

JAK inhibitors for asthma



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Asthma is an inflammatory disease of the airways characterized by intermittent episodes of wheezing, chest tightness, and cough. Many of the inflammatory pathways implicated in asthma involve cytokines and growth factors that activate Janus kinases (JAKs). The discovery of the JAK/signal transducer and activator of transcription (STAT) signaling pathway was a major breakthrough that revolutionized our understanding of cell growth and differentiation. JAK inhibitors are under active investigation for immune and inflammatory diseases, and they have demonstrated clinical efficacy in diseases such as rheumatoid arthritis and atopic dermatitis. Substantial preclinical data support the idea that inhibiting JAKs will ameliorate airway inflammation and hyperreactivity in asthma. Here, we review the rationale for use of JAK inhibitors in different asthma endotypes as well as the preclinical and early clinical evidence supporting such use. We review preclinical data from the use of systemic and inhaled JAK inhibitors in animal models of asthma and safety data based on the use of JAK inhibitors in other diseases. We conclude that JAK inhibitors have the potential to usher in a new era of anti-inflammatory treatment for asthma. (*J Allergy Clin Immunol* 2021;148:953-63.)

Key words: Asthma, severe asthma, Janus kinase, JAK/STAT pathway, JAK inhibitor

RATIONALE FOR JAK INHIBITORS IN ASTHMA

Asthma is an inflammatory disease of the airways characterized by intermittent episodes of wheezing, chest tightness, and cough. Asthma affects approximately 300 million people worldwide and more than 25 million people in the United States. Severe asthma affects 5% to 15% of the population with adult asthma and is associated with increased mortality, increased hospitalizations, significant burden of symptoms, health care costs, and missed

Abbreviations used

AHR: Airway hyperresponsiveness
 ASM: Airway smooth muscle
 BAL: Bronchoalveolar lavage
 DMARD: Disease-modifying antirheumatic drug
 EPO: Erythropoietin
 FDA: US Food and Drug Administration
 FENO: Fractional exhaled nitric oxide
 IC₅₀: Inhibitory concentration of 50%
 ICS: Inhaled corticosteroid
 ILC2: Group 2 innate lymphoid cell
 iNOS: Inducible nitric oxide synthase
 JAK: Janus kinase
 Ki: Inhibitory constant
 OVA: Ovalbumin
 RA: Rheumatoid arthritis
 STAT: Signal transducer and activator of transcription
 Treg: Regulatory T
 TSLP: Thymic stromal lymphopoietin
 VTE: Venous thromboembolism

work and school. In the United States alone, more than \$80 billion in direct and indirect costs are spent on asthma annually, with most of that amount spent on patients with severe asthma. Severe asthma represents a subset of difficult-to-treat asthma and occurs in patients whose disease remains uncontrolled despite the use of high doses of inhaled corticosteroids (ICSs) combined with long-acting β -agonists or other controllers.¹ Even with the approval of several targeted biologics in the past few years, many patients continue to experience exacerbations or uncontrolled disease, indicating a need for more novel therapies.

Over the past several decades, it has become clear that asthma is a heterogeneous disease in terms of its clinical presentation, physiologic characteristics, and responses to therapy. Much of this variability is due to differences in gene-by-environment

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interactions, resulting in complex biochemical pathways underlying the disease. As we have gained a better understanding of pathophysiologic mechanisms of asthma, we have shifted from a phenotypic classification system focused on specific triggers and clinical presentation to a classification system focused on endotypes, particular pathophysiologic mechanisms, and related clinical biomarkers associated with a given patient.² There are 2 major endotypes in asthma: type 2 and non-type 2. The type 2 pathway is defined by activation of cytokines derived from T_H2 cells and group 2 innate lymphoid cells (ILC2s); these include IL-4, IL-5, and IL-13 that cause airway inflammation by activating eosinophils, B cells, airway epithelial cells, and other cell types. Biomarkers of type 2 asthma include blood/sputum eosinophilia and elevated levels of fractional exhaled nitric oxide (FENO) and IgE. The type 2–low pathway is characterized by absence of type 2–high cytokines and biomarkers, and it manifests either increased levels of neutrophils in the airways or a paucigranulocytic profile, with normal levels of airway neutrophils and eosinophils.³ Type 2–low asthma is currently not well understood, and it likely encompasses multiple distinct endotypes.

Most of the inflammatory pathways implicated in different asthma endotypes involve cytokines and growth factors that signal via receptors coupled to Janus kinases (JAKs). The JAK family comprises the following 4 members of intracellular tyrosine kinases: JAK1, JAK2, JAK3, and Tyk2. JAK family members phosphorylate and activate intracellular signal transducer and activator of transcription (STAT) proteins, which regulate the transcription of their downstream target genes. The discovery of the JAK/STAT signaling pathway was a major breakthrough that revolutionized our understanding of cell growth and differentiation.⁴ Experiments using cell culture and genetically modified mouse models revealed an essential role for JAK/STAT family members in immune-mediated diseases, including asthma.

Cytokines and growth factors implicated in asthma pathophysiology that signal via JAK-coupled receptors include IL-4, IL-5, IL-6, IL-9, IL-13, GM-CSF, IFN- α , IFN- β , IFN- γ , and thymic stromal lymphopoietin (TSLP). Notable exceptions to this list include TNF, IL-1, IL-17 members, and IL-33.^{5,6} Different JAK family members couple to cytokine receptors in a combinatorial manner. However, it is important to note that not all JAK family members participate equally in signal transduction, despite association with a given receptor. For example, IL-13–induced phosphorylation of STAT6 was preferentially inhibited by compounds targeting JAK1 (but not JAK2 or Tyk2) in the BEAS-2B airway epithelial cell line.⁷ Similarly, IL-6 recruits and activates JAK1, JAK2, and Tyk2, but IL-6–dependent STAT3 phosphorylation was preferentially inhibited by JAK1 (but not Tyk2) inhibitors in human cell lines.⁸

Small molecule JAK inhibitors are under active development and have been approved to treat rheumatoid arthritis (RA) and other conditions.^{9,10} In this review, we summarize the rationale for targeting JAK-dependent signaling pathways in patients with type 2–high and type 2–low asthma, review available literature reporting preclinical studies in asthma models of asthma, and review a recently published human clinical trial that demonstrated proof of concept of target engagement in asthma.¹¹ We conclude by considering the safety profile of JAK inhibitors and speculate on the positioning of these therapies in the context of other asthma treatments.

TABLE I. Currently approved JAK inhibitors for various indications

Compound	Indication
Tofacitinib	RA, PsA, UC
Ruxolitinib	PCV, MF
Baricitinib	RA
Upadacitinib	RA
Fedratinib	MF

MF, Myelofibrosis; PCV, polycythemia vera; PsA, psoriatic arthritis; UC, ulcerative colitis.

JAK INHIBITOR SPECIFICITY AND ROUTE OF DELIVERY

Rapid progress is being made in the development of orally available small molecule JAK inhibitors.^{9,12,13} There are currently 5 US Food and Drug Administration (FDA)-approved compounds for clinical use, 2 for use in hematologic disorders (ruxolitinib and fedratinib) and 3 for use in inflammatory diseases (tofacitinib, baricitinib, and upadacitinib [Table I]), and many others are in phase II/III studies for a variety of conditions, including RA, ulcerative colitis, psoriasis, and atopic dermatitis. Ruxolitinib and tofacitinib are considered pan-JAK inhibitors (inhibiting JAK1, JAK 2, and JAK3), although tofacitinib has lower affinity for JAK2 than ruxolitinib does. All of the currently approved JAK inhibitors cause dose-dependent myelosuppression, which is usually mild and reversible and thought to be due to inhibition of JAK2-dependent hematopoietic growth factors.¹⁴ For treatment of inflammatory diseases, there has been recent emphasis on developing targeted antagonists that spare JAK2-dependent growth factors in order to avoid bone marrow suppression. However, there is significant overlap in the chemical structure of JAK isoforms, and although inhibitors with selectivity for certain JAK family members have been developed, it is not clear whether narrow specificity is desirable for a complex inflammatory disease such as asthma. Substrate specificity for a given JAK inhibitor can be determined by *in vitro* biochemical assays (ie, the inhibitory constant [K_i] for ATP-competitive inhibition with use of purified substrates) or cell-based assays (eg, the inhibitory concentration of 50% [IC₅₀] for cytokine-induced STAT phosphorylation in reference cell lines). Although these approaches usually result in comparable and reproducible rank order potencies under controlled conditions,⁸ it is clear that specificity for a given JAK family member can also vary depending on cell type and cytokine(s) studied.^{15,16} Therefore, the idea of JAK isoform specificity should be considered a relative concept. It is clear that we have more to learn about how these compounds work in human subjects, and clinicians and investigators need to remain vigilant for unexpected and off-target effects in human clinical trials.

Particularly exciting for asthma is the development of inhaled JAK inhibitors, with chemical structures that facilitate lung retention and minimize systemic exposure.^{16–20} This is an active area of research that was recently reviewed comprehensively.²¹ Local deposition and retention of inhaled JAK inhibitors might obviate some of the concerns raised with systemic administration, such as JAK2-dependent myelosuppression. We review some of the preclinical studies with these novel compounds in asthma in Table II^{16,18,19,22–27} and in the section on decreased lymphocyte and neutrophil counts later in this article.

TABLE II. JAK inhibitors used in animal models of lung inflammation

Compound	JAK specificity	Route	Species	Allergen/model	Findings	Reference
Tofacitinib	Pan-JAK	Subcutaneous via osmotic minipumps (1.5-15 mg/kg/d)	Mouse	OVA i.p. + alum, then OVA aerosol	Reduced BAL eosinophilia, eotaxin, and IL-13 levels when administered during both sensitization and challenge or during the challenge phase alone	22
P6	Pan-JAK	i.p.*	Mouse	OVA i.p. + alum, then OVA aerosol	Decreased airway resistance, BAL eosinophilia, and Muc5AC, but only if PLGA-encapsulated and administered during challenge No effect if administered during sensitization and challenge	23
R256†	JAK1/3	Oral gavage (10-50 mg/kg)	Mouse	OVA i.p. + alum then OVA aerosol	Decreased airway resistance, BAL eosinophilia, mucus production Decreased T _H 2 cytokines only if administered during sensitization	24
Ruxolitinib	Pan-JAK	Intragastric (180 mg/kg)‡	Mouse	OVA + low-dose LPS inhaled then OVA aerosol	Reduced BAL eosinophil, neutrophil, and IL-17 levels	25
iJak-381	JAK1	i.n., dry powder inhaler	Mouse Guinea pig	OVA i.p. + alum then OVA aerosol Aspergillus, Alternaria, and house dust mite (AAH)	Reduced BAL eosinophilia, CCL11, airway resistance, and Muc5AC in OVA-challenged mice Reduced BAL eosinophilia, neutrophilia, CCL11, and CXCL1 in AAH-challenged mice Reduced lung inflammation in OVA-challenged guinea pigs	18
LAS194046	Pan-JAK§	i.t. (0.3-1 mg/kg)	Rat	OVA i.p. + alum then OVA aerosol	Reduced BAL eosinophilia and neutrophilia, and AHR (Penh)	16
iJAK-001	Not stated	i.t. (0.03-1 mg/kg) or dry powder inhaler (10 mg)	Rat Sheep	Alternaria extract i.t. Ascaris suum	Reduced Alternaria-induced BAL eosinophilia in mice Reduced Ascaris-induced airway resistance in sheep	19
Tofacitinib		Aerosol (1-2 mg/kg)	Mouse	House dust mite extract	Reduced eosinophil and total protein levels	26
Tofacitinib		i.p. (5 mg/kg)	Mouse	OVA i.p. + alum then OVA i.n.	Reduced numbers of tissue eosinophils, polypoid-like lesions, T _H 2 cytokines, and phospho-STAT6	27

AAH, *Aspergillus*, *Alternaria*, and house dust mite; i.n., intranasal; i.p., intraperitoneal; i.t., intratracheal, PLGA, poly D,L-lactic-co-glycolic acid.

*Alone (3 mg/kg) or in PLGA-encapsulated nanoparticles (60 mg/kg).

†This compound is apparently now known as AZD0449.

‡Timing of ruxolitinib administration is not clear.

§This compound was also found to inhibit Flt3 and TrkA.

JAK-STAT AND TYPE 2-HIGH ASTHMA

The JAK/STAT pathway has a critical role in the development and signaling of T_H2 cytokine-driven immune responses. IL-4 and IL-13 activate STAT6 in their target cells in a JAK-dependent manner, and experiments using gene-targeted knockout mice confirmed a key role for STAT6 in IgE production, bronchial hyperresponsiveness, airway remodeling, and mucus metaplasia after allergen sensitization.^{28,29} Other type 2 asthma-associated cytokines also signal through JAK/STAT proteins, including IL-5 and TSLP. Because each of these key type 2 asthma cytokines (IL4, IL5, IL13, and TSLP) signal via JAK-dependent pathways, there is a strong potential role for JAK inhibitors in the treatment of type 2 asthma.

Development and maintenance of type 2 immune responses in asthma

JAK signaling pathways are critically important for the differentiation of CD4⁺ T_H2 cells from naive precursors. Key cytokines implicated in this process include IL-2 and IL-4, which activate the transcription factors STAT5 and STAT6, respectively, via cytokine receptors coupled to JAK1 and JAK3.³⁰ However, the acute production of cytokines from already-differentiated T_H2 cells occurs in a JAK-independent manner via signals from the T-cell receptor (TCR) and costimulatory molecules. Proof of principle that inhibiting JAK1/3 attenuates T_H2 cell differentiation was reported by Ashino et al, who used a compound called R526.²⁴ Interestingly, R526 had no effect on the differentiation

of T_H1 or T_H17 cells, possibly reflecting the requirement for Tyk2-dependent signals downstream of IL-12 and/or IL-23 receptors in these cells. As expected, R526 did not inhibit T_H2 cytokine production from already-differentiated T cells restimulated via the TCR. This compound also blocked eosinophilic airway inflammation in a mouse model of asthma (see later and Table II). A phase I clinical trial of R526, now known as AZD0449, was recently completed, with (it is hoped) results to be reported soon (ClinicalTrials.gov identifier NCT03766399).

Although less is currently known about the molecular regulation of T_H2 cell gene expression in ILC2s, a recent study demonstrated that JAK inhibitors can block cytokine production from these cells. Human lung ILC2s were obtained by bronchoalveolar lavage (BAL) and cultured in the presence of the pan-JAK inhibitor tofacitinib. Interestingly, the production of IL-5 by ILC2s was resistant to the effects of dexamethasone, whereas tofacitinib (alone and in combination with dexamethasone) suppressed IL-5 production by ILC2s, probably by attenuating the effects of TSLP present in BAL fluids.³¹ This and other studies suggest the possibility that JAK inhibitors may be particularly effective in subjects with steroid-resistant T_H2 cell–high asthma, especially if characterized by expansion of pathogenic ILC2s.^{32,33}

In addition to blocking the differentiation or activation of T_H2 cells and ILC2s, JAK-dependent signals may be important for the homeostasis of T_H2 cells and ILC2s. In support of this possibility, IL-2 (which also signals via JAK1 and JAK3) was recently shown to play a critical role in the maintenance of allergen-specific tissue-resident memory (Trm) T_H2 cells in the lung in a mouse model of asthma.³⁴ JAK inhibitors might therefore decrease the persistence of these pathogenic T cells (which contribute to mucus metaplasia and airway hyperresponsiveness (AHR)] in the lung.³⁵ However, this potential beneficial effect of JAK inhibitors in asthma would need to be balanced with the inhibition of protective tissue-resident memory T cells that reside in the lung following respiratory viral infections.³⁶ It is worth noting that IL-2 is a pleiotropic cytokine and regulates the differentiation and survival of regulatory T (Treg) cells in a JAK/STAT-dependent manner.³⁷ Although this raises the possibility that JAK inhibitors might interfere with the function of Treg cells, tofacitinib was found to spare Treg cell function in kidney transplant recipients.³⁸ In summary, JAK inhibitors may be useful in subjects with T_H2 cell–high asthma by inhibiting the development, activation, or homeostasis of $CD4^+$ T_H2 cells, as well as ILC2s. Potentially of greater importance will be the ability of JAK inhibitors to block the downstream signals of T_H2 cytokines on their target cells.

Type 2 cytokine signal transduction as a therapeutic target in asthma

IL-4 binds IL-4R α and γ_c , which couple to JAK1 and JAK3, whereas IL-4 and IL-13 bind IL-4R α and IL-13R $\alpha1$, which couple to JAK1, JAK2, and Tyk2. Support for the idea that blocking IL-4R α signal transduction is an effective therapeutic strategy in type 2–high asthma comes from the clinical experience with the IL-4R α antagonist dupilumab.³⁹ IL-4 and IL-13 have pleiotropic effects on virtually every cell type relevant to asthma pathophysiology; particularly important targets in the lung include airway epithelial cells and ASM cells.⁴⁰ In airway epithelial cells, IL-4 and IL-13 induce a characteristic pattern of gene expression in a STAT6-dependent manner, resulting in the

production of eosinophil-attracting chemokines, mucus metaplasia, and inducible nitric oxide synthase (iNOS).^{28,29,41,42} Guided by structure-activity relationships, Zak et al developed a family of new specific JAK1 inhibitors that blocked IL-13–induced STAT6 phosphorylation in the BEAS-2B airway epithelial cell line.⁷ TSLP-induced eotaxin-1 (CCL11) was also inhibited by the JAK1/2 inhibitor CYT387 in human nasal epithelial cells (HNECs) from subjects with chronic rhinosinusitis with nasal polyps.⁴³ In another study, tofacitinib blocked CCL5 production (also known as RANTES) in BEAS2B cells induced by the double-stranded RNA poly(I:C), and it did so more potently than fluticasone did.⁴⁴ Thus, JAK inhibitors can block the production of epithelial chemokines relevant to asthma in response to a variety of stimuli. Taken together, these exciting results suggest that JAK inhibitors will inhibit cytokine-driven epithelial activation in asthma. STAT6 does not appear to be a major target of glucocorticoids in the airway,⁴⁵ which raises the possibility that JAK inhibitors will also be effective in subjects with type 2–high and steroid-unresponsive disease.⁴⁶ Because IL-4 and IL-13 also induce production of iNOS in airway epithelial cells, FENO can be used as a pharmacodynamic biomarker of target engagement. Interestingly, Braithwaite et al recently conducted a phase I clinical trial of the JAK inhibitor GDC-0214; they showed proof of concept that FENO levels are indeed reduced by this compound¹¹ (see later).

In addition to inducing epithelial chemokine production, mucus metaplasia, and iNOS expression, T_H2 cytokines also cause epithelial barrier dysfunction. Barrier dysfunction in this context refers to dysfunction of tight junction complexes that normally help cells form a tight monolayer, and it is increasingly recognized as a feature of severe asthma and other diseases associated with chronic mucosal inflammation.^{47,48} In 16HBE epithelial cells, IL-4– and IL-13–induced barrier dysfunction was attenuated by a pan-JAK inhibitor.⁴⁹ Support for the idea that inhibiting JAK signaling can potentiate barrier function *in vivo* comes from a study by Amano et al, who reported that the JAK inhibitor JTE-052 improved skin barrier function in a mouse model.⁵⁰ It will be interesting in future studies to determine whether JAK inhibitors facilitate the restoration of barrier function in asthma, which might help break the “vicious cycle of leak.”⁴⁷

ASM cells are another potential target of IL-4 and IL-13 in the airway.^{51–54} Manson et al recently reported that IL-4 and IL-13 induced AHR in a dexamethasone-insensitive manner in isolated human small airways.⁵⁵ This was associated with increased expression of the histamine H1 receptor, but interestingly, it was not blocked by a STAT6 inhibitor.⁵⁵ Future studies will be needed to define the role of specific JAK family members in type 2 cytokine–driven ASM responses, and it remains to be seen whether JAK inhibitors might have direct effects on ASM contractility and AHR, independent of their anti-inflammatory effects.

IL-5 plays a critical role in eosinophil biology, and it promotes the growth and survival of eosinophils via the IL-5 α and IL-5R β_c (CSF2RB) receptor complex, which signals via JAK2. The IL-5 pathway–targeted biologics mepolizumab, reslizumab, and benralizumab are now widely used to treat severe and exacerbation-prone asthma.⁵⁶ Therefore, it stands to reason that JAK2 antagonists might have antieosinophilic effects that are potentially of clinical utility in asthma. However, JAK2 is also involved in signal transduction downstream of erythropoietin

(EPO), thrombopoietin (TPO), and myeloid growth factors (ie, GM-CSF and G-CSF), and bone marrow suppression is a recognized side effect of JAK2 inhibition.¹⁴ Inhaled JAK2 inhibitors that are not active systemically might be able to suppress cytokine-dependent eosinophil activation within the lung without suppressing hematopoiesis. It is important to note that the JAK inhibitors tofacitinib, ruxolitinib, and baricitinib have all demonstrated efficacy in the treatment of idiopathic hypereosinophilic syndrome, eosinophilic esophagitis, and eosinophilic fasciitis.⁵⁷⁻⁵⁹ Efficacy in hypereosinophilic syndrome and related conditions could be due to inhibition of the JAK-dependent production of cytokines that drive pathologic eosinophilia, as well as JAK-coupled cytokine receptors on eosinophils. In future studies of JAK inhibitors in asthma, it will be important to investigate whether inhibiting airway eosinophils is associated with, or necessary for, clinical efficacy.

JAK-STAT AND TYPE 2-LOW ASTHMA

Although the immune and inflammatory mechanisms underlying type 2–low asthma are not currently well understood,¹⁻³ it seems likely that JAK inhibitors may also be useful in some subjects with type 2–low asthma. Here we review and speculate on the potential role for JAK inhibitors in endotypes potentially associated with type 2–low asthma.

IL-6

Circulating levels of IL-6 are increased in some subjects with type 2–low asthma in association with metabolic disturbances and systemic inflammation.⁶⁰⁻⁶² A gene signature of IL-6–driven epithelial activation is apparent in many individuals with severe asthma independent of type 2 inflammation.⁶³ An IL-6 antagonist is currently under investigation in individuals with severe asthma and elevated plasma IL-6 levels in the National Heart, Lung and Blood Institute–sponsored PrecISE Asthma Network⁶⁴ (ClinicalTrials.gov identifier NCT04129931). IL-6 receptor signaling activates JAK1, JAK2, and Tyk2, but IL-6–dependent STAT3 phosphorylation was preferentially inhibited by JAK1 inhibitors in studies using reference human cell lines.⁸ Therefore, JAK1 inhibitors might be beneficial in subjects with IL-6–high asthma. Measuring IL-6–dependent biomarkers such as C-reactive protein (CRP) could provide a biomarker of target engagement in these subjects.

IFN- γ

Increased frequency of IFN- γ –expressing lymphocytes has been observed in subjects with severe asthma, and IFN- γ signaling was required for AHR in a mouse model of severe asthma.⁶⁵⁻⁶⁷ Taken together, these observations suggest that blocking JAK1 or JAK2 activation downstream of IFN- γ receptors may be useful in subjects with this asthma endotype. At present, whether these subjects can be identified with an easily measured biomarker is not clear, but reduced expression of secretory leukocyte peptidase inhibitor (SLPI) in sputum or lung lavage samples might be useful in this regard.

IL-17

In mouse models, T_H17 cells are important for airway neutrophilia and AHR, which can be inhibited by neutralizing IL-17A.⁶⁸

However, the IL-17 receptor antagonist brodalumab did not meet the clinical end points in a controlled trial in individuals with moderate-to-severe asthma,⁶⁹ and whether blocking IL-17 family members in subsets of individuals with asthma is clinically useful remains to be seen. Although IL-17A signals independently of JAK family members, IL-17A–secreting T_H17 cells develop in response to JAK-coupled cytokines.^{8,70} In mouse models, there is heterogeneity among T_H17 cells, with some subsets having potentially protective effects in promoting tissue integrity, whereas pathologic T_H17 cell subsets cause tissue inflammation and injury.⁷¹⁻⁷³ Because pathologic T_H17 cell subsets are dependent on IL-23, which preferentially signals via Tyk2,⁸ Tyk2 antagonists might be effective in diseases mediated by pathologic T_H17 cells.

Mast cells

Mast cell activation characterizes many subjects with severe asthma, and the tyrosine kinase inhibitor imatinib decreased AHR, mast cell counts, and tryptase release in a pilot study of individuals with severe and refractory asthma,⁷⁴ probably by targeting the receptor tyrosine kinase c-Kit, which activates JAK2 and STAT1.⁷⁵ STAT5 is critical for mast cell growth and survival,⁷⁶ and it was recently shown to be involved in TSLP-induced skin mast cell degranulation.⁷⁷ The role of JAK family members in mast cell biology is under active investigation. In human mast cell lines, ruxolitinib blocked degranulation and cytokine production.⁷⁸ These observations raise the possibility that JAK inhibitors might block mast cell activation in asthma, which can be monitored by measuring serum and lung tryptase levels and other readouts.

PRECLINICAL STUDIES IN ANIMAL MODELS

Several preclinical studies have demonstrated efficacy of systemic or topical JAK inhibitors in animal models of asthma (Table II).^{16,18,19,22-27,79} In an early study, Kudlacz et al administered tofacitinib (known then as CP-690550) via subcutaneous osmotic minipumps (1.5–15 mg/kg per day) to mice during either sensitization with ovalbumin (OVA) plus alum intraperitoneally, or only during challenge with an OVA aerosol. Tofacitinib inhibited BAL levels of eosinophils, eotaxin, and IL-13 in a dose-dependent manner when administered during sensitization and challenge, as well as during challenge alone.²² Matsunaga et al studied the effect of the pan-JAK inhibitor P6 on airway inflammation and AHR in OVA-challenged mice.²³ P6 inhibited AHR and some aspects of airway inflammation, especially if administered by intraperitoneal injection encapsulated with poly D,L-lactic-co-glycolic acid (PLGA) during the challenge phase with OVA aerosols. Surprisingly, P6 was *not* effective when administered during both sensitization and challenge with aerosolized OVA.

Ashino et al administered the JAK1/3 inhibitor R256 to mice and compared its effects when administered during the sensitization or challenge phases with use of OVA as a model allergen.²⁴ When administered by oral gavage only during sensitization with OVA plus alum administered intraperitoneally, R256 inhibited the AHR, BAL eosinophilia, periodic acid–Schiff–positive airway cells, and T_H2 cytokine production that developed during OVA aerosol challenge 21 days later. When administered only during OVA challenge, R256 also inhibited AHR, BAL eosinophilia, and mucus metaplasia; however, T_H2 cytokine production was

unaffected. This mimicked the effect of this compound on T_H2 cell differentiation and cytokine production *in vitro*, in which case R526 inhibited cytokine-induced T-cell differentiation but not OVA-driven recall cytokine production in already-differentiated cells.

Dengler et al reported on the development of the novel inhaled pyrazolopyrimidine JAK inhibitor iJAK-381, which was very effective in different animal models of allergic airway inflammation.¹⁸ In kinase inhibition assays, iJAK-381 inhibited JAK1 (K_i = 0.26 nM) more than JAK2 (K_i = 0.62 nM) did, and it was less active against JAK3 (K_i = 20.8 nM) and Tyk2 (K_i = 3.15 nM). Although iJAK-381 inhibited IL-13-induced STAT6 phosphorylation, as well as JAK2-dependent EPO and IL-5 signaling in cell lines, when administered *in vivo* it was specific for STAT6 inhibition in the lung. Inhaled iJAK-381 blocked eosinophilic and neutrophilic airway inflammation and reactivity in 2 different mouse models with either OVA or a combination of *Aspergillus*, *Alternaria*, and house dust mite extracts used as model allergens. Inhaled iJAK-381 had no apparent systemic effects on spleen cellularity or natural killer cell count, which is a sensitive marker of systemic JAK inhibition.⁸⁰ Inhaled iJAK-381 also blocked OVA-induced airway inflammation in guinea pigs. Taken together, the data from this important study demonstrated proof of concept that a selective inhaled JAK inhibitor has therapeutic potential in asthma.

A new class of pyridone JAK inhibitors was recently developed; it demonstrated good selectivity, long lung retention time, and low oral bioavailability, suggesting that these compounds would be effective when administered via inhalation.²⁰ Calbet et al reported that 1 member of this class, LAS194046, suppressed OVA-driven airway inflammation and airway reactivity (as measured by enhanced pause [Penh]) in rats.¹⁶ LAS194046 was a potent pan-JAK inhibitor, with IC₅₀ values of 5.46, 0.4, and 2.07 nM, respectively, for JAK1, JAK2, and JAK3, and 21.8 nM for Tyk2, in *in vitro* kinase inhibition assays. LAS194046 demonstrated target engagement in the lung, as indicated by reduced levels of phospho-STAT1 and phospho-STAT6 in lung homogenates in the absence of pharmacologically relevant systemic plasma levels.

Caniga et al studied a new inhaled JAK inhibitor called iJAK-001 in a rat model of *Alternaria* extract-induced lung inflammation and found that when dosed intratracheally before challenge, it inhibited BAL eosinophilia in a dose-dependent manner.¹⁹ Substrate specificity for iJAK-001 was not described in the report. A single dose of iJAK-001 delivered 48 hours before challenge was also effective. iJAK-001-treated animals demonstrated improved lung retention and a greater lung-to-plasma ratio than did the more water-soluble JAK inhibitor tofacitinib. In sheep challenged with *Ascaris suum*, iJAK-001 (when delivered via a dry powder inhaler) inhibited the increase in airway resistance during both the early- and late-phase airway responses. Importantly, intravenously administered iJAK-001 (delivered to match the systemic exposure with the inhaled compound) had no effect in this model. This study adds further support to the idea that lung-restricted JAK inhibition is sufficient to achieve physiologically relevant end points in asthma models.

Younis et al developed a method to aerosolize tofacitinib, with a particle size estimated to deposit efficiently in the lung (1.2 ± 0.2-μm median mass aerodynamic diameter). They found that nebulized tofacitinib was significantly effective in reducing house dust mite-induced airway eosinophilia and also reduced BAL

protein levels, a marker of barrier dysfunction.²⁶ Their findings demonstrate that tofacitinib can be successfully nebulized at a droplet size suitable for apparent therapeutic efficacy.

Joo et al developed a mouse model of chronic rhinosinusitis by topical application of intranasal OVA in sensitized mice and compared the effect of intraperitoneally administered tofacitinib with that of triamcinolone.²⁷ Tofacitinib effectively inhibited nasal inflammation, as determined by reduced numbers of tissue eosinophils and T_H2 cytokines, and it was associated with target engagement, as determined by reduction in phospho-STAT6. Thus, JAK inhibitors can block both upper and lower airway pathology in mouse models of allergic airway inflammation.

In addition to these studies of allergen-driven airway inflammation, which involves effector cells of the adaptive immune system, JAK inhibitors have also been shown to dampen LPS-induced lung inflammation, which is mediated by the innate immune system. For example, Calama et al administered aerosolized LPS (0.1 mg/mL) and studied the effect that orally administered tofacitinib (3–30 mg/kg) administered 1 hour prior has on lung inflammation.⁸¹ Tofacitinib effectively suppressed lung phospho-STAT3 levels and BAL neutrophilia, and it selectively attenuated production of IL-6, MIP-1α, IL-1β, and MIP-3α without inhibiting TNF-α or CXCL1 levels in BAL fluids. Therefore, JAK inhibitors have the potential to suppress innate and adaptive immunity in the lung.

HUMAN CLINICAL STUDIES

The inhaled selective, small molecule JAK inhibitor GDC-0214 was recently evaluated by Braithwaite et al in a double-blind, randomized, placebo-controlled, phase I proof-of-activity study.¹¹ GDC-0214 is a relative JAK1 inhibitor of the pyrazolopyrimidine class. In biochemical studies, GDC-0214 has a K_i for JAK1 inhibition of 0.40 nM, which is 2.3-fold more selective than for JAK2, 20-fold more selective than for JAK3, and 3-fold more selective than for Tyk2. In cell-based assays of cytokine-induced STAT phosphorylation, GDC-0214 has an IC₅₀ value of 17 nM for JAK1 (IL-13-induced phosphorylation of STAT6), which is 12-fold more selective than for JAK2 (EPO-induced phosphorylation of JAK2). The trial included adults with mild asthma and a baseline FENO level greater than 40 ppb. All subjects completed a 4-day, single-blind placebo period followed by a 10-day, double-blind treatment period of either placebo or GDC-0214. The treatment arm comprised 4 sequential ascending dose cohorts of patients who were given 1 mg once daily, 4 mg once daily, 15 mg once daily, and 15 mg twice daily. Each dose-allocated cohort completed the treatment period before the subsequent higher-dosed cohort was treated. Mild asthma was defined as a physician-made diagnosis of 6 months or more and an FEV₁ value greater than 70% predicted. Patients who had used ICSs within the past 60 days, had poor asthma control, or had a significant smoking history were excluded. A total of 36 patients were enrolled; their mean age was 28 years and their ages ranged from 18 to 56 years. The majority of patients were female (56%), predominately White (64%), and not of Hispanic or Latino origin (89%). Their mean baseline FENO level, established during the blinded placebo period, was 93 ppb, with a wide range of variation from 32 to 190 ppb. The primary outcome to establish drug activity was placebo-corrected percent reduction in FENO level from baseline to the end of the 10-day active treatment period.

TABLE III. Clinical trials of JAK inhibitors in asthma listed on [ClinicalTrials.gov](https://clinicaltrials.gov) (search date July 10, 2021)

Name of compound (route)	Study phase	Sponsor	Target	ClinicalTrials.gov identifier
AZD0449 (inhaled)	Completed	AstraZeneca	JAK1	NCT03766399
VR588 (in vitro study)	Completed	Imperial College London	Pan-JAK	NCT02740049
AZD4604 (inhaled)	Not yet recruiting	AstraZeneca	Not specified	NCT04769869
TD-8236 (inhaled)	Completed	Theravance Biopharma	Pan-JAK	NCT04150341
TD-8236 (inhaled)	Completed	Theravance Biopharma	Pan-JAK	NCT03652038

Although changes in FENO level were nonsignificant for the 2 lowest doses of 1 mg daily and 4 mg daily,¹¹ significant reductions in FENO level were observed in the cohorts treated with the 2 highest doses of 15 mg daily and 15 mg twice daily. At the end of the active treatment period, the placebo-corrected absolute reductions in FENO level for the 15-mg daily group and 15-mg twice-daily group were 18 ppb and 44 ppb, respectively. The changes in treatment group FENO level were placebo-corrected by 1.1 ppb (± 20), which was the small difference observed in the pooled placebo sample between baseline and end of the treatment period. Following the treatment period, there was a 28-day safety monitoring follow-up period during which no deaths or serious adverse events were noted. Most patients (88.9%) reported at least 1 adverse event, but only 1 adverse event (thirst) was thought to be attributable to GDC-0214. Upper respiratory tract infections were the most commonly reported adverse outcome (30.6%), but the infection rate was not significantly different between the placebo and treatment groups. Low systemic exposure to GDC-0214 was expected on the basis of preclinical data and demonstrated by unaffected eosinophil counts, as well as by low plasma levels of the drug. Plasma levels of GDC-0214 measured at multiple time points during the study period were at least 15-fold less than concentrations expected to inhibit systemic JAK1. This important study established that local targeting of JAK1 is a feasible approach, and it opens the door to a new era of pharmacotherapy in asthma.

Ongoing studies of other JAK inhibitors in asthma that are currently listed on [ClinicalTrials.gov](https://clinicaltrials.gov) are reported in [Table III](#).

SAFETY

An important aspect of considering JAK inhibitors in asthma is balancing their potential efficacy in reducing lung inflammation and AHR with safety concerns. The safety of JAK inhibitors has been intensively studied and scrutinized, especially in subjects with rheumatic diseases treated with tofacitinib,⁸²⁻⁸⁴ with ongoing postmarketing and surveillance studies that will lead to a better understanding of the long-term safety profile of these compounds in the coming years. Although early studies of tofacitinib were conducted in subjects treated with other disease-modifying antirheumatic drugs (DMARDs [eg, methotrexate]), which have their own potential side effects, subsequent clinical experience with JAK inhibitors has indicated serious potential safety concerns that will need to be carefully considered in clinical trials in asthma. Here we review 6 potential safety concerns of systemic JAK inhibitors: (1) opportunistic and respiratory viral infections, (2) viral reactivation, (3) malignancy and lymphoproliferative disorders, (4) decreased lymphocyte and neutrophil counts, (5) lipid profile and cardiovascular disease, and (6) venous thromboembolism (VTE). To date, there is insufficient experience with the use of topical JAK inhibitors, but it seems likely that most

of these concerns will be significantly lessened in patients treated with inhaled JAK inhibitors.

Opportunistic and respiratory viral infections.

Because JAK inhibitors are immunosuppressive, infection should be considered as a potential risk of these agents. Serious and sometimes fatal infections due to bacterial, mycobacterial, invasive fungal, viral, or other opportunistic pathogens have been reported in patients with RA who are receiving tofacitinib.⁸²⁻⁸⁴ Infections were also among the most common adverse events in studies with baricitinib.⁸⁵ The risk of serious infections in patients treated with JAK inhibitors needs to be considered in context with other populations. In patients with RA treated with tofacitinib the rate of serious infections is 2.7 per 100 patient years, whereas in general asthma populations the risk of serious infections is 1.8 per 100 patient years, and in individuals with asthma who are taking oral steroids the rate is 7.8 per 100 patient years.⁸⁶ Therefore, the risk of serious infections in patients with RA treated with tofacitinib is less than that observed in individuals with severe, corticosteroid-dependent asthma. Because of the potential risk of infections with the administration of any JAK inhibitor, subjects with acute and chronic infections or a history of recurrent infections and/or latent infections should be excluded from studies with these compounds. All subjects treated with JAK inhibitors should be monitored for signs and symptoms of infection during treatment, with appropriate antimicrobial therapies initiated promptly when indicated.

Respiratory viral infections are of special concern in asthma given their association with asthma exacerbations. Neither respiratory viral infections nor asthma exacerbations have been reported with increased frequency in clinical trials of JAK inhibitors in other diseases to date, although these studies did not specifically enroll subjects with severe asthma or carefully monitor asthma exacerbations. Additionally, the striking clinical efficacy of different JAK inhibitors in patients with COVID-19 pneumonia is encouraging⁸⁷ and indicates that these compounds can suppress deleterious lung inflammation associated with a life-threatening respiratory virus. On the other hand, individuals with severe asthma may be particularly susceptible to respiratory viral infections, and future clinical trials in asthma will need to carefully monitor viral infections and exacerbation rates in asthma.

Viral reactivation. Viral reactivation has been reported with JAK inhibitors and appears to be a class effect with these compounds. Cases of herpes virus reactivation (eg, herpes zoster), including disseminated disease, were observed in clinical studies with tofacitinib and other JAK inhibitors.^{84,88} In patients with RA, the risk of herpes zoster is increased by concomitant use of prednisone (>7.5 mg per day), as well as other immunosuppressive agents (eg, methotrexate). In the general population, the incidence rate of herpes zoster per 100 person years ranges from 0.31 to 0.55, which is similar to the incidence rate in individuals with

asthma who are not taking oral corticosteroids (0.47). In individuals with asthma who are taking 5 mg or more of prednisone per day, the incidence rate is 0.72 per 100 person years.⁸⁶ In patients with RA who are taking tofacitinib alone, the incidence rate is 2.2, whereas it increases to 3.8 in subjects using tofacitinib and oral corticosteroids and to between 4.4 and 5.4 in subjects using tofacitinib plus other immunosuppressive agents. Thus, subjects experiencing recurrent or disseminated herpes zoster, as well as subjects with other active viral infections (eg, hepatitis B and C), should be excluded from studies with JAK inhibitors. Appropriate antiviral vaccines (including zoster) should be administered to subjects before initiation of JAK inhibitor therapy where appropriate.

Malignancy and lymphoproliferative disorders. Lymphoma and other malignancies have been observed in patients treated with tofacitinib.⁸³ EBV-associated posttransplant lymphoproliferative disorder has been observed at an increased rate in renal transplant patients treated with tofacitinib and concomitant immunosuppressive medications.⁸⁹ In controlled clinical trials and postmarketing studies in subjects with RA and subjects with psoriatic arthritis, higher rates of malignancy were observed in subjects treated with tofacitinib with or without another DMARD than in subjects treated with placebo with or without a DMARD.⁸³ Although malignancies were observed in these observational studies, a meta-analysis comparing the risk of malignancies in patients with RA treated with tofacitinib and other DMARDs concluded that tofacitinib use did not increase the risk of malignancies.⁹⁰ Furthermore, it is estimated that the rate of malignancy in the general asthma population (1.6-1.9 per 100 patient years) is comparable to the rate of malignancy in patients with RA treated with tofacitinib (0.8-1.8 per 100 patient years).⁹¹ The ORAL Surveillance study ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02092467) identifier NCT02092467) is an FDA-mandated postmarketing safety study intended to demonstrate noninferiority of tofacitinib to TNF inhibitors among older patients with RA from the standpoint of risk of cardiovascular events and malignancies. In February 2021, on the basis of preliminary results from the ORAL Surveillance study, the FDA issued a safety alert warning about the risk of cancer in older subjects taking tofacitinib for RA. The final results of this and other studies will need to be monitored to help assess the risk-benefit ratio of using JAK inhibitors in other inflammatory diseases.

Decreased lymphocyte and neutrophil counts. Tofacitinib has been associated with initial lymphocytosis at 1 month of exposure, followed by a gradual decrease in mean lymphocyte counts of approximately 10% during 12 months of therapy. Counts less than 500 cells/mm³ were associated with an increased incidence of treated and serious infections. Tofacitinib has also been associated with an increased incidence of neutropenia (<2000 cells/mm³) versus the incidence with placebo.⁸⁴ In 2 phase 3 clinical studies of ruxolitinib, 1% of patients had to have their dose reduced or their treatment stopped because of neutropenia.⁹² Dose-dependent decreases in neutrophil counts were also observed in patients treated with baricitinib, but infections were rare even in subjects with neutropenia.⁹³ Lymphocyte and neutrophil counts should be monitored closely in subjects taking JAK inhibitors.

Lipid profile and cardiovascular disease. JAK blockade by tofacitinib was associated with increases in lipid parameters, including total cholesterol, low-density lipoprotein, and high-density lipoprotein levels. Maximum effects were

generally observed within 6 weeks. Elevated lipid levels were also observed in subjects treated with ruxolitinib and baricitinib. In ongoing studies in subjects with RA and psoriasis, the alterations in lipid profiles have not translated into an increased incidence of major cardiac adverse events.⁹⁴⁻⁹⁶ However, in 2021 the FDA announced that on the basis of preliminary analyses of the ORAL Surveillance study ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02092467) identifier NCT02092467), older RA subjects treated with tofacitinib experienced more major cardiovascular events than did those treated with TNF inhibitors. RA is known to be an independent risk factor for cardiovascular disease, and final results from this study will be needed to help us understand the cardiovascular risk profile of JAK inhibitors in different patient populations.

VTE. Concerns were raised about the risk of VTE in subjects treated with higher doses of JAK inhibitors, and upadacitinib, tofacitinib, and baricitinib all carry black box warnings about the risk of thrombosis. Whether or how JAK inhibitors induce a prothrombotic state is not known, and observational studies are confounded by the fact that many immune and inflammatory diseases are associated with increased risk of VTE. Transient increases in platelet counts have been observed following initiation of JAK inhibitors,⁹⁷ but these increases were not associated with thrombotic events. A recent metaanalysis concluded that the risk of VTE associated with JAK inhibitor use was low.⁹⁸ More information clarifying the potential prothrombotic risks of systemic JAK inhibition should be forthcoming soon.

Other safety considerations. Other safety considerations with JAK inhibitors include anemia and elevation of liver enzyme levels, which occurred in 1% to 2% of patients. Most of these abnormalities have occurred in studies with background DMARD (primarily methotrexate) therapy.

CONCLUSIONS

JAK inhibitors are a new class of anti-inflammatory agents that have strong therapeutic potential in asthma. On the basis of their broad mechanism of action, JAK inhibitors should be clinically useful in multiple different asthma endotypes, including in subjects with type 2-high and type 2-low asthma. An obvious unmet need is in individuals with severe asthma whose disease is poorly controlled despite high-dose ICSs plus other controllers, in which case JAK inhibitors could represent a more convenient (via pill or inhaler) and less expensive therapy than biologics. JAK inhibitors have the potential to improve symptom control, reduce exacerbations, and minimize the use of systemic corticosteroids. The benefits of JAK inhibitors will need to be carefully balanced with potential risks. The ongoing development of inhaled JAK inhibitors is particularly exciting because these compounds should have a better safety profile than systemic JAK inhibitors in asthma.

As we await more efficacy and safety data from studies of JAK inhibitors in other inflammatory diseases, we can speculate about where to position these therapies in the asthma armamentarium. Systemic JAK inhibitors should be investigated in individuals with severe and/or exacerbation-prone asthma, who require maintenance or frequent bursts of systemic steroids to achieve control. In these subjects, even a single course of systemic glucocorticoids is associated with serious side effects, and maintenance steroids are associated with substantial morbidity and mortality.⁹⁹⁻¹⁰² The safety profile of systemic JAK inhibitors

reported to date seems to be at least comparable to that of systemic glucocorticoids. Because JAK inhibitors have a completely different mechanism of action, they may be particularly effective in individuals with asthma who are refractory or resistant to the anti-inflammatory effects of glucocorticoids. Inhaled JAK inhibitors should be studied in subjects with severe and mild-to-moderate asthma, both as maintenance inhalers and on demand during periods of loss of control. As JAK inhibitors enter clinical trials in asthma, these compounds should also provide insights into disease pathobiology. An important area for future research will be identifying biomarkers that predict response to therapy and can be used to monitor response to therapy. This will help us tailor therapy to the best-responding subset of patients and achieve the goal of personalized medicine in asthma.

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