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Translating JAKs to Jakinibs

Massimo Gadina,* Danielle A. Chisolm,† Rachael L. Philips,† Iain B. McInnes,‡ Paul S. Changelian,§ and John J. O’Shea†

The discovery of JAKs and STATs and their roles in cytokine and IFN action represented a significant basic advance and a new paradigm in cell signaling. This was quickly followed by discoveries pointing to their essential functions, including identification of JAK3 mutations as a cause of SCID. This and other findings predicted the use of therapeutically targeting JAKs as a new strategy for treating immune and inflammatory diseases. This now is a reality with seven approved jakinibs being used to treat multiple forms of arthritis, inflammatory bowel disease, and myeloproliferative neoplasms, and numerous ongoing clinical trials in other settings. This story provides interesting insights into the process of translating basic discoveries and also reveals the need to return to basic work to fill gaps that now become apparent. The Journal of Immunology, 2020, 204: 2011–2020.

Interferons, cytokines, and growth factors were first identified more than half a century ago; however, the advances in molecular biology techniques, the pace of discovery in this field exploded in the 1980s (1). The elucidation of the diversity of these factors provided enormous insights into hematopoiesis and immunoregulation, making it hard to overstate the clinical significance of these advances. Immunologists can be appropriately very proud of the efficiency by which the avalanche of their basic discoveries was quickly translated into many new, effective, and safe drugs. Cytokines themselves were quickly used to treat hematologic, oncologic, and infectious diseases. This was quickly followed by targeting cytokines using mAbs to treat autoimmune disease. Indeed, the era of “biologics” undeniably revolutionized the treatment of autoimmune diseases, from rheumatoid arthritis (RA) and other inflammatory arthropathies to inflammatory bowel disease (IBD) and psoriasis (2–5). Despite the success of biologics, not all patients respond to these drugs, and others do not respond completely. This necessitated consideration of newer strategies, including investigations into whether targeting cytokine signaling might provide a safe and effective alternative. In this short review, our goal is to tell the story of the discovery of JAKs, how their role in cytokine signaling was elucidated, and how a new class of drugs was conceived and developed. We will discuss drugs that are approved and in development and how we expect this class of drugs will be used in the future. We would also be remiss if we did not point out the basic science gaps and what is needed to understand cytokine signaling more completely for even more therapeutic opportunities. As a caveat, this is a fast-moving field. We have tried our best to be as current as possible; however, for clinical issues, be sure to check the research yourself.

Discovery of Janus kinases and their role in cytokine signaling

The elucidation of the function of JAKs began with the discovery of IFNs by Isaacs and Lindenmann (6) and the recognition that IFNs induced rapid transcriptional programs in the antiviral response (7). This area attracted the attention of a number of investigators, as it provoked the corollary that rapid membrane-to-nucleus signaling occurred to engage the transcriptional apparatus. George Stark and colleagues (8) tackled this problem by generating a series of mutant cell lines resistant to the actions of IFNs. A key finding came from George’s colleague, Sandra Pellegrini (9), who showed that one IFN-resistant line was overcome by complementation with a gene encoding a structurally distinct kinase, tyrosine kinase 2 (TYK2), which had been cloned by Krolewski and colleagues shortly before (10). Fortunately, right around this time, the broad importance of tyrosine kinases in signal transduction was becoming increasingly clear, and the race was on to discover new kinases using different screening strategies. Shortly after the discovery of TYK2 came the discovery of two other kinases, termed JAK1 and JAK2 (11). These kinases were named by Andrew Wilks for the Roman god Janus, the two-faced god of doorways, based on the presence of tandem kinase-like and kinase domains (a remarkably prescient designation, as it turns out). Using the different Stark lines, it became clear that TYK2 and JAK1 were both required for type I IFN signaling, and IFN-γ signaling used JAK1 and JAK2 (9–12). Later, we and others discovered a fourth member of the family, JAK3, which...
was found to be activated by IL-2 (13). Later, IL-12 was also found to signal via TYK2 (14). The completion of the human genome ultimately revealed that out of a total of 518 kinases, only these four kinases had the characteristic tandem kinase/kinase-like domains.

JAKs play a critical role in cytokine signaling (Fig. 1). JAKs bind to a subset of cytokine receptors (βc cytokines, γc cytokines, IFNs, gp130 cytokines, IL-10 family, TS LP, IL-12, IL-13, and IL-17), and upon cytokine-activation, JAKs use ATP to phosphorylate themselves as well as the intracellular tail of the receptor subunits. This creates docking sites for the recruitment of downstream signaling molecules. A critical discovery in understanding cytokine signaling was the identification of another class of JAK substrates by members of Jim Darnell’s laboratory (15, 16), the tyrosine-phosphorylated STAT family of DNA binding proteins. These transcription factors carry out the rapid transcriptional programs, thus solving a large part of the mystery of how IFNs could so quickly mediate changes in gene expression.

In vivo function of JAKs: Mendelian disorders, mutations, and knockouts

The efforts of Stark, Darnell, and colleagues provided striking and tantalizing evidence for a new paradigm in cell signaling: a direct membrane-to-nucleus scheme that explained how cytokines could rapidly and directly impact gene transcription. What was less clear was the role of the four JAKs in vivo. During this time, new cytokines continued to be discovered, and the question was how important were JAKs for all these factors. Spoiler alert — we now know that there are 57 cytokines that signal by the four JAKs (Table I; more on this later).

The first in vivo proof of the criticality of JAKs came from the recognition of humans with a primary immunodeficiency. This appreciation began with the discovery that mutations of the common γ-chain, γc (encoded by IL2RG), a shared receptor subunit that pairs with ligand-specific subunits to form receptors for IL-2, 4, 7, 9, 15, and 21, underlies the disorder X-linked SCID (17). The absence of signaling by all these cytokines has dramatic effects on lymphocyte development and function. With the recognition that JAK3 specifically associates with γc, it was predicted that mutations of JAK3 might be a cause of autosomal recessive SCID. In fact, this turned out to be the case; patients with mutations of IL2RG and JAK3 phenocopy one another in that T and NK cells are lacking and B cells are dysfunctional in both diseases (Table I) (18, 19). Of note, patients with immunodeficiency due to JAK3 or IL2RG mutations do not have clinical problems beyond immunodeficiency, and stem cell transplantation is curative. This points to selective functions of JAK3 and was the rationale for efforts to therapeutically target this kinase. Of note, somatic JAK3 mutations were recently identified in NK cell enteropathy (20).

Shortly after the discovery of JAK3–SCID, knockout mice targeting Jak3, jak2, jak1, and Tyk2 were reported. Deletion of Jak3 in mice results in a phenotype mirroring human autosomal SCID, with immune deficits but not global abnormalities beyond immune cells (21). In contrast to Jak3 knockout mice, germline deletion of Jak2 in mice has embryonically lethal results because of impaired definitive hematopoiesis and the essential function of Jak2 in erythropoietin (EPO) signaling (22, 23). Conditional Jak2 deletion in young adult mice also severely affects thrombopoiesis, granulopoiesis, and monocytopenia (24). Selective deletion of Jak2 in platelets and megakaryocytes does not cause thrombocytopenia but rather causes thrombocytosis and increased circulating thrombopoietin (25). Conversely, somatic GOF mutations in the JAK2 kinase-like domain are associated with myeloproliferative neoplasms (MPN), including polycythemia rubra vera, essential thrombocytopenia, and myelofibrosis, the most common being the V617F mutation (26). Of note, the importance of activating mutations in the JAK kinase-like domain in flies anticipated disease in humans, as a mutation termed tumorous lethal of the Drosophila JAK, Hopscotch, caused leukemia (27). Germline deletion of Jak1 causes perinatal lethality, as Jak1 is essential for signaling by all class II cytokine receptors, γc using cytokines, and gp130 using cytokines (Table I). Biallelic germline mutations identified in a single patient were associated with atypical mycobacterial infection and early onset metastatic bladder cancer, although the patient had normal numbers of T, B, and NK cells (28).
Genome-wide association studies have implicated JAK1 in humans cause multiple system immune disease (29), and polymorphisms of JAK1 are associated with juvenile idiopathic arthritis (30). Consistent with Pellegrini’s seminal work using the Stark cell lines, Tyk2−/− mice have impaired responses of IFNs and IL-12 (31, 32). Humans with TYK2 deficiency have severe infections with bacterial, viral, and fungal pathogens (33–35). In addition, these patients have variable allergic disease and infections with bacterial, viral, and fungal pathogens (33–35).

Gain-of-function mutations of JAK1 in humans cause multiple system immune disease (29), and polymorphisms of JAK1 are associated with juvenile idiopathic arthritis (30). Consistent with Pellegrini’s seminal work using the Stark cell lines, Tyk2−/− mice have impaired responses of IFNs and IL-12 (31, 32). Humans with TYK2 deficiency have severe infections with bacterial, viral, and fungal pathogens (33–35). In addition, these patients have variable allergic disease and elevation of IgE and variable impairment of IL-6 signaling. Genome-wide association studies have implicated TYK2 in susceptibility to autoimmune diseases, including systemic lupus erythematosus (SLE), Crohn’s disease, ulcerative colitis (UC), psoriasis, multiple sclerosis, systemic sclerosis, inflammatory myopathies, primary biliary cirrhosis, and type 1 diabetes (36). Importantly, some protein-coding variants in TYK2 have been reported to provide protection against various autoimmune diseases, including multiple sclerosis, RA, psoriasis, and SLE (37–39).

### Jakinibs as a new class of drugs for autoimmune disease

The dramatic impact of JAK3 mutations in humans led to the proposal that targeting JAKs with small-molecule kinase inhibitors could represent a new strategy in the treatment of immune-mediated diseases (19) (Fig. 1). The verification of the critical but distinct role of JAKs in different mouse models provided a strong rationale for pursuing the development of jakinibs. The first reported in vivo use of a jakinib showed that tofacitinib (also known as CP 690,550; Anatomical Therapeutic Chemical [ATC] code: L04AA29) effectively blocked allograft rejection (40). Later, tofacitinib and other jakinibs were tested in an array of preclinical models of immune-mediated diseases, including arthritis, allergy, graft-versus-host disease (GVHD), psoriasis, and lupus. First-generation jakinibs were all found to inhibit more than one JAK.

Based on genetic and preclinical data, the expectation was that such drugs would be efficacious; what was less clear was whether they would be safe. Today, seven jakinibs are approved for various indications, including RA, psoriatic arthritis, UC, MPN, acute GVHD and (canine) allergic dermatitis. Six jakinibs are approved for humans (tofacitinib, ruxolitinib [ATC code: L01XE18], baricitinib [ATC code: L01XE37], peficitinib, fedratinib [ATC code: L01XE57], and upadacitinib [ATC code: L04AA44]) and one is approved for dogs (oclacitinib; ATCvet code: QD11AH90) (Fig. 1). The safety and efficacy of first-generation nonselective jakinibs have been established in numerous clinical studies, and numerous clinical trials are ongoing for the treatment of many other diseases. Notably, the first selective jakinib, upadacitinib, was recently approved for the treatment of RA (discussed in next section).

### Arthritis

Tofacitinib and baricitinib are approved for the treatment of patients with RA with inadequate response to conventional therapies. Tofacitinib and baricitinib can be used as monotherapy or with methotrexate, and the combination is equivalent to or even statistically superior to anti-TNF mAb comparators. Jakinibs are also effective in patients who have had an inadequate response to biologics other than TNF inhibitors. Peficitinib was approved in Japan for the treatment of RA. Tofacitinib has also been approved for the treatment of psoriatic arthritis. The longstanding efficacy and safety of tofacitinib has been documented for 9.5 y (41), and recent long-term extension studies suggest that baricitinib enjoys similar tolerance. Whereas jakinibs clearly affect inflammation, jakinibs also appear to control pain independent of inflammation (42).

How cytokines influence pain and inflammation as distinct but related parameters is clearly an area in need of further investigation. Preclinical models support the use of jakinibs in spondyloarthritis (43), and recently, tofacitinib was shown to offer benefit in axial spondyloarthritis (43–45).

### Inflammatory bowel disease

Tofacitinib is approved for moderate-to-severe UC, showing an increase in the likelihood of a steroid free remission. However, tofacitinib was not effective in Crohn’s

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### Table I. Summary of usage of JAKs by various cytokines

<table>
<thead>
<tr>
<th>JAK</th>
<th>Cytokine</th>
<th>Mouse Knockout</th>
<th>Human LOF</th>
<th>Human GOF</th>
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<tbody>
<tr>
<td>JAK1</td>
<td>γc cytokines (IL-2, IL-4, IL-7, IL-9, IL-15, IL-13, and IL-21)</td>
<td>Perinatal lethality, T” B^NK” SCID</td>
<td>Atypical mycobacterial infection, metastatic bladder cancer</td>
<td>Systemic immune dysregulation</td>
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<td></td>
<td>TSLP gp130 cytokine family: (IL-6, IL-11, IL-31, OSM, CNTF, LIF, CT-1, and NNT-1)</td>
<td>IFN-γ, IFN-α, IFN-β, and IFN-λ</td>
<td></td>
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<td></td>
<td>IL-10–like cytokines: (IL-10, IL-19, IL-20, IL-22, IL-24, and IL-26)</td>
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<td></td>
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<tr>
<td>JAK2</td>
<td>βc cytokines (IL-3, IL-5, and GM-CSF)</td>
<td>Embryonic lethal, anemia</td>
<td>Not reported</td>
<td>MPN leukemia, lymphoma</td>
</tr>
<tr>
<td></td>
<td>TSLP gp130 cytokine family: (IL-6, IL-11, IL-31, OSM, CNTF, LIF, CT-1, and NNT-1)</td>
<td>IFN-γ, IFN-α, IFN-β, and IFN-λ</td>
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<td></td>
<td>IL-12, IL-23</td>
<td></td>
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<tr>
<td>JAK3</td>
<td>γc cytokines (IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21)</td>
<td>T” B^NK” SCID</td>
<td>Primary immunodeficiency, variants protect against autoimmunity</td>
<td>leukemia</td>
</tr>
<tr>
<td>TYK2</td>
<td>IFN-α, IFN-β, and IFN-λ</td>
<td>Viral susceptibility, diminished responses to type 1 IFNs, IL-12, and IL-23</td>
<td></td>
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<td>gp 130 cytokines</td>
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<td>IL-10-like cytokines (IL-10, IL-19, IL-20, IL-22, IL-24, and IL-26)</td>
<td>IL-13</td>
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<td>IL-12, IL-23, and IL-27</td>
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*Some evidence points to a role for TYK2 in signaling by gp130 and other cytokines; however, the cell, cell-state, and species-specific requirements for gp130 cytokines and likely many other cytokines are incompletely understood.*

CNTF, ciliary neurotrophic factor; CT-1, cardiotropin-1; G-CSF, G-CSF (encoded by CSF3); GH, growth hormone; LIF, leukemia inhibitor factor; NNT-1, neurotrophin-1 (encoded by CLEC71 gene); OSM, oncostatin M; TPO, thrombopoietin; TSLP, thymic stromal lymphopoietin.
disease, but other jakinibs are being studied in this disease (see below). In a phase 2 study, peficitinib showed no dose response in patients with moderate-to-severe UC, but evidence of efficacy was suggested at dosages $\geq 75$ mg/d (47). Primary biliary cholangitis is an autoimmune disease affecting the liver, and baricitinib is being tested in this disease (NCT03742973).

*Dermatological diseases.* Allergic dermatitis (eczema) is a common disorder affecting humans (and dogs), but at present, the only jakinib approved for this indication, oclacitinib, is approved for dogs. However, a phase 2 trial has been conducted showing that significantly more patients with atopic dermatitis who received baricitinib (4 mg/d) improved compared with controls (48). A phase 3 trial with baricitinib is currently underway (NCT03334422) (Table II). In addition, topical tofacitinib and delgotcinib (JTE-052) have been tested in allergic dermatitis with promising results (JapicCTI-152887) (49–52). One prominent feature of allergic dermatitis is pruritus, resulting in an itch–scratch cycle. Cytokines have also been linked to the molecular pathogenesis of pruritus through expression of IL-4Rs on neurons (53), as such treatment with jakinibs has the potential to break the scratch–itch cycle.

Alopecia areata is an autoimmune disease manifested by hair loss that affects roughly 2% of the population. Preclinical and small open-label studies have shown jakinibs to be useful in this disorder (54–56). Both ruxolitinib and tofacitinib were found to be safe and efficacious for the treatment of moderate-to-severe and severe alopecia areata, respectively (55, 56). This is another setting in which topical jakinibs may be useful (57). One of the most striking findings is that JAK inhibition not only limits immune responses but may also promote hair growth (58). If this is the case, jakinibs might be useful for treating hair loss beyond loss caused by autoimmune mechanisms.

Psoriasis is another dermatological disease in which cytokines are known to play a prominent role (59). Tofacitinib has shown efficacy in late-phase clinical trials for the treatment of this disease. Only the 10 mg, twice daily dosage of tofacitinib showed noninferiority to TNF inhibitors, and the Food and Drug Administration issued a complete response letter indicating that it would not be able to approve tofacitinib for psoriasis. Baricitinib, was found to be efficacious in a phase II study that tested patients with moderate-to-severe psoriasis, but only at dosages $\geq 8$ mg/d (60).

GVHD is a critical complication of allogenic stem cell transplant for patients with cancer or hematologic/immunologic genetic disorders; in fact, ruxolitinib is approved for acute GVHD. Skin disease is one manifestation of GVHD, and corticosteroids are the standard first-line treatment for GVHD (61). However, some patients become steroid-refractory or steroid-dependent. Therefore, some trials found jakinibs as a promising new treatment option for GVHD (62). Other immune diseases with prominent skin involvement for which jakinibs are being studied include the following: systemic sclerosis (63), vitiligo (62), actinic dermatitis (62), sarcoidosis (64), palmoplantar pustulosis (65), and amyopathic dermatomyositis-associated lung disease (66).

*Interferonopathies.* SLE is a disorder in which IFNs and other cytokines appear to be important contributors to pathogenesis. In a randomized double-blind placebo-controlled trial, 314 patients who received baricitinib at 4 mg/d (not the 2 mg/d dosage) had significant improvement in signs and symptoms of active SLE (67). In a small study in patients with familial chilblain lupus due to TREX1 mutations, baricitinib showed efficacy (68). Dermatomyositis has also been shown to be associated with an “IFN signature,” and tofacitinib is being studied for this indication (NCT03002649).

Autoinflammatory diseases, such as chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature and stimulator of IFN genes (STING)–associated vasculopathy with onset in infancy are monogenic interferonopathies and have been treated with jakinibs (69–72). A trial is ongoing in Aicardi–Goutié`res syndrome (NCT03921554). Down syndrome has also been argued to be an interferonopathy, and jakinibs have been suggested as a possible therapy.
Table III. Major side effects of jakinibs

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<td>Infection</td>
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More recently, JAK inhibition has been reported to be efficacious in a child with interferonopathy due to USP18 deficiency (74).

Myeloproliferative neoplasms and cancer. Mutations in the kinase-like domain of JAK2 are a common pathogenetic mechanism underlying the spectrum of diseases encompassing polycythemia vera, essential thrombocytosis, and myelofibrosis. Ruxolitinib and fedratinib are approved for the treatment of these disorders, and momelotinib (another JAK1/JAK2 inhibitor) is being studied in MPNs (75, 76) (NCT02124746, NCT02101268, NCT01969838, NCT02515630, NCT03441113, NCT01236638, and NCT00935987).

In addition, many types of cancers, from leukemias and lymphomas to solid organ malignancies, exhibit activation of the JAK/STAT pathway (77). This can either be due to mutation of JAKs or, indirectly, by autocrine cytokine production. Many trials are ongoing to test the efficacy of jakinibs in this setting (NCT03117751 and NCT04021082). In addition, preclinical data has shown that targeting JAKs can modulate the tumor microenvironment and facilitate immunotoxin access to malignant cells (78). This could be yet another strategy in which jakinibs might be useful in the treatment of cancer.

Side effects of first-generation jakinibs

Given that first-generation jakinibs block multiple JAKs and therefore block many cytokines, it is not surprising that these drugs have side effects, especially when used at higher doses. Common side effects include infection, anemia and hematological effects, hyperlipidemia, and other effects (Table III).

Infection. The essential function of JAKs in host defense is well-established, so it was no surprise that infections are a common adverse event in patients treated with jakinibs. In fact, serious infections, such as tuberculosis, fungal, and opportunistic infections, are more frequently seen in patients on jakinibs. Generally though, the incidence of infections in jakinib-treated patients is equivalent to that observed in patients on TNF-blockers and other biological therapies (79–82). One reason why infections are not more common in patients taking jakinibs versus biologics is that not all cytokines are JAK dependent (e.g., TNF, IL-1, IL-17, and IL-8). The second reason relates to dose. In some patients with inherited autoinflammatory disease, the doses required to control symptoms were high, and treatment was associated with BK viremia (83). A third consideration is that in some settings multiple immunosuppressive drugs are used, thus increasing the incidence of infection (84). In transplant trials, tofacitinib was found effective compared with cyclosporin A; however, these patients were also treated with mycophenolate and steroids. Perhaps unsurprisingly, there was a higher incidence of infections and some patients developed posttransplant Epstein–Barr virus–related lymphoproliferative disease (84).

An infectious complication that is commonly associated with jakinibs is reactivation of varicella zoster virus (herpes zoster) (81, 85–87). Overall, the rate of zoster associated with tofacitinib is roughly the rate seen with biologics (88). Because of the increased risk, vaccination is recommended (88–91). There has been one case of fatal John Cunningham virus infection with ruxolitinib (92).

Anemia and other hematological effects. Treatment with first-generation jakinibs leads to a decrease of hemoglobin. Presumably this is related to inhibition of JAK2, which interferes with the action of EPO and related cytokines that are dependent upon JAK2 (93). Other changes include decreased numbers of lymphocytes, NK cells, neutrophils, and platelets (41, 94). Especially with higher doses of jakinibs, there may be an increased risk of venous thrombosis and pulmonary embolism.

Hyperlipidemia. Jakinibs alter cholesterol metabolism and have modest but statistically significant increases in high- and low-density lipoproteins and triglyceride levels. However, the ratio of low density lipoprotein-C to high density lipoprotein-C is not altered, and whether these changes increase the risk of cardiovascular disease is unclear (95–97).

Other effects. Jakinibs also reduce creatinine clearance (a measure of kidney function) (98), increase creatine phosphokinase levels without causing muscle disease (89), and increase levels of liver enzymes (99–101). Gastrointestinal perforations have been reported, possibly because of the blockade of IL-6 signaling, but the cytokines involved in this adverse event have not been definitively clarified. Lymphoma and other malignancies have been observed in patients treated with jakinibs, but autoimmune diseases, such as RA, are themselves associated with an increased incidence of cancers, especially lymphoma.

Next-generation, selective jakinibs

Whereas first-generation jakinibs have been shown to be efficacious and reasonably safe, they inhibit many cytokines. This raises the obvious question as to whether targeting a single JAK might provide efficacy with fewer side effects because a narrower spectrum of cytokines would be inhibited. Of course, this presupposes that we are aware of the essential cytokine profiles that mediate the core effector biology of the major immune-mediated diseases and, moreover, that this will translate across to tractable JAK selectivity. Multiple second-generation jakinibs have been developed, with one approved (upadacitinib) and others at various phases of clinical trials.

JAK1 inhibitors. Whereas many cytokines signal via JAK1, the spectrum of cytokines blocked with a JAK1 antagonist is narrower than first-generation jakinibs, and avoidance of JAK2 inhibition in particular could be advantageous. Upadacitinib, filgotinib (ATC code: L04AA45), and abrocitinib are selective JAK1 inhibitors in which results of phase 3 clinical trials have been completed (102–106). As indicated, upadacitinib has been approved for RA. In patients with inadequate response to methotrexate, upadacitinib was statistically superior to the TNF inhibitor adalimumab (103). Filgotinib showed efficacy in RA patients with inadequate responses to biologics (106) and showed efficacy in a phase 2 trials in ankylosing
spondylitis and psoriatic arthritis (46, 107). In a phase 2 dose-ranging study, abrocitinib was found to improve psoriasis and atopic dermatitis (108, 109). Itacitinib is being studied in phase 3 trials in GVHD (NCT03139604) and malignancies (hematologic and solid tumor) as well as RA, UC, and psoriasis (NCT01634087). Other JAK1-selective drugs being tested include SHR0302 for IBD (NCT03675477 and NCT03677648) and INCB054707 for hydradenitis suprativa (NCT03569371 and NCT03607487). Overall, a substantial number of JAK1-selective inhibitors have been generated and are being studied in a wide range of diseases.

Adverse events found with JAK1-selective inhibitors are increased lipids and infections, including herpes zoster and dyslipidemia. Upadacitinib was also associated with anemia and thromboembolic events, suggesting that at higher doses upadacitinib might inhibit JAK2. Abrocitinib is associated with neutropenia, possibly related to inhibition of IL-6 signaling, and thrombocytopenia, also possibly related to JAK2 inhibition.

Inhaled jakinibs are in development and testing (e.g., AZD0449 and NCT03766399). For example, a selective JAK1 inhibitor has been optimized for inhaled delivery and reverses pathology in a rodent asthma model (110).

**TYK2 inhibitors.** TYK2 is used by a relatively narrow spectrum of cytokines, including IL-12, IL-23, and type I and type III IFNs (Table 1). The demonstrated role of these cytokines in autoimmunity, along with data pointing to the protective effect of inactivating TYK2 coding variants, provides a rationale for selectively targeting TYK2 (111). BMS-986165 is a selective TYK2 inhibitor, and its efficacy has been documented in a dose-ranging phase 2 trial in psoriasis (112). Adverse events included upper respiratory tract infection, acne, and one case of melanoma; no anemia, neutropenia, or thrombocytopenia were reported (113). Of note, BMS-986165 does not target the catalytic domain; rather, it targets the kinase-like domain (Fig. 1) (113). It is being studied in SLE, psoriasis, psoriatic arthritis, and IBD. Breprocitinib (PF-06700841) is a TYK2/JAK1 inhibitor being tested in psoriasis, IBD, and alopecia areata.

**JAK3 inhibitors.** Based on genetic data, a selective JAK3 inhibitor would be expected to only inhibit the six γc cytokines (IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21), a much narrower spectrum of action compared with other jakinibs. Decernotinib was reported to be a selective inhibitor of JAK3 and phase 2 trials showed efficacy in RA; however, drug-drug interactions were found, limiting the use of decernotinib (114–117). Decernotinib was associated with neutropenia, suggesting the possibility of activity toward JAK1 or JAK2 and incomplete selectivity for JAK3 (118).

**JAK2 inhibitors.** Inhibitors, like fedratinib, with greater selectivity for JAK2 have been developed primarily for the treatment of MPN [NCT03755518 (119)]. Gandotinib (LY2784544) is a potent inhibitor of JAK2 activity that also shows increased potency for the JAK2^V617F mutant kinase (120).

**Combination therapy.** Although there is an effort to narrow the spectrum of kinase inhibition, there are also advantages in targeting JAKs along with other kinases. PF-06651600 is a combined JAK3/Tec family kinase inhibitor (121). The Tec family of tyrosine kinases encompasses Itk, Bruton tyrosine kinase (Btk), Rlk, and Bmx, which have important immunological functions. Btk has been widely studied because of its critical role in B cell development and evidence showing that pharmacologic blockade Btk is effective in ameliorating lymphoma progression and arthritis models. PF-06651600 is being studied in RA, alopecia areata, Crohn’s disease, and UC (122, 123) (NCT02974868, NCT02958865, NCT02969044, NCT03395184, and NCT03978520). SYK is a nonreceptor tyrosine kinase highly expressed in hematopoietic cells and plays important roles in immune cell differentiation and signaling. Targeting SYK alongside JAKs may also extend the range of cytokines targeted for therapies. Gusacitinib (ASN002) is an oral dual JAK/SYK inhibitor shown to have effects on the treatment of moderate-to-severe atopic dermatitis by diminishing cellular infiltrates, ameliorating epidermal barrier abnormalities, and suppressing inflammation (124).

Cancers are one circumstance in which multikinase inhibitors might be especially attractive. Cerdulatinib is another SYK/JAK inhibitor being studied in lymphoma and leukemia (125) (NCT04021082) but could also be useful for immune and inflammatory disorders. Pacritinib is a JAK2/FLT3 inhibitor being investigated in myelofibrosis, leukemia, small cell lung cancer, and GVHD.

An alternative strategy is to use jakinibs with other kinase inhibitors. Itacitinib (ATC code: L04AA46) is being tested in combination with the PI3Kδ inhibitor parsaclisib (64, 126) and ibritumomab (ATC code: L01XE27; NCT02760485). AZD4205, a JAK1-selective inhibitor, is being studied with the EGFR inhibitor osimertinib (ATC code: L01XE35) for cancer (NCT03450330). Still another strategy is to use selective jakinibs in combination; for instance, itacitinib is being tested with low-dose ruxolitinib (NCT03144687).

In cancer, one could imagine that jakinibs could represent a double-edged sword, as they could antagonize the production and actions of IFN-γ, which helps eliminate tumors. Indeed, somatic LOF mutations of JAK1 can arise in tumor cells and are associated with resistance to IFN-γ and cancer evasion. However, blocking IFN signaling with jakinibs also has the capacity to reverse expression of inhibitory receptors and in this way can promote responsiveness to checkpoint blockade therapy (127). A phase 2 study is ongoing in hematological malignancies using anti-PD1 immunotherapy (pembrolizumab) and ruxolitinib (NCT04016116) (128).

**Conclusions**

Targeting cytokines both inside and outside the cell is now a reality. Both strategies have generated remarkably effective and reasonably safe drugs that represent extraordinary advances in the treatment of autoimmune diseases. The drugs are highly efficacious but are not without side effects. There is clearly a great deal of work to be done to identify the best drug or combination of drugs and the best route of administration for the right disease at the right time. Aside from topical and inhaled jakinibs, TD-1473 is a nonabsorbable pan-jakinib being studied in IBD (NCT03758443, NCT03920254).

Looking back, it is worth considering some of the lessons that the story of JAKs and jakinibs have provided. First, it is certainly true that strong basic efforts in combination with human and mouse genetics predicted efficacy (i.e., the criticality...
of JAK3 was dramatically established by the elucidation of SCID due to JAK3 mutations). It is not surprising that jakinibs are efficacious in treating autoimmune disease; what was less clear was safety. Knocking out Jak1 and Jak2 is lethal, and patients with JAK3 and TYK2 mutations have serious infections. Therefore, it was much less clear whether jakinibs would be safe. It took clinical trials with careful attention to dose to arrive at reasonably safe regimens. However, whereas traditional clinical trials establish doses that are safe and effective in large populations of individuals, it would be ideal if we had reliable biomarkers to accurately measure inflammation, knowing that individuals represent diverse collections of polymorphisms that affect the biology of cytokines during different aspects of their disease. We also know that gene regulation represents the integration of multiple signaling pathways, and experience shows that use of combined therapies are often inevitable for many diseases. Genes encoding cytokines, cytokine receptors, and transcription factors all reside within genomic hubs called “superenhancers,” indicative of the exquisite regulation that governs these key factors. Jakinibs preferentially impact genes with superenhancer architecture (129). Hence, more precise, real-time measurements of interdicting signaling hopefully will provide clues as to how to use combined therapies most effectively. An obvious unresolved question is whether biologics and jakinibs can be used safely in combination, even if for a short term as induction therapy. A few case reports have been presented; however, there are no formal trials thus far.

Although there is still a great deal of work ahead therapeutically, it would also be misleading to imply that we really understand cytokine signaling as well as we should. Remarkably, there are still gaps in conditional knockout genetic models. For instance, to this day, no conditional JAK3 knockout mouse has been generated, so a complete understanding of the tissue-specific functions of JAK3 is lacking. Selective drugs can help fill the gaps in our knowledge, but more genetic tools will be important to confirm findings.

Structural understanding of JAKs has improved (130), but even more detailed structural and cell biology insights into cytokine receptor/JAK interactions and mechanisms of signaling are needed, and to date there is no crystal structure of an entire JAK molecule. Targeting the pseudokinase domain of TYK2 is an exciting advance, but a complete understanding of the structure of cytokines/cytokine receptors/JAKs should provide many more opportunities. With the advances in imaging from cryogenic electron microscopy to superresolution microscopy, we will likely have a more sophisticated view of cytokine signaling and cell biology. As technologies change, there is little doubt that we will envision cytokine signal transduction and gene regulation will change significantly. With this, keep your eyes open for many new therapeutic opportunities along the way.

Acknowledgments

We regret that, because of space constraints, many relevant publications were omitted from this review.

Disclosures

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