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Type I/II cytokines, JAKs, and new strategies for treating autoimmune diseases

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Abstract

Cytokines are major drivers of autoimmunity, and biologic agents targeting cytokines have revolutionized the treatment of immune-mediated diseases. Despite the effectiveness of these drugs, they do not induce complete remission in all patients, prompting the development of alternative strategies—including targeting of intracellular signal transduction pathways downstream of cytokines. Many cytokines that bind type I and type II cytokine receptors are critical regulators of immune-mediated diseases and employ the Janus kinase (JAK) and signal transducer and activator of transcription (STAT) pathway to exert their effect. Pharmacological inhibition of JAKs block the actions of type I/II cytokines, and within the past 3 years therapeutic JAK inhibitors, or Jakinibs, have become available to rheumatologists. Jakinibs have proven effective for the treatment of rheumatoid arthritis and other inflammatory diseases. Adverse effects of these agents are largely related to their mode of action and include infections and hyperlipidemia. Jakinibs are currently being investigated for a number of new indications, and second-generation selective Jakinibs are being developed and tested. Targeting STATs could be a future avenue for the treatment of rheumatic diseases, although substantial challenges remain. Nonetheless, the ability to therapeutically target intracellular signalling pathways has already created a new paradigm for the treatment of rheumatologic disease.

Introduction

A fundamental insight gained over more than three decades is that diverse cytokines are pivotal drivers of rheumatoid arthritis (RA) and other autoimmune diseases[1–3]. Cytokine-targeting therapies developed on the basis of these discoveries have dramatically changed the treatment options for patients with RA, psoriasis, inflammatory bowel disease (IBD) and

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Competing interests

J.OS. declares that he and the US Government receive royalties based on patents related to targeting Janus kinases. JOS and M.G. and the US Government have had longstanding Cooperative Research and Development Agreements with Pfizer, which produces tofacitinib, a Janus kinase inhibitor.

Author contributions

D.M.S., M.B., M.G. and JOS researched the data, provided a substantial contribution to discussions of the content, contributed to writing the article, and reviewed/edited the manuscript before submission.

other disorders[4–6] . Clearly, however, not all patients respond adequately to these therapies, complete remission is not as common as one would hope, and ‘cure’ remains an elusive goal. These challenges suggest that alternatives to targeting cytokines extracellularly should be considered. Indeed, the prospect of targeting intracellular pathways activated by cytokines is now a reality.

In considering the cytokines that drive autoimmunity, it is important to understand that the term ‘cytokine’ encompasses structurally distinct factors that bind cellular receptors belonging to at least seven families, which signal through very different pathways (Figure 1). In this Review, we focus on cytokines that drive autoimmune disease and that employ one particular pathway, the Janus kinase (JAK) and signal transducer and activator of transcription (STAT) pathway[7, 8]. Despite their importance in host defense and immune-mediated disease, cytokines such as TNF, IL-1 and IL-17 do not signal via JAKs and STATs. However, JAK–STAT-dependent cytokines can induce these cytokines and in this way also affect immune-mediated disease. In the first part of the Review, we will discuss the roles of JAK-dependent cytokines in normal and aberrant immune responses, and then briefly review JAK-STAT signalling. The second portion of the Review focuses on the emergence of therapies targeting the JAK-STAT signalling pathway.

Roles of Type I/II Cytokines in Health and Disease

Cytokines that bind type I and type II receptors include interleukins, interferons, interferon-like cytokines, colony-stimulating factors, hormones, and growth factors (Table 1). Some of these cytokines share receptor subunits and can be grouped on this basis.

Type I cytokines in autoimmunity

The common γ-chain (γc, also known as IL-2 receptor γ subunit) cytokines include IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21. The fundamental role of these cytokines in lymphocyte development is illustrated by primary immunodeficiency that arises from inactivating mutations of γc (encoded by *IL2RG*), termed X-linked severe combined immunodeficiency (X-SCID). Many γc cytokines also have been implicated in diverse autoimmune diseases. IL-2 is the prototypic T-cell growth factor and has both proinflammatory and anti-inflammatory effects. This cytokine is important for effector T cells, upregulating production of other cytokines and augmenting the cytolytic activity of CD8 T cells and natural killer (NK) cells. Blockade of the IL-2 receptor with daclizumab is approved for the treatment of transplant rejection and seems to be efficacious in multiple sclerosis (MS)[9, 10]. IL-4 is found at elevated levels in the synovial fluid of patients with early RA but is absent in late disease[11]; generally, though, IL-4 is thought to be more important in driving allergic diseases. Both IL-7 and IL-15 are important for lymphocyte homeostasis and T-cell memory[11, 12] IL-15 also regulates NK cells, whereas IL-7 is important for the homeostasis of other innate lymphoid cells[13]. Thymic stromal lymphopoietin (TSLP) shares the IL-7 receptor alpha chain as a receptor component; it has a role in T cell development and allergy[12]. IL-9 is involved in atopy and IBD[14]. IL-21 promotes differentiation of follicular helper T (T_{FH}) cells, which act on B cells in germinal centres to promote antibody class-switching[15]. Inactivating mutations of *IL21* and *IL21R* result in immunodeficiency[16]. Because of their role in class switching, T_{FH} cells are thought to be important for diverse

autoantibody-linked autoimmune diseases including RA and systemic lupus erythematosus (SLE). Levels of IL-21 are increased in the synovium and serum of patients with RA[17]. Targeting IL-21 has been investigated in phase 1 trials of RA and Crohn's disease although results have not yet been reported[18].

The common β-chain cytokines include IL-3, IL-5, and granulocyte-macrophage colony-stimulating factor (GM-CSF). Encoded by *CSF2*, GM-CSF is a proinflammatory growth factor with a key role in the generation, survival, and activation of myeloid cells. It is produced by pathogenic T cells, and a body of data points to its importance in driving autoimmunity[19]. GM-CSF blockade has been effective for RA in phase 2 clinical trials[20].

IL-6 is the prototypic proinflammatory cytokine, which, like a number of related cytokines, signals through the gp130 receptor subunit (encoded by *IL6ST*). Among its many actions, IL-6 drives the production of TNF and IL-1 and induces the differentiation of T helper (T_H)17 cells. These cells are the major producers of IL-17, which acts synergistically with TNF to promote activation of chondrocytes and fibroblast-like synoviocytes, production of metalloproteinases, and joint destruction in RA[1]. Tocilizumab and many other agents in development target IL-6 (Table I). Clinical trials are ongoing for multiple indications beyond RA, such as vasculitis and myositis (ClinicalTrials.gov identifiers NCT01450137 and NCT02043548, respectively)[21]. Other gp130 cytokines include IL-11, IL-27, and IL-31. IL-11 is involved in haematopoiesis and bone remodelling. IL-31 is produced by T_H2 cells, mast cells and other leukocytes; this cytokine has a role in skin barrier function and myeloid development[22]. IL-27 is also a gp130 cytokine but primarily limits inflammation, including antagonizing IL-17 production[23–25] (see below)

Related to the gp130 cytokines is the dimeric cytokine family, which includes IL-12, IL-23, and IL-35. IL-12 promotes the differentiation of IFN-γ-producing T_H1 cells, whereas IL-23 promotes IL-17 production and generation of T_H17 cells. IL-23 is important in the pathogenesis of psoriasis, IBD, and spondyloarthritis[2]. Targeting IL-23 and IL-12 with ustekinumab is effective for psoriasis[26] and has shown promise as a treatment for IBD[27].

Type II cytokines in autoimmunity

The type II cytokines comprise a large group of >30 signalling molecules including the type I, II, and III interferons (IFN-ασ, IFN-β, IFN- γ, IL-28, and IL-29) and IL-10-related cytokines (IL-10, IL-19, IL-20, IL-22, IL-24, and IL-26). A large body of evidence implicates type I interferons in the development of autoimmunity: multiple rheumatic diseases including RA, SLE, systemic sclerosis (SSc), and myositis are characterized by activation of type I interferon signalling[28, 29]. Mendelian disorders associated with upregulation of type I interferon signalling also present with severe inflammation and autoimmunity[30]. Paradoxically, though, IFN-β is an approved therapy for MS (as discussed below).

The type II interferon IFN-γ also contributes to the pathogenesis of autoimmune diseases, although the relative contribution of T_H1 versus T_H17 cells is the subject of ongoing research. T_H1 cells, the main producers of IFN-γ, have been implicated in the development of RA and in animal models of collagen-induced arthritis (CIA)[11]. However, mice lacking

the *Ifng* and *Ifngr1* genes are not protected from CIA, and IFN-γ deficiency can even exacerbate the disease. IL-20 is produced by keratinocytes, neutrophils and fibroblast-like synoviocytes[31], has been implicated in synoviocyte migration and osteoclast formation, and is upregulated in RA synovium[32]. Phase 2 clinical trials have shown encouraging results in the treatment of RA[32]. IL-20 produced by keratinocytes has been implicated in the pathogenesis of psoriatic skin disease[31]. Other type II cytokines, such as IL-10 and IL-22, have different actions, bringing us to the 'good guy' cytokines.

Anti-inflammatory actions of cytokines

Although cytokines are clearly positive effectors of immune responses and drivers of autoimmunity, many type I and II cytokines also have anti-inflammatory actions that need to be considered in the treatment of autoimmune disease. IL-2 is a prime example: the importance of this cytokine in promoting immune tolerance through upregulation of FOXP3 protein would seem to indicate that IL-2 inhibition might induce, rather than treat, autoimmunity,[33] but the efficacy of targeting IL-2 in MS provides a very different view[9, 10]. Whilst daclizumab is efficacious in the treatment of MS, this therapy can be complicated by rash and alopecia, indicative of exacerbation of autoimmunity[9, 10, 34].

IL-2 is not alone in having complex actions; many cytokines have both proinflammatory and anti-inflammatory properties. Other cytokines with anti-inflammatory properties include IL-10, IL-22, and IL-35. IL-10 is perhaps the best characterized of these anti-inflammatory cytokines: it suppresses T-cell–dendritic cell/macrophage interactions[11, 35] and negatively regulates NLRP3 inflammasome activation to ameliorate synovial inflammation in animal models[36]. Mutations of *IL10* and *IL10R* cause severe early-onset IBD[37]. IL-22 is involved in epithelial barrier function and can augment IL-17 function; elevated levels of this cytokine are associated with increased disease activity in RA[38]. IL-27 can enhance T_H1 cell differentiation, but also antagonizes IL-2 production, promotes IL-10 production, and inhibits T_H17 cell responses[23–25]. IL-27 is found at increased levels in the serum and synovial fluid of patients with RA, but whether its role is predominantly proinflammatory or anti-inflammatory is unclear[25, 39]. However, recent data suggest that IL-27 prevents the development of ectopic lymphoid-like structures in arthritic joints[40]. Even the interferons, which are crucial for immune responses to viral infections and heavily implicated in the pathogenesis of autoimmune diseases, have immunosuppressive effects; in fact, IFN-β is an approved treatment for MS[41]. Other "good guy" functions of cytokines include the role of growth factors and hormones that regulate homeostasis and haematopoiesis. Erythropoietin, thrombopoietin, granulocyte colony-stimulating factor (G-CSF), growth hormone, and leptin, among others signal via JAKs[42]. All of these factors must be considered carefully when designing small-molecule inhibitors of JAKs, which will simultaneously and potently block the actions of multiple factors.

Overview of JAK–STAT signalling

The receptors bound by type I and type II cytokines lack intrinsic catalytic activity; rather, these receptors have an extracellular cytokine-binding domain and a cytoplasmic domain that associates with JAKs. There are four JAKs—JAK1, JAK2, JAK3, and TYK2 (tyrosine

kinase 2)—each associates with different cytokine receptors (Table I)[8, 42]. JAKs are kinases or phosphotransferases, meaning they transfer phosphate from ATP to tyrosine residues of their substrates, an important activation event within cells. One crucial attribute of JAKs is their ability to deposit a phosphate on themselves (autophosphorylation) or on other JAKs (transphosphorylation). Cytokines induce JAK enzymatic activity by binding the extracellular portion of their cognate receptors. This modification enables a variety of signalling molecules to recognize the 'activated' receptor.

When one considers the number of cytokines that signal through JAKs, it is not surprising that mutations and polymorphisms of genes encoding these kinases have been linked to diseases of immunity. For example, JAK3 mediates signaling for all the γc cytokines; thus, inactivating mutations of *JAK3* recapitulate the phenotype of γc deficiency and cause SCID[43, 44]. *TYK2* loss-of-function causes a milder immunodeficiency[45]. A large body of evidence from genome-wide association studies has implicated JAKs in the pathogenesis of autoimmune diseases. *TYK2* is linked to SLE and Crohn disease[46, 47], and *JAK2* polymorphisms are associated with Behcet disease[48].

STATs comprise an important class of molecules that transmit signals from type I and II cytokine receptors to the nucleus. STATs reside in the cytosol prior to activation. Upon stimulation by cytokines, STATs bind active, phosphorylated receptors and are, in turn, phosphorylated by JAKs. This modification causes STATs to translocate to the nucleus, where they bind DNA and activate the transcription of target genes. There are seven STATS: STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, and STAT6. Like JAKs, the various STATs transmit signals for different cytokine receptors. However, a degree of functional promiscuity exists: in certain situations, one particular STAT protein can transmit signals for cytokines that would usually signal through another STAT protein. Both activating and inactivating mutations of STATs can cause abnormalities in immune function. Loss-of-function mutations *STAT1* and *STAT2* cause immunodeficiency[42]. *STAT1* gain-of-function mutations lead to chronic mucocutaneous candidiasis via inhibition of IL-17 production[42] . Autoimmunity has also been reported in association with *STAT1* gain-of-function mutations likely due to increased signaling downstream of IFNγ, IL-21, and IL-6[49]. Dominant negative mutations of *STAT3* cause hyper IgE syndrome (also known as Job syndrome), which is characterized by dermatitis, elevated levels of IgE, susceptibility to bacterial pulmonary infection, connective tissue disorders, and bone abnormalities[50]. Conversely, gain-of-function *STAT3* mutations cause early-onset lymphoproliferative disease and autoimmunity[51]. *STAT5* deficiency manifests with immunodeficiency and autoimmunity, as well as dwarfism attributable to the role of STAT5 in mediating growth-hormone signalling[52, 53]. Genome-wide association studies have established links between *STAT3* and Behcet disease[48], Crohn's disease[54], and psoriasis[54], whereas *STAT4* polymorphisms are associated with RA and SLE[55]. Finally, polymorphisms in *STAT6*, which mediates IL-4 signalling, are associated with atopy and asthma[56]. Thus, a large body of evidence points to a critical role for JAKs and STATs in the pathogenesis of rare and common disorders of human immunity.

Jakinibs in autoimmune diseases

The success of 'biologic therapies' in treating autoimmunity notwithstanding, the prospect of new alternatives—namely blocking key cytokines by targeting their signal transduction pathways with small molecules—offers a novel and exciting opportunity for treating disease (Figure 2). This possibility was recognized in 1995[44], but the development of JAK inhibitors (Jakinibs) faced several important challenges. One important consideration is that most Jakinibs act as competitive inhibitors of ATP (Figure 3)[42] and all kinases have some degree of homology. Despite such challenges, differences in kinase structure can be capitalized upon to generate inhibitors that exhibit some degree of specificity. First-generation Jakinibs (tofacitinib, ruxolitinib, baricitinib and oclacitinib) inhibit more than one JAK but seem to have reasonable kinome selectivity. Interestingly, none of the first-generation Jakinibs are good TYK2 inhibitors[57].

Tofacitinib

The first Jakinib developed for the treatment of autoimmune diseases was tofacitinib, which inhibits JAK1, JAK3 and, to a lesser extent, JAK2[58]. After promising results in preclinical and early-phase clinical trials for various immune-mediated diseases[42], tofacitinib was extensively evaluated in pivotal trials in 2012. Multiple phase 2 and 3 studies demonstrated that tofacitinib was effective for RA as monotherapy or in combination with DMARDs, for treatment-naive or DMARD-refractory disease, and even for disease refractory to treatment with biologic agents[59–63]. Tofacitinib was superior to methotrexate in the treatment of naive patients[64] and as effective as adalimumab[63]. Two clinical trials assessing MRI and radiographic responses in nearly 1,000 patients indicated that tofacitinib halts structural damage and erosive disease[62, 64, 65]. Based on these findings, tofacitinib was approved by the US FDA for the treatment of patients with RA with inadequate response to methotrexate.

Tofacitinib has also been found to be effective in the treatment of psoriasis and psoriatic arthritis[66], being noninferior to etanercept[66–68]. However, the FDA has declined to approve tofacitinib for psoriasis. This may be because higher doses of tofacitinib (10 mg twice daily) than the approved dose approved for RA (5 mg twice daily) were required optimal efficacy. The higher dose (10 mg twice daily) was not approved by the FDA because of concerns regarding adverse events[69].. Studies also indicate that continuous therapy results in more durable remissions, although up to 60% of patients can recapture a response when treated intermittently[70]. Tofacitinib is also an efficacious treatment for ulcerative colitis[71] and possibly for Crohn disease, although results in the latter are inconsistent[72]. In prophylaxis of kidney transplant rejection, tofacitinib preserves renal function better than standard-of-care with calcineurin inhibitors, by preventing tubular atrophy and interstitial fibrosis[73].

Ruxolitinib and baricitinib

The JAK1 and JAK2 inhibitor ruxolitinib was actually the first FDA-approved Jakinib, for the treatment of myeloproliferative disease[42]. However, ruxolitinib seems to be efficacious in models of preclinical arthritis[74] and results of a phase II trial in RA are promising (ClinicalTrials.gov identifier NCT00550043).[21]

Another JAK1 and JAK2 inhibitor, baricitinib, has shown efficacy in the treatment of RA, in which setting—like tofacitinib—it is effective in patients with disease refractory to treatment with DMARDs and TNF inhibitors[75–78]. Baricitinib halts radiographic disease progression, with significant differences in erosions and joint-space narrowing, particularly at the higher dose of 4 mg twice daily, and to a lesser degree with 2 mg twice daily.[75–78]

Oclacitinib

Oclacitinib is a nonselective Jakinib approved for the treatment of eczema in dogs. Orally administered Jakinibs are not currently being tested for the treatment of allergic diseases; however, the efficacy of oclacitinib in canine atopic dermatitis[79] demonstrates that inhibition of JAKs can disrupt key signalling pathways that drive allergic inflammation[80]. These findings have paved the way for clinical trials of topical tofacitinib for the treatment of atopic skin disease in humans (ClinicalTrials.gov identifier NCT02001181).[21]

Adverse effects of Jakinibs

As with other effective therapies in autoimmune disease, Jakinibs are not without side effects. Many of the adverse effects of Jakinibs can, like their therapeutic efficacy, be directly traced to their mechanism of action: blocking JAK-dependent cytokine signalling. Because more patients have been treated with tofacitinib than other agents, we know the most about this drug; however, the adverse effect profile of baricitinib seems comparable[75–78].

Infection

An obvious concern with the use of any immunomodulatory drug is the risk of infection, and in clinical trials increased rates of infections have been demonstrated in tofacitinib-treated patients. These infections were mainly upper respiratory tract infections, urinary tract infections, and viral gastroenteritis. However, serious cases of tuberculosis, fungal infection, *Pneumocystis jirovecii* pneumonia, and bacterial pneumonia were reported as well[66, 73, 81–84]. Given the broad range of cytokines that can be affected by tofacitinib, this outcome was not unexpected.

The risk of infection with tofacitinib use is comparable to that with other biologic agents[83, 84], with one exception: the risk of varicella zoster virus (VZV) infection seems to be increased[83, 85]. This increase could be attributable to the effects of tofacitinib on interferon or IL-15 signalling, the latter being a key cytokine for NK-cell homeostasis[86]. However, NK cell counts are only moderately reduced in tofacitinib-treated patients and do not correlate with infection risk[87, 87, 88]. Tofacitinib-treated patients also have impaired responses to vaccination[89], so clinicians are encouraged to immunize patients against pneumococcus and VZV prior to initiating this therapy, as is also recommended with other biologic agents. In renal transplant recipients, higher rates of BK virus infection were seen in those treated with tofacitinib than with ciclosporin, although the drugs were used in the setting of combination immunosuppressive therapy[90]. Whilst most of these infections do not result in nephropathy, a trend towards higher rates of BK virus-associated nephropathy in tofacitinib-treated patients was observed.[90] Clinical trials and long-term extension studies

have not revealed similar issues in patients with RA[83, 84], but clearly this potential risk must be monitored as new Jakinibs emerge for various clinical indications and as part of combined treatment regimens.

Lipid effects

Increases in levels of total cholesterol, LDL cholesterol, and HDL cholesterol have been noted in clinical trials of tofacitinib[42, 83, 84] This side effect is also seen with IL-6 receptor (IL-6R) blocker tocilizumab[91,92] IL-6 promotes insulin resistance and redistributes fatty acids from the blood to peripheral tissues, thereby decreasing serum lipids[92, 93]. Thus, treatment-induced changes in lipid levels may or may not correlate with rates of cardiovascular disease. The results of clinical trials and long-term extension data do not indicate an increased risk of cardiovascular disease with tofacitinib use[83, 93]. On the contrary, tofacitinib can improve arterial stiffness, suggesting that it might improve cardiovascular outcomes in patients with RA[94]. In this context, tofacitinib-induced hypercholesterolemia could be cardioprotective. Indeed, patients with RA commonly have low total, HDL, and LDL cholesterol levels and a discordantly increased risk of cardiovascular disease[93]. Moreover, Jakinibs alter lipoprotein kinetics[95] and increase LDL particle size[95,95]. Jakinibs also confer metabolic properties of brown fat to white adipocytes by uncoupling the respiratory chain and increasing mitochondrial activity[96], a novel and provocative mechanism by which JAK blockade might reduce cardiovascular risk. Needless to say, the metabolic effects of JAK blockade are complex and deserve further attention.

Other Effects

Because all first-generation Jakinibs inhibit JAK2, which mediates erythropoietin, thrombopoietin, IL-11, G-CSF and GM-CSF signalling, patients treated with these agents can develop cytopaenias, especially anaemia; generally, this effect is not necessarily a cause for discontinuation of treatment. Other adverse effects reported in clinical trials include minimal elevations in serum transaminases[42] and reductions in glomerular filtration rate[81]. Neither of these laboratory abnormalities seems to indicate any substantial end-organ damage, with the possible exception of BK nephropathy[83].

Other considerations

Although the adverse events attributed to Jakinibs in clinical trials for rheumatic diseases indicate an acceptable safety profile, several additional factors warrant consideration. Cytokines such as IL-10, IL-22 and IL-27 have important anti-inflammatory actions; other cytokines such as IL-2 have both inflammatory and immunosuppressive actions. Whether suppression of these anti-inflammatory actions will be clinically relevant in the context of inflammatory cytokine blockade remains to be determined.

Finally, the potential role of JAK inhibition in tumour development warrants discussion. Because interferons, IL-12, and other cytokines contribute to the elimination and destruction of tumours, prolonged blockade of JAK–STAT signalling might promote malignancy[97]. However, as is the case with biologic therapies, whether Jakinib-treated patients are truly at increased risk of cancer is unclear. In RA trials, the risk of malignancy in tofacitinib-treated

patients was not significantly increased[83]. Although tofacitinib is associated with an increased risk of nonmelanoma skin cancer, the effect size is similar to that seen with other biologic agents such as TNF inhibitors[82]. When Jakinibs have been used for transplant-rejection prophylaxis in combination with other immunosuppressive drugs, the risk of developing post-transplant lymphoproliferative disease (PTLD) is increased.

Other uses of Jakinibs

The future of therapies that target JAKs and the cytokines that signal through them is only starting to emerge. One need only consider the example of tocilizumab to see the potential applicability of JAK blockade to a wide variety of immune-mediated diseases. Initially used for the treatment of RA, tocilizumab is now approved for use in polyarticular juvenile idiopathic arthritis (JIA) and systemic-onset JIA[98, 99] and has been used successfully to treat large-vessel vasculitis[100], polymyalgia rheumatica[101], AA amyloidosis[102], spondyloarthritis[103], SSc[104], and myositis[105], among other rheumatic diseases. As IL-6 is clearly blocked by existing Jakinibs, these diseases should be reasonable settings in which to test JAK blockade.

Alopecia areata is characterized by an IFN-γ and γc cytokine gene signature[89], or upregulation of these genes. Tofacitinib, ruxolitinib, and baricitinib have all been used successfully to treat alopecia areata and alopecia universalis[106–109], and clinical trials in these diseases are ongoing (ClinicalTrials.gov identifiers NCT02299297, NCT02197455, NCT02312882, NCT01950780)[21]. Preliminary evidence indicates that tofacitinib could be equally effective in the treatment of vitiligo[110], and that inhibition of JAK-STAT signaling causes hair growth through initiation of anagen[111].

The presence of the type I inteferon gene signature in SLE, myositis, primary Sjogren's syndrome, and SSc, and perhaps a subset of RA, suggests that Jakinibs might be useful in these settings[28, 29, 112, 113]. A case of refractory dermatomyositis was indeed reported to respond to treatment with ruxolitinib[114]. The potential for Jakinibs to treat interferon-mediated diseases has also been suggested by preliminary data in patients with interferonopathies—monogenic diseases characterized by uncontrolled upregulation of type I intereferons[115]. A 'compassionate use' study of baricitinib in chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE) and STING-associated vasculopathy with onset in infancy (SAVI) is ongoing[116] (ClinicalTrials.gov identifier NCT01724580)[21].

An important consideration involves the optimal dosing and route of administration for Jakinibs. Although the current paradigm of continuous low-level dosing is comparable to that of DMARDs and other maintenance therapies, it is possible that Jakinibs might be most effective when used in high doses to induce remission followed by tapering to much lower doses. Moreover, topical formulations of tofacitinib improve symptoms of dry eye disease[117] and are being investigated for psoriasis and eczema (ClinicalTrials.gov identifiers NCT01831466, NCT02193815, NCT02001181, and NCT00678561)[21]. If these formulations prove as efficacious as systemic therapy, they could fundamentally alter the treatment of

diseases that affect a single organ system—including pulmonary and gastrointestinal diseases.

Jakinibs: the next generation

The assessment of the adverse events associated with tofacitinib led the European Medical Agency to refuse marketing approval for tofacitinib for the treatment of RA. Other agencies had a different view of the relative risks and benefits of this drug. As with any immunomodulatory drug, the goal is to identify regimens that provide efficacy at the minimal effective dose. As more experience is gained with tofacitinib and other Jakinibs in a range of disorders, we should have a better understanding of the risks and benefits of these agents and perhaps more insights into optimal dosing and regimens in real-world usage. Whether the assessment of the relative benefits and risks of baricitinib and other agents in development will or will not be viewed similarly to those of tofacitinib remains to be seen.

However, with the demonstrated efficacy of multiple first-generation Jakinibs that exhibit kinome selectivity but target multiple JAKs, the question naturally arises of whether targeting a single JAK would be superior. Might such agents exhibit similar efficacy with fewer adverse events? Conversely though, agents that block fewer JAKs by definition block fewer inflammatory cytokines, which could negatively affect their therapeutic efficacy. Indeed, development of second-generation Jakinibs with a greater degree of JAK selectivity is underway. Phase 1 and 2 trials seem to indicate that the JAK1 inhibitor filgotinib[118] and the JAK3 inhibitor decernotinib[119, 120] are effective for the treatment of RA. Several important questions will need to be answered as these and other selective Jakinibs emerge on the clinical scene. The apparent selectivity of these agents needs to be verified *in vivo*, by examining lymphoid, myeloid, and erythroid compartments for signs that other JAKs—especially JAK2—are being affected. Furthermore, even if an inhibitor is exquisitely selective for one JAK over another, selectivity is not necessarily ensured across the kinome. This was the case for a recently developed class of reversible covalent JAK3 inhibitors that also targeted several other tyrosine kinases[121]. At present, ~20 Jakinibs are in clinical trials for various autoimmune conditions including autoinflammatory disease, RA, psoriasis and psoriatic arthritis, SLE, JIA, spondyloarthritis, discoid lupus, ulcerative colitis, atopic dermatitis, and diabetic nephropathy (ClinicalTrials.gov identifiers NCT01458574, NCT01724580, NCT01687309, NCT01500551, NCT01597050, and NCT02001181)[21].

Targeting STATs?

In principle, cytokine signalling could also by blocked by inhibiting STAT activation, interrupting STAT–receptor interactions, blocking STAT dimerization, or interfering with STAT–DNA binding[42, 122]. These strategies have been considered especially in cancer, in which constitutive JAK–STAT activation is a common event[42]. However, unlike JAKs, STATs are not enzymes, and generating drug candidates suitable for clinical use has been difficult owing to challenges of bioavailability, *in vivo* efficacy, and selectivity; for instance, STAT3, a heavily investigated target in the treatment of solid-organ malignancy, has a high degree of homology with STAT1. Moreover, STATs represent convergent endpoints for multiple signalling pathways with critical roles in tumour prevention and host defense[123].

These issues must be considered if STATs are to be targeted in the treatment of rheumatic diseases.

Conclusions

Cytokines that signal through JAKs and STATs are fundamental drivers of host defense, immunity, and inflammation. Advances in the understanding of cytokine biology, and of the links between cytokine regulation and autoimmunity, have sparked a revolution in the treatment of rheumatic diseases over the past 15 years. However, the continuing burden of immune-mediated diseases clearly demonstrates that novel forms of therapy are needed. The emergence of small-molecule targeted therapies represents a new phase in targeted therapy owing to the ability of these agents to simultaneously block multiple signalling pathways. Tofacitinib is the first targeted small molecule to be approved for use in rheumatologic diseases and is likely to be joined by a number of other Jakinibs. A new chapter in the treatment of immune-mediated diseases has begun, in which the paradigm has been fundamentally shifted yet again. The next 5 years will be a crucial time, as we learn how to best use Jakinibs and other small-molecule inhibitors for the treatment of the vast array of autoimmune diseases encountered by clinicians.

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Biographies

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Daniella Schwartz graduated from Rice University. She obtained her MD from Wake Forest University School of Medicine, NC, USA, and then trained in Internal Medicine at Virginia Commonwealth University, VA, USA. She completed her rheumatology fellowship at the NIH in 2015 and is currently a Metzger Scholar in Translational Research. She is interested in the epigenetic factors that determine T cell lineage commitment, and the role of these factors in driving autoimmunity.

John Joseph O’Shea

John J. O’Shea graduated Phi Beta Kappa from St Lawrence University with a BSc degree, and then gained a Doctor of Medicine degree from the University of Cincinnati. He completed a residency in Internal Medicine at the State University of New York Upstate Medical University, subspecialty training at the National Institute of Allergy and Infectious Diseases, NIH, and further postdoctoral training at the National Institute of Child Health and Human Development. He is currently the Director of the Intramural Research Program at the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), NIH. Dr. O’Shea served as the Acting Director of the NIH Center for Regenerative Medicine 2009–2011. Dr O’Shea cloned *JAK3* and his interest for the past 20 years has been cytokine

signalling. He is a member of the National Academy of Medicine and has won numerous prizes including the Ross Prize in Molecular Medicine.

Michael Bonelli

Dr Michael Bonelli obtained his MD from the Medical University of Vienna, Austria. He finished his training in Internal Medicine at the general hospital of Vienna, where he is currently a Rheumatology fellow. He started his scientific career at the Department of Rheumatology at the Medical University of Vienna, where he investigated the role of regulatory T cells in patients with systemic lupus erythematosus. Between 2011 and 2015 he worked in the laboratory of Dr John O'Shea, where he analyzed the plasticity of regulatory T cells under inflammatory conditions. Currently, he is working at the Medical University of Vienna exploring the role of T cells in inflammatory and autoimmune diseases.

Massimo Gadina

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Key points

Cytokines are major drivers of autoimmunity, and targeting cytokines has revolutionized the treatment of rheumatologic diseases

- Despite their success, biologic agents that target key cytokines are not completely effective in all patients
- Type I and II cytokines signal through the JAK–STAT pathway, and pharmacological inhibition of this signal transduction pathway with small molecules can block the actions of these cytokines
- JAK inhibitors, or Jakinibs, are effective for rheumatoid arthritis and other immune-mediated diseases
- Many of the adverse effects of Jakinibs can be linked to action of the cytokines that are blocked
- Jakinibs are currently being investigated for a number of new indications, and second-generation selective Jakinibs are being developed and tested

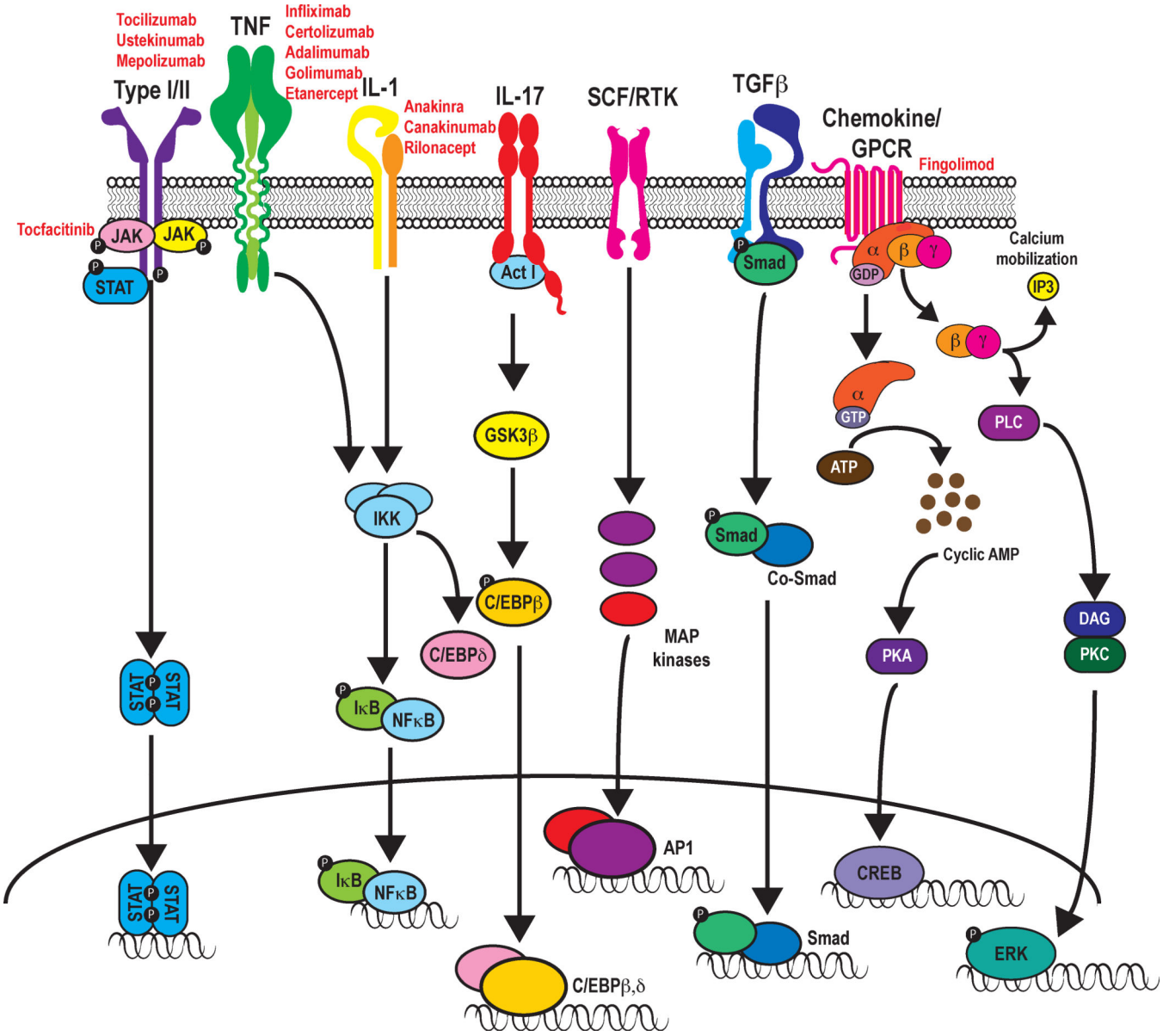

Figure 1.

Cytokines are grouped into superfamilies based on shared structural elements of the receptors they bind. The major cytokine families are: the type I/II cytokines, the IL-1 family, the TNF family, the IL-17 cytokines, the RTK/SCF cytokines, the TGF-β family cytokines, and chemokines. The type I/II cytokine families signal through JAKs and STATs. When a cytokine binds to its cognate receptor, the receptor becomes activated. JAKs autophosphorylate and transphosphorylate, causing STATs to be recruited to the activated receptor where they, in turn, are phosphorylated. The STATs then dimerize and translocate to the nucleus where they initiate transcription. FDA-approved medications blocking the different classes of cytokine receptor are denoted in red.

Abbreviations: JAK, Janus kinase; RTF, receptor tyrosine kinase; SCF, stem cell factor; STAT, TGF-β, transforming growth factor β.

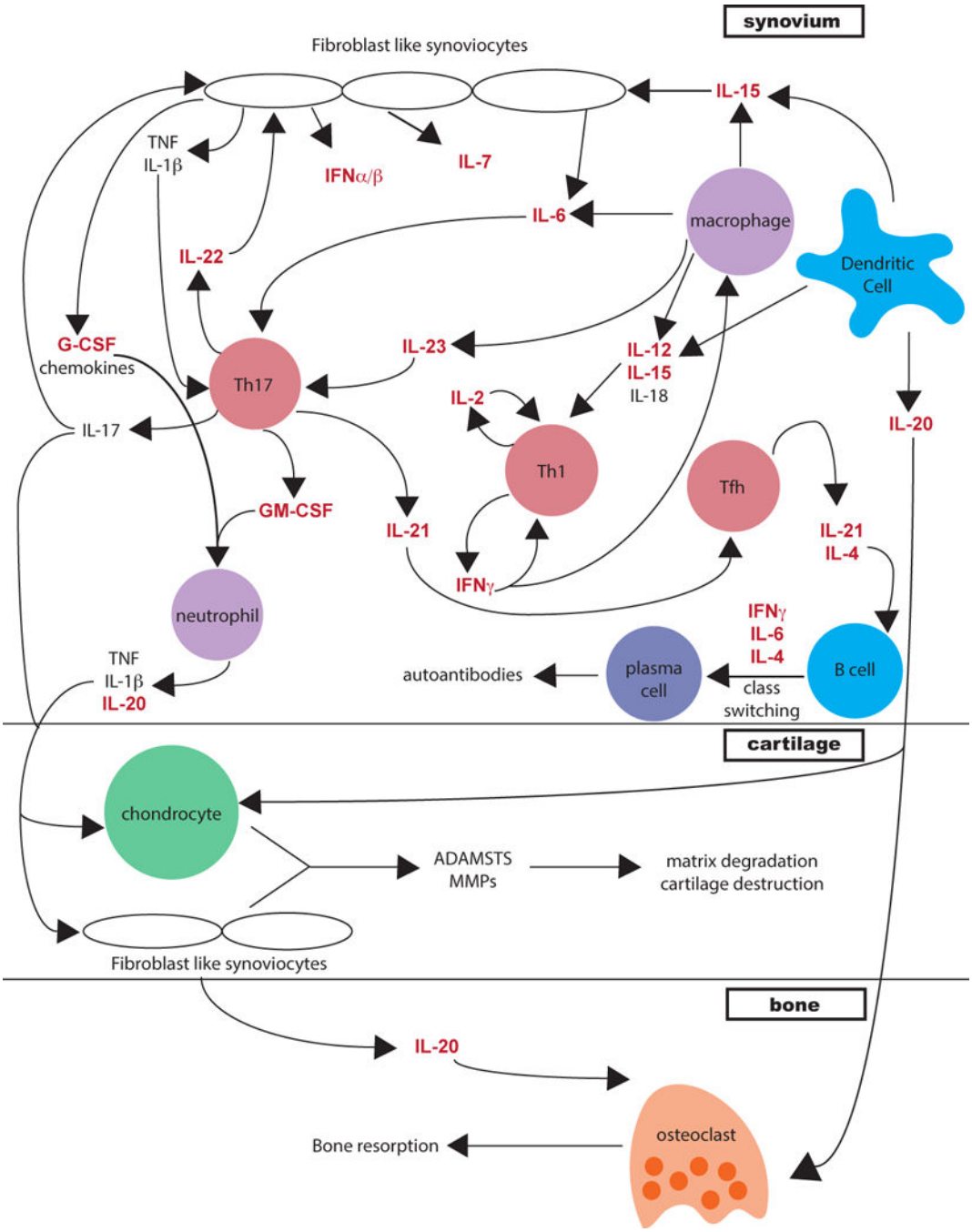

Figure 2.

Type I and II cytokines (red), which are blocked by JAKinibs, are major drivers of autoimmune diseases such as RA. Within the RA joint, FLSs are major sources of TNF, IL-1β, IL-6, and G-CSF. IL-6, along with IL1β, and IL-23, promotes the differentiation of pathogenic T_H17 cells. Pathogenic T_H17 cells produce the neutrophil growth factor GM-CSF. G-CSF and GM-CSF drive neutrophil-derived production of TNF and IL-1β. T_H17 cells are also the major producers of IL-17. IL-17, TNF, and IL-1β induce production of MMPs and ADAMTS, which degrade the cartilage matrix. Similarly, production of the type

I cytokine IL-12 by macrophages and DCs promotes the differentiation of pathogenic T_H1 cells. T_H1 cells produce IFN-γ, activates macrophages. IL-20 and other cytokines promote bone resorption by osteoclasts. DCs also produce IL-15, which promotes FLS survival and T_H1 differentiation, and IL-21, which promotes T_{FH} cell differentiation and antibody production.

Abbreviations; ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; DC, dendritic cell; FLS, fibroblast-like synoviocyte; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocuyte-macrophage colony-stimulating factor; IFN-γ, interferon-γ; MMP, matrix metalloproteinase; T_{FH} cell, follicular helper T cell; T_H17 cell, type 17 helper T cell.

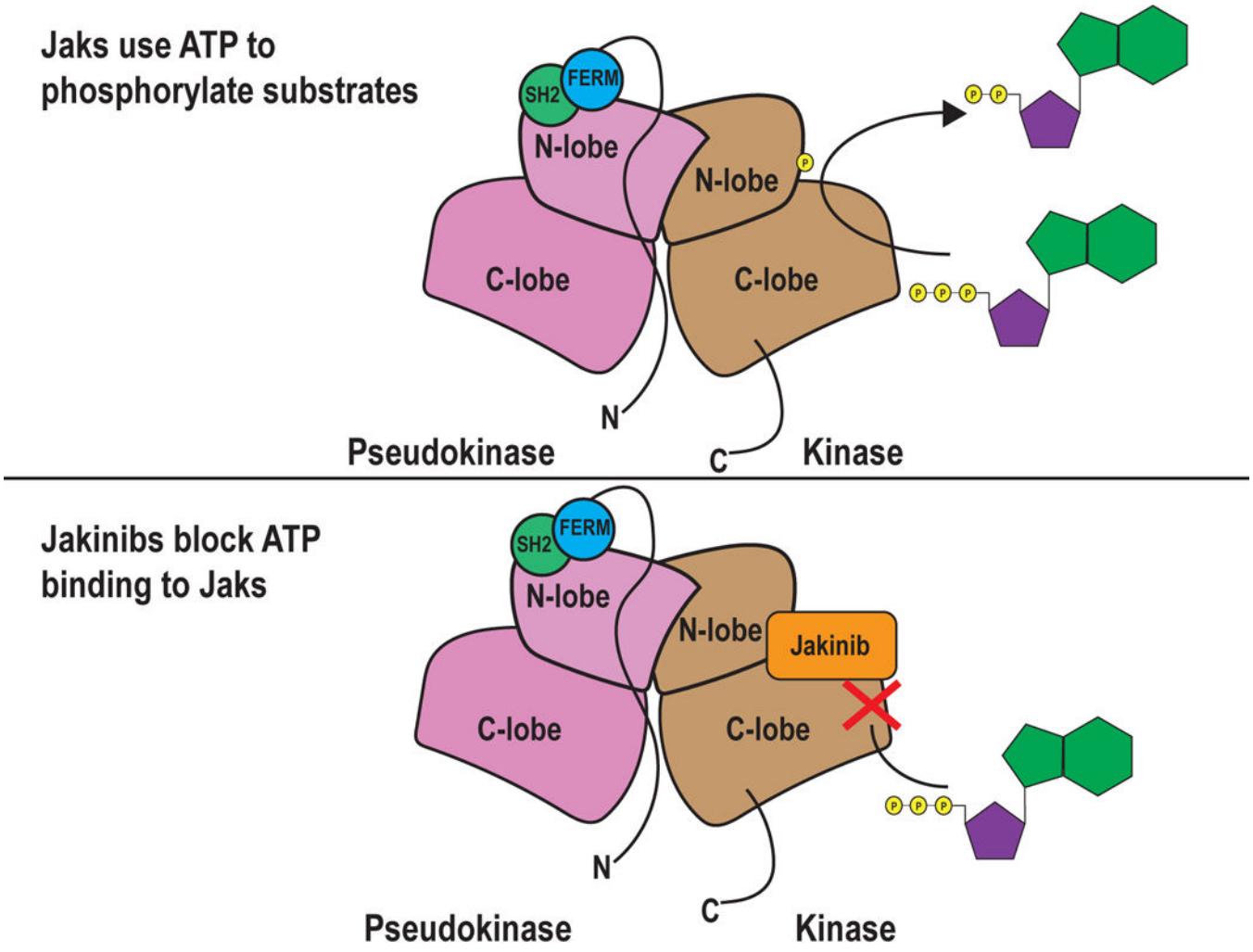

Figure 3.

JAKs are composed of several key domains including a tyrosine kinase domain, pseudokinase domain, FERM (band four-point-one, ezrin, radixin, moesin) domain, and an SH2 (Src homology 2) domain. The tyrosine kinase domain binds ATP hydrolyzing it to ADP and catalyzes both autophosphorylation and other substrates including STATs. Jakinibs bind to the pocket ordinarily occupied by ATP, thereby preventing JAKs from using ATP and phosphorylating their substrates.

Table I

Selected Type I/II Cytokines Important for Hematopoiesis, Immune Homeostasis, and Immune-mediated Disease

TYPE I CYTOKINES	Signaling JAKs	Signaling STATs	Immune function and potential role in Immune mediated Disease	Blocking Agent (FDA-Approved or in late phase Clinical Trials) Clinical Indication
Common γc				
IL-2	JAK1, JAK3	3+5	Enhances effector and regulatory responses	Daclizumab **Transplant, MS**
IL-4	JAK1, JAK3	6	Allergies and asthma – Th2-mediated diseases	Dupilumab* **Asthma**
IL-7	JAK1, JAK3	3+5	T cell development and homeostasis	Not applicable
IL-9	JAK1, JAK3	1+3+5	Atopic disease, IBD	Not applicable
IL-13**	JAK1, JAK3, JAK2 (???),TYK2	6	Airway hyperresponsiveness, goblet cell metaplasia and mucus hypersecretion, fibrosis	Lebrikizumab **Asthma** Dupilumab* **Asthma**
IL-15	JAK1, JAK3	3+5	Memory T cells, NK cells, induction of cell proliferation	Not applicable
IL-21	JAK1, JAK3	1+3+5	Tfh cells	Not applicable
Common βχ				
GM-CSF	JAK2	3+5	Macrophages, T cells, mast cells, NK cells, endothelial cells and fibroblasts, stem cell stimulation and differentiation	Not applicable
IL-3	JAK2	3+5+6	Differentiation of multipotent hematopoietic stem cells, proliferation of all cells in the myeloid lineage	Not applicable
IL-5	JAK2	3+5+6	Allergies, asthma, eosinophilic disease	Mepolizumab **Asthma** Benralizumab, reslizumab
gp130				
IL-6	JAK1, JAK2, TYK2	1+3	Prototypic proinflammatory cytokine Broadly relevant for many autoimmune disease	Tocilizumab[120] **RA, sJIA, pJIA** Sarilumab, ALX-0061, Olokizumab, Sirukinumab, Siltuximab, Clazakinumab
IL-11	JAK1, JAK2, TYK2	3	Hematopoiesis, bone remodeling	Not applicable
IL-27	JAK1, JAK2, TYK2	1+2+3+4+5	Regulation of the activity of B- and T-lymphocytes	Not applicable
Dimeric				
IL-12	JAK2, TYK2	4	Th1-associated disease	Ustekinumab ** **Psoriasis, PsA**
IL-23	JAK2, TYK2	3+4	Th17-mediated disease	Ustekinumab,** **Psoriasis, PsA** Tildrakizumab, guselkumab
IL-27	JAK1, JAK2	1+3	Enhance Th1 and inhibit Th17 responses	Not applicable
IL-35	JAK1, JAK2	1+4	Anti-inflammatory responses	Not applicable

TYPE I CYTOKINES	**Signaling JAKs**	**Signaling STATs**	**Immune function and potential role in Immune mediated Disease**	**Blocking Agent (FDA-Approved or in late phase Clinical Trials) Clinical Indication**
Hormone-like				
EPO	JAK2	5	Control of erythropoiesis	Adverse effects of Jakinibs
TPO	JAK2	1+3+5	Regulation of the differentiation of megakaryocytes and platelets	Adverse effects of Jakinibs
G-CSF	JAK2	5	Bone marrow stimulation to produce stem cells and granulocytes	Adverse effects of Jakinibs
Growth hormone	JAK2	3+5a	Stimulation of cell division of chondrocytes and IGF-1	Not applicable
Leptin	JAK2	3+5a	Coordination of energy homeostasis, increases satiety	Not applicable
TYPE II CYTOKINES	Signaling JAKs	Signaling STATs	Immune function and potential role in Immune mediated Disease	Blocking Agent or Agonist (FDA-Approved or in late phase Clinical Trials) **Clinical Indication**
IFN family				
IFNα/β	JAK1, TYK2	**1+2**+3+4+5	Enhance immunity against infections and drive autoimmunity	Anifrolumab Medi-546 Systemic Sclerosis SLE
IFNγ	JAK1, JAK2	1	Enhance immunity against infections and drive autoimmunity	Not applicable
IL-28	JAK1, TYK2	1+2-3-4-5	Enhance immunity against infections	Not applicable
IL-29	JAK1, TYK2	1+2-3-4-5	Enhance immunity against infections	Not applicable
IL-10 family				
IL-10	JAK1, JAK2, TYK2	1+3+5	Anti-inflammatory actions	Not applicable
IL-19	JAK1, JAK2, TYK2	3	B cell activation Antibody production	Not applicable
IL-20	JAK1, JAK2, TYK2	3	Synoviocyte migration Osteoclast formation	Not applicable
IL-22	JAK1, JAK2, TYK2	1+3+5	Promote barrier immunity Augment IL-17 function	Not applicable

Table I provides examples of key Type I/II cytokines, the JAKs and STATs through which they signal, and selected targeted therapies that block their actions, and are either FDA-approved or in late phase clinical trials for rheumatic or other diseases. Cytokines are grouped based on shared structure/subunits.

* Of note, IL-13 is grouped with the gamma chain cytokines, but IL-13 does not share the common γ χηαιn; rather it shares the IL-4 receptor alpha subunit (IL4Rα). Dupilumab targets IL4Rα and therefore blocks both IL-4 and IL-13.

** IL-12 and IL-23 both are composed of p40 subunit. Ustekinumab, which targets p40, blocks both cytokines.

Abbreviations: RA = rheumatoid arthritis, AS – ankylosing spondylitis, HLH = hemophagocytic lymphohistiocytosis, sJIA = systemic juvenile idiopathic arthritis, pJIA = polyarticular juvenile idiopathic arthritis, MS = multiple sclerosis, AML = acute myelogenous leukemia, MDS = myelodysplastic syndroems, HES = hypereosinophilic syndrome, AD = atopic dermatitis, EE = eosinophilic esophagitis, GPA = granulomatosis with polyangiitis, eGPA = eosinophilic granulomatosis with piolyangiitis, COPD = chronic obstructive pulmonary disease, LVV = large vessel vasculitis, GVHD = Graft-versus-host disease, PsA = psoriatic arthritis, UC = ulcerative colitis, IBD = inflammatory bowel disease, SpA = spondyloarthropathy, Ssc = systemic sclerosis, pSS = primary Sjogren's syndrome, OA = osteoarthritis, HIV = human immunodeficiency virus, IPF = idiopathic pulmonary fibrosis.

Clinical trials can be found at www.clinicaltrials.gov: NCT01463644, NCT00538434, NCT00527566, NCT02238353, NCT02101333, NCT02034474, NCT01297699, NCT02453256, NCT01209689, NCT02477059, NCT01782235, NCT01649375, NCT01961609, NCT01806597, NCT01640938, NCT01392326, NCT01266811, NCT01663441, NCT01369342, NCT02407236, NCT02407223, NCT01274338, NCT02199327, NCT00002882, NCT00003924, NCT00125814, NCT00167583, NCT00002238, NCT00249860, NCT00344253, NCT00070187, NCT00538811, NCT02415127, NCT00076635, NCT01270490, NCT00004402

Table II

JAKs and disease

JAK	Human disease: gain of function	Human disease: loss of function
JAK1	ALL, AML, solid organ malignancies	Not applicable
JAK2	PCV*, MF*, ET*, hypercoagulable state, somatic mutations associated with acute and chronic hematologic malignancies	Not applicable
JAK3	Acute megakaryoblastic leukemia, T cell leukemias and lymphomas	Severe combined immunodeficiency (SCID), Jacobsen syndrome
TYK2	Cutaneous lymphoproliferative disorders, T cell leukemias	Primary immunodeficiency

* PCV = polycythemia vera, MF = myelofibrosis, ET = essential thrombocythemia

Table II describes known associations of JAK mutations with human disease. Rare JAK mutations are associated with disorders of immune function, and JAKs can be activated in a variety of cancers

Table III

STATs and disease

STAT	Human disease: gain of function	Human disease: loss of function	Human disease: polymorphism
STAT1	Chronic mucocutaneous candidiasis	Susceptibility to mycobacterial and viral infections	Not applicable
STAT2	Not applicable	Increased susceptibility to viral infections	Not applicable
STAT3	Lymphoproliferative disease and autoimmunity, Large granulocytic leukemia (LGL)	Autosomal-dominant Hyper IgE (Job's) syndrome	Behcet's disease
STAT4	Not applicable	Not applicable	RA, SLE
STAT5a/STAT5b	Somatic mutations associated with LGL	Autoimmunity, bleeding diathesis, immunodeficiency and dwarfism	Not applicable
STAT6	Not applicable	Not applicable	Asthma, atopy, high serum IgE

Table III describes known associations of STAT mutations with human disease. Rare STAT mutations are associated with disorders of immune function, and STATs are commonly activated in a variety of cancers

Table IV

Jakinibs being investigated for rheumatic diseases

Drug	Target	Status	Diseases
Ruxolitinib (INC424)	JAK1, JAK2	*FDA approved* Phase II Phase IIb	RA Various cancers Psoriasis (topical)
Tofacitinib (CP690550)	JAK3>JAK1>> (JAK2)	*FDA approved* Phase III Phase II	RA Psoriasis, Ulcerative colitis Spondyloarthritis, JIA Transplant rejection
Oclacitinib (PF03394197)	JAK1	*FDA approved*	Canine allergic dermatitis
ABT494	JAK1	Phase II	RA, Crohn's
Baricitinib (INCB28050, LY3009104)	JAK1, JAK2	Phase II	RA, Psoriasis, Diabetic nephropathy, autoinflammatory disease
Filgotinib (GLPG0634)	JAK1	Phase II	RA, Crohn's disease
INCB039110	JAK1, JAK2	Phase II	Psoriasis, RA
Peficitinib (ASP015K)	pan-JAK	Phase II	Psoriasis, RA
R333	JAK/SYK	Phase II	Discoid lupus (topical)
GLG0778	JAK1	Phase II	SLE
GSK2586184	JAK1	Phase II	SLE, Psoriasis
Decernotinib (VX509)	JAK3	Phase IIb	RA

Table IV denotes JAKinibs that are FDA-approved for, or in clinical trials for, the treatment of autoimmune diseases.

Abbreviations: RA = rheumatoid arthritis, JIA = juvenile idiopathic arthritis, SYK = spleen tyrosine kinase