anus kinases to jakinibs: from basic insights to clinical practice

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Abstract

Rheumatology key messages

- Cytokines are critical for host defence but are major drivers of autoimmune disease.
- Janus kinases are essential for signalling by a large family of cytokines.
- Janus kinase inhibitors represent a new strategy for the treatment of immune and inflammatory diseases.

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A very brief history of cytokines and cytokine signalling

With the current reality of Janus kinase (JAK) inhibitors (jakinibs) being used to treat thousands of patients for a wide array of diseases, it may seem anachronistic to go back in time and review the history of cytokines. However, as more agents are developed and more patients are treated, it becomes increasingly important to consider the broad range of factors potentially impacted by the inhibition of JAKs. While it is far beyond the scope of a brief review to examine this topic in great detail, it is necessary to understand which cytokines are JAK dependent and which are not as we consider both efficacy and side effects of jakinibs. Indeed, there are >200 factors that can be referred to as cytokines, and while the term cytokine can be a useful shorthand, in other ways it may be misleading. Furthermore, some cytokines have names that do not readily suggest their JAK dependency. For all these reasons, a brief review of cytokines is in order.

Discovered in 1957, it has been argued that interferons (IFNs) are the first recognized cytokines. In reality, much earlier, in 1905, it was appreciated that the production of red blood cells was controlled by what was referred to as a hormone. By 1957 it was understood that the kidney produces erythropoietin (EPO) [1]. Similarly, two other hormones, prolactin (PRL) and growth hormone (GH), were discovered in the 1930 s and 1950 s, respectively, with no hint that they were related to IFNs. In 1969, the term lymphokine first came into use to denote products of lymphocytes with immunoregulatory function. Later, the terms IL and cytokine became more widely used when it was better appreciated that numerous cells beyond lymphocytes produce immunoregulatory factors. So, while many secreted factors that regulate growth and differentiation of diverse cells were rapidly being identified, what was not appreciated was the similarity and differences among these factors, the receptors they employed and the modes by which they signalled.

The molecular biology revolution that began in the 1980 s led to the cloning of IFNs, ILs and various growth factors and was followed shortly thereafter by the cloning of cytokine receptors. As numerous factors were being identified with a dizzying array of names denoting diverse putative properties, the cloning of cytokines began to reveal their relatedness and differences as well as homologies of their cognate receptors. We now understand that one important group of structurally

related cytokines includes IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-11, IL-12, IL-13, IL-15, IL-21, IL-23, IL-27, IL-31 and IL-35. Also included in this group are GH, PRL, EPO, thrombopoietin (TPO), G-CSF, GM-CSF, leptin, leukaemia inhibitory factor, oncostatin M (OSM), ciliary neurotrophic factor, cardiotropin-1 (CT1), cardiotropin-like cytokine factor 1 (also called neurotrophin-1 or NNT-1) and thymic stromal lymphopoietin (a total of 29 type I cytokines). Added to this list are the type I IFNs, including IFNs encoded within the chromosome 9 cluster: IFN-α (*IFNA1, 2, 4, 5, 6, 7, 8, 10, 13, 14, 16, 17, 21*), IFN-β (*IFNB*), IFN-ε (*IFNE*), IFN-κ (*IFNK*), type II IFN or IFN-γ, type III IFNs [IFN-λ1 (*IFNL1*, aka IL-29), *IFNL2* (IL-28A), *IFN3* (IL-28B), *IFNL4*, IFN-Ω (*IFNW1*)] and IL-10-related cytokines (IL-10, IL-19, IL-20, IL-22, IL-24 and IL-26) (28 type II cytokines). These cytokines have a conserved structure and are referred to as 4-α-helical cytokines [2].

In thinking about the clinical utility of jakinibs and their side effects, it is easy to focus on cytokines we commonly associate with autoimmune disease; however, we still have much to learn about other members of this family. While rheumatologists are very cognizant of the role of cytokines like IL-6, IL-12 and IFN-γ in inflammation, other factors like OSM may be less familiar, in part because of its name. Yet evidence points to OSM as an important driver of IBD [3]. Similarly, the function of thymic stromal lymphopoietin as an important driver of allergic disease is also not obvious because of its name [4, 5]. Importantly, both thymic stromal lymphopoietin and OSM are dependent upon JAKs and so may be very relevant to patients on jakinibs.

The second reason for grouping these cytokines together is that receptors used by these seemingly diverse factors are also structurally related. Hemopoietin or type I cytokine receptors are transmembrane molecules that bind many of the ILs, colony-stimulating factors and hormone cytokines like EPO, TPO, GH, PRL and leptin (Table 1). These receptors have a conserved amino acid motif (WSXWS), which defines membership in this family. Closely related are the type II cytokine receptors, which include receptors for IFNs and IL-10 [6]. Although structurally similar to type I cytokine receptors, type II receptors lack the WSXWS motif; nonetheless, it is useful to lump type I and II receptors together because the intracellular domain of all these receptors binds JAKs and relies on these enzymes for signalling.

TABLE 1

Summary of usage of JAKs by various cytokines

JAK	Cytokine
JAK1	γc cytokines
	IL-13
	TSLP

JAK	Cytokine
	gp130 cytokines
	IL-10 family cytokines
	IFN-γ, IFN-α, IFN-β, IFN-λ
JAK2	βc cytokines
	TSLP
	gp130 cytokines
	Leptin, GH, PRL, EPO, TPO
	IFN-γ
	IL-12, IL-13, IL-23
JAK3	γc cytokines
TYK2	IFN-α, IFN-β, IFN-λ

JAK	Cytokine
	gp130 cytokines*
	IL-10 family cytokines
	IL-12, IL-13, IL-23, IL-27

Summary of usage of JAKs by various cytokines. IFN-λ1, IFN-λ2 and IFN-λ3 are also known as IL-29, IL-28a and IL-28b, respectively. βc cytokines: IL-3, IL-5 and GM-CSF. IL-10 family cytokines: IL-10, IL-19, IL-20, IL-22, IL-24 and IL-26. γc cytokines: IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21. Gp130 family cytokines: IL-6, IL-27, oncostatin M, ciliary neurotrophic factor, leukaemia inhibitor factor, cardiotropin-1 and neurotrophin-1 (encoded by *CLCF1* gene). *In some systems, TYK2 seems to be important for signalling 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.

G-CSF is encoded by CSF3; CLCF1: cardiotropin-like cytokine factor 1; TSLP: thymic stromal lymphopoietin.

More on the details of the JAKs in a moment, but in reflecting on the spectrum of cytokines that use this mode of signalling, it is clear that nearly every biologic process is affected, from the growth, differentiation and metabolism of diverse cells and tissues to hematopoiesis, host defence, anti-viral responses and immunoregulation. Again, a detailed review of phenotypes associated with deficiency of each of these 57 cytokines would be unwieldy; nonetheless, the biology of these factors needs to be kept in mind when considering the positive and negative actions of jakinibs. A few illustrative examples will be provided, especially as the phenotypes associated with the different JAKs are considered.

Equally important to understand as we consider blocking intracellular signalling is that the term cytokine encompasses a large number of factors that bind multiple classes of receptors structurally unrelated to type I/II cytokine receptors (Fig. 2). While many are very important in terms of host defence and immunopathology, these receptors do not signal via JAKs and employ distinct modes of intracellular signalling. This is important not only considering the circumstances in which jakinibs may not be efficacious, but also in terms of understanding why they are safer than one might imagine, especially with respect to immunosuppression.

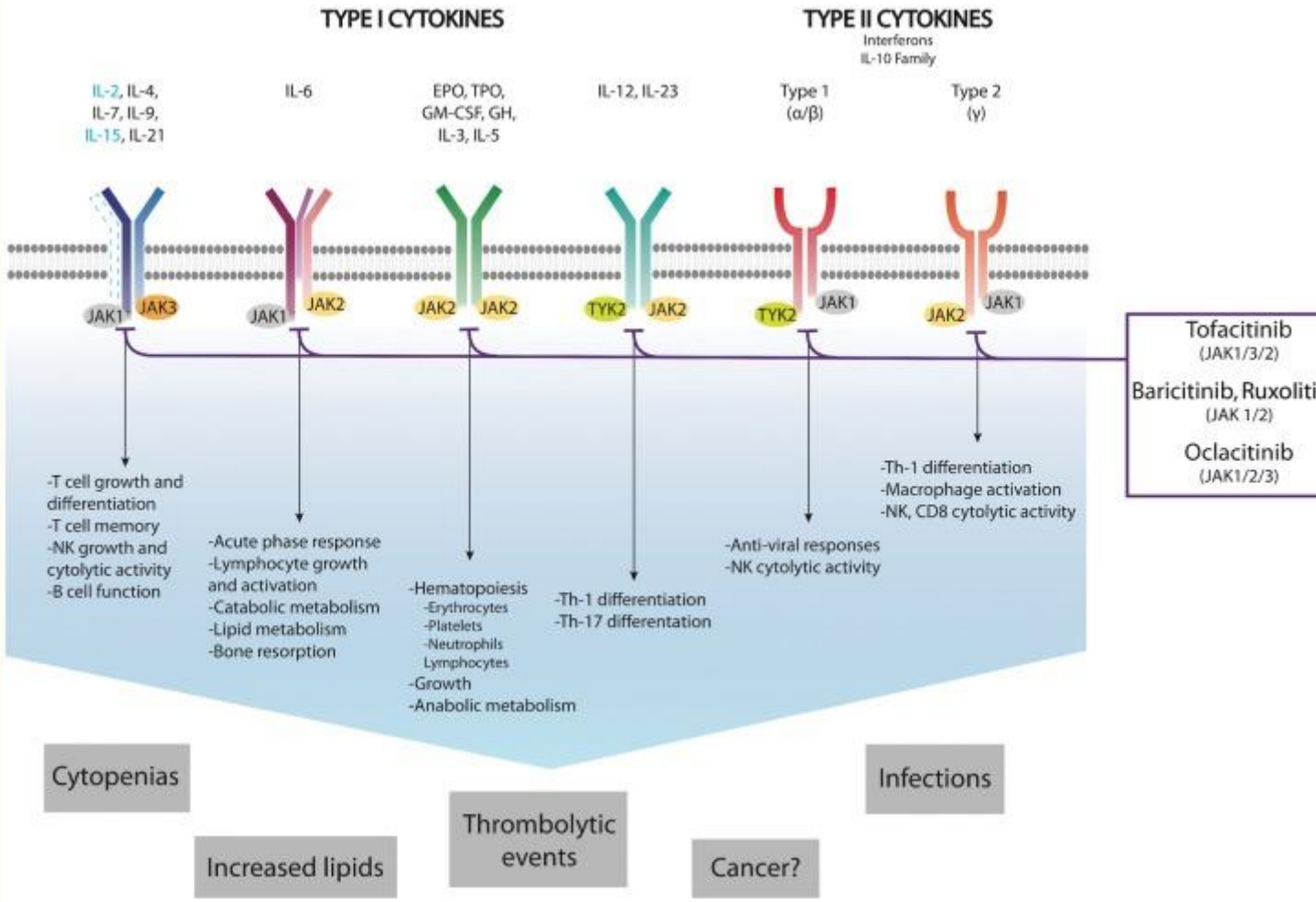

FIG. 2

JAK usage and putative relationship to adverse events

Different cytokine receptors bind different combinations of JAKs to activate different programs in cells. First-generation jakinibs broadly impact many cytokines, whereas selective inhibition of JAKs has a more restricted action and in principle is likely to have a narrow spectrum of side effects.

Some cytokines bind transmembrane receptors with intrinsic kinase domains such as those that bind receptor tyrosine kinases. Examples include stem cell factor, macrophage colony-stimulating factor and platelet-derived growth factor. Architecturally similar is the serine–threonine kinase family of receptors that bind transforming growth factor and related factors. TNF and the 18 other members of the TNF superfamily are key drivers of immune and inflammatory diseases, which bind to 18 structurally conserved receptors [7]. TNF superfamily receptors signal via adapter molecules that link the receptor to the kinases that activate nuclear factor κB (NF-κB) transcription factor and to cysteine proteases (caspases). The prototypical pro-inflammatory cytokine IL-1 binds to a different class of receptors (IL-1R), comprised of 11 members, which also signals through NF-κB [8]. Yet another family, the IL-17R family, is comprised of five members and also employs NF-κB, in addition to other intracellular signal transduction pathways [9]. Lastly, IL-8 and other chemokines bind to seven transmembrane receptors. It has been argued that chemokine receptors can engage JAKs, but the dependence upon JAKs *vs* classic modes of G protein-coupled signalling has not been fully resolved [10].

It is perhaps surprising, given the numerous cytokines that rely on JAKs, that this family is comprised of just four members: JAK1, JAK2, JAK3 and TYK2 [11, 12]. The carboxy terminus

represents the catalytic domain, which is homologous to the other 518 kinases in the human genome. Adjacent to the kinase domain is a key feature of the JAKs that gives them their names, a regulatory kinase-like domain, also referred to as the pseudokinase domain; the kinase and kinase-like domains represent the two faces of JAKs. Much experimental work has pointed to the importance of this regulatory domain, but its critical role is dramatically illustrated by mutations seen in the spectrum of diseases termed myeloproliferative neoplasms (MPNs; see below) [13]. For these reasons, inhibitors are in development that target this domain [14, 15]. The amino-terminal FERM (band 4.1, ezrin, radixin, moiesin) domain is a region through which JAKs interact with the cytosolic domain of cytokine receptors.

JAKs are phosphotransferases and their enzymatic function is activated by receptor engagement by cytokines [11, 12]. Cytokine-activated JAKs use adenosine triphosphate (ATP) to phosphorylate each others' tyrosine residues as well as the intracellular tail of the receptor subunits, creating docking sites that recruit downstream signalling molecules (Fig. 1). A critical subset of substrates that bind to phosphorylated cytokine receptors is the signal transducer and activator of transcription (STAT) family of DNA binding proteins [16]. Receptor-bound STATs are themselves tyrosine phosphorylated by JAKs. STAT phosphorylation mediates dimerization and translocation to the nucleus. Nuclear accumulation of the STATs allows DNA binding and activation of gene transcription and thus establishes a remarkably direct, evolutionarily conserved membrane-to-nucleus signalling pathway. There are seven mammalian STATs: STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B and STAT6. By virtue of selective binding to cytokine receptors, different cytokines have the capacity to preferentially recruit different STATs.

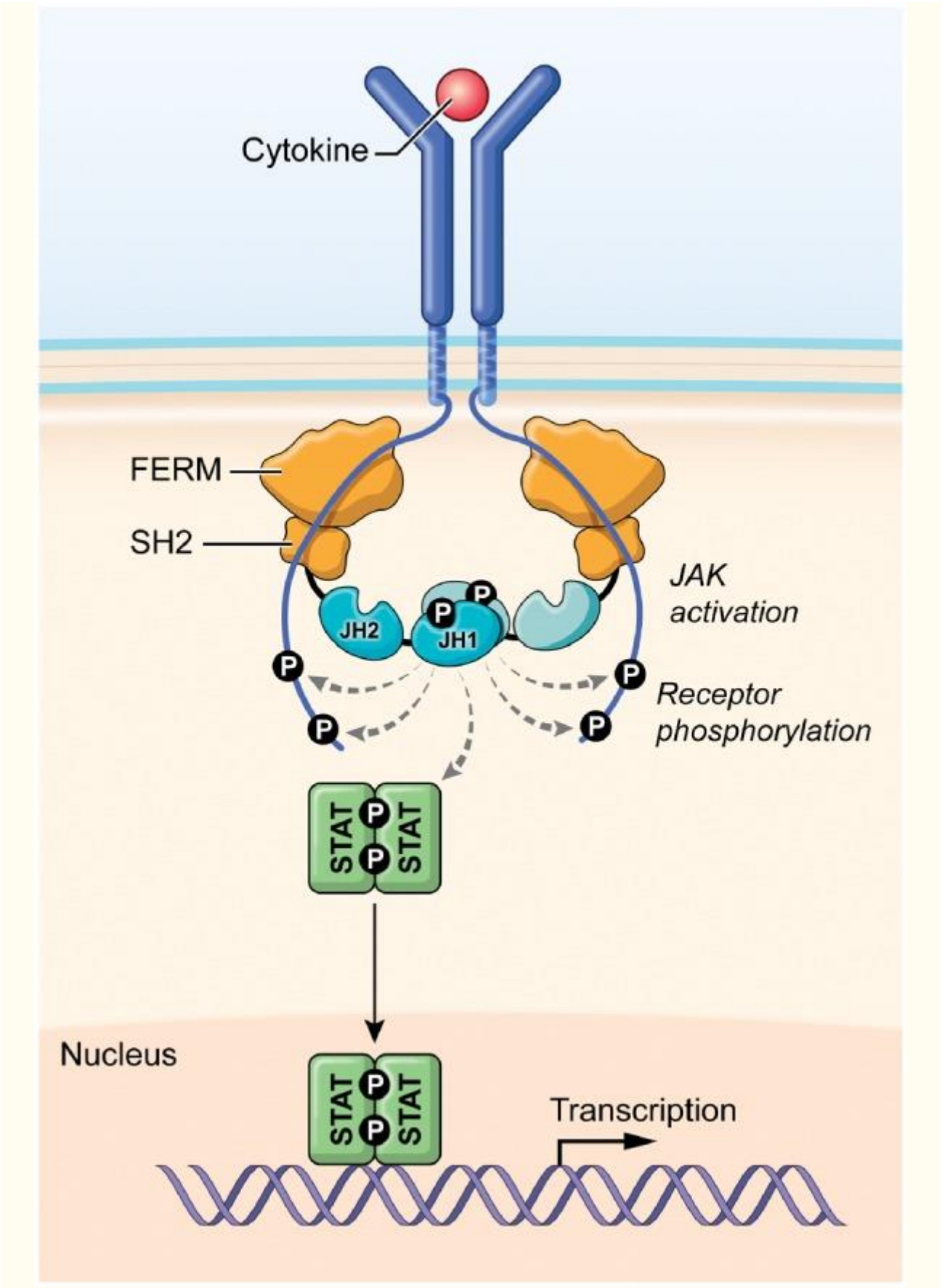
Cytokine
FERM
SH2
JH2
JH1
P
JAK
activation
Receptor
phosphorylation
STAT
STAT
Nucleus
Transcription

FIG. 1

Schematic of signalling by type I/II cytokines

Cytokines that bind the type I/II family of receptors activate receptor-bound JAKs, which catalyse ATP and phosphorylate each other and receptor subunits. Signalling molecules with SH2 domains bind the phosphorylated receptors and are themselves phosphorylated and activated. One such class of molecules is the signal transducer and activator of transcription family of DNA binding proteins, which upon phosphorylation, translocate to the nucleus and regulate gene expression. SH2: src homology 2.

Establishing the criticality of JAKs

The importance of JAKs was first revealed by the elegant use of a series of mutagenized cell lines that failed to respond to IFNs [16]. Pioneering work from the Pellegrini and Stark laboratories established that signalling by type I IFNs was restored by genetic reconstitution of one line with *TYK2*, a kinase whose function was unknown until this key experiment [17]. Of note, IFN-γ signalling was normal in this mutant cell line, a finding that was clarified when it was revealed that this IFN uses different JAKs (see below). These mutant cell lines also revealed the importance of STATs in IFN and cytokine signalling [16].

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 severe combined immunodeficiency (X-SCID) [18]. The absence of signalling 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, with the discovery of JAK3-SCID, it became clear that X-SCID and JAK3-SCID phenocopy each other in that T and NK cells are lacking and B cells are dysfunctional in both diseases [11, 19–21]. Of note, patients with mutations in *JAK3* or *IL2RG* do not have defects beyond immunodeficiency following therapy with stem cell transplantation. This points to selective functions of JAK3 and was the rationale for efforts to therapeutically target this kinase [19].

Shortly after the discovery of JAK3-SCID, knockout mice targeting *Jak3*, *Jak1*, *Jak2* and *Tyk2* were reported. Deletion of *Jak3* in mice results in a phenotype mimicking human autosomal SCID and immune deficits but not global abnormalities beyond immune cells. Since *Jak3* knockout mice are viable and fertile, one can study these mice in detail and the dominant function of JAK3 appears to be in immune cells. However, no conditional *Jak3* knockout mice have been generated, so a complete understanding of the tissue-specific functions of JAK3 is lacking.

In contrast to *Jak3* knockout mice, deletion of *Jak2* is not viable. Mouse germline deletion of *Jak2* results in lethality at embryonic day 12.5 due to impaired definitive hematopoiesis, reflecting the essential, non-redundant function of JAK2 in EPO signalling. As expected, complete deficiency of *Jak2* in humans has not been reported. Because of the lethality associated with germline deletion of *Jak2*, conditional knockout mice have been generated and *Jak2* deletion after birth results in rapid loss of hematopoietic stem cells and progenitors, leading to bone marrow failure and subsequent lethality in adult mice. Conditional *Jak2* deletion in young adult mice also severely affects thrombopoiesis, granulopoiesis and monocytopoiesis but apparently has no effect on lymphopoiesis [22, 23]. Perhaps unexpectedly, selective deletion of *Jak2* in platelets and megakaryocytes did not cause thrombocytopenia; in contrast, this causes thrombocytosis. Deletion of *Jak2* revealed a role in the regulation of circulating TPO levels by controlling removal of TPO by platelets and megakaryocytes. Thus JAK2 is required for production of platelets from hematopoietic precursors but is not required for platelet production by megakaryocytes. Deletion of Jak2 in platelets and megakaryocytes results in greater availability of TPO and consequently early

megakaryocyte precursors [24, 25]. It is tempting to speculate that this may be true for many other cytokines.

JAK2 is critical for PRL signalling and accordingly, conditional deletion of *Jak2* impacts mammary gland development and function. In addition, deletion of *Jak2* in gonadotropin-releasing hormone neurons results in delayed puberty and impaired fertility [26]. Despite these profound developmental effects, deletion of *Jak2* in adult mice apparently has no effect on the heart, kidney, lung or brain.

Germline deletion of *Jak1* is also lethal; *Jak1*$^{-/-}$ mice are small at birth, fail to nurse and die perinatally. Using cells from these mice, it was established that JAK1 is essential for signalling by all class II cytokine receptors, γc using cytokines and gp130 using cytokines (Table 1 and **Fig. 2**) [27]. JAK1 conditional knockout mice have been generated [28], so we will have the opportunity to learn more about tissue-specific functions of JAK1; no doubt we will be surprised. As might be expected based on the mouse knockout, JAK1-deficient humans have not been reported.

A number of cytokines have been reported to activate TYK2 in various settings (**Fig. 2**), but mice lacking TYK2 are viable and have relatively limited impairment of responses, principally affecting IFNs, IL-12 and IL-23. IL-12 promotes the production of IFN-γ in T and NK cells and IL-23 promotes IL-17 production in T cells and innate lymphoid cells. In conjunction with the success of biologics targeting IL-12 and IL-23, a strong argument can be made for the utility of targeting TYK2 in autoimmune disease. However, TYK2 is required for IL-10 and IL-22, cytokines that have important protective effects, especially in the gut [29, 30].

Humans with TYK2 deficiency have been reported and their phenotypes differ. The first child had atopic dermatitis and moderately elevated IgE as well as severe infections with bacterial, viral and fungal pathogens. The cells from this child had impaired IL-6 signalling. However, other patients were reported to have neurobrucellosis and herpes simplex infections (one had severe BCG infection), with only very minor IgE elevation and no atopy or fungal skin infections. Interestingly, no impairment in IL-6 signalling was noted in this group of patients [31]. Taken together with data from mouse models and given all the cytokines reported to use TYK2, it is probably most accurate to conclude that targeting TYK2 likely has merit, but a degree of uncertainty remains regarding the cell-specific assignment of dependency of cytokines upon TYK2.

Given the importance of the JAK–STAT pathway in normal cells, the identification of a link between activation of JAKs and cancer was no surprise [32–35]. The list of cancers with somatic mutations resulting in activation of JAKs and STATs is broad. In addition, there are many examples of cancers with constitutive activation of the pathway due to autocrine and paracrine production of cytokines or gain-of-function (GOF) mutations of upstream receptors, kinases or other signalling molecules [36–38]. Somatic GOF mutations in the JAK2 kinase-like domain are associated with myeloproliferative diseases, including polycythemia vera, essential thrombocytopenia and myelofibrosis, the most common being the V617F mutation [39, 40, 41]. Of note, before the human V617F mutation was identified, an activating mutation of *Drosophila* JAK, Hopscotch, denoted tumorous-lethal, was found to underlie a leukemic-like disorder in flies. Constitutive activation of JAK1, JAK2, JAK3 and TYK2, either through GOF mutations or by constitutive cytokine production are associated with a variety haematological and solid organ malignancies [42–44].

In addition to evidence provided by mutations, polymorphisms of JAK genes link their function with a variety of human diseases [45]. *JAK1* polymorphisms are associated with juvenile idiopathic arthritis [46]. Several genome-wide association studies have implicated *TYK2* in susceptibility to autoimmune diseases, including lupus, Crohn's disease, ulcerative colitis, psoriasis, multiple sclerosis, systemic sclerosis, inflammatory myopathies, primary biliary cirrhosis and type 1 diabetes [47]. TYK2 variants P1104A (rs34536443) and I684S (rs12720356) have been shown to be

catalytically impaired but rescued signalling in response to IFN-α/β, IL-6 and IL-10 [48]. Rs34536443 was found to be associated with protection against endometriosis-related infertility [49]. Furthermore, three protein-coding variants in *TYK2* independently protect against RA and SLE—P1104A (rs34536443), A928V (rs35018800) and I684S (rs12720356)—while two of the *TYK2* variants (P1104A and A928V) may also be protective in the case of IBD [50]. Notably, the P1104A but not the I684S variant results in reduced type I IFN-dependent STAT phosphorylation. In a humanized mouse model carrying the orthologous murine mutation (P1124A), TYK2 phosphorylation was also reduced in response to several cytokines. These animals also showed protection to experimental autoimmune encephalomyelitis [51].

Taken together, genetic data clearly establish that type I/II cytokines are entirely dependent upon JAKs for signal transduction. Nonetheless, a few caveats are in order.

While the concept of the JAK–STAT pathway is useful shorthand, STATs can be activated by factors that do not signal via JAKs. One example is epidermal growth factor, which signals via a receptor tyrosine kinase. The proto-oncogene c-Src also induces STAT activation. Also, while it is easy to consider signal transduction pathways from distinct receptors as independent entities, it must be borne in mind that crosstalk exists. For instance, IFN-γ can modify actions of Fc receptor signalling; conversely, IFN-γR signalling is affected by Fcγ signalling for the induction of a subset of IFN-γ-specific antimicrobial functions [10, 52]. This crosstalk could influence the efficacy of jakinibs but might also relate to adverse events.

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Clinical use of first-generation JAK inhibitors

The rationale for targeting JAKs arose from the vast amounts of data implicating cytokines in autoimmune disease, the success of biologics and the unequivocal evidence for the requisite role of JAKs in cytokine signalling, both *in vitro* and *in vivo*. The first reported *in vivo* use of a jakinib as an immunomodulatory drug described its use in blocking allograft rejection [53]. Subsequently, numerous compounds have shown efficacy in preclinical models and there are now four approved jakinibs (three for humans and one for dogs; Table 2): ruxolitinib, a JAK1 and JAK2 inhibitor, approved for MPNs; tofacitinib, a JAK1, JAK2 and JAK3 inhibitor, approved for RA, PsA and ulcerative colitis; baricitinib, a JAK1 and JAK2 inhibitor, approved for RA; and oclacitinib, a JAK1 and JAK2 inhibitor, approved for allergic dermatitis in dogs. In addition, jakinibs are being studied in a wide variety of other autoimmune diseases (Table 3). Preclinical studies have shown that tofacitinib can ameliorate immunopathology seen in murine lupus models [54]. Trials in lupus in humans have commenced using tofacitinib and baricitinib and, for the latter, a dose of 4 mg was associated with an improvement in symptoms (http://scientific.sparx-ip.net/archiveeular/? searchfor = baricitinib&c = a&view = 1&item = 2018OP0019). Numerous trials, including phase 3 trials, in a variety of dermatological disorders have been completed, including psoriasis, allergic dermatitis (in humans), alopecia areata, dermatomyositis and graft-*vs*-host disease (GVHD).

TABLE 2

Approved jakinibs

Drug	Target	Indication
Tofacitinib	JAK1, JAK3, JAK2	RA, PsA, ulcerative colitis
Ruxolitinib	JAK1, JAK2	Polycythemia vera, intermediate–high risk myelofibrosis
Baricitinib	JAK1, JAK2	RA
Oclacitinib	Multiple JAKs	Atopic dermatitis (dogs)

TABLE 3

Ongoing clinical trials with jakinibs

Juvenile arthritis
Ankylosing spondylitis
Vasculitis
Crohn’s disease
Ulcerative colitis
Uveitis

Sjogren’s syndrome

Psoriasis

Alopecia areata (topical and systemic),

Atopic dermatitis (topical and systemic)

Vitiligo

Scleroderma

Lupus

Vasculitis

MPN

GVHD (topical and systemic)

Leukaemia

Lymphoma

Solid organ malignancy

Given the association between JAK activation and cancer, many trials are testing jakinibs in haematologic and solid organ malignancies. Cancers are one circumstance in which multikinase inhibitors that block JAKs along with other pathways might have superior efficacy. For instance, cerdulatinib is an SYK/JAK inhibitor being studied in lymphoma and leukaemia [55]. Similarly, pacritinib is a JAK2/FLT3 inhibitor being investigated in myelofibrosis, leukaemia, small cell lung cancer and GVHD.

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Side effects of jakinibs: mostly, but not all predictable

Since approved jakinibs target more than one JAK, thus targeting a wide spectrum of cytokines, it is not surprising that adverse events are seen with these drugs. Side effects observed with various jakinibs may be related to off-target effects; however, given the 57 cytokines are potentially affected by first-generation jakinibs, it is a worthwhile exercise to at least consider how common side effects might be related to a cytokine (Fig. 2). Indeed, most adverse events can be readily explained by the known mechanism of action of selected cytokines, but a few are more difficult to explain.

Infections

In view of the role of cytokines in host defence, the complication of infection was certainly expected. Serious infections and opportunistic infections are increased with the use of jakinibs; however, the incidence of infections is roughly equivalent to that observed in patients on biologic therapies, including TNF blockers [56–59]. Indeed, considering all the cytokines that are blocked by jakinibs, it may seem surprising that infections are not more of a problem. However, as discussed above, one explanation for this may be that many cytokines like TNF, IL-1, IL-8 and IL-17 are not dependent upon JAKs.

Reactivation of varicella zoster virus is one of the more common infectious complications in jakinib-treated patients, occurring at twice the rate seen in patients on biologics [60–64]. The risk of this complication is increased by the use of jakinibs with steroids and MTX [61, 65–68]. Given the antiviral role of IFNs, the increase in herpes zoster is not entirely surprising, but exactly why the risk of this particular viral infection is increased is less clear; however, sifalimumab and anifrolumab (anti-IFN-α and anti-IFN-α receptor mAbs) are also associated with an increased risk of herpes zoster. Similar to what has already been recommended with other biologic DMARDs, it is advisable to vaccinate patients against herpes zoster before starting therapy with tofacitinib or other jakinibs.

Lymphopaenia and haematologic alterations

Treatment with jakinibs can cause anaemia and decreased numbers of lymphocytes, NK cells, neutrophils and platelets. Owing to the importance of cytokines in hematopoiesis and lymphocytosis, these side effects can be viewed as related to inhibition of signalling by cytokines that use JAK2 (e.g. EPO, TPO) and other hematopoietic growth factors such as IL-6 and IL-11. Changes in lymphocytes and neutrophils have not been reported to be associated with serious infections or malignancy [69–72].

Thromboembolic events

Concern for the possibility of increased risk of thromboembolic events has been raised with the use of baricitinib [73]. While the risk of deep venous thrombosis and pulmonary embolism is generally increased in patients with RA, relating this complication to the action of a specific cytokine or group of cytokines is difficult. Clearly JAK2 inhibition perturbs TPO signalling and platelet homeostasis, but how this relates to thrombosis is unclear at present and this side effect may also be related to off-target effects of the drug. As discussed above, the thrombocytopenia frequently seen with JAK inhibitor treatment is not due to JAK2 inhibition in platelets and megakaryocytes *per se*, but rather to JAK2 inhibition in stem/progenitor cells [24]. Inhibition of JAK2 could thus lead to increased TPO levels and in principle might adversely influence thromboembolic events. However, this is largely speculation and it will need to be examined rigorously. Alternatively, in considering unexpected or seemingly paradoxical adverse, one needs to bear in mind the crosstalk between signalling pathways (e.g. IFN-γ and FcR signalling interactions described above).

Dyslipidemia

The use of jakinibs is associated with an increase in total cholesterol low-density lipoprotein (LDL) and high-density lipoprotein (HDL) particles [74]. The exact mechanism for this side effect is also somewhat unclear, although targeting IL-6 is also associated with hyperlipidemia. Active RA patients have increased catabolism of LDL particles, and IL-6 appears to be one of the major drivers of this hypercatabolism. Treatment of patients with the IL-6R antibody tocilizumab results in normalization of this excessive LDL catabolism, resulting in increased LDL an average of 20%. In addition, tocilizumab also reduced LDL production [75]. Whether these changes increase the risk of cardiovascular disease has also not been determined [74, 76]. In the case of tofacitinib given as monotherapy, LDL, HDL and total cholesterol all increased, obscuring the impact on cardiovascular risk; interestingly, these increases were dose dependent. In patients receiving baricitinib, a shift in the size of LDL particles was observed, with lower numbers of small, dense LDL particles. In contrast, HDL particles were increased. These HDLs also had reduced content of serum amyloid A, which is known to negatively affect the capability of HDL in mediating cholesterol efflux. Therefore administration of the jakinib resulted in a more efficient reverse cholesterol transport with potentially beneficial effects [77]. These data raise the possibility that jakinibs can limit vascular damage in patients with autoimmune disease by decreasing inflammation despite increasing cholesterol [78].

Liver functions and other metabolic alterations

Mild increases in creatine phosphokinase levels have been observed, which have not been associated with overt muscle disease [79]. However, the importance of JAKs in metabolism has become increasingly recognized [80, 81].

The use of jakinibs can be associated with increased liver enzymes and gastrointestinal perforation [82–84]. Although jakinibs are useful in the treatment of IBD, other cytokines, including IL-10 and IL-22, are important for gut homeostasis. Whether blockade of these cytokines relates to gastrointestinal adverse events has not been established.

Malignancies

Although jakinibs are being used to treat cancer, lymphoma and other malignancies have been observed in patients treated with these drugs. RA patients generally have an increased incidence of cancers, including lymphoma, and the rate of cancer in jakinib-treated patients is similar to that of patients on biologic therapies. Still, there are a number of reasons to suspect that use of jakinibs

might be a risk for the development of cancer. Epstein–Barr virus–associated post-transplant lymphoproliferative disorder was observed at an increased rate in renal transplant patients treated with tofacitinib, but these patients also received additional, concomitant immunosuppressive medications [85].

Given the importance of cytokines, including IFN-γ, in controlling cancer, it is certainly plausible that JAK inhibition could be linked to an increased risk of cancer [86, 87].

In summary, it is remarkable to consider that in patients on jakinibs, factors ranging from GH, leptin, cardiotropin and ciliary neurotrophic factor to EPO, IL-6 and IFN-γ are all blocked. Nonetheless, jakinibs have been used by thousands of patients relatively safely. However, when confronted with complaints in any given patient taking this class of drugs, it is useful to bear in mind the broad consequences of JAK inhibition.

Next-generation selective jakinibs

The broad impact of jakinibs on cytokines evokes the question of whether more selective agents might be advantageous (Table 4). Conversely, inhibition of a narrow spectrum of cytokines could also limit efficacy. Selective targeting of JAK1 would block many pro-inflammatory mechanisms but also spare interfering with many of JAK2-dependent cytokines that promote hematopoiesis factors, including EPO, TPO, G-CSF, GM-CSF, IL-3 and IL-11. Multiple JAK1 selective inhibitors, including filgotinib, upadacitinib, itacitinib and PF-04965842, are being developed and tested in a wide variety of disorders [88–93]. Phase 3 studies of upadacitinib in RA show that these drugs are clearly efficacious [94, 95]. For some of these drugs, anaemia is still seen, arguing for incomplete selectivity and residual JAK2 inhibition; in this respect, the selectivity may be relative and not absolute.

TABLE 4

Next-generation selective jakinibs

Filgotinib	JAK1
Upadacitinib	JAK1
Itacitinib	JAK1
PF-04965842	JAK1
Decernotinib	JAK3

PF-06651600 JAK3

BMS-986165 TYK2

PF-06700841 TYK2, JAK1

TYK2 inhibition would also impact relatively few cytokines. The efficacy of ustekinumab (anti-IL-12 and IL-23), tildrakizumab and guselkumab (anti-IL-23) and anifrolumab (anti-IFNAR) provides a rationale for the development of TYK2 inhibitors. BMS-986165 and PF-06700841 are selective TYK2 inhibitors being studied in SLE, psoriasis, PsA and IBD [96]. Of interest, BMS-986165 shows high specificity for TYK2 but does not target the tyrosine kinase domain. Rather, it binds to the ATP-binding pocket of the TYK2 kinase-like domain [15].

Given the importance of γc cytokines (IL-2, IL-4, IL-7, IL-9 and IL-21) and the striking phenotype of JAK3-SCID patients, a selective JAK3 inhibitor would also be expected to be efficacious. Decernotinib is reportedly a selective JAK3 inhibitor that showed efficacy in phase 2 trials [97–99]. A side effect of decernotinib was neutropenia, a finding that is harder to reconcile with the phenotype of patients with JAK3-SCID and *Jak3* knockout mice. A possible explanation could be that, at the doses used, decernotinib interferes with another JAK and possibly IL-6 signalling. Decernotinib also exhibited CYP3A4-mediated drug–drug interactions, thereby affecting the metabolism of statins and other drugs [100]. PF-06651600 is a highly selective covalent JAK3 inhibitor being studied in RA, alopecia areata and IBD [101, 102]. It will be important to determine whether neutropenia is seen with this compound. Other JAK3-selective jakinibs are also in development [103, 104]. Peficitinib is a pan-JAK inhibitor, but it is also reported to have some selectivity for JAK3. Interestingly, its use in RA was associated with increased haemoglobin [105].

Challenges and opportunities ahead

While multiple jakinibs have been approved and many more are in the pipeline for a vast array of indications, there are a number of unanswered questions, many of which will require rigorous clinical investigation.

In principle, jakinibs could be used in nearly any circumstance in which inflammation driven by JAK-dependent cytokine plays a part in the pathophysiology. For instance, JAK inhibition was reported to have efficacy in a preclinical model of stroke [106]. Similarly, IFN-γ and STAT1 reportedly promote myocardial cell death, and conceivably the inhibition of signalling could limit death [107]. Considering the range of settings in which steroids have salutatory effects, the circumstances in which jakinibs might have utility are equally broad. The extent to which jakinibs can be used as a replacement or adjunct to steroids will also need to be considered.

Exactly how to maximize efficacy and minimize adverse events will require a better understanding and measurement of the optimal degree of JAK inhibition required for an individual patient with any given disease and in a distinct phase of that illness. Modulating the dose or using a broad spectrum initially to induce response followed by selective jakinibs for maintenance therapy might

be a strategy. In fact, in the case of tofacitinib use in ulcerative colitis, approval has been given for an initial treatment at 10 mg twice a day followed by a maintenance dose of 5 mg twice a day. This approach could be particularly useful in the setting of severe and life-threatening autoimmune diseases or considered in the circumstance of multikinase inhibitors. Given the depth of our knowledge related to cytokine signalling and gene expression, multiple biomarkers should be available to guide therapy, but this field is far less mature than desirable. In principle, failure to respond to jakinibs could imply inadequate dosing or that the disease is not mediated by JAK-dependent cytokines. The identification of appropriate biomarkers to distinguish among these possibilities is certainly an area in need of investigation. In this respect, the precise tissue-specific roles of JAKs are incompletely understood. Selective jakinibs are likely to provide new mechanistic insights, but more effective use of conditional *Jak* knockouts and the generation of additional models are needed to better understand the mechanism of action of JAK inhibitors.

Oral, systemic dosing is the mode of administration of current approved jakinibs, consistent with the view that autoimmune diseases are systemic in nature. That being said, certain autoimmune diseases present opportunities for more focused dosing. Topical treatment for dermatological disease, inhaled therapy for pulmonary disease (e.g. asthma) and non-absorbable jakinibs for IBD are possibilities being tested.

For many disorders, from cancer to autoimmunity, combination chemotherapy is required for effective therapy and remission. Jakinibs can be safely used with MTX, but they are also being used with biologics [108]. The safety and efficacy of this strategy will need to be established. We now appreciate the complexity of regulation of key genes such as those encoding pro-inflammatory cytokines and their receptors; such highly regulated genes reside within loci with superenhancer architecture. We know that jakinibs have a profound impact on the epigenome and preferentially impact genes with superenhancer loci, but an important unanswered question is how can combination chemotherapeutic strategies be used to effectively but safely regulate these critical loci? In the setting of cancer, it could be useful to employ agents that directly impact chromatin. In a mouse MPN model, use of the bromodomain and extra-terminal motif inhibitor JQ1 in conjunction with ruxolitinib showed improved efficacy [109]. In patients with MPN, mutations of *JAK2* and the metabolic enzyme isocitrate dehydrogenase have a poor outcome. Combined inhibition of JAK2 and isocitrate dehydrogenase have utility in a murine model [110]. Such a combination might be useful to induce remission in severe autoimmune disease. For chronic phases of disease rather than simply blocking cytokines or just turning genes on or off, more sophisticated targeting of the numerous enhancers that control key genes might allow more subtle modulation and fine-tuning of gene expression, thus providing better balance of control of autoimmunity and preservation of host defence.

Our understanding of the detailed molecular structure of JAKs and their cytokine receptors remains quite limited and is hampered by the fact that the crystal structure of the entire JAK molecule has yet to be solved. Nonetheless, advances are being made [111]. Improved modalities for imaging should offer opportunities for the development of new classes of inhibitors with even greater selectivity. Targeting the regulatory pseudokinase (kinase-like) domain of JAKs could enhance the selectivity of JAK inhibitors [15]. With respect to treating disorders in which JAKs are mutated, selectively targeting the mutant version of the kinase could provide advantages. Gandotinib, for instance, appears to have selectivity for the V617F version of JAK2 and is being studied in myeloproliferative disorders [112].

An important aspect of regulation of JAKs and cytokine signalling is mediated by suppressor of cytokine signalling (SOCS) proteins and some progress has been made in this area. Structural insights on the SOCS mechanism of action are emerging [113, 114]. The kinase inhibitory region of SOCS1 targets the substrate-binding groove of JAK without the need for JAK phosphorylation,

resulting in the blockage of JAKs phosphotransferase activity. SOCS1 differs from the closely related protein SOCS3, another potent inhibitor of IFN-γ signaling, in that SOCS1 does not interact directly with the IFN-γ receptor via its SH2 domain [113]. These new findings will hopefully provide novel therapeutic opportunities to modulate cytokine signalling [114].

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