrhea [36].

Other JAK inhibitors

Other JAK inhibitors are also in development and clinical trials for oncologic [37-49] and autoimmune indications [50]are ongoing (see Table 2). The comparative efficacy and toxicity of the various JAK inhibitors will be important to follow in longer-term studies.

Targeting other kinases

Despite their scientific appeal, multiple attempts to generate clinically useful p38 MAPK inhibitors have generally failed, either due to toxicity or inadequate efficacy. Only one compound, VX-702, yielded a modest effect on clinical signs and symptoms in rheumatoid arthritis and a transient effect on biomarkers of inflammation [51].

Zap70 or Syk mediate signals from receptors that contain immunoreceptor tyrosine-based motifs (ITAMs) (Figure 1). Like JAK3, Zap70 deficiency also causes SCID, but in this case there is preferential loss of CD8+ T cells [52]. For this reason, Zap70 is a rational target; unfortunately, a clinically useful compound has not emerged. In contrast, the Syk

Table 2. Kinase inhibitors in development for autoimmunity

Agent	Targets for therapeutic activity	Indication/Phase
Tofacitinib	JAK3/JAK1/JAK2	RA/Phase III Psoriasis/Phase II IBD/Phase II
VX-509	JAK3	RA/Phase II
R-348	JAK3	RA/Phase I
Ruxolitinib	JAK1/JAK2	Psoriasis/Phase II
INCB-028050	JAK1/JAK2	RA/Phase II
GLPG-0634	JAK1/JAK2/TYK2	RA/Phase II
AC-430	JAK2	RA/Phase I Lymphoma/Phase I
Lestaurtinib	FLT3/TrkA/JAK2	AML/Phase III Psoriasis/Phase II Pancreatic cancer/Phase II
Fostamatinib	Syk/FLT3/KIT/LCK	RA/Phase III NHL/Phase I/II CLL/Phase I/II
ACU-XSP-001	Syk	Asthma/Phase II
R-343	Syk	Asthma/Phase I
PRT-062607	Syk	RA/Phase I CLL/Phase I NHL/Phase I
Sotrastaurin (AEB071)	PKC	Psoriasis/Phase II IBD/Phase II Solid organ transplantation/Phase II Diffuse Large B-Cell Lymphoma/Phase I
PLX 5622	CSF1R (Fms)	RA/Phase I

Abbreviations: ACU-XSP-001, small interfering RNA that silences Syk gene; CLL, chronic lymphoid leukemia; CSF1, colony stimulating factor 1 receptor; FLT3, ms-related tyrosine kinase 3; NHL, non-Hodgkin lymphoma; PKC, protein kinase C; TrkA, aka high affinity nerve growth factor receptor or neurotrophic tyrosine kinase receptor type 1 or TRK1-transforming tyrosine kinase protein.

inhibitor, fostamatinib, has been found to have efficacy in a Phase II study in rheumatoid arthritis [53]. Fostamatinib also targets FLT3, KIT, LCK among other kinases, possibly contributing to its clinical benefit [54]. Toxicities include diarrhea, infections, neutropenia and hypertension. A subsequent study, involving patients refractory to biologics, failed to meet its primary endpoint; however, this was attributed to study design issues [55]. Because of its critical role in B cell function, another logical target is Tec family member Btk. Such inhibitors are first being used in the setting of B cell lymphoma, but could be useful in autoimmune diseases as well.

Members of the PKC family are activated downstream of a variety of key immunologic receptors. One PKC inhibitor, sotrastaurin, has been tested in kidney allograft rejection, and showed moderate efficacy but less nephrotoxicity compared with calcineurin inhibitor regimens [56]. Phase I and II trials on inflammatory bowel disease, psoriasis [57] and Diffuse Large B-Cell Lymphoma are ongoing.

Colony stimulating factor 1 receptor (c-Fms) is a transmembrane receptor tyrosine kinase. A phase 1 trial of a CSF1R inhibitor (PLX5622) in rheumatoid arthritis is ongoing.

Conclusions and future prospects

The last few years have produced great advances in deciphering the mechanisms involved in intracellular signaling pathways. Consequently, our understanding of the molecular basis of immune cell activation is vastly more sophisticated. These advances have been associated with the identification of effective, safe kinase inhibitors. Many of these agents were developed for the treatment of cancer, and the pleiotropic effects of kinase inhibitors, initially thought of as a disadvantage, have proved to be beneficial. The use of kinase inhibitors has expanded beyond malignancies to autoimmune diseases with favourable safety profile. Also, multikinase inhibitors that have broad effects have been less problematic than one might have envisioned, but it is too early for us to know how useful such inhibitors will be in the treatment of immune-mediated disease. Highly selective kinase inhibitors, such as p38 MAPK inhibitors, have been disappointing in the treatment of autoimmune diseases, either due to toxicity and/or lack of efficacy. Whether broad spectrum, multi-kinase inhibitors or highly selective second and third generation kinase inhibitors will ultimately be more efficacious and safe remains to be established. The issue of acquired resistance, while a very real problem in oncology, presumably will not be a major issue in autoimmune diseases.

At this point, it seems likely that we will see the development of many more immunosuppressants that inhibit kinases expressed in immune cells. It will also not be a surprise if many drugs that enter clinical use as treatments for cancer are found to be efficacious in the treatment of autoimmune disease or transplant rejection. There is ample precedent for this with drugs such as cyclophosphamide, azathioprine and methotrexate. It also bears pointing out that not all kinase inhibitors exert their effect by competing for ATP in the kinase domain. A prime example is the drug rapamycin (also called sirolimus), as an approved immunosuppressant effective for allograft rejection and graft versus host disease [58-61]. It binds FK binding protein 12 and mammalian target of rapamycin (mTOR) complex 1 and indirectly inhibits the kinase mTOR [62-65], a kinase that is activated by a number of growth factor receptors and cytokines [66]. Thus, indirectly inhibiting kinases by targeting their associated complexes is another effective strategy for developing drugs. Regardless, the number of kinase inhibitors and the range of clinical indications are likely to expand dramatically in the next few years. Precisely how these drugs are used in combination with or in place of other therapies such as biologics, steroids, etc. remains to be determined.

Abbreviations

TCR, T cell receptor; BCR, B cell receptor; NK, natural killer; PKC, protein kinase C; JAK, Janus kinase; IL, interleukin; TNF, tumor necrosis factor; CML, chronic myeloid leukemia; SCID, severe combined immunodeficiency; MAPK, mitogen-activated protein kinase; Syk, spleen tyrosine kinase; ITAMs, immunoreceptor tyrosine based motifs; mTOR, mammalian target of rapamycin.

Competing Interests

J.O'S and National Institutes of Health (NIH) hold patents related to targeting JAKs as targets for immunomodulatory agents. J. O'S and the NIH have a Collaborative Research Agreement and Development Award with Pfizer.

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