



The potential use of tyrosine kinase inhibitors in severe asthma

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Purpose of review

Severe asthma comprises heterogeneous phenotypes that share in common a poor response to traditional therapies. Recent and ongoing work with tyrosine kinase inhibitors suggests a potential beneficial role in treatment of severe asthma.

Recent findings

Various receptor and nonreceptor tyrosine kinase pathways contribute to aspects of airway inflammation, airway hyperresponsiveness, and remodeling of asthma. Selective and nonselective tyrosine kinase inhibitors may be useful to block pathways that are pathologically overactive or overexpressed in severe asthma. Recent in-vivo studies have demonstrated the utility of inhibitors against specific tyrosine kinases (epidermal growth factor receptor, c-kit/platelet derived growth factor receptor, vascular endothelial growth factor receptor, spleen tyrosine kinase, and janus kinase) in altering key aspects of severe asthma.

Summary

Asthma and even severe asthma does not consist of a single phenotype. Targeting key inflammatory and remodeling pathways engaged across subphenotypes with tyrosine kinase inhibitors appears to hold promise.

Keywords

airway remodeling, asthma, targeted treatment, tyrosine kinase inhibitors

INTRODUCTION

Asthma is a heterogeneous respiratory disorder with clinical symptoms induced by airway inflammation, airway hyperresponsiveness (AHR), and airway remodeling. This umbrella definition captures many syndromes in which disease is caused by interactions between various environmental insults (allergens, viruses, pollutants, environmental tobacco smoke, specific drugs, hormones, etc.) and locally expressed susceptibility genes. Dividing asthma into distinct phenotypes may provide a greater understanding of underlying pathologies and consequently allow more effective targeted therapy. Severe or refractory asthma is seen in up to 10% of asthmatic patients and results in higher morbidity, mortality, and a disproportionate use of healthcare costs [1]. These patients may not respond appropriately to continuous and high-dose corticosteroid therapy, underscoring a need for novel targeted treatments that could improve their clinical symptoms and lung function. On a molecular basis, one strategy is to inhibit key intermediate cellular signaling pathways induced by inhaled insults that activate inflammatory cells and alter structural cells

of the lung. Protein tyrosine kinases are intimately involved in signal transduction underlying asthma pathogenesis. Selectively targeting tyrosine kinases that are overactive or overexpressed with small molecule inhibitors may represent a new approach in the treatment of severe asthma.

SEVERE ASTHMA: DEFINITION AND MECHANISMS

To date, there is not a single uniformly agreed upon definition of severe asthma, although major consensus statements have been issued by the American

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KEY POINTS

- Severe asthma comprises several subphenotypes.
- Tyrosine kinases play key roles in several mechanisms (i.e. airway inflammation, airway hyperresponsiveness, and airway remodeling) leading to the subphenotypes of severe asthma.
- Inhibitors of tyrosine kinases slow or thwart the three key features of severe asthma *in vitro*.
- The most promising data, which may lead to therapeutic options for severe asthma, are seen with inhibitors of the receptor tyrosine kinases (i.e. epidermal growth factor receptor, c-kit/platelet derived growth factor receptor, vascular endothelial growth factor receptor), and the nonreceptor tyrosine kinases (i.e. spleen tyrosine kinase and janus kinase).

Thoracic Society workshop on refractory asthma [1,2] and the WHO [3[¶]]. These working definitions in large part focus on clinical symptomology and/or inappropriate response to medications and underscore the concept that multiple subphenotypes of severe asthma exist, with varying physiologic, pathologic, and molecular abnormalities [4[¶],5]. On a molecular basis, more recent attempts to stratify asthma severity in adults used sputum granulocytes to differentiate between four phenotypes (<2% eosinophils and either < or ≥40% neutrophils; ≥2% eosinophils and either < or ≥40% neutrophils) with different clinical characteristics, lung function, and inflammatory mediators [4[¶]]. These additional data demonstrate that severe asthma is not a single-disease entity. Having a therapeutic strategy that could broadly target key or interlinked inflammatory and remodeling pathways likely to be engaged across subphenotypes deserves exploration.

The primary molecular mechanism underlying asthma has traditionally been thought to be driven by aeroallergens activating T-helper type 2 (Th2) lymphocytes that subsequently orchestrate the allergic inflammatory cascade. Production of allergen-specific IgE and maturation, recruitment, and activation of mast cells, basophils, and eosinophils contribute to inflammation, AHR, and remodeling. In severe asthma, there is deviation from this classical profile, with a mixed Th2/Th1/Th17 phenotype resulting in increased neutrophils, tissue injury, and remodeling [6[¶],7,8]. Whereas granulocytic inflammation undoubtedly is a major player that needs to be addressed in severe asthma, ongoing epithelial injury/repair and bronchial hyperresponsiveness also merit attention.

The airway epithelium is being increasingly viewed as a key player in asthma pathogenesis. The epithelium is not a passive barrier; by virtue of its location between the external environment and host, it directs both inflammatory and remodeling responses. In susceptible individuals, the lower airway epithelium is both structurally and functionally defective. After injury, these defects lead to aberrant communication with the underlying mesenchyme. The epithelial mesenchymal trophic unit may be persistently activated, changing the airway microenvironment to favor remodeling and chronically reducing lung function [9[¶]]. Histologically, this is reflected as epithelial metaplasia, goblet cell hyperplasia, hypertrophy and hyperplasia of the airway smooth muscle (ASM), angiogenesis, and deposition of extracellular matrix (ECM) including collagen. Clinically, this is manifested as decline in lung function and refractoriness to standard therapies.

AHR is a cardinal feature of asthma that worsens in proportion to disease severity, yet its mechanisms remain poorly explained [9[¶]]. Postulated mechanisms of AHR include interactions between locally infiltrated mast cells and ASM, an overall increase in ASM thickness and altered biophysical properties [10–12]. Asthma therapy with inhaled corticosteroids guided by measurements of AHR results in a more favorable impact on symptoms, lung function, and airway biopsies, when compared with conventional assessment [13].

Taken together, these data underscore the concept that even in severe asthma, there are many different pathologic differences with likely distinct (albeit overlapping) clinical, inflammatory, and reparative/remodeling processes. It is grossly insufficient to focus all efforts on developing treatments to address established inflammation, as remodeling and AHR could also be modified to improve clinical and functional outcome measures.

OVERVIEW OF TYROSINE KINASES AND THEIR INHIBITORS

Tyrosine kinases, critical enzymes in signal transduction pathways, transfer a phosphate group from ATP to a target protein's tyrosine residue. This protein phosphorylation functions as an on/off switch for a variety of cellular activities. Tyrosine kinases are further subclassified into receptor tyrosine kinases (RTKs), which are enzymes involved in transmembrane signaling, and nonreceptor tyrosine kinases (non-RTKs), which are enzymes not associated with cell surface receptors but rather are intracellular. Both RTKs and non-RTKs play a role in cellular communication and regulation of activity

in a variety of cell types in asthma. RTKs on responsive cells are engaged by external inflammatory signals (e.g. allergen, virus, cytokine, or growth factor) and activate intracellular protein kinases that amplify and propagate the signal by further activating downstream enzymes and transcription factors [14]. These pathways control a wide variety of transcription factors capable of regulating gene expression that can affect the growth and activation of inflammatory cells such as mast cells and eosinophils, apoptosis, epithelial cell function, smooth muscle proliferation, and angiogenesis [14,15]. Some of the key RTKs in asthma include epidermal growth factor receptor (EGFR), c-kit (a stem cell factor receptor), platelet-derived growth factor receptor (PDGFR), and vascular endothelial growth factor receptor (VEGFR). Non-RTKs, including spleen tyrosine kinase (Syk), Src, and janus kinase (JAK), are important for signaling in immune cells relevant to asthma [14].

Given the central role of RTKs and non-RTKs in signaling pathways of inflammation and remodeling, one can surmise the role of tyrosine kinase inhibitors (TKIs). TKIs could inhibit inflammatory mediators and adhesion molecules, regulate T-cell, macrophage and epithelial cell function, blunt AHR, and diminish airway remodeling relevant to asthma pathogenesis [16]. In-vitro and in-vivo studies in asthmatic animal models and human asthmatic patients suggest that TKIs hold promise as a therapeutic option for severe asthma.

SPECIFIC RECEPTOR TYROSINE KINASES AND THEIR INHIBITORS

Several important receptor tyrosine kinases (e.g. EGFR, c-kit, PDGFR, VEGFR) and their roles in asthma pathophysiology will be discussed in this section. Ligands for RTKs are primarily growth factors that are responsible for repair and remodeling of airways in asthmatic patients.

EPIDERMAL GROWTH FACTOR RECEPTOR

Epithelial cells and ASM cells are sources of inflammatory mediators and growth factors [17,18,19]. With epithelial injury, the underlying mesenchyme is activated to participate in the reparative process, secreting a variety of profibrogenic growth factors such as epidermal growth factor [20]. Additionally, the airway epithelium can upregulate expression of EGFR, as has been demonstrated specifically in severe chronic asthmatic airways [21–23,24²⁵,25–28]. As a result, mesenchymal cells undergo transformation to myofibroblasts and secrete ECM components, contributing to pathological remodeling

of the airways. In severe asthma, airflow limitation from exaggerated mucus production may result from metaplasia of goblet cells, which is in part driven by neutrophil-dependent EGFR signaling [29]. Signaling through EGFR is also thought to increase recruitment of eosinophils and contribute to AHR [24³⁰,30].

Tyrosine kinase inhibition of EGFR demonstrates favorable in-vitro effects relevant to severe asthma [26,31³²,32]. The investigational TKIs, tyrphostin AG1478, and BIBX1522, inhibit EGFR [33]; blockade of EGFR would have potential pharmacologic effects such as reduction of smooth muscle [34] and epithelial [35] cell proliferation, decreasing of mucin synthesis by MUC5AC [31³⁶,36], and improvement of airway remodeling of asthma [26].

In-vivo animal studies confirm benefit of TKIs targeting EGFR. An ovalbumin (OVA)-induced mouse asthma model demonstrated reduced collagen deposition and goblet cell proliferation in response to tyrphostin AG1478 [37]. Erlotinib treatment of mice with house dust mite-induced chronic allergic asthma suppressed airway remodeling (specifically airway smooth muscle hypertrophy and goblet cell metaplasia) and AHR [24³⁸]. Interestingly, neither study showed a beneficial reduction in eosinophilic airway inflammation. Gefitinib, a small-molecule EGFR inhibitor, has been found to suppress mucus secretion in animal studies [38].

C-KIT

Stem cell factor (SCF), the ligand for c-kit, is an important growth factor and activator of mast cells and eosinophils [15,39]. Serum and airway SCF concentrations have been shown to correlate with asthma severity and airway mast cell localization in humans [40,41]; c-kit mRNA is also increased in airway biopsies from asthmatic patients [42]. In murine studies, SCF/c-kit activation plays a role in airway remodeling by recruiting bone marrow-derived fibroblasts capable of promoting lung myofibroblastic transformation [43]. Upregulation of c-kit on dendritic cells suppresses Th1 responses and promotes Th2 and Th17 responses in mice [44].

Inhibiting the SCF/c-kit pathway *in vivo* may curtail the effects of mast cells and eosinophils in the pathophysiology of asthma. TKIs targeting c-kit blunt AHR improve lung function parameters, and reduce airway inflammation in a variety of asthma animal models [45–47]. Mouse models of asthma treated with imatinib and sunitinib demonstrated reduced markers of airway inflammation (airway eosinophils; Th2 cytokines IL-4, and IL-13; chemokines CCL2, CCL5, CCL6; and serum IgE) and AHR [45,46]. In a murine model of chronic allergic

asthma, treatment with imatinib resulted in diminished total lung collagen production and deposition [48], suggesting that c-kit inhibition prevents airway remodeling. Using the unique feline chronic asthma model (induced using Bermuda grass allergen in an outbred species that spontaneously develops an asthma syndrome similar to humans [49[■],50]), our group evaluated the effects of chronic (1 month) treatment with oral masitinib. We demonstrated reduction in airway eosinophilia; additionally, there was significant improvement in measures of overall lung compliance but not large airway resistance (i.e. EC200Raw), as measured after methacholine challenge [47,51[■]]. Masitinib in humans with corticosteroid-dependent severe asthma (the only c-kit TKI evaluated to date in a human clinical trial) showed improved asthma symptom scores, despite a lack of effect on steroid weaning or forced expiratory volume in the first second, a marker of airway obstruction [52].

PLATELET-DERIVED GROWTH FACTOR RECEPTOR

Allergen exposure in experimental asthma leads to upregulation of PDGF, with signaling through this pathway leading to ASM proliferation and enhanced AHR [53]. Altered fibroblast proliferation and activities (in part mediated by PDGF) are linked to airway remodeling. Human ASM cell hyperplasia in asthmatic patients may be a result of synergistic activity between fibroblast growth factor-2 (FGF-2) and TGF- β 1, and stimulated by PDGFR α activation [54]. Higher expression of the PDGFR α gene may also be associated with severe forms of the disease in children [55,56]. Endobronchial biopsy-derived fibroblasts from adult patients with severe asthma express upregulation of PDGFR- β [57]. There is evidence that PDGF contributes to β -adrenoreceptor desensitization [58], which can further complicate asthma therapy.

In vitro, the PDGFR TKIs, tyrphostin AG1296 and PDGFR inhibitor III, suppressed the synergism between FGF-2 and TGF- β 1, postulated to be responsible for ASM cell hyperplasia of asthmatic airway remodeling [54]. These data suggest a role of PDGFR inhibition in abating ASM hyperplasia and fibrosis in asthma.

Imatinib, masitinib, and sunitinib, discussed previously as c-kit inhibitors, also inhibit PDGFR; it is unclear which of their beneficial effects in asthma can be ascribed directly to inhibition of PDGFR versus other tyrosine kinase pathways. Although associations have been established between PDGFR expression, its genetic polymorphisms, and asthmatic airways, a clear role for

inhibiting the PDGFR in the treatment of clinically severe asthma has yet to be demonstrated in humans.

VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR

VEGF is a potent angiogenic factor involved in vascular and extravascular remodeling in the asthmatic patient's lung [59–61]. Induced sputum VEGF levels were elevated in asthmatic patients and correlated with degree of airway narrowing and airway vascular permeability [62]. Increased serum VEGF has been documented in pediatric patients with a more severe asthmatic phenotype [63[■]] and there is evidence that genetic polymorphisms in VEGF are linked to a decline in lung function [64]. Moderate asthmatic patients with attenuation of airway remodeling and bronchoconstriction secondary to formoterol/budesonide treatment demonstrated reduction in VEGF and VEGFR1 [65], suggesting an association between reduction in VEGF/VEGFR1 and improved features of asthma. In a murine noneosinophilic asthma model, Th1 and Th17 responses were suggested to be dependent on VEGFR-induced T-cell priming [66[■]].

Whereas sunitinib (a multitargeted TKI inhibiting VEGF, c-kit, PDGFR, etc.) dampened airway inflammation, AHR and airway remodeling in a murine chronic asthma model, only expression of phosphorylated c-kit protein was evaluated and specific assessment of vascular remodeling was not performed [46]. It is possible that the VEGFR pathway may have mediated some of the extravascular remodeling changes [61]. Inhibition of VEGFR via semaxinib (SU5416) in a murine asthma model induced by lipopolysaccharide-containing allergens inhibited T-cell priming and differentiation into Th1 and Th17 pathways, suggesting a role for anti-VEGFR treatment in severe noneosinophilic asthma [66[■],67].

MISCELLANEOUS RECEPTOR TYROSINE KINASES

FGF is increased in bronchoalveolar lavage fluid (BALF) from mild and moderately atopic human asthmatic patients compared with nonasthmatic controls [68] and the FGF/FGFR pathway has been implicated in remodeling in the asthmatic patient's lung [59,69]. Nerve growth factor, increased in human asthmatic bronchi [70], has been linked to airway inflammation [71] and AHR [72] in asthma models. Insulin-like growth factor-1 has also been implicated in airway remodeling, but its role is less clear [73,74].

NONRECEPTOR TYROSINE KINASES AND THEIR INHIBITORS

A host of non-RTKs including Syk, Src, and JAK, among others are involved in signaling of immune receptors [e.g. T-cell, B-cell, and high affinity IgE receptors (FC ϵ R)], and inflammatory cell cytokine and chemokine receptors. Engagement of these signaling pathways affects cellular proliferation, differentiation, activation, recruitment, and survival, which, in turn, promote and perpetuate airway inflammation, AHR, and remodeling [75].

SPLEEN TYROSINE KINASE

Syk kinase is involved in antigen-receptor signaling in T lymphocytes and B lymphocytes, signaling of FC ϵ RI in mast cells to trigger mast cell degranulation and histamine release [76], and eosinophil survival in response to interleukin-5 (IL-5) and granulocyte

macrophage-colony stimulating factor [77,78]. Piceatannol, a Syk TKI, inhibited histamine release and attenuated in-vitro bronchial contraction of guinea pig airway muscle [79]. Although Syk inhibition blunts histamine release, it does not seem to regulate IgE-dependent activation of mast cells [80]. In a rat model, the Syk TKI BAY 61–3606 reduced airway inflammatory cells, bronchial edema, and, possibly, improved bronchoconstriction, as measured by BALF cell analysis, Evans blue dye extravasation from cells, and airway pressures measurements, respectively [81].

Src

Src family kinases are functional in signaling pathways for growth factors and their cell surface receptors [82], some of which have been shown to be highly expressed in ASM cells, wherein they may

Table 1. Animal and human studies with tyrosine kinases inhibitors

Target	Compound ^a	<i>In vitro</i> / <i>In vivo</i>	Study species	Outcome
EGFR	Tyrphostin AG1478	<i>In vitro</i>	Human	Reduction of smooth muscle and epithelial cell proliferation [26,34,35] and mucin synthesis [31 [■] ,36]
		<i>In vivo</i>	Mouse	Decreased collagen deposition and goblet cell proliferation [37]
EGFR	BIBX1522	<i>In vitro</i>	Human	Reduction of smooth muscle and epithelial cell proliferation [26,34,35] and mucin synthesis [31 [■] ,36]
EGFR	Erlotinib	<i>In vivo</i>	Mouse	Diminished airway remodeling and AHR [24 [■]]
EGFR	Gefitinib	<i>In vivo</i>	Rat	Suppression of mucus secretion [38]
c-kit	Imatinib	<i>In vivo</i>	Mouse	Reduction of airway eosinophilia and IL-4, IL-13, CCL2, CCL5, CCL6 [45]; decreased AHR [45,48]; decreased airway remodeling [48]
c-kit	Sunitinib	<i>In vivo</i>	Mouse	Reduction of airway eosinophilia, AHR, serum allergen-specific IgE, IL-4 and IL-13 [46]
c-kit	Masitinib	<i>In vivo</i>	Cat	Reduction of airway eosinophilia and improvement in compliance [47,51 [■]]
c-kit	Masitinib	<i>In vivo</i>	Human	Improvement in asthma symptom scores [52]
PDGFR	Tyrphostin AG1296	<i>In vitro</i>	Human	Diminished bronchial smooth muscle proliferation [54]
PDGFR	Imatinib, masitinib, sunitinib			See above
VEGFR	Semaxinib (SU5416)	<i>In vivo</i>	Mouse	Inhibited T cell priming and differentiation into Th1 and Th17 pathways [67]
SYK	Piceatannol	<i>In vitro</i>	Guinea pig	Inhibited histamine release; attenuated bronchial smooth muscle contraction [79]
SYK	BAY 61-3606	<i>In vivo</i>	Rat	Reduced airway inflammation and edema; diminished airway pressures [81]
Src	SU6656	<i>In vivo</i>	Rat	Inhibition of angiotensin II induced bronchial smooth muscle hyperresponsiveness [85]
JAK	P6	<i>In vivo</i>	Mouse	Suppressed airway eosinophilia and AHR [89 [■]]
	Tofacitinib			Impaired Th2 and Th17 cell differentiation [90]; and reduced airway eosinophils, IL-13 and eotaxin [87]

AHR, airway hyperresponsiveness; EGFR, epidermal growth factor receptor; JAK, janus kinase; PDGFR, platelet-derived growth factor receptor; SYK, spleen tyrosine kinase; VEGFR, vascular endothelial growth factor receptor.

^aIt is prudent to note that many/most tyrosine kinases inhibitors work via targeting multiple receptor tyrosine kinases (or nonreceptor tyrosine kinases). The table reflects the outcome for the major target listed.

play a role in AHR [83]. However, one src TKI in an in vitro co-culture (cytokine stimulated ASM cells and hematopoietic progenitor cells) was ineffective in attenuating eosinophil differentiation, as did inhibitors of other kinases [84]. These experiments suggest that although src TKI may have a role in airway smooth muscle contraction and AHR, it may not be mediated via the eosinophil pathway [84]. Treatment of rat bronchial smooth muscle (BSM) with SU6656, a selective Src family TKI, attenuated angiotensin II (Ang II, a known activator of Src kinases) mediated BSM contraction [85]; Ang II is also known to be associated with severe acute asthma [86].

JANUS KINASE

The janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway is involved in Th2 responses via cytokine signaling, making it a desirable target in allergic asthma [87]. It is additionally involved in signaling for the IL-23 Th17 axis, a pathway key in promoting neutrophilic inflammation in severe asthma [88[■]]. The pan-JAK inhibitor pyridine 6 (P6; inhibitor of JAK-1–3 and Tyk2) suppressed airway eosinophilia and AHR in OVA-challenged asthmatic mice; however, the inhibitory effects on Th2 responses appeared to be accompanied by an increase in Th17 cells [89[■]]. Tofacitinib (CP-690550), a JAK-1 and JAK-3 inhibitor, impaired murine IL-4-dependent Th2 cell differentiation and IL-23-dependent Th17 cell differentiation [90]. In a murine model of allergic pulmonary inflammation, tofacitinib diminished BALF eosinophilia, IL-13, and eotaxin [87].

CONCLUSION

Several studies identified specific tyrosine kinase dependent pathways responsible for elements of severe asthma (Table 1). In-vitro inhibition of tyrosine kinases has been shown to interrupt these pathways. Animal studies validated in-vitro findings, with a few TKIs minimizing features of severe asthma phenotypes: reduced airway inflammation, decreased AHR (c-kit/PDGFR inhibitors, JAK inhibitor P6), and lessened airway remodeling (imatinib, erlotinib). Studies of pathophysiology have not yet translated to clinical applicability in severe human asthmatic patients, primarily due to the complex nature of the disease. Clinically successful TKIs will likely be nonspecific and target several pathways.

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Conflicts of interest

C.R.R. had received support to conduct research from AB Science (Paris, France) in the amount of US\$60K, and from Pfizer (Kalamazoo, MI) in the amount of US\$124K. Both financial disclosures involved research in TKIs, and therefore may be considered as potential conflicts. V.P.G. has no financial disclosures or conflicts to declare.

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