



Asthma 2

Targeting the interleukin pathway in the treatment of asthma

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See Editorial page 1014

This is the second in a Series of two papers about Asthma

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Asthma is a common heterogeneous disease with a complex pathophysiology. Current therapies based on inhaled corticosteroids and longacting β_2 agonists are effective in controlling asthma in most, but not all patients, with a few patients falling into the severe asthma category. Severe asthma is characterised by poor asthma control, recurrent exacerbations, and chronic airflow obstruction despite adequate and, in many cases, high-dose treatments. There is strong evidence supporting the role for interleukins derived from T-helper-2 (Th2) cells and innate lymphoid cells, such as interleukins 4, 5, and 13, as underlying the eosinophilic and allergic inflammatory processes in nearly half of these patients. An anti-IgE antibody, omalizumab, which binds to circulating IgE, a product of B cells from the actions of interleukin 4 and interleukin 13, is used as treatment for severe allergic asthma. Studies examining cytokine blockers such as anti-interleukin-5, anti-interleukin-4R α , and anti-interleukin-13 monoclonal antibodies in patients with severe asthma with recurrent exacerbations and high blood eosinophil counts despite use of inhaled corticosteroids have reported improved outcomes in terms of exacerbations, asthma control, and forced expiratory volume in 1 s. The US Food and Drug Administration's recommendation to use an anti-interleukin-5 antibody for the treatment of severe eosinophilic asthma suggests that there will be a therapeutic place for these anti-Th2 agents. Biomarkers should be used to identify the right patients for such targeted approaches. More guidance will be needed as to which patients should receive each of these classes of selective antibody-based treatments. Currently, there is no treatment that targets the cytokines driving asthma associated with non-eosinophilic inflammation and low Th2 expression.

Introduction

Asthma is the 14th most important chronic disease in the world in terms of the prevalence, extent, and duration of disability, affecting 334 million individuals of all ages.¹ Although mortality from asthma has been falling, the disease still accounts for 90 and 170 deaths per million in female and male individuals, respectively. The economic costs of asthma are made up of direct costs such as hospital care and treatment, and indirect costs such as time lost from work. In Europe, the total cost per patient has been estimated to range from €509 for controlled asthma to €2281 for uncontrolled asthma.²

Current management of asthma consists of non-pharmacological approaches, such as allergen avoidance, and pharmacological approaches.³ Daily inhaled corticosteroids combined with longacting β_2 agonists are the mainstay of asthma treatment. For patients who remain uncontrolled on this combination, other controller drugs such as leukotriene-receptor antagonists,

slow-release theophylline, and longacting anticholinergics can be added. Some patients can be offered daily treatment with oral corticosteroids, whereas treatment with the monoclonal anti-IgE antibody omalizumab may be indicated in those with an allergic background. 5–10% of patients need such high-level treatments⁴ and have severe asthma.^{5,6}

To understand the processes that increase asthma severity, research has continued into the pathophysiology of asthma, and has focused on the interleukins or cytokines and their potential role in the disease.⁷ (In this Series paper, cytokines and interleukins are terms used interchangeably to indicate a broad and loose category of small proteins that are important in activating cell signalling in the context of the inflammatory process of asthma.) This Series paper will focus on how these cytokines drive the inflammatory processes and pathophysiological mechanisms of asthma, and the results of blocking the effects of cytokines on asthma outcome measures in patients with severe asthma, particularly with approaches that block anticytokine antibodies. It will also highlight the diversity of the inflammatory process in severe asthma such that it becomes important to define the phenotype that will respond best to each specifically targeted treatment.

Search strategy and selection criteria

Data for this Series paper were identified by searches in MEDLINE and PubMed and references from relevant articles and reviews in English, using the search terms “asthma” or “asthma treatments” with “interleukins” and “cytokines”, refining the search to specific interleukins or cytokines of interest between Jan 1, 2005 and June 30, 2015. Search was also made in the abstract publications from the European Respiratory Congress held in 2014, and the American Thoracic Meeting held in May, 2014, and Sept, 2015.

Airway inflammation, cytokines and interleukins, and molecular phenotyping

Asthma is a multicellular disease. It is a chronic inflammatory disease of the airways characterised by infiltration of eosinophils, basophils, mast cells, and CD4 T-helper cells into the airway submucosa. This

inflammatory process is associated with mucus hypersecretion, airway wall remodelling, and airway hyper-responsiveness, which may all contribute to chronic airway obstruction. One important initiating component is the airway epithelium, along with dendritic cells and airway macrophages, responding to external environmental factors such as allergens, pollutants, and infectious agents (eg, viruses). This initial response leads to the transmission of signals to other cells such as T cells, B cells, and mast cells, with activation of airway structural cells such as airway smooth muscle cells, mucus goblet cells, and fibroblasts⁸ (figure 1). Cytokines are important in the promotion of chronic airway inflammation and remodelling^{9,10} by their actions on inflammatory and structural cells and by affecting and promoting cell-cell interactions.^{11,12} T-helper-2 (Th2) cells and type 2 innate lymphoid cells (ILC2s) are now recognised as important cells underlying

allergic eosinophilic asthma. They produce interleukins 4, 5, and 13, which leads to an increase in IgE production and eosinophilic inflammation, whereas innate immune cytokines such as interleukins 1, 25, and 33, and thymic stromal lymphopoietin (TSLP) released from airway epithelial cells can activate Th2 cells or ILC2s, or both.⁹

The heterogeneity of asthma has long been recognised, both in terms of clinical outcomes and response to available treatments. Unsupervised clustering methods with use of clinical features and inflammatory biomarkers have identified phenotypes described by asthma control and severity, age of onset of disease, obesity, and sputum eosinophil count.^{13,14} This phenotypic diversity can be accounted for by different inflammatory mechanisms underpinned by different molecular pathways.¹⁵ Because Th2 cells have been recognised as an important part of the mechanisms underlying asthma, a Th2-high asthma has

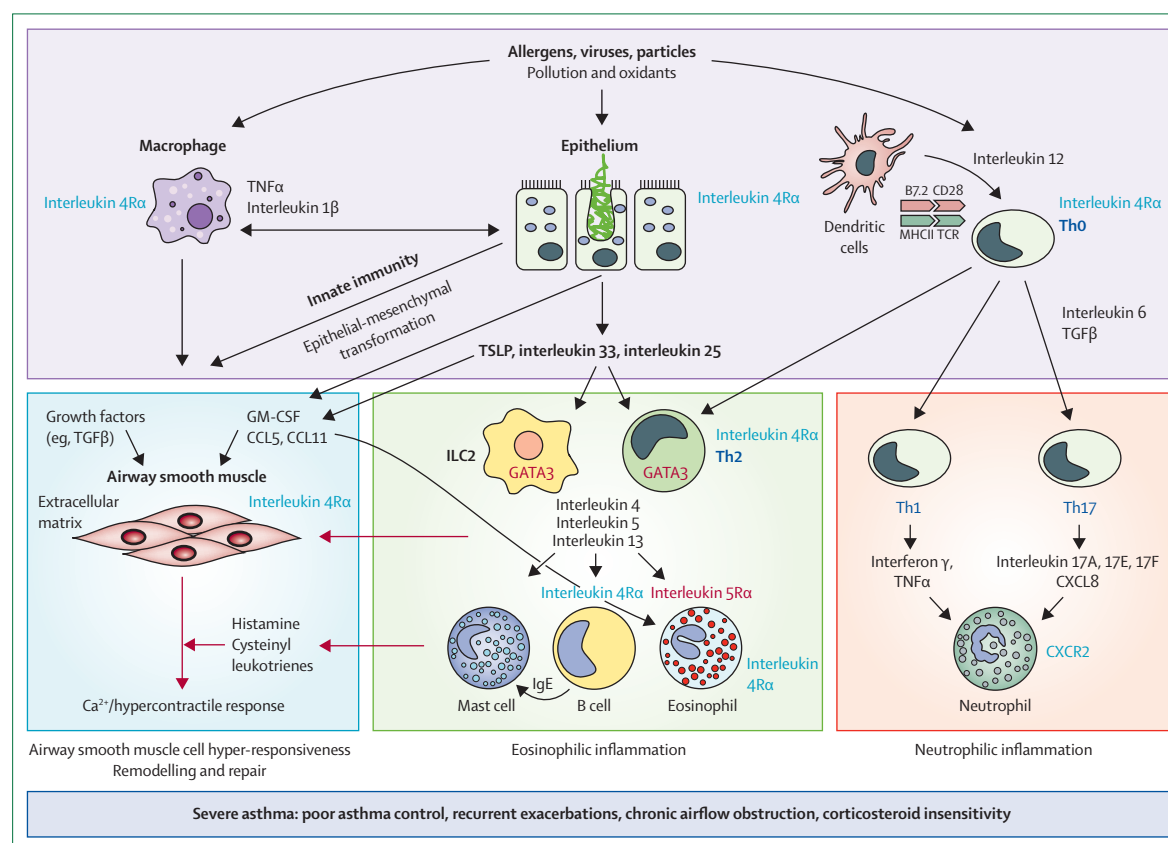


Figure 1: Pathophysiological mechanisms underlying severe asthma and targets for anti-interleukin therapy

Interactions between environmental factors and innate and adaptive immune and inflammatory responses are initiated at the level of the airway epithelium, in particular alveolar macrophages and dendritic cells, with the release of TSLP, interleukin 33, and interleukin 25 from the airway epithelium. Generation of Th2 cells and ILC2s leads to the elaboration of interleukins 4, 5, and 13, which are important for the generation of an allergic eosinophilic inflammation with eosinophil recruitment and activation, and the production of IgE. Activation of Th1 and Th17 cells can also be a feature of asthma, possibly contributing to neutrophilic inflammation. Airway wall remodelling and repair driven by epithelial-mesenchymal transformation and the effects of interleukins on airway structural cells such as airway smooth muscle cells and epithelial cells contribute to chronic airflow obstruction and bronchial hyper-responsiveness. Targets for interleukin intervention against interleukins 4, 5, and 13 have included GATA3 in Th2 and ILC2 cells and antibody-directed inhibition against each individual interleukin or against relevant receptors such as interleukin 4Ra or interleukin 5Ra. ILC2=type 2 innate lymphoid cells. TCR=T-cell receptor. Th1=T-helper-1 cell; Th2=T-helper-2 cell. Th17=T-helper-17 cell. TGFβ=transforming growth factor β. TNFα=tumour necrosis factor α. TSLP=thymic stromal lymphopoietin. GM-CSF=granulocyte-macrophage colony-stimulating factor.

been defined by use of genes expressed in airway epithelial brushings, consisting of a set of interleukin-13-inducible genes comprising *POSTN*, *CLCA1*, and *SERPINB2*.¹⁶ Only half of a group of patients with mild-to-moderate asthma had a Th2-high asthma phenotype that was characterised by a greater degree of bronchial hyper-responsiveness, higher serum IgE concentrations, and increased blood and airway eosinophilia.¹⁷ This finding has led to the dichotomisation of asthma into a Th2-high and a Th2-low endotype.¹⁸ This categorisation has implications for testing new compounds that target specific molecular pathways in the clinic, particularly in identifying patients most likely to respond to these treatments.¹⁹ In the first clinical trial of the anti-interleukin-5 antibody mepolizumab, which was used to block the effects of the Th2-derived cytokine interleukin 5, the drug proved ineffective in patients with asthma recruited solely according to severity;²⁰ however, in a subsequent study in patients with severe asthma with a history of frequent exacerbations and eosinophilia in sputum or blood, mepolizumab treatment resulted in an important reduction in exacerbations compared with placebo.²¹

Biomarkers that are accessible in the clinic can be used to stratify patients, especially for identification of those who will respond to blocking antibodies directed towards interleukins 4, 5, and 13. Eosinophil counts in blood are positively correlated to eosinophil counts in sputum; raised blood eosinophil counts of more than 220 cells per μL are present in 53% of patients with severe asthma and sputum eosinophilia of more than 2% is present in 55% of patients with severe asthma.²² In patients with mild-to-severe asthma, blood eosinophil count was also reported to have the highest accuracy in identifying sputum eosinophilia in asthma, compared to nitric oxide concentration in exhaled breath, or serum periostin.²³ A sputum eosinophilia of more than 2% was totally specific but had a poor sensitivity for airway Th2 inflammation.²⁴ These findings suggest that blood eosinophil counts alone might be useful in selecting individuals with Th2-high asthma, and this approach has been used to indicate a population of patients with severe asthma who respond to interleukin-5 blocking agents.²⁵ Another biomarker, serum periostin, has been reported to be a better predictor of airway eosinophilic inflammation in patients with severe asthma²⁶ and has successfully identified patients with moderate-to-severe asthma who show an improvement in forced expiratory volume in 1 s (FEV₁) after treatment with an anti-interleukin-13 antibody.²⁷

Anti-interleukin approaches in asthma

Targeting the Th2 pathway

Activation of the Th2 pathway is initiated from a complex interaction between the innate and adaptive immune responses, starting with the differentiation of Th2 cells from uncommitted T cells, which requires the action of co-stimulatory molecules and cytokines expressed by dendritic cells and inflammatory cells. For the activation

of Th2 cells, synthesis of innate cytokines, such as TSLP, interleukin 25, and interleukin 33 from the epithelium,²⁸ can be induced by external stimuli such as lipopolysaccharide, pollutants, and viruses.²⁹ Additionally, these cytokines activate dendritic cells resulting in further differentiation and clonal expansion of Th2 cells with further activation of Th2 cells and tissue eosinophilia.

Effector Th2 cells release interleukins 4, 5, and 13, which are expressed in the bronchial submucosa of patients with asthma. An alternative source of interleukin 5 and interleukin 13 is ILC2s, which express no conventional lymphocyte or dendritic-cell phenotypic markers. ILC2s require GATA3 transcription factor for their development and function; they are also stimulated by TSLP and interleukins 25 and 33 to produce interleukins 5 and 13.²³ High concentrations of ILC2s have been seen in eosinophilic nasal polyps, as well as in peripheral blood of patients with asthma.^{26,27}

Although IgE is not considered an interleukin, its role in allergic inflammation is crucial and its production from B cells is a result of the effect of interleukin 4 and interleukin 13 in causing a class switch in immunoglobulin synthesis towards IgE. After IgE binds to high-affinity receptors on mast cells and basophils, cross-linking of IgE receptors leads to subsequent activation of these cells to release preformed mediators and cause synthesis of cysteinyl leukotrienes and pro-inflammatory cytokines. Thus, IgE-activated mast cells secrete interleukins, such as interleukins 3, 4, and 5, and granulocyte-macrophage colony-stimulating factor, which recruit or activate eosinophils.

Omalizumab is a humanised antibody that binds to the C ϵ 3 domain of the free IgE heavy chain. When it binds to free circulating IgE, there is a reduction in IgE available for binding to high-affinity IgE receptors (Fc ϵ R1) and also a reduction in high-affinity Fc ϵ R1 receptors present on mast cells and basophils.³⁰ These events lead to an interruption of the allergic cascade and prevention of mast-cell degranulation with a reduction in eosinophilic inflammation.³⁰ Furthermore, omalizumab decreases early and late asthmatic responses, sputum and tissue eosinophil counts, and submucosal cells positive for IgE and Fc ϵ R1.^{31–33} Omalizumab improved asthma-related quality-of-life measures and reduced exacerbation rates, with small improvements in FEV₁ in patients with moderate-to-severe asthma.^{4,34} In severe persistent allergic asthma, omalizumab as an add-on therapy decreased clinically significant asthma exacerbations by 26%, compared with placebo.³⁵ Biomarkers of therapeutic efficacy for omalizumab in reducing exacerbations are high concentrations of nitric oxide in exhaled breath and high blood eosinophil counts.³⁶ A trial of omalizumab in both adults and children with severe allergic asthma is recommended.⁴

More potent anti-IgE antibodies are in development. QGE031 (Novartis, Basel, Switzerland) is a humanised anti-IgE antibody with a 50-fold higher affinity for IgE than omalizumab. QGE031 reduced circulating

concentrations of IgE, FcεR1, surface IgE, and skin prick responses to aeroallergens to a greater extent than omalizumab in allergic patients with well-controlled asthma.³⁷

Production of IgE can be inhibited by quilizumab (Genentech, San Francisco, CA, USA), a humanised monoclonal antibody directed against an extracellular 52-aminoacid segment termed M1 prime of human membrane IgE through reduction of new IgE-producing plasma cells. Quilizumab reduces total serum IgE concentrations and allergen-specific IgE, an effect that can be longlasting. In patients with allergic asthma undergoing allergen challenge, quilizumab blocked the generation of IgE and reduced allergen-induced early and late asthmatic airway responses, with a non-significant reduction in allergen-induced increases in sputum eosinophil counts.³⁸ Clinical development of quilizumab for allergic asthma has been discontinued.

Anti-interleukin-4Rα antibody

Both interleukin 4 and interleukin 13 bind to the heterodimeric combination of the α1 chain of the interleukin-13 receptor (interleukin 13Rα1) and the α chain of the interleukin-4 receptor (interleukin 4Rα), which leads to the signalling of both interleukin 4 and interleukin 13.³⁹ Blocking interleukin 4Rα with an antibody targeted to this receptor chain would block the effects of both interleukin 4 and interleukin 13 (figure 2).

Interleukin 4 has an important role in Th2-cell differentiation from naive T cells. Interleukin 4, together with interleukin 13, causes isotype class-switching of B cells towards IgE synthesis and is involved in mast-cell recruitment. Interleukin 4 also upregulates high-affinity IgE receptors on mast cells and low-affinity receptors on B cells and mononuclear phagocytic cells. Interleukin 4 and interleukin 13 upregulate vascular cell adhesion molecules on endothelial cells, thus facilitating transmigration of eosinophils, T lymphocytes, monocytes, and basophils.

Interleukin 13 shares 30% homology with interleukin 4 and is secreted by Th2 cells, ILC2s, mast cells, basophils, and eosinophils. In asthma, the effects of interleukin 13 include increases in goblet-cell differentiation, activation of fibroblasts, an increase in bronchial hyper-responsiveness, and switching of B-cell antibody production towards IgE.⁴⁰

Pitrakinra, a mutated form of human interleukin 4 with a high affinity for interleukin 4Rα, inhibits the downstream effects of both interleukin 4 and interleukin 13 by acting as a competitive antagonist. In phase 2 trials, pitrakinra attenuated the late-phase asthmatic response to allergen challenge in patients with mild atopic asthma.⁴¹ A reduction in exacerbation frequency during withdrawal of inhaled corticosteroids was reported in subgroups of patients with eosinophilic asthma, raised exhaled nitric oxide concentrations, and with specific interleukin-4 receptor polymorphisms.^{42,43}

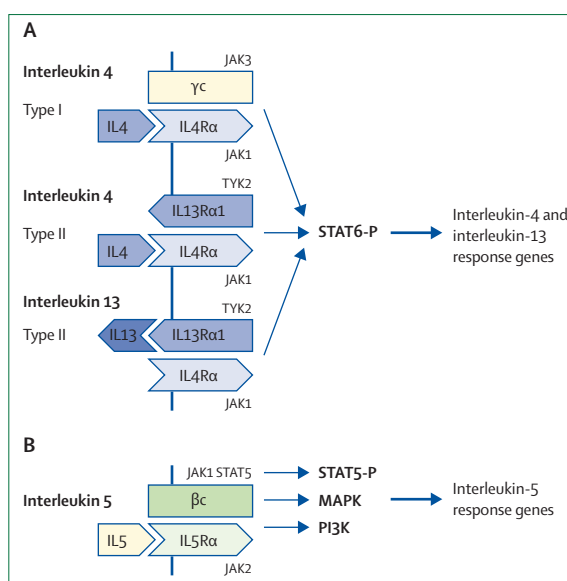


Figure 2: Signalling pathways of (A) interleukins 4 and 13, and (B) interleukin 5

(A) Interleukin 4 signals through type I or type II interleukin-4Rα receptors, whereas interleukin 13 signals through type II interleukin-4Rα receptor through binding to interleukin 13Rα1. These interactions lead to the phosphorylation of STAT6 with subsequent transcription of interleukin-4 and interleukin-13 response genes. (B) Interleukin-5 engagement of interleukin 5Rα leads to recruitment of βc, resulting in the activation of the JAK/STAT pathway, MAPK cascade, and PI3K pathway. IL=interleukin. JAK=janus kinase. MAPK=mitogen-activated protein kinase. PI3K=phosphoinositide-3-kinase. STAT=signal transducers and activators of transcription. TYK=tyrosine kinase. βc=common β chain. γc=common γ chain. STAT5-P=phosphorylated STAT-5. STAT-6P=phosphorylated STAT-6.

There has been no further development of pitrakinra in asthma.

AMG 317 (Amgen, Thousand Oaks, CA, USA) is a human monoclonal antibody against interleukin 4Rα that blocks both interleukin-4 and interleukin-13 pathways. In a study of patients with moderate-to-severe asthma, AMG 317 did not change Asthma Control Questionnaire (ACQ) scores from baseline and had no effect on exacerbations compared with placebo (table).⁴⁴

In patients with moderate asthma with evidence of either sputum or blood eosinophilia, a human monoclonal antibody that binds to interleukin 4Rα, dupilumab (Regeneron, Tarrytown, NY, USA), reduced asthma exacerbations and improved asthma control and lung function compared with placebo, when longacting β₂ agonist was discontinued and inhaled corticosteroid dose reduced and stopped.⁴⁵ There was also an associated reduction in fractional exhaled nitric oxide with reduced serum concentrations of Th2-associated inflammatory markers such as CCL17 (TARC), CCL26 (eotaxin-3), and IgE. In a second phase 2 study of adults with uncontrolled asthma on medium-dose to high-dose inhaled corticosteroids and longacting β₂ agonists, dupilumab (200 mg or 300 mg every 2 weeks or every 4 weeks) improved FEV₁ at week 12 in patients with blood eosinophil

count of 300 cells per μL or more, although not when given as a dose of 200 mg every 4 weeks.⁴⁶ Dupilumab also reduced annualised severe exacerbation rates irrespective of baseline eosinophil counts, apart from when given as a dose of 300 mg every 4 weeks. These findings might raise questions about the role of blood eosinophils as a predictive biomarker of response to this treatment.

Anti-interleukin-4 antibody

Inhaled recombinant human soluble interleukin-4 receptor has been used to antagonise interleukin 4. A

single nebulised dose was shown to be effective in moderate asthma and was associated with improvement in FEV_1 , lower use of β_2 agonists, and reduced concentrations of exhaled nitric oxide compared with placebo.⁶¹ In a dosing study over 12 weeks, there was significant effect in preventing reduction in FEV_1 but not on exacerbation rates following reduction of maintenance doses of inhaled corticosteroids, in moderate persistent asthma.⁶² However, no benefits were reported in subsequent clinical trials. There has been no further development of this soluble interleukin-4 receptor in asthma.

	Antibody	Patient characteristics and biomarkers	Effects of antibody treatment
Interleukin 4R α ⁴⁴	AMG 317	Patients with moderate-to-severe asthma	No effect on ACQ scores or exacerbations
Interleukin 4R α ⁴⁵	Dupilumab	Patients with moderate-to-severe asthma with sputum eosinophilia ($\geq 3\%$) or blood eosinophilia (≥ 300 cells per μL). LABA was discontinued and ICS dose tapered and stopped	Reduction in exacerbations of 89% (6% of patients had exacerbations in dupilumab group vs 44% in placebo group). Significant improvement in FEV_1 (0.27 L compared with placebo) and in ACQ-5 score (-0.73 points compared with placebo)
Interleukin 4R α ⁴⁶	Dupilumab	Patients with uncontrolled asthma on medium-dose to high-dose ICS plus LABA. Stratified according to blood eosinophil count <300 or ≥ 300 cells per μL . Dupilumab given as 200 mg or 300 mg every 2 weeks or every 4 weeks	Improved FEV_1 in patients with blood eosinophil count of ≥ 300 cells per μL (apart from dose of 200 mg every 4 weeks). Reduced annualised severe exacerbations rates irrespective of baseline eosinophil count (apart from dose of 300 mg every 4 weeks)
Interleukin 13 ⁴⁷	Lebrikizumab	Patients with mild asthma undergoing allergen challenge	Non-significant reduction in late-phase response; those with increased blood eosinophil counts, serum IgE, or periostin had a greater reduction. Decrease in serum IgE and chemokine ligands 13 and 17
Interleukin 13 ²⁷	Lebrikizumab	Patients with moderate-to-severe asthma not well controlled on ICS with or without LABA with mean FEV_1 of 65% of predicted. 80% were on LABA. Stratification according to serum periostin concentration	Mean FEV_1 improved by 5.5% in patients treated with lebrikizumab, with patients with high serum periostin concentration showing 8.2% improvement vs 1.6% in those with low periostin. No effect on ACQ-5 or exacerbations
Interleukin 13 ⁴⁸	Lebrikizumab	Patients with uncontrolled asthma despite daily use of high-dose ICS and a second asthma controller. Two studies testing 37.5 mg, 125 mg, 250 mg, or placebo given subcutaneously every 4 weeks	Lower exacerbation rates reported in lebrikizumab group than in placebo group, with 60% reduction in patients with high periostin concentration vs 5% in those with low periostin. No dose response evident. Treatment with lebrikizumab improved FEV_1 by 9.1% in patients with high periostin vs 2.6% in patients with low periostin. (Study discontinued in middle of study period with no definitive statistics)
Interleukin 13 ⁴⁹	Tralokinumab	Patients with moderate-to-severe uncontrolled asthma despite controller therapies with ACQ-6 >1.5 and one or more exacerbations in past year	No change in ACQ-6 at 13 weeks. Increase in FEV_1 of 0.21 L with tralokinumab vs 0.06 L with placebo ($p=0.072$). β_2 agonist use decrease of -0.68 puffs per day with tralokinumab vs -0.10 puffs per day with placebo ($p=0.020$). Better response in those with higher interleukin-13 concentrations in sputum
Interleukin 13 ⁵⁰	Tralokinumab	Patients with severe uncontrolled asthma on ICS plus LABA plus other controllers including oral corticosteroids	No effect on primary endpoint of annualised exacerbations. Significant increase in FEV_1 in those receiving treatments every 2 weeks, with greater improvements in those with high serum concentrations of periostin or DPP-4
Interleukin 13 ⁵¹	GSK679586	Patients with severe asthma	No improvements in asthma control, pulmonary function, or exacerbations
Interleukin 5 ⁵²	Mepolizumab	Patients with mild asthma undergoing allergen challenge	No effect on late-phase response or bronchial hyper-responsiveness despite reduction in blood and sputum eosinophil counts
Interleukin 5 ²⁰	Mepolizumab	Patients with asthma with persistent symptoms despite ICS (400–1000 μg per day of beclometasone or equivalent)	Reduced blood and sputum eosinophil counts. No statistically significant effect on clinical endpoints. A trend towards reduced exacerbation rates
Interleukin 5 ⁵³	Mepolizumab	Patients with refractory eosinophilic asthma with recurrent severe exacerbations	Reduction in exacerbation rate (exacerbations per patient, 2.0 in mepolizumab group vs 3.4 in placebo group, relative risk ratio 0.57) and improvement in AQLQ (0.55 in mepolizumab group vs 0.19 in placebo group). No effect on FEV_1 or bronchial hyper-responsiveness
Interleukin 5 ⁵⁴	Mepolizumab	Patients with severe asthma on OCS with persistent sputum eosinophilia	Reduction in exacerbations and in prednisolone dose with improved asthma control and FEV_1
Interleukin 5 ²¹	Mepolizumab	Patients with asthma with recurrent severe asthma exacerbations and evidence of eosinophilic inflammation (raised blood or sputum eosinophil counts or raised FE_{NO}) despite 880 μg fluticasone or equivalent per day. Mepolizumab given intravenously at doses of 75 mg, 250 mg, or 750 mg	All doses of mepolizumab reduced exacerbations: reduction from 2.40 per patient to 1.24 per patient (48% reduction) with 75 mg; reduction to 1.46 per patient per year (39% reduction) with 250 mg; and reduction to 1.15 per patient per year (52% reduction) with 750 mg. No effect on ACQ, AQLQ, or FEV_1
Interleukin 5 ⁵⁵	Mepolizumab	Patients with severe asthma with recurrent exacerbations on high-dose ICS and evidence of eosinophil count ≥ 150 cells per μL at screening or ≥ 300 cells per μL in past year. Mepolizumab given either 75 mg intravenously or 100 mg subcutaneously	Rate of exacerbation reduced by 47% with intravenous mepolizumab and by 53% with subcutaneous mepolizumab. Baseline FEV_1 increased by a mean of 100 mL with intravenous mepolizumab, and 98 mL with subcutaneous mepolizumab. ACQ-5 improved by 0.42 and 0.44 points, respectively

(Table continues on next page)

Antibody		Patient characteristics and biomarkers	Effects of antibody treatment
(Continued from previous page)			
Interleukin 5 ⁵⁶	Mepolizumab	Patients with severe asthma on ICS and LABA and OCS; blood eosinophil count ≥ 150 cells per μL at screening or ≥ 300 cells per μL in past year. Mepolizumab dose 100 mg subcutaneously	Primary endpoint is decrease in dose of OCS. Likelihood of decrease in corticosteroid dose was 2.39 times greater with mepolizumab than with placebo, (median percentage reduction in the mepolizumab-treated group of 50%). Reduced exacerbation rates by 32% (1.44 in mepolizumab group vs 2.12 in placebo group). ACQ-5 improved by 0.52 points. Trend towards greater changes from baseline of FEV ₁ in mepolizumab group
Interleukin 5 ⁵⁷	Reslizumab	Patients with severe asthma on ICS plus LABA with sputum eosinophilia of $>3\%$	Improved ACQ score by -0.7 in reslizumab group compared with -0.3 in placebo group (p=0.054). FEV ₁ improved with a change of 0.18 L after reslizumab compared with -0.08 L after placebo (p=0.002). Reduction in sputum eosinophil count. Trend towards reduced exacerbations in reslizumab group
Interleukin 5 ⁵⁸	Reslizumab	Patients inadequately controlled on ICS with a blood eosinophil count of >400 cells per μL with one or more exacerbations in the previous year. Two duplicate studies reported	Significantly reduced exacerbations with a rate ratio of 0.50 and 0.41 in these two studies. Improvement in FEV ₁ , AQLQ scores, and ACQ-7 scores. Improvement in FEV ₁ increased with disease severity: mean increase of 0.113 L for those on ICS plus LABA only. Proportion of patients with minimally important differences of ≥ 0.5 -point improvement from baseline to endpoint in AQLQ was 74% in reslizumab group vs 65% in placebo group (p=0.03)
Interleukin 5R α ⁵⁹	Benralizumab	Patients on high-dose ICS, with 2–6 exacerbations per year, raised blood eosinophil count, blood eosinophil index and FE _{NO} >50 ppb. Three doses of benralizumab tested: 2 mg, 20 mg, and 100 mg doses in patients with eosinophilic asthma, and only 100 mg dose in patients with non-eosinophilic asthma	Decrease in asthma exacerbations in patients with baseline blood eosinophil count >300 cells per μL at 20 mg and 100 mg doses of benralizumab (0.30 vs 0.68 compared with placebo with 57% reduction; 0.38 vs 0.68 with 43% protection, respectively). In eosinophilic asthma, mean FEV ₁ and ACQ-6 improved compared with baseline with 0.10 L for FEV ₁ and -0.26 units for ACQ-6
Interleukin 5R α ⁶⁰	Benralizumab	Patients presenting with acute exacerbation of asthma in emergency room. Single infusion of benralizumab	Reduced subsequent exacerbation rate by 49%, including those needing hospital admission by 60%

ACQ=Asthma Control Questionnaire. AQLQ=Asthma Quality of Life Questionnaire. DPP-4=dipeptidyl peptidase 4. FE_{NO}=exhaled nitric oxide concentration. FEV₁=forced expiratory volume in 1 s. ICS=inhaled corticosteroid; LABA=long acting β_2 agonist. OCS=oral corticosteroid. ppb=parts per billion.

Table: T-helper-2 (Th2)-directed monoclonal antibodies in severe asthma

Anti-interleukin-13 antibody

Lebrikizumab (Genentech, San Francisco, CA, USA) is an IgG4 humanised monoclonal antibody that blocks binding of interleukin 13 to interleukin 4R α to prevent signalling through the interleukin-4R α and interleukin-13R α 1 heterodimer (figure 2). In patients with mild asthma who underwent bronchial allergen challenge, lebrikizumab reduced the late asthmatic response compared with placebo, although not significantly, but did not affect the early-phase response.⁴⁷ In patients with uncontrolled asthma despite use of inhaled corticosteroids and longacting β_2 agonists, treatment with lebrikizumab improved FEV₁ by 8.2% above the baseline value in patients with a high concentration of serum periostin, whereas patients with a low concentration of serum periostin showed no FEV₁ response.²⁷ By contrast, Noonan and colleagues⁶³ enrolled patients with asthma who had never received treatment with inhaled corticosteroids and reported no improvement in FEV₁ with lebrikizumab in individuals with a high concentration of serum periostin and a minor increase in those with low periostin concentration.

In two studies that examined the effect on exacerbations as a primary endpoint, lebrikizumab reduced the rate of asthma exacerbations in patients with uncontrolled asthma despite daily use of high-dose inhaled corticosteroid and a second asthma controller, with the reduction in exacerbation rate being more pronounced in patients with high periostin expression than in those with low periostin expression.⁴⁸ There was also an improvement in FEV₁ in patients with high periostin

expression who were treated with lebrikizumab. However, because a host-cell impurity in the study drug was discovered during the study, treatment was discontinued such that only a mean duration of treatment of 24 weeks was available for analysis.⁴⁸

Tralokinumab (MedImmune, Cambridge, UK) is an interleukin-13-neutralising IgG4 monoclonal antibody that blocks the binding of interleukin 13 to interleukin 13R α 1 and interleukin 13R α 2. In patients with moderate-to-severe uncontrolled asthma, tralokinumab did not improve symptoms but resulted in a non-significant increase in FEV₁ compared with placebo, with greater effects in patients with detectable sputum interleukin-13 concentrations.⁴⁹ In patients with uncontrolled asthma on high-dose inhaled corticosteroids and longacting β_2 agonists with or without other controller drugs, tralokinumab had no effect on exacerbation rates, and subgroup analysis showed a trend towards a reduction in exacerbations in those with high serum concentrations of periostin and dipeptidyl peptidase 4.⁵⁰ There was a significant increase in FEV₁ from baseline in patients treated every 2 weeks, but not in those treated every 4 weeks, after an initial 2-weekly treatment period, but no change in ACQ-6 or Asthma Quality of Life Questionnaire (AQLQ) scores for both treatment categories.

GSK679586 (GlaxoSmithKline, Greater London, UK) blocks the binding of interleukin 13 to interleukin 13R α 1. Treatment with GSK679586 during a 3-month period did not result in clinically meaningful improvements in asthma control, pulmonary function, or exacerbations in patients with severe asthma compared with placebo.⁵¹

Anti-interleukin-5 antibody and anti-interleukin-5 receptor antibody

Interleukin 5, produced by Th2 cells and ILC2s, is a cytokine specifically targeted at eosinophils; it is important for their terminal differentiation and maturation. Together with CCL11 (eotaxin), interleukin 5 mobilises eosinophils and eosinophil precursors into the circulation. Interleukin 5 primes eosinophils for increased survival and increased cytotoxicity. Eosinophil infiltration and degranulation lead to the release of cytotoxic products including major basic protein, eosinophil cationic protein, eosinophil-derived neurotoxin, and eosinophil peroxidase, which contribute to airway epithelial damage, mucus hypersecretion from goblet cells, bronchial hyper-responsiveness, and impaired ciliary beating. Eosinophils also secrete cysteinyl leukotrienes (LTC₄, LTD₄, and LTE₄).

Mepolizumab (GlaxoSmithKline, Greater London, UK) is a humanised monoclonal IgG1 antibody to interleukin 5. In two early studies, mepolizumab reduced sputum and blood eosinophil counts compared with placebo,^{20,52} but had no effect on response after allergen challenge. Additionally, in a cohort of adults with moderately severe asthma recruited on the basis of asthma severity, mepolizumab had no beneficial effects on symptom control or exacerbations.²⁰ In later studies in a specific cohort of patients with severe asthma on a combination of inhaled corticosteroids and longacting β_2 agonists with a history of exacerbations and persistent sputum or blood eosinophilia, mepolizumab decreased exacerbations, reduced use of oral corticosteroids, and improved symptoms and lung function compared with placebo.^{53,64} Subsequently, in a phase 2 study in patients with recurrent severe asthma exacerbations and eosinophilic inflammation, mepolizumab was effective in reducing exacerbation rates, but did not improve FEV₁ or quality of life.²¹ In this study, a blood eosinophil count of 150 cells per μ L or more was associated with a reduction in exacerbation rate of 72% compared with a reduction of 30% in those with a blood eosinophil count of less than 150 cells per μ L. Patients with a sputum eosinophil count of 3% or more showed similar reductions to those with a count of less than 3%. However, the reduction in exacerbation rate in either group was more than 66%.²⁵

In subsequent studies in patients with recurrent exacerbations of asthma (more than two exacerbations per year) despite high-dose inhaled corticosteroids and eosinophilic inflammation (>300 cells per μ L blood in the past year), use of mepolizumab led to a substantial reduction in exacerbations and improved asthma control.^{55,56} There was also an improvement in FEV₁ at 32 weeks with mepolizumab compared with placebo.⁵⁵ Furthermore, in patients with severe eosinophilic asthma on oral corticosteroid therapy, there was a median reduction in corticosteroid dose of 50% from baseline in the mepolizumab group compared with no reduction in the placebo group. This reduction in corticosteroid dose was accompanied by a reduction in the rate of

exacerbations and in asthma symptoms as measured on ACQ-5.⁵⁶ Not all patients in this study responded to mepolizumab: 36% of patients treated with mepolizumab reported no reduction in oral corticosteroid dose, or a lack of asthma control, or withdrew from the study, compared with 56% in the placebo group.

Reslizumab (Teva, Philadelphia, PA, USA) is an IgG κ humanised monoclonal antibody against interleukin 5. In patients with severe asthma on inhaled corticosteroids and longacting β_2 agonists with sputum eosinophilia of more than 3%, reslizumab improved asthma control and FEV₁ with a trend towards fewer exacerbations than in those receiving placebo.⁵⁷ In two phase 3 studies in patients inadequately controlled on inhaled corticosteroids with a blood eosinophil count of more than 400 cells per μ L and one or more exacerbations in the previous year, reslizumab significantly reduced the frequency of exacerbations compared with placebo, with rate ratios of 0.50 and 0.41 in the two studies respectively.⁵⁸ There was also an improvement in FEV₁, AQLQ score, and ACQ-7 score.

Benralizumab (MedImmune, Gaithersburg, MD, UK) is a recombinant IgG1 antibody directed against the α chain of the interleukin-5 receptor. The removal of a fucose sugar residue in the CH2 region of human IgG1 has allowed for an increased affinity for the activating Fc γ receptor on cells such as eosinophils; there is evidence that this alteration results in amplification of eosinophil apoptosis.⁶⁵ In patients with uncontrolled asthma using medium-dose to high-dose inhaled corticosteroids, with between two and six exacerbations per year and with eosinophilic inflammation defined by an algorithm to predict raised sputum eosinophil counts, subcutaneous injection of benralizumab at 20 mg or 100 mg doses reduced asthma exacerbations by 36% and 41%, respectively, compared with placebo.⁵⁹ Patients receiving benralizumab also had improvements in mean FEV₁ compared with baseline at 1 year (mean difference in change from baseline for 100 mg dose vs placebo 0.10 L for FEV₁ and -0.26 for ACQ-6 score). This finding was supported by results from another study in which patients who were recruited after being treated for an exacerbation of asthma received a single infusion of benralizumab. Compared with placebo, benralizumab reduced asthma exacerbation rates by 49% and exacerbations needing hospital admission by 60%.⁶⁰

Anti-TSLP antibody

TSLP is an interleukin-7-related cytokine secreted by airway epithelial cells on allergen and environmental exposure;^{66,67} it activates dendritic cells to release chemokines that recruit and activate Th2 cells.⁶⁸ Expression of interleukin 33 and TSLP is increased in the airway epithelium of patients with asthma, particularly in those with severe asthma.^{69,70} TSLP may also be involved in airway wall remodelling by acting on lung fibroblasts.⁷¹

AMG 157 (Amgen, Thousand Oaks, CA, USA), a human anti-TSLP monoclonal antibody, attenuated allergen-induced early and late asthmatic responses in mild allergic asthma. It also reduced blood and sputum eosinophil counts before and after allergen challenge, and fractional exhaled nitric oxide concentrations.⁷²

Antagonists of CRTh2

Prostaglandin D₂, released during allergen-induced reactions in the airways, activates a G-protein-coupled receptor, chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTh2, also known as DP2 receptor), present on Th2 cells, ILC2s, and eosinophils, and induces the chemotaxis of these cells.⁷³ Production of Th2-type cytokines by ILC2s is also stimulated by prostaglandin D₂ via activation of its CRTh2 receptor,⁷⁴ and also by leukotriene D₄ (LTD₄).⁷⁵

In patients with moderate persistent asthma who were not taking inhaled corticosteroids, the CRTh2 antagonist OC000459 (Oxagen, Abingdon, UK) improved FEV₁, asthma-related quality of life scores, and night-time symptoms, without affecting sputum eosinophil counts, compared with placebo.⁷⁴ The CRTh2 antagonist AMG 853 (Amgen, Thousand Oaks, CA, USA), used as an add-on to inhaled corticosteroid therapy, did not improve asthma symptoms or lung function in patients with uncontrolled moderate-to-severe disease.⁷⁵ Another CRTh2 antagonist, BI671800 (Boehringer Ingelheim, Ingelheim am Rhein, Germany), caused a small improvement in FEV₁ in symptomatic patients who had not previously used controller treatment and in patients on inhaled corticosteroids.⁷⁶ In a phase 2a study in patients with mild-to-severe asthma with sputum eosinophil counts of more than 2%, treatment with another CRTh2 antagonist, QAW039 (Novartis, Basel, Switzerland), resulted in a 3.5-fold reduction in sputum eosinophil count compared with placebo, accompanied by a 2.3-fold reduction in eosinophil count in bronchial biopsies.⁷⁷ QAW039 improved AQLQ score, but not ACQ-7 score, compared with placebo.

Targeting non-Th2 targets

Th17 cells are CD4 T cells that express interleukins 17A, 17E, 17F, and 22, and are able to mediate neutrophil activation via the production of CXCL8 (interleukin 8).^{78,79} Allergens and environmental stimuli such as cigarette smoke and diesel exhaust particles can trigger Th17-mediated airway inflammation in asthma.⁸⁰ Overexpression of interleukin 17A and interleukin 17F has been shown in lung tissue from patients with asthma, with expression levels correlating with asthma severity, especially in patients with neutrophilic corticosteroid-resistant disease.⁸¹ Interleukin 17A or interleukin 17F, or both, stimulate airway structural cells, including bronchial epithelial cells and subepithelial fibroblasts, to secrete neutrophil chemoattractants such as CXCL8 and CXCL1.

Brodalumab (Amgen, Thousand Oaks, CA, USA), a human anti-interleukin-17RA monoclonal antibody, had no effect on asthma control scores, symptom-free days, and FEV₁ in patients with inadequately controlled moderate-to-severe asthma who were receiving inhaled corticosteroid therapy. Furthermore, analysis of nine prespecified patient subgroups showed a high-reversibility subgroup with a post-bronchodilator FEV₁ improvement of 20% or more who showed a significant improvement in ACQ score at the 210 mg but not at the 280 mg dose.⁸² A follow-up phase 2b study focusing on this phenotype was stopped because of a lack of reported efficacy in an interim analysis. There has been no further development of this antibody in asthma.

CXCL8 is a chemokine (chemoattractant cytokine) involved in the chemoattraction and activation of neutrophils through the CXCR2 receptor. SCH527123 (Merck, Whitehouse Station, NJ, USA), a CXCR2 antagonist, inhibited ozone-induced sputum neutrophilia in patients without asthma.⁸³ It also reduced sputum neutrophilia in adults with severe asthma, with a modest reduction in mild exacerbations, but did not improve asthma control.⁸⁴

Tumour necrosis factor α (TNF α) is a pro-inflammatory Th1 cytokine that induces airway inflammation and hyper-responsiveness, mucus hypersecretion, and activation of macrophages. In two small, placebo-controlled, double-blind, crossover studies of patients with corticosteroid-refractory asthma, treatment with etanercept (Pfizer, Collegeville, PA, USA) (a soluble recombinant dimer protein consisting of two human TNF α receptors fused with the Fc domain of human IgG1) resulted in clinical improvements in PC20 (the concentration of methacholine required to provoke a 20% decrease in FEV₁), quality of life scores, and lung function in one study,⁸⁵ and improvements in ACQ scores with reductions in the use of nebulised β_2 agonists in the other.⁸⁶ However, in a subsequent larger study in adults with uncontrolled severe persistent asthma, the anti-TNF α antibody golimumab (Centocor, Malvern, PA, USA) had no overall beneficial effects. A post-hoc analysis suggested that patients with substantial bronchodilator reversibility had fewer exacerbations while on golimumab.⁸⁷ Serious side-effects with golimumab included an increased frequency of infections and malignancies compared with placebo, which probably represents the importance of TNF α in the innate immune response to infections and cancer.

Summary and conclusions

Blockade of the effects of interleukin 5 with a monoclonal antibody directed towards interleukin 5 or its receptor, interleukin 5R α , in patients with severe asthma with eosinophilia in blood or sputum led to a reduction in asthma exacerbation rates and an improvement in asthma control and FEV₁ (table). Blocking the effects of interleukin 13 with anti-interleukin-13 antibody has led to an improvement in FEV₁, particularly in patients with a

high serum concentration of periostin, with a reduction in exacerbations with lebrikizumab, but not with tralokinumab. Blocking interleukin 4R α with dupilumab, which neutralises the effects of interleukin 4 and interleukin 13, reduced exacerbations irrespective of baseline eosinophil count, improved FEV₁ in those with high blood eosinophil count, and reduced serum IgE concentrations. Interleukin 5, therefore, has a most important pathogenic role in exacerbations through its role in activating eosinophils, and the modest improvement in airflow obstruction after antibody treatment perhaps results from disruption of the interaction between eosinophils and airway smooth muscle cells. Alternatively, the improvement in FEV₁ with anti-interleukin-13 antibody might result from direct inhibition of airway smooth muscle contractility and bronchial hyper-responsiveness. The reduction in exacerbations noted with anti-interleukin-4R α antibody might result from inhibition of both interleukin 4 and interleukin 13, which could implicate the biomarkers that were inhibited—namely, IgE and the chemokines CCL17 and CCL26.

With the recent recommendation by the US Food and Drug Administration for use of mepolizumab as an add-on maintenance treatment in patients aged 18 years or older with severe eosinophilic asthma, other antibody-based therapies will also be approved for severe asthma. Inevitably, the question will be asked as to what are the most appropriate antibody-based treatments (including anti-IgE therapy) for patients with severe uncontrolled asthma associated with eosinophilia. One might assume that patients with recurrent exacerbations would benefit most from an anti-interleukin-5 approach, whereas patients with more chronic airflow obstruction without recurrent exacerbations would benefit from an anti-interleukin-13 approach, and patients with both chronic airflow obstruction and recurrent exacerbations might benefit from an anti-interleukin-5 or anti-interleukin-4R α treatment. Alternatively, one could resort to the current concept of biomarkers and the knowledge gained so far from the clinical trials of these blocking antibodies. Thus, for anti-IgE treatment, it would be necessary to demonstrate raised total serum IgE and the presence of circulating specific IgEs, and a high concentration of serum periostin perhaps an indication for use of anti-interleukin-13 antibody. Direct comparison of the effects of one antibody treatment against another and a search for better biomarkers or combinations of biomarkers that will predict response and efficacy may be needed.

There has been less success with targets outside the interleukin-4, interleukin-5, and interleukin-13 cytokines. The importance of the non-Th2 or low-Th2 expressors remains unclear and there could be diverse mechanisms at play. What sputum neutrophilia is an indicator of is not entirely clear, and endotyping of non-Th2 mechanisms in severe asthma is necessary. Non-Th2 mechanisms might involve excessive activation of the innate inflammatory response, such as that induced by

bacterial or viral infections, and linked to corticosteroid insensitivity, and also to airway wall remodelling. Additionally, there might be interactions between Th2 and non-Th2 mechanisms.

Targeting the interleukin pathway has led to the formulation of antibody therapies against interleukins 4, 5, and 13, which are important cytokines underlying the eosinophilic inflammation in asthma. These treatments will hopefully become available for patients with severe asthma in the coming decade and will represent an important milestone in the management of severe asthma.

Contributors

I am sole author and contributor.

Declaration of interests

I have received honoraria for participating in advisory board meetings regarding treatments for asthma and chronic obstructive pulmonary disease for GlaxoSmithKline, AstraZeneca, Novartis, and Johnson & Johnson, and have received research grant funding through my institution from Pfizer, GSK, and Merck. I have also been remunerated for speaking engagements by AstraZeneca, Merck, and Novartis.

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