

Asthma phenotyping: a necessity for improved therapeutic precision and new targeted therapies

■ Kian Fan Chung^{1,2}

From the ¹Airway Disease Section, National Heart and Lung Institute, Imperial College London; and ²National Institute for Health Research (NIHR), Respiratory Biomedical Research Unit, Royal Brompton & Harefield NHS Trust and Imperial College London, London, UK

Abstract. Chung KF (Airway Disease Section, National Heart and Lung Institute, Imperial College London, London, UK; National Institute for Health Research (NIHR), Respiratory Biomedical Research Unit, Royal Brompton & Harefield NHS Trust and Imperial College London, London, UK). Asthma phenotyping: a necessity for improved therapeutic precision and new targeted therapies (Review). *J Intern Med* 2015; doi: 10.1111/joim.12382.

Asthma is a common heterogeneous disease with a complex pathophysiology that carries a significant mortality rate and high morbidity. Current therapies based on inhaled corticosteroids and long-acting β -agonists remain effective in a large proportion of patients with asthma, but ~10% (considered to have 'severe asthma') do not respond to these treatments even at high doses or with the use of oral corticosteroids. Analytical clustering methods have revealed phenotypes that include dependence on high-dose corticosteroid treatment, severe airflow obstruction and recurrent exacerbations associated with an allergic background and late onset of disease. One severe phenotype is eosinophilic inflammation-predominant asthma, with late-onset disease, rhinosinusitis, aspirin

sensitivity and exacerbations. Blood and sputum eosinophilia have been used to distinguish patients with high Th2 inflammation and to predict therapeutic response to treatments targeted towards Th2-associated cytokines. New therapies in the form of humanized antibodies against Th2 targets, such as anti-IgE, anti-IL4R α , anti-IL-5 and anti-IL-13 antibodies, have shown encouraging results in terms of reduction in exacerbations and improvement in airflow in patients with a 'Th2-high' expression profile and blood eosinophilia. Research efforts are now focusing on elucidating the phenotypes underlying the non-Th2-high (or Th2-low) group, which constitutes ~50% of severe asthma cases. There is an increasing need to use biomarkers to indicate the group of patients who will respond to a specifically targeted treatment. The use of improved tools to measure activity of disease, a better definition of severe asthma and the delineation of inflammatory pathways with omics analyses using computational tools, will lead to better-defined phenotypes for specific therapies.

Keywords: asthma clusters, asthma phenotyping, asthma treatments, eosinophils, neutrophils, T helper type 2 (Th2).

Introduction

Asthma is a common disease that affects individuals of all ages. It usually arises in childhood and may persist into adulthood, but can begin at any age. Worldwide, asthma affects up to 300 million individuals, with a reported annual death rate of 250 000, and nearly 10 million children and adults below 45 years of age have asthma in Europe. The prevalence increased substantially between 1950 and 2000, but the increase has levelled off in the past decade. With the introduction of inhaled corticosteroid (ICS) therapies, death from asthma has decreased, but the impact of the disease remains substantial. In Europe, the

economic costs of asthma consisting of direct costs, such as hospital care and medication, and indirect costs, such as time lost from work, range from 509 Euros for controlled asthma to 2281 Euros for uncontrolled asthma per patient [1]. The total cost of asthma treatment for individuals between 15 and 64 years of age may amount to 19.3 billion euros.

The rapid rise in the prevalence of childhood asthma in many countries in recent decades indicates that important environmental determinants such as exposure to allergens, infective agents or pollutants may act on a genetically susceptible population through gene–environment interactions.

The driving factors remain unclear and one compelling hypothesis is that they are associated with a 'Western' environment, reflecting urbanization and reduced exposure to microorganisms that protect against allergies and asthma (the 'hygiene hypothesis') [2].

This review will focus on the new approaches to understanding asthma heterogeneity and pathogenic mechanisms, in terms of defining the various phenotypes of asthma and linking the phenotypes to underlying mechanisms, so that improved biomarkers and treatments can be developed for patients with asthma who do not respond to currently available treatments.

Asthma as a syndrome consisting of several phenotypes

Asthma is a syndrome characterized by symptoms of wheeze, shortness of breath, chest tightness and cough and by variable expiratory airflow limitation. It has long been recognized that asthma consists of different phenotypes. Over the years, different types of asthma have been described by experienced clinicians supported by long-term studies in cohorts of patients with asthma, particularly in children. Several features have been recognized, including the onset of asthma either in childhood or during later years, the presence of atopy, the effect of upper respiratory tract viral infections in inducing exacerbations, disease induction by ingestion of aspirin or nonsteroidal anti-inflammatory agents and the concomitant presence of comorbidities such as rhinosinusitis or obesity. The presence of eosinophils in sputum samples was found to be associated with asthma and indicated a good therapeutic response to inhaled or oral corticosteroid therapy [3–5].

A phenotype is defined as the composite observable characteristics or traits of an organism that result from genetic as well as environmental influences. For a chronic disease such as asthma, phenotyping may be useful in providing long-term prediction of outcomes and determining specific treatments for selected phenotypes, such that these differences between individual patients can be exploited to relate to clinically meaningful outcomes. Cluster analysis of clinical and physiological features is increasingly used to define phenotypes of asthma (Table 1). In the Severe Asthma Research Program (SARP) and the UK Leicester adult cohorts, hierarchical cluster analysis of populations with a range of asthma severities was used [6, 7]. Patient

clusters were either identified with minimal airflow obstruction and low disease activity, early disease onset and an atopic background or associated with adult-onset and active disease (more severe group of patients with asthma) (Table 1). Thus, age at disease onset, lung function and atopic state featured highly in these clusters or phenotypes, as also observed in cohorts from Korea and Japan [8, 9]. Clusters related to more severe disease were represented in the adult SARP cohort by individuals on high-dose ICS therapy, often combined with oral corticosteroid treatment associated with severe airflow obstruction. In the TENOR study, a cluster described in adolescents and adults was associated with aspirin sensitivity, in primarily White, female and atopic patients with late-onset asthma, and these patients were more likely to experience exacerbations [10].

The obesity component in severe asthma defined in both the SARP and Leicester cohorts has also been described in other analyses [11, 12], with obese uncontrolled and obese well-controlled clusters that differed from one another with regard to age of asthma onset, measures of asthma symptoms and control, exhaled nitric oxide concentration (FeNO) and airway hyper-responsiveness but were similar with regard to measures of lung function, airway eosinophilia and serum IgE [11]. A cluster of obese women with late-onset asthma and frequent symptoms with high healthcare use but with low sputum eosinophil counts has also been described [13].

Pathophysiology of asthma

Understanding of the inflammatory mechanisms of asthma has increased in the last 25 years with the delineation of the importance of the airway epithelium in responding to external stimuli such as allergens, pollutants and infectious agents (e.g. viruses) to cause the recruitment and/or activation of innate and adaptive cells (e.g. dendritic cells, mast cells and innate lymphoid cells).

Th2 mechanisms

The Th2 cytokine pathway has become one of the main targets for new treatments for asthma (Fig. 1) since the demonstration that Th2 cytokines, including IL-3, IL-4, IL-5, IL-9 and IL-13, are expressed in bronchial submucosa of patients with the disease [14, 15]. These cytokines contribute to allergic airways inflammation, triggering the

Table 1 *Clusters of asthma determined by cluster analysis*

Severe asthma research program (SARP) [6]

Cluster 1	Early-onset atopic asthma with normal lung function treated with up to two controller medications and minimal healthcare utilization
Cluster 2	Early-onset atopic asthma and preserved lung function but increased medication requirements and healthcare utilization
Cluster 3	Older obese women with late-onset nonatopic asthma, moderate reductions in FEV ₁ and frequent oral corticosteroid use to manage exacerbations
Clusters 4 and 5	Severe airflow obstruction with bronchodilator responsiveness but differences in their ability to attain normal lung function, age of asthma onset, atopic status and use of oral corticosteroids

Leicester cohort [7]

Cluster 1	Early-onset atopic asthma, with airway dysfunction and eosinophilic inflammation; increased number of hospitalizations
Cluster 2	Noneosinophilic inflammation; obese, female predominant
Cluster 3	Early onset, symptom predominant with minimal eosinophilic disease
Cluster 4	Eosinophilic inflammation predominant with few symptoms, late-onset disease

FEV₁, forced expiratory volume in 1 s.

activation and recruitment of IgE antibody-producing B cells, mast cells, basophils and eosinophils. Hence, a 'Th2-high' expression profile is associated with high levels of circulating IgE and atopy, and eosinophilia. More recently, the presence of group 2 innate lymphoid cells that are also a source of IL-4, IL-5 and IL-13 has been described [16], and whether they could constitute an alternative pathway for the production of these cyto-

kines or indeed contribute to the Th2-high profile remains unclear. The airway epithelium appears to be an important upstream controller of the regulation of Th2 cytokines through the production of cytokines such as thymic stromal lymphopoietin (TSLP), IL-25 and IL-33, which can be stimulated by exposure of epithelial cells to external stimuli including pollutants, viruses and allergens [17]. IL-33 is a member of the IL-1 cytokine family and an inducer of and chemoattractant for Th2 cells. TSLP is an IL-7-related cytokine secreted by airway epithelial cells that activates dendritic cells to release chemokines that attract and activate Th2 cells. Expression levels of both IL-33 and TSLP are increased in airway epithelium of patients with asthma, particularly in those with severe asthma [18, 19].

Th1 and Th17 mechanisms

Th1 CD4⁺ T cells are characterized by the production of interferon-gamma (IFN γ) which play a role in controlling intracellular infections, particularly due to viruses, and in autoimmunity. It has been shown that Th1 cells expressing IFN γ are increased in bronchoalveolar lavage fluid, sputum cells and bronchial biopsies from patients with asthma [20–22]. Furthermore, in a mouse model, a neutralizing antibody to IFN γ inhibited the allergen-induced bronchial hyper-responsiveness [23].

Th17 cells are CD4⁺ T cells that express IL-17A, IL-17E, IL-17F and IL-22 and are able to mediate neutrophil activation via the production of IL-8 [24, 25]. Th17 cells may mediate corticosteroid-resistant airway inflammation and airway hyper-responsiveness in mice [26], and the Th17-associated cytokines IL-17A and IL-17F have been localized in the airways of patients with severe asthma [27].

Airway remodelling changes

Airway remodelling is characterized by an increase in airway smooth muscle mass, subepithelial fibrosis and increased numbers of mucous glands and goblet cells (Fig. 2). These structural abnormalities can alter airway mechanics and contribute to bronchial hyper-responsiveness, whilst structural cells can also contribute to inflammatory processes through release of cytokines, chemokines, growth factors and extracellular matrix elements [28]. The epithelium in severe asthma is reported to be thicker than in mild-to-moderate asthma [29], with increased

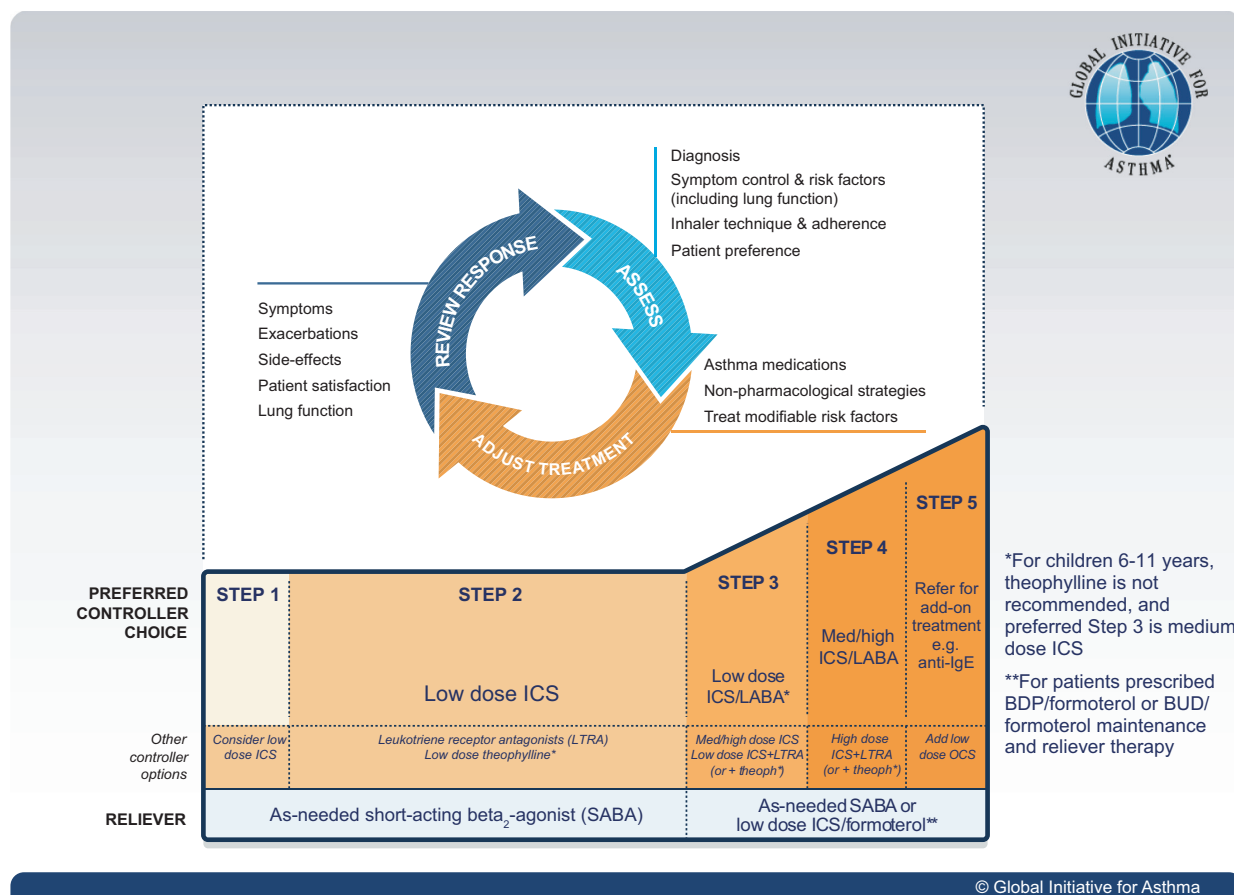


Fig. 1 Stepwise approach to asthma treatments with the use of controller and reliever medications according to the Global Initiative for Asthma (GINA). Combination therapy with inhaled corticosteroids (ICSs) and long-acting β -agonists (LABAs) is used at steps 3 and 4, with other add-on medications. At Step 5, add-on treatments may consist of anti-IgE therapy. BDP: beclomethasone dipropionate; BUD: budesonide; LTRA: leukotriene receptor antagonist. Reproduced with permission from GINA (http://www.ginasthma.org/local/uploads/files/GINA_Report_2014_Aug12.pdf).

proliferation, apoptosis and release of pro-inflammatory factors [29]. There is an increased amount of airway smooth muscle associated with airflow obstruction and bronchial hyper-responsiveness, with an increased proliferation rate [30–33]. Finally, fibrocytes, which can differentiate into myofibroblasts, are increased in blood and in smooth muscle bundles in patients with asthma with fixed airway obstruction and/or severe disease [34–36]. Subepithelial thickening of the bronchial reticular layer is a feature of asthma of all severities [30, 37, 38]. Patients with severe asthma also have increased the expression of transforming growth factor- β isoforms and collagen deposition, compared to those with mild asthma, in association with eosinophilia [39, 40].

Eosinophilic and noneosinophilic (neutrophilic) asthma phenotypes

The presence of eosinophils measured in blood or in sputum samples may identify a phenotype of asthma termed 'eosinophilic'. Using a cut-off point of blood eosinophil count of $>220/\text{mm}^3$, up to 53% of a group of patients with severe asthma were found to have an elevated eosinophil count [41]. Using sputum eosinophil counts of either $\geq 2\%$ or 3% , 17% of ICS-treated subjects [42], 25% of hospital clinic patients with asthma [43] and 55% of patients with severe asthma [41] were defined as having eosinophilic asthma. Blood and sputum eosinophil counts correlated well [41, 44], with a blood eosinophil count of $>188/\text{mm}^3$ able to identify a sputum eosinophil count of $>3\%$ with a

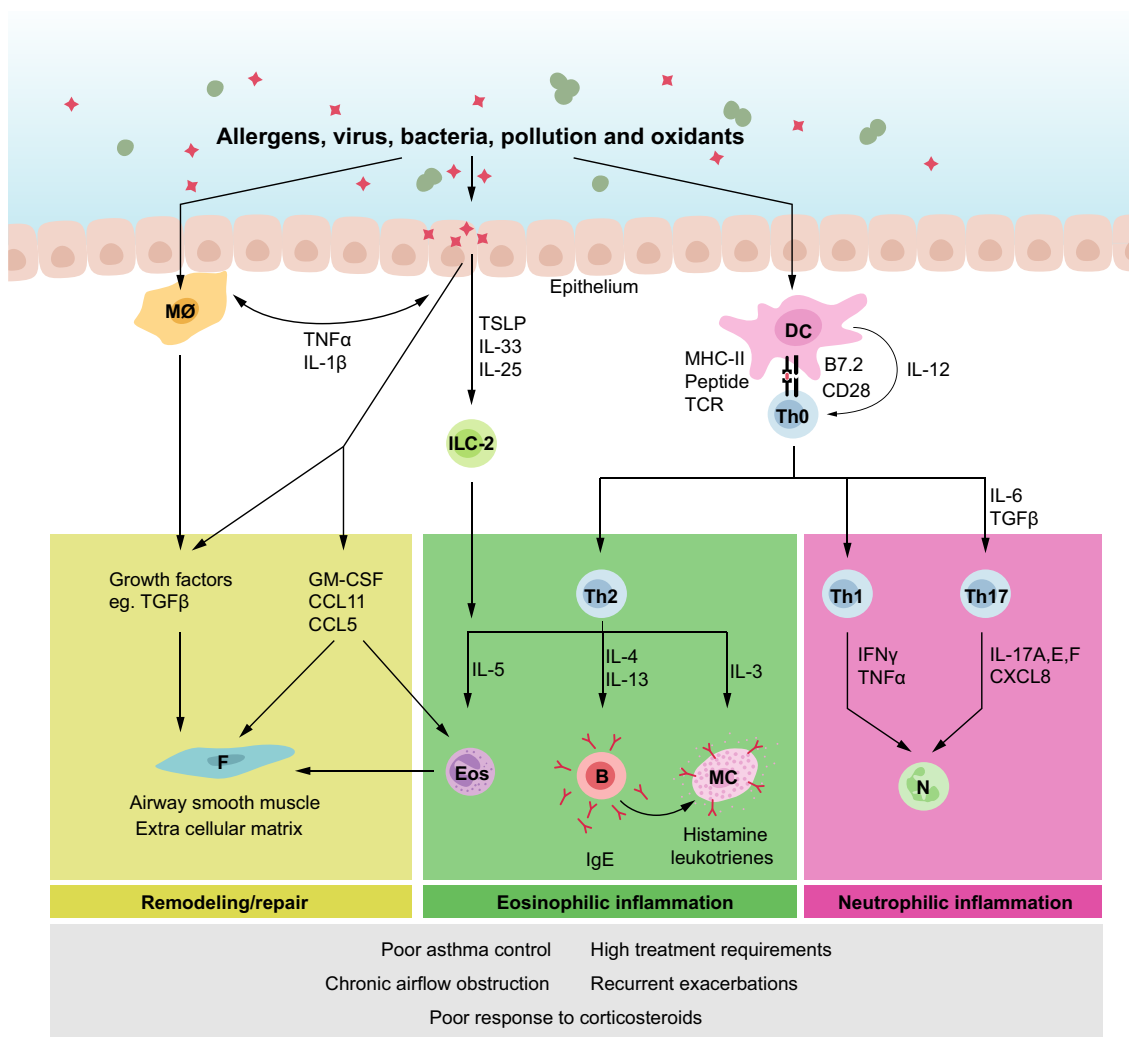


Fig. 2 Pathophysiological mechanisms in asthma illustrated by the interactions between resident and inflammatory cells and the external environment. The importance of the T helper type 2 (Th2) cells is shown by the contribution of Th2 cytokines IL-4, IL-5 and IL-13 to eosinophilic inflammation and IgE production. Innate lymphoid cell type 2 (ILC-2) may also contribute to the production of IL-5 and IL-13, and Th1 and Th17 cells to a more neutrophilic inflammation. Airway wall remodelling and repair are also important processes that could be driven by Th2 cytokines as well as by growth factors and cytokines derived from epithelial cells and macrophages.

sensitivity and specificity of 72% and 73%, respectively [41].

Noneosinophilic asthma has been defined as asthma with a sputum eosinophil count of less than either $\leq 2\%$ or $\leq 3\%$, whilst neutrophilic inflammation has been defined with cut-off points varying from $\geq 60\%$ to $>76\%$. Noneosinophilic asthma was in fact the most predominant form of the disease in many described asthma cohorts [42, 43, 45, 46], and neutrophilic asthma constituted $\sim 20\%$ [41,

45]. In the SARP cohort, individuals with moderate-to-severe asthma were characterized by a predominantly neutrophil or mixed neutrophil/eosinophil inflammation [47].

Th2-high asthma has been defined using the gene signature of airway epithelial brushings of a set of IL-13-inducible genes consisting of periostin, chloride channel regulator 1 and serpin peptidase inhibitor [48]. Patients with the Th2-high asthma phenotype had a greater degree of bronchial

hyper-responsiveness, higher serum IgE levels and increased blood and airway eosinophilia, subepithelial fibrosis and airway mucin gene expression [49]; in addition, they responded well to ICS therapy. Those with a 'Th2-low' signature showed little or no response to ICS treatment. Th2 gene signatures can also be obtained by performing polymerase chain reaction measurement of Th2-associated genes in sputum cells and used to denote patients with Th2-high asthma [50].

The mechanisms underlying airway neutrophilia are less clear. Neutrophilia may reflect a non-Th2-driven mechanism and, possibly, a steroid-insensitive asthma phenotype. Corticosteroid treatment can contribute to the neutrophilia and even Th1 factors such as the release of tumour necrosis factor alpha (TNF α) may play a role. Th17 cells have been implicated as a cause of neutrophilia in severe asthma [51]. Bacterial colonization in the airways of patients with severe asthma with defective phagocytosis of bacteria by macrophages could also contribute to neutrophilia [52–54].

Heterogeneous responses to corticosteroid therapy

Poor response to treatments particularly with corticosteroids is recognized, and low-dose ICS therapy improves lung function in only a proportion of patients with asthma [55–57]. Only 78% of patients with mild-to-moderate asthma and 62% of those with moderate-to-severe asthma achieved good disease control with a combination of ICS and long-acting β -agonists (LABAs) (fluticasone propionate and salmeterol) [58]. Another aspect of poor response to corticosteroid therapy is observed in patients with severe asthma. By definition, patients with severe asthma are corticosteroid insensitive as they do not respond to treatment, although they do respond to very high doses of corticosteroid therapy such as with triamcinolone [59].

Sputum eosinophilia and high levels of FeNO [60], as well as a Th2-high gene expression in the airway epithelium [48], can predict a favourable clinical response to treatment with ICS. Among patients with severe asthma, there is a well-defined group with persistently high blood or sputum eosinophil counts and frequent exacerbations that could represent an eosinophilic corticosteroid-insensitive phenotype [7, 61]. Other factors that could be

associated with corticosteroid insensitivity include cigarette smoking, bacterial infections with an altered microbiome, obesity and deficiency of vitamin D [62].

New targeted treatments for asthma

Increased understanding of the pathophysiology of asthma has led to new treatments being developed mainly based on targeting constituents of the Th2 pathway. In addition, there is an increasing need to use biomarkers to indicate the phenotype of patients who are likely to respond therapeutically. This is a novel approach to the treatment of asthma that will provide the first step towards more personalized therapy [63]. Fewer treatments are available in terms of targeting the non-Th2 and noneosinophilic pathways.

Targeting the Th2 pathway

Anti-IgE antibody. Omalizumab, the first approved biological agent recommended for asthma, is currently available for the treatment of patients with severe allergic asthma (Table 2). Omalizumab is a humanized antibody that binds to the C ϵ 3 domain of the free IgE heavy chain and will therefore bind to free circulating IgE. This treatment also decreases the level of the high-affinity Fc ϵ R1 receptor present on mast cells and basophils [64]. Through these two actions, there is an interruption of the allergic cascade and prevention of mast cell degranulation with suppression of free IgE and eosinophilic inflammation [64]. In a meta-analysis, omalizumab improved disease symptoms and asthma-related quality of life measures and reduced exacerbation rates [65, 66]. In severe persistent allergic asthma, omalizumab as an add-on therapy decreased clinically significant asthma exacerbations by 26%, compared to the effect of placebo [67]. Omalizumab also improved asthma-related quality of life, morning peak expiratory flow and asthma symptom scores [66]. Biomarkers of therapeutic efficacy for omalizumab in reducing exacerbations include high levels of nitric oxide in exhaled breath (FeNO) and high eosinophil blood counts [68]. The European Respiratory Society and American Thoracic Society recommend a therapeutic trial of omalizumab in both adults and children with severe allergic asthma [66].

QGE031 is another humanized anti-IgE antibody that has a 50-fold higher affinity for IgE than

Table 2 *Th2-directed therapies based on monoclonal antibodies in clinical trials for asthma*

Th2 target	Drug	Patient characteristics and biomarkers	Main response	Reference
Free IgE	Omalizumab	Severe asthma on ICS+LABA; atopic status, serum IgE 30–700 IU mL ⁻¹	Reduced asthma exacerbations; reduction in maintenance OCS	[67]
IL-4R α	AMG-317	Moderate-to-severe asthma	Reduction in exacerbation at the highest dose	[73]
IL-4R α	Dupilumab	Moderate-to-severe asthma with sputum eosinophilia ($\geq 3\%$) or blood eosinophilia (≥ 300 /mL)	Asthma control and FEV ₁ maintained as dose of ICS tapered or stopped	[74]
IL-5	Mepolizumab	Severe asthma on ICS and LABA $\pm$ OCS; blood eosinophils ≥ 150 /mm ³ at screening or ≥ 300 /mm ³ in past year	Reduced exacerbation rates Decrease in maintenance OCS Improvement in FEV ₁	[78, 79]
IL-5	Reslizumab	Severe asthma on ICS+LABA; sputum eosinophilia $>3\%$	Decrease in asthma exacerbations Improvement in FEV ₁	[76]
IL-5R α	Benralizumab	High-dose ICS, 2–6 exacerbations/year; blood eosinophil count; blood eosinophil index and FeNO >50 ppb	Some decrease in asthma exacerbations	[80]
IL-13	Lebrikizumab	Not well controlled on ICS+LABA; blood eosinophils and serum IgE; serum periostin	Improvement in FEV ₁	[81]
IL-13	Talokinumab	Moderate-to-severe uncontrolled asthma despite controller therapies; atopic status; blood eosinophils; sputum IL-13	Small improvement in FEV ₁	[83]

FeNO, nitric oxide in the exhaled breath; FEV₁, forced expiratory volume in 1 s; ICS, inhaled corticosteroids; LABA, long-acting β -agonist; OCS, oral corticosteroids; ppb: parts per billion.

omalizumab. QGE031 reduced circulating levels of IgE, Fc ϵ R1, surface IgE and skin prick responses to aeroallergens to a greater extent compared to omalizumab [69]. This more potent anti-IgE antibody may be suitable for individuals with levels of serum IgE >1300 IU mL⁻¹.

Another approach is to inhibit the production of IgE. Quilizumab is a humanized monoclonal antibody directed against an extracellular 52-amino acid segment termed M1 prime of human membrane IgE. This antibody reduced the generation of new IgE-producing plasma cells, with consequent

reduction in serum IgE levels [70]. Quilizumab caused a long-lasting reduction in total and allergen-specific serum IgE [70]. In allergic asthma subjects undergoing allergen challenge, this antibody blocked the generation of new IgE and reduced allergen-induced early and late asthmatic airway responses, and there was a nonsignificant reduction in allergen-induced increases in sputum eosinophil count [71].

Anti-IL-4R α antibody. IL-4 activates Th2 cells, causes isotype class-switching of B cells towards IgE synthesis and is involved in mast cell recruitment. IL-4 binds to IL-4R α via two different types of receptors (type I and type II), which leads to the signalling of both IL-4 and IL-13 [72]. AMG 317, a human monoclonal antibody against IL-4R α that blocks both IL-4 and IL-13 pathways, showed some clinical efficacy in patients with moderate-to-severe asthma in reducing exacerbations at the highest dose used [73]. However, in patients with persistent, moderate-to-severe asthma and elevated eosinophil levels in either blood or sputum who were treated with ICS and LABAs, dupilumab, a human monoclonal antibody against IL-4R α , was associated with fewer asthma exacerbations when LABAs and ICS were withdrawn, with improved lung function and reduced levels of Th2-associated inflammatory markers [74].

Anti-IL-5 antibody. IL-5 is a Th2 cytokine that is essential for the terminal differentiation, maturation and survival of eosinophils. In an unselected cohort of adult patients with asthma, it was found that the anti-IL5 antibody mepolizumab was not effective [75]. However, in later studies in patients with severe asthma on a combination of ICS and LABA with persistent sputum or blood eosinophilia, two anti-IL-5 antibodies, mepolizumab and reslizumab, were found to decrease exacerbations, reduce oral corticosteroid use and improve symptoms and lung function [61, 76, 77]. In patients with recurrent severe asthma exacerbations and eosinophilic inflammation, mepolizumab was effective in reducing exacerbation rates, but without improvement in forced expiratory volume in 1 s (FEV₁) or quality of life [78]. In subsequent larger studies in patients with recurrent exacerbations of asthma (more than two per year) despite high-dose inhaled steroids and eosinophilic inflammation (>300/mm³ blood in past year), mepolizumab caused a significant reduction in oral corticosteroid dosage, reduced exacerbations and improved asthma control [78, 79]. There

was also an improvement in FEV₁ at 32 weeks with mepolizumab compared to placebo [78].

Benralizumab is an antibody against the α -chain of the IL-5 receptor. In patients with uncontrolled asthma using medium- to high-dose ICS, with between two and six exacerbations per year and a baseline blood eosinophil count >300/mm³, there was a nonsignificant trend towards a reduction in exacerbations with benralizumab [80].

Anti-IL-13 antibody. Lebrikizumab is an IgG4 humanized monoclonal antibody that binds to IL-13. Corren *et al.* showed that in patients with asthma that was not well controlled on ICS and LABA, treatment with lebrikizumab improved FEV₁ by 8.2% above the baseline value in those with a high serum periostin level, whereas patients with a low serum periostin level showed no FEV₁ response [81]. By contrast, Noonan *et al.* enrolled patients with asthma who had never received ICS therapy and found no improvement in FEV₁ in individuals with high periostin levels and a minor increase in those with low periostin levels [82]. Another anti-IL-13 antibody, tralokinumab, did not improve symptoms but resulted in a nonsignificant increase in FEV₁ compared to placebo, with greater effects in patients with detectable sputum IL-13 levels [83].

The findings in the Corren *et al.* study indicated that serum periostin levels could be used as a biomarker of response to lebrikizumab [81]. Periostin levels have been shown to correlate with airway eosinophilia [84]. On the other hand, high serum periostin levels may also define patients with chronic airflow obstruction [85] and a cluster of well-controlled asthmatic individuals with a rapid decline in FEV₁ [86].

Antagonists of chemoattractant homologous receptor expressed on Th2 cells. Prostaglandin D₂, which is released during allergen-induced reactions in the airways, activates a G-protein-coupled receptor, chemoattractant homologous receptor expressed on Th2 cells (CRTh2; also known as DP2 receptor), present on Th2 cells and eosinophils and induces the chemotaxis of these cells [87]. CRTh2 antagonists have been developed for the treatment of asthma. In patients with moderate persistent asthma not on ICS therapy, the CRTh2 antagonist OC000459 improved FEV₁, asthma-related quality of life scores and night-time symptoms, without affecting sputum eosinophil counts

[88]. Another CRTh2 antagonist, AMG 853, used as an add-on to ICS therapy, did not improve asthma symptoms or lung function in patients with uncontrolled moderate-to-severe disease [89].

Anti-TSLP antibody. AMG 157, a human anti-TSLP monoclonal antibody, attenuated allergen-induced early and late asthmatic responses. It also reduced levels of blood and sputum eosinophils before and after allergen challenge, and FeNO levels [90].

Targeting non-Th2 targets

Anti-IL-17RA. Brodalumab, a human anti-IL-17RA monoclonal antibody, had no effect on asthma control scores, symptom-free days and FEV₁ in subjects with inadequately controlled moderate-to-severe asthma receiving ICS therapy. Furthermore, a *post hoc* examination of prespecified patient subgroups did not reveal any particular group with a significant response to this treatment [91].

Anti-CD25 antibody. The humanized IgG1 monoclonal antibody, daclizumab, which binds specifically to the α -subunit (Tac, CD25) of the high-affinity IL-2R of activated lymphocytes, inhibits IL-2 binding. Daclizumab has been shown to improve FEV₁ and asthma control in adults with moderate-to-severe asthma inadequately controlled on ICS [92]. It is unclear whether this therapy would be effective specifically in patients with evidence of IL-2 or high Th2 activity underlying the asthmatic process.

CXCR2 antagonist. CXCL8 (IL-8) is a chemokine involved in the chemoattraction and activation of neutrophils through the CXCR2 receptor, particularly in severe asthma. The CXCR2 antagonist SCH527123 inhibited ozone-induced sputum neutrophilia in nonasthmatic subjects [93]. SCH527123 also reduced sputum neutrophilia in adults with severe asthma, with a modest reduction in mild exacerbations, but without any improvement in asthma control [94].

Anti-TNF α antibody. TNF α is a pro-inflammatory Th1 cytokine that induces airway inflammation and hyper-responsiveness, mucus hypersecretion and activation of macrophages. In a study of adults with uncontrolled severe persistent asthma, the anti-TNF α antibody golimumab had no treatment effect; however, a *post hoc* analysis

suggested that patients with significant bronchodilator reversibility experienced fewer exacerbations whilst on golimumab [95]. Serious side effects included an increased prevalence of infections and malignancies in the treated group, which is likely to represent the importance of TNF α in the innate immune response to infections and cancer in the lungs.

Macrolide antibiotic therapy. Using the 16S ribosomal RNA technique to assess the microbiome in the lower airways, it was found that bacterial diversity and bacterial burden were greater in patients with ongoing asthma compared to non-asthmatic healthy control subjects [96, 97]. Macrophage phagocytosis of bacteria is impaired in severe asthma [52]. Macrolide antibiotics have been used for treating asthma associated with *Chlamydia pneumoniae* or *Mycoplasma pneumoniae* infections [98]. Clarithromycin added to ICS therapy in patients with severe asthma reduced sputum levels of neutrophils and IL-8, together with an improvement in quality-of-life measures but with no effect on FEV₁ [99]. In a study of patients with mild-to-moderate persistent asthma that was suboptimally controlled by low-dose ICS, the addition of clarithromycin did not lead to better asthma control, although bronchial hyper-responsiveness was improved [100]. Finally, in a *post hoc* analysis in a cohort with exacerbation-prone severe asthma, azithromycin caused a lower rate of severe exacerbations and a reduction in respiratory tract infections requiring treatment with antibiotics compared to placebo in subjects with noneosinophilic severe asthma (defined as a blood eosinophil count $\leq 200/\mu\text{L}$) [101]. This indicated that azithromycin could be beneficial in patient with noneosinophilic severe asthma.

Bronchial thermoplasty. Bronchial thermoplasty is administered via a bronchoscopic procedure during which the large airways are heated to 65 °C. In a blinded, sham treatment-controlled study, bronchial thermoplasty led to a reduction in severe asthma exacerbations, with a significant improvement in asthma-specific quality of life with a reduction in days lost from school or work because of asthma. Sham treatment itself had a beneficial effect on quality-of-life measures, and 6% of patients undergoing the active procedure were hospitalized during the treatment period [102]. In an open study in patients with severe refractory asthma, thermoplasty improved FEV₁ and asthma control [103]. The mechanisms by

which this treatment is effective are unknown, and its efficacy and side effects in the treatment of patients with severe asthma remain to be determined. It is unclear which asthma phenotype would benefit most from this treatment.

Conclusions

The clinical, physiological and inflammatory presentation of the asthma syndrome is complex, but there have been advances in our understanding and interpretation of this complexity through the analysis of inflammatory markers in well-defined patient cohorts. The characterization of a Th2-high molecular phenotype has enabled the development of specific targets towards components of the Th2 inflammatory cascade. The challenge now is how to phenotype Th2-low asthma. To advance personalized medicine for asthma, it is important to develop better tools, such as validated measures of disease activity and severity, and to define severe asthma using control measures of disease and through the analysis of inflammatory pathways as well as omics technologies using computational tools [104, 105]. More 'complex' phenotypes may be obtained using different advanced computational and mathematical modelling that may be more powerful in predicting outcomes to very highly targeted therapies. This will lead the way to a more personalized medicine approach for a disease as complex as asthma. Phenotyping will ensure that targeted therapeutic agents will be tailored and delivered to the 'right' patient at the 'right' time.

Conflict of interest statement

The author has received honoraria for participating in Advisory Board meetings regarding treatments for asthma and chronic obstructive pulmonary disease for GlaxoSmithKline, Astra-Zeneca, Merck, Novartis and Gilead. The author has also been on Speakers' Bureaus of GlaxoSmithKline, Boehringer-Ingelheim, Astra-Zeneca, Merck and Novartis.

Acknowledgements

Work carried out in the author's laboratory is funded by the UK Medical Research Council, National Environmental Research Council UK, the US National Institute for Environmental Health Sciences and the NIHR Respiratory Biomedical Research Unit, Royal Brompton & Harefield NHS Trust and Imperial College London, UK.

References

- 1 Accordini S, Corsico AG, Braggion M *et al*. The cost of persistent asthma in Europe: an international population-based study in adults. *Int Arch Allergy Immunol* 2013; **160**: 93–101.
- 2 Strachan DP. Hay fever, hygiene, and household size. *BMJ* 1989; **299**: 1259–60.
- 3 Brown HM. Treatment of chronic asthma with prednisolone; significance of eosinophils in the sputum. *Lancet* 1958; **2**: 1245–7.
- 4 Berry MA, Shaw DE, Green RH, Brightling CE, Wardlaw AJ, Pavord ID. The use of exhaled nitric oxide concentration to identify eosinophilic airway inflammation: an observational study in adults with asthma. *Clin Exp Allergy* 2005; **35**: 1175–9.
- 5 Deykin A, Lazarus SC, Fahy JV *et al*. Sputum eosinophil counts predict asthma control after discontinuation of inhaled corticosteroids. *J Allergy Clin Immunol* 2005; **115**: 720–7.
- 6 Moore WC, Meyers DA, Wenzel SE *et al*. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. *Am J Respir Crit Care Med* 2010; **181**: 315–23.
- 7 Haldar P, Pavord ID, Shaw DE *et al*. Cluster analysis and clinical asthma phenotypes. *Am J Respir Crit Care Med* 2008; **178**: 218–24.
- 8 Kim TB, Jang AS, Kwon HS *et al*. Identification of asthma clusters in two independent Korean adult asthma cohorts. *Eur Respir J* 2013; **41**: 1308–14.
- 9 Kaneko Y, Masuko H, Sakamoto T *et al*. Asthma phenotypes in Japanese adults – their associations with the CCL5 and ADRB2 genotypes. *Allergol Int* 2013; **62**: 113–21.
- 10 Schatz M, Hsu JW, Zeiger RS *et al*. Phenotypes determined by cluster analysis in severe or difficult-to-treat asthma. *J Allergy Clin Immunol* 2013; **133**: 1549–56.
- 11 Sutherland ER, Goleva E, King TS *et al*. Cluster analysis of obesity and asthma phenotypes. *PLoS ONE* 2012; **7**: e36631.
- 12 Gibeon D, Batuwita K, Osmond M *et al*. Obesity-associated severe asthma represents a distinct clinical phenotype: analysis of the British Thoracic Society Difficult Asthma Registry Patient cohort according to BMI. *Chest* 2013; **143**: 406–14.
- 13 Amelink M, de Nijs SB, de Groot JC *et al*. Three phenotypes of adult-onset asthma. *Allergy* 2013; **68**: 674–80.
- 14 Robinson DS, Ying S, Bentley AM *et al*. Relationships among numbers of bronchoalveolar lavage cells expressing messenger ribonucleic acid for cytokines, asthma symptoms, and airway methacholine responsiveness in atopic asthma. *J Allergy Clin Immunol* 1993; **92**: 397–403.
- 15 Bentley AM, Meng Q, Robinson DS, Hamid Q, Kay AB, Durham SR. Increases in activated T lymphocytes, eosinophils and cytokine mRNA expression for interleukin-5 and granulocyte/macrophage colony-stimulating factor in bronchial biopsies after allergen inhalation challenge in atopic asthmatics. *Am J Respir Cell Mol Biol* 1993; **8**: 35–42.
- 16 McKenzie AN, Spits H, Eberl G. Innate lymphoid cells in inflammation and immunity. *Immunity* 2014; **41**: 366–74.
- 17 Licona-Limon P, Kim LK, Palm NW, Flavell RA. TH2, allergy and group 2 innate lymphoid cells. *Nat Immunol* 2013; **14**: 536–42.

- 18 Prefontaine D, Nadigel J, Chouiali F *et al*. Increased IL-33 expression by epithelial cells in bronchial asthma. *J Allergy Clin Immunol* 2010; **125**: 752–4.
- 19 Ying S, O'Connor B, Ratoff J *et al*. Expression and cellular provenance of thymic stromal lymphopoietin and chemokines in patients with severe asthma and chronic obstructive pulmonary disease. *J Immunol* 2008; **181**: 2790–8.
- 20 Krug N, Madden J, Redington AE *et al*. T-cell cytokine profile evaluated at the single cell level in BAL and blood in allergic asthma. *Am J Respir Cell Mol Biol* 1996; **14**: 319–26.
- 21 Boniface S, Koscher V, Mamessier E *et al*. Assessment of T lymphocyte cytokine production in induced sputum from asthmatics: a flow cytometry study. *Clin Exp Allergy* 2003; **33**: 1238–43.
- 22 Cho SH, Stanciu LA, Holgate ST, Johnston SL. Increased interleukin-4, interleukin-5, and interferon-gamma in airway CD4+ and CD8+ T cells in atopic asthma. *Am J Respir Crit Care Med* 2005; **171**: 224–30.
- 23 Kumar RK, Herbert C, Webb DC, Li L, Foster PS. Effects of anticytokine therapy in a mouse model of chronic asthma. *Am J Respir Crit Care Med* 2004; **170**: 1043–8.
- 24 Roussel L, Houle F, Chan C *et al*. IL-17 promotes p38 MAPK-dependent endothelial activation enhancing neutrophil recruitment to sites of inflammation. *J Immunol* 2010; **184**: 4531–7.
- 25 Halwani R, Al-Muhsen S, Hamid Q. T helper 17 cells in airway diseases: from laboratory bench to bedside. *Chest* 2013; **143**: 494–501.
- 26 McKinley L, Alcorn JF, Peterson A *et al*. TH17 cells mediate steroid-resistant airway inflammation and airway hyperresponsiveness in mice. *J Immunol* 2008; **181**: 4089–97.
- 27 Doe C, Bafadhel M, Siddiqui S *et al*. Expression of the T helper 17-associated cytokines IL-17A and IL-17F in asthma and COPD. *Chest* 2010; **138**: 1140–7.
- 28 Tillie-Leblond I, de Blic J, Jaubert F, Wallaert B, Scheinmann P, Gosset P. Airway remodeling is correlated with obstruction in children with severe asthma. *Allergy* 2008; **63**: 533–41.
- 29 Cohen L, Tarsi J *et al*. Epithelial cell proliferation contributes to airway remodeling in severe asthma. *Am J Respir Crit Care Med* 2007; **176**: 138–45.
- 30 Macedo P, Hew M, Torrego A *et al*. Inflammatory biomarkers in airways of patients with severe asthma compared with non-severe asthma. *Clin Exp Allergy* 2009; **39**: 1668–76.
- 31 James AL, Bai TR, Mauad T *et al*. Airway smooth muscle thickness in asthma is related to severity but not duration of asthma. *Eur Respir J* 2009; **34**: 1040–5.
- 32 Kaminska M, Foley S, Maghni K *et al*. Airway remodeling in subjects with severe asthma with or without chronic persistent airflow obstruction. *J Allergy Clin Immunol* 2009; **124**: 45–51.e1–4.
- 33 Komakula S, Khatri S, Mermis J *et al*. Body mass index is associated with reduced exhaled nitric oxide and higher exhaled 8-isoprostanes in asthmatics. *Respir Res* 2007; **8**: 32.
- 34 Saunders R, Siddiqui S, Kaur D *et al*. Fibrocyte localization to the airway smooth muscle is a feature of asthma. *J Allergy Clin Immunol* 2009; **123**: 376–84.
- 35 Wang CH, Huang CD, Lin HC *et al*. Increased circulating fibrocytes in asthma with chronic airflow obstruction. *Am J Respir Crit Care Med* 2008; **178**: 583–91.
- 36 Lo CY, Michaeloudes C, Bhavsar PK *et al*. Increased phenotypic differentiation and reduced corticosteroid sensitivity of fibrocytes in severe asthma. *J Allergy Clin Immunol* 2015; in press.
- 37 Wenzel SE, Schwartz LB, Langmack EL *et al*. Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. *Am J Respir Crit Care Med* 1999; **160**: 1001–8.
- 38 Bourdin A, Neveu D, Vachier I, Paganin F, Godard P, Chanez P. Specificity of basement membrane thickening in severe asthma. *J Allergy Clin Immunol* 2007; **119**: 1367–74.
- 39 Balzar S, Chu HW, Silkoff P *et al*. Increased TGF-beta2 in severe asthma with eosinophilia. *J Allergy Clin Immunol* 2005; **115**: 110–7.
- 40 Xie S, Sukkar MB, Issa R, Khorasani NM, Chung KF. Mechanisms of induction of airway smooth muscle hyperplasia by transforming growth factor-beta. *Am J Physiol Lung Cell Mol Physiol* 2007; **293**: L245–53.
- 41 Schleich F, Brusselle G, Louis R *et al*. Heterogeneity of phenotypes in severe asthmatics. The Belgian Severe Asthma Registry (BSAR). *Respir Med* 2014; **108**: 1723–32.
- 42 McGrath KW, Icitovic N, Boushey HA *et al*. A large subgroup of mild-to-moderate asthma is persistently noneosinophilic. *Am J Respir Crit Care Med* 2012; **185**: 612–9.
- 43 Schleich FN, Chevrement A, Paulus V *et al*. Importance of concomitant local and systemic eosinophilia in uncontrolled asthma. *Eur Respir J* 2014; **44**: 97–108.
- 44 Zhang XY, Simpson JL, Powell H *et al*. Full blood count parameters for the detection of asthma inflammatory phenotypes. *Clin Exp Allergy* 2014; **44**: 1137–45.
- 45 Simpson JL, Scott R, Boyle MJ, Gibson PG. Inflammatory subtypes in asthma: assessment and identification using induced sputum. *Respirology* 2006; **11**: 54–61.
- 46 Jatakanon A, Mohamed JB, Lim S, Maziak W, Chung KF, Barnes PJ. Neutrophils may contribute to the pathogenesis of airway inflammation in steroid dependent intractable asthma. *Am J Respir Crit Care Med* 1998; **157**: A875.
- 47 Moore WC, Hastie AT, Li X *et al*. Sputum neutrophil counts are associated with more severe asthma phenotypes using cluster analysis. *J Allergy Clin Immunol* 2014; **133**: 1557–63.e5.
- 48 Woodruff PG, Boushey HA, Dolganov GM *et al*. Genome-wide profiling identifies epithelial cell genes associated with asthma and with treatment response to corticosteroids. *Proc Natl Acad Sci U S A* 2007; **104**: 15858–63.
- 49 Woodruff PG, Modrek B, Choy DF *et al*. T-helper type 2-driven inflammation defines major subphenotypes of asthma. *Am J Respir Crit Care Med* 2009; **180**: 388–95.
- 50 Peters MC, Mekonnen ZK, Yuan S, Bhakta NR, Woodruff PG, Fahy JV. Measures of gene expression in sputum cells can identify TH2-high and TH2-low subtypes of asthma. *J Allergy Clin Immunol* 2014; **133**: 388–94.e5.
- 51 Al-Ramli W, Prefontaine D, Chouiali F *et al*. T(H)17-associated cytokines (IL-17A and IL-17F) in severe asthma. *J Allergy Clin Immunol* 2009; **123**: 1185–7.
- 52 Liang Z, Zhang Q, Thomas CM *et al*. Impaired macrophage phagocytosis of bacteria in severe asthma. *Respir Res* 2014; **15**: 72.
- 53 Zhang Q, Illing R, Hui CK *et al*. Bacteria in sputum of stable severe asthma and increased airway wall thickness. *Respir Res* 2012; **13**: 35.

- 54 Wood LG, Simpson JL, Hansbro PM, Gibson PG. Potentially pathogenic bacteria cultured from the sputum of stable asthmatics are associated with increased 8-isoprostane and airway neutrophilia. *Free Radical Res* 2010; **44**: 146–54.
- 55 Baumgartner RA, Martinez G, Edelman JM *et al.* Distribution of therapeutic response in asthma control between oral montelukast and inhaled beclomethasone. *Eur Respir J* 2003; **21**: 123–8.
- 56 Israel E, Chervinsky PS, Friedman B *et al.* Effects of montelukast and beclomethasone on airway function and asthma control. *J Allergy Clin Immunol* 2002; **110**: 847–54.
- 57 Martin RJ, Szefer SJ, King TS *et al.* The predicting response to inhaled corticosteroid efficacy (PRICE) trial. *J Allergy Clin Immunol* 2007; **119**: 73–80.
- 58 Bateman ED, Boushey HA, Bousquet J *et al.* Can guideline-defined asthma control be achieved? The gaining optimal asthma control study. *Am J Respir Crit Care Med* 2004; **170**: 836–44.
- 59 ten Brinke A, Zwinderman AH, Sterk PJ, Rabe KF, Bel EH. “Refractory” eosinophilic airway inflammation in severe asthma: effect of parenteral corticosteroids. *Am J Respir Crit Care Med* 2004; **170**: 601–5.
- 60 Cowan DC, Taylor DR, Peterson LE *et al.* Biomarker-based asthma phenotypes of corticosteroid response. *J Allergy Clin Immunol* 2015; **135**: 877–83.
- 61 Haldar P, Brightling CE, Hargadon B *et al.* Mepolizumab and exacerbations of refractory eosinophilic asthma. *N Engl J Med* 2015; **135**: 877–883.
- 62 Hew M, Chung KF. Corticosteroid insensitivity in severe asthma: significance, mechanisms and aetiology. *Intern Med J* 2010; **40**: 323–34.
- 63 Chung KF. Defining phenotypes in asthma: a step towards personalized medicine. *Drugs* 2014; **74**: 719–28.
- 64 Djukanovic R, Wilson SJ, Kraft M *et al.* Effects of treatment with anti-immunoglobulin E antibody omalizumab on airway inflammation in allergic asthma. *Am J Respir Crit Care Med* 2004; **170**: 583–93.
- 65 Normansell R, Walker S, Milan SJ, Walters EH, Nair P. Omalizumab for asthma in adults and children. *Cochrane Database Syst Rev* 2014; **1**: Cd003559.
- 66 Chung KF, Wenzel SE, Brozek JL *et al.* International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur Respir J* 2014; **43**: 343–73.
- 67 Humbert M, Beasley R, Ayres J *et al.* Benefits of omalizumab as add-on therapy in patients with severe persistent asthma who are inadequately controlled despite best available therapy (GINA 2002 step 4 treatment): INNOVATE. *Allergy* 2005; **60**: 309–16.
- 68 Hanania NA, Wenzel S, Rosen K *et al.* Exploring the effects of omalizumab in allergic asthma: an analysis of biomarkers in the EXTRA study. *Am J Respir Crit Care Med* 2013; **187**: 804–11.
- 69 Arm JP, Bottoli I, Skerjanec A *et al.* Pharmacokinetics, pharmacodynamics and safety of QGE031 (ligelizumab), a novel high-affinity anti-IgE antibody, in atopic subjects. *Clin Exp Allergy* 2014; **44**: 1371–85.
- 70 Brightbill HD, Jeet S, Lin Z *et al.* Antibodies specific for a segment of human membrane IgE deplete IgE-producing B cells in humanized mice. *J Clin Invest* 2010; **120**: 2218–29.
- 71 Gauvreau GM, Harris JM, Boulet LP *et al.* Targeting membrane-expressed IgE B cell receptor with an antibody to the M1 prime epitope reduces IgE production. *Sci Transl Med* 2014; **6**: 243ra85.
- 72 Maes T, Joos GF, Brusselle GG. Targeting interleukin-4 in asthma: lost in translation? *Am J Respir Cell Mol Biol* 2012; **47**: 261–70.
- 73 Corren J, Busse W, Meltzer EO *et al.* A randomized, controlled, phase 2 study of AMG 317, an IL-4R α antagonist, in patients with asthma. *Am J Respir Crit Care Med* 2010; **181**: 788–96.
- 74 Wenzel S, Ford L, Pearlman D *et al.* Dupilumab in persistent asthma with elevated eosinophil levels. *N Engl J Med* 2013; **368**: 2455–66.
- 75 Flood-Page P, Swenson C, Faierman I *et al.* A study to evaluate safety and efficacy of mepolizumab in patients with moderate persistent asthma. *Am J Respir Crit Care Med* 2007; **176**: 1062–71.
- 76 Castro M, Mathur S, Hargreave F *et al.* Reslizumab for poorly controlled, eosinophilic asthma: a randomized, placebo-controlled study. *Am J Respir Crit Care Med* 2011; **184**: 1125–32.
- 77 Nair P, Pizzichini MM, Kjarsgaard M *et al.* Mepolizumab for prednisone-dependent asthma with sputum eosinophilia. *N Engl J Med* 2009; **360**: 985–93.
- 78 Ortega HG, Liu MC, Pavord ID *et al.* Mepolizumab treatment in patients with severe eosinophilic asthma. *N Engl J Med* 2014; **371**: 1198–207.
- 79 Bel EH, Wenzel SE, Thompson PJ *et al.* Oral glucocorticoid-sparing effect of mepolizumab in eosinophilic asthma. *N Engl J Med* 2014; **371**: 1189–97.
- 80 Castro M, Wenzel SE, Bleeker ER *et al.* Benralizumab, an anti-interleukin 5 receptor α monoclonal antibody, versus placebo for uncontrolled eosinophilic asthma: a phase 2b randomised dose-ranging study. *Lancet Respir Med* 2014; **2**: 879–90.
- 81 Corren J, Lemanske RF, Hanania NA *et al.* Lebrikizumab treatment in adults with asthma. *N Engl J Med* 2011; **365**: 1088–98.
- 82 Noonan M, Korenblat P, Mosesova S *et al.* Dose-ranging study of lebrikizumab in asthmatic patients not receiving inhaled steroids. *J Allergy Clin Immunol* 2013; **132**: 567–74.e12.
- 83 Piper E, Brightling C, Niven R *et al.* A phase II placebo-controlled study of tralokinumab in moderate-to-severe asthma. *Eur Respir J* 2013; **41**: 330–8.
- 84 Jia G, Erickson RW, Choy DF *et al.* Periostin is a systemic biomarker of eosinophilic airway inflammation in asthmatic patients. *J Allergy Clin Immunol* 2012; **130**: 647–54.e10.
- 85 Kanemitsu Y, Matsumoto H, Izuhara K *et al.* Increased periostin associates with greater airflow limitation in patients receiving inhaled corticosteroids. *J Allergy Clin Immunol* 2013; **132**: 305–12.e3.
- 86 Nagasaki T, Matsumoto H, Kanemitsu Y *et al.* Integrating longitudinal information on pulmonary function and inflammation using asthma phenotypes. *J Allergy Clin Immunol* 2014; **133**: 1474–7, 7.e1–2.
- 87 Pettipher R, Hansel TT, Armer R. Antagonism of the prostaglandin D2 receptors DP1 and CRTH2 as an approach to treat allergic diseases. *Nat Rev Drug Discov* 2007; **6**: 313–25.
- 88 Barnes N, Pavord I, Chuchalin A *et al.* A randomized, double-blind, placebo-controlled study of the CRTH2

- antagonist OC000459 in moderate persistent asthma. *Clin Exp Allergy* 2012; **42**: 38–48.
- 89 Busse WW, Wenzel SE, Meltzer EO *et al.* Safety and efficacy of the prostaglandin D2 receptor antagonist AMG 853 in asthmatic patients. *J Allergy Clin Immunol* 2013; **131**: 339–45.
 - 90 Gauvreau GM, O'Byrne PM, Boulet LP *et al.* Effects of an anti-TSLP antibody on allergen-induced asthmatic responses. *N Engl J Med* 2014; **370**: 2102–10.
 - 91 Busse WW, Holgate S, Kerwin E *et al.* Randomized, double-blind, placebo-controlled study of brodalumab, a human anti-IL-17 receptor monoclonal antibody, in moderate to severe asthma. *Am J Respir Crit Care Med* 2013; **188**: 1294–302.
 - 92 Busse WW, Israel E, Nelson HS *et al.* Daclizumab improves asthma control in patients with moderate to severe persistent asthma: a randomized, controlled trial. *Am J Respir Crit Care Med* 2008; **178**: 1002–8.
 - 93 Holz O, Khalilieh S, Ludwig-Sengpiel A *et al.* SCH527123, a novel CXCR2 antagonist, inhibits ozone-induced neutrophilia in healthy subjects. *Eur Respir J* 2010; **35**: 564–70.
 - 94 Nair P, Gaga M, Zervas E *et al.* Safety and efficacy of a CXCR2 antagonist in patients with severe asthma and sputum neutrophils: a randomized, placebo-controlled clinical trial. *Clin Exp Allergy* 2012; **42**: 1097–103.
 - 95 Wenzel SE, Barnes PJ, Bleecker ER *et al.* A randomized, double-blind, placebo-controlled study of tumor necrosis factor- α blockade in severe persistent asthma. *Am J Respir Crit Care Med* 2009; **179**: 549–58.
 - 96 Hilty M, Burke C, Pedro H *et al.* Disordered microbial communities in asthmatic airways. *PLoS ONE* 2010; **5**: e8578.
 - 97 Huang YJ, Nelson CE, Brodie EL *et al.* Airway microbiota and bronchial hyperresponsiveness in patients with suboptimally controlled asthma. *J Allergy Clin Immunol* 2011; **127**: 372–81.e1–3.
 - 98 Kraft M, Cassell GH, Pak J, Martin RJ. Mycoplasma pneumoniae and Chlamydia pneumoniae in asthma: effect of clarithromycin. *Chest* 2002; **121**: 1782–8.
 - 99 Simpson JL, Powell H, Boyle MJ, Scott RJ, Gibson PG. Clarithromycin targets neutrophilic airway inflammation in refractory asthma. *Am J Respir Crit Care Med* 2008; **177**: 148–55.
 - 100 Sutherland ER, King TS, Icitovic N *et al.* A trial of clarithromycin for the treatment of suboptimally controlled asthma. *J Allergy Clin Immunol* 2010; **126**: 747–53.
 - 101 Brusselle GG, Vanderstichele C, Jordens P *et al.* Azithromycin for prevention of exacerbations in severe asthma (AZISAST): a multicentre randomised double-blind placebo-controlled trial. *Thorax* 2013; **68**: 322–9.
 - 102 Castro M, Rubin AS, Laviolette M *et al.* Effectiveness and safety of bronchial thermoplasty in the treatment of severe asthma: a multicenter, randomized, double-blind, sham-controlled clinical trial. *Am J Respir Crit Care Med* 2010; **181**: 116–24.
 - 103 Pavord ID, Cox G, Thomson NC *et al.* Safety and efficacy of bronchial thermoplasty in symptomatic, severe asthma. *Am J Respir Crit Care Med* 2007; **176**: 1185–91.
 - 104 Auffray C, Adcock IM, Chung KF, Djukanovic R, Pison C, Sterk PJ. An integrative systems biology approach to understanding pulmonary diseases. *Chest* 2010; **137**: 1410–6.
 - 105 Wheelock CE, Goss VM, Balgoma D *et al.* Application of 'omics technologies to biomarker discovery in inflammatory lung diseases. *Eur Respir J* 2013; **42**: 802–25.

Correspondence: Professor Kian Fan Chung, MD, DSc, National Heart and Lung Institute, Imperial College London, Dovehouse St, London SW3 6LY, UK.
(fax: +442075947954; e-mail: f.chung@imperial.ac.uk). ■