From phenotypes to endotypes to asthma treatment

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Purpose of review
The current guidelines for asthma diagnosis and management do not recognize that different phenotypes of asthma exist, with significant variations in the manifestation of airway inflammation, symptoms, severity, and response to treatment. This article will critically review new approaches to classify asthma together with the emerging endotype-driven therapeutic strategies.

Recent findings
Several new approaches for classifying asthma are available, from precision and deep phenotyping to identification of novel causal pathways and translation of biomarkers into pathway-specific diagnostic tests. New phenotypes, such as epigenetic phenotypes, asthmatic granulomatosis, or neurophenotypes are described. Large clinical trials testing the endotype-driven approach are increasingly successful, but the dissociated effect and the drug efficacy at the target site remain unsolved issues. Profiling the Th2 low and the resident cell compartment of asthma are major unmet needs in asthma endotyping.

Summary
Each of the hallmark characteristics of asthma (inflammation, remodeling, airway hyperreactivity) is the expression of a complex network of molecules, very diverse both within any given patient in time and between any two patients. Some of these networks are repetitive across individuals with asthma and specific for clinical expression, gene–environment interaction and inflammatory cell profiles represent novel endotype-specific diagnostic and therapeutic strategies.

Keywords
asthma phenotypes and endotypes, biomarkers, mechanistic pathways of asthma

INTRODUCTION

Asthma is a complex heritable disease, characterized by inflammation, airway wall remodeling and airway hyperresponsiveness associated with marked heterogeneity in regard to severity, symptoms, and disease management.

A classification of asthma control and severity based on symptoms, lung function, exacerbations, limitation of physical activity and need for rescue medications guides asthma treatment today. This approach has significantly reduced asthma mortality and morbidity; however, all over the world, the disease continues to increase in both prevalence and severity. Current treatment has little or no effect on the natural history of asthma, and certainly does not cure the disease.

Recent evidence highlights several cellular and molecular endotypes of asthma driven by significant differences in the types and expression levels of key pathogenetic players. Disregarding asthma endotypes might lead to unsuccessful clinical, genetic, or epidemiological trials due to a bulk patient selection [1▪▪].

STRATEGIES USED TO CLASSIFY ASTHMA

Clinicians are already familiar with the phenotyping exercise consisting of identifying visible properties associated with asthma [1▪▪]. Yet, phenotypic descriptions in clinical notes and medical publications are often imprecise and frequently over-lapping.

Deep phenotyping can be defined as the precise and comprehensive analysis of phenotypic abnormalities based on stratification into disease subclasses with a common biological basis. Use of computational resources to capture, store, and exchange phenotypic data followed by sophisticated algorithms to integrate it with genomic variation, omics profiles, and other clinical information is essential [2]. Mathematical techniques such as

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KEY POINTS

- Repetitive and relevant complex network of molecules governing the hallmark characteristics of asthma are validated as disease-specific endotypes.
- New methods to disentangle asthma heterogeneity, from complex mathematical algorithms to systems biology and pharmacology are becoming increasingly available.
- The response to a targeted intervention in asthma may vary between individuals or for the same individual in relation to the outcome measures (dissociated effect).
- Endotype-driven treatment of asthma has improved the response rate to targeted treatment but did not solve completely the dissociated effect of the intervention nor the variability in response due to drug efficacy at target site.
- Further deep phenotyping of airway smooth muscle and epithelial components of asthma, as well as of Th2 low inflammation is needed to move the field forward.

factor or cluster analysis allow the organization of large amounts of interrelated data in an unbiased way. Latent class growth analysis can model multiple phenotypes and identify both time-invariant (sex) and time-varying (environmental exposure) risk factors. The technique can accommodate questionnaire data collected at irregularly spaced age intervals and can simultaneously identify multiple trajectories of health outcomes [3▪].

Better discriminatory analyses may ensue from incorporating longitudinal data, such as exacerbation rate, fluctuation analysis of lung function or fractional exhaled nitric oxide (FeNO), or lung function decline [4▪,5▪] and of other biomarkers apart from inflammation such as imaging, transcriptomics, and proteomics data (Fig. 1). Approaches to classify asthma based on endotypes reveal a multitude of mechanisms and drug targets [1▪▪]. Innovative techniques such as bipartite network analysis and visual analytics introduce a new dimension to data analysis meant to identify underlying mechanistic pathways [6]. New clinical trials examining the holistic response of a phenotype-dependent pathway to drug perturbation, as defined by systems pharmacology, are expected [7▪]. Application of systems biology approaches to asthma pharmacogenomics will improve the likelihood of predicting a response to a given asthma treatment [8].

EPIGENETIC PHENOTYPES

Epigenetic biomarkers (DNA methylation, modification of histone tails and noncoding RNAs) from easily obtained samples (buccal, nasal, or peripheral blood cells) or from bronchial biopsies might identify individuals patients at risk for asthma, phenotypes driven by environmental stress, or the likelihood of response to environmental or therapeutic interventions. Targeting epigenetic biomarkers might lead to novel anti-inflammatory therapies, especially for corticosteroid-resistant asthma.

In the Lovelace Smokers Cohort a synergistic interaction increasing odds for asthma was noted between methylated genes measured in sputum DNA. Methylated genes identified a symptomatically more severe asthma phenotype [9▪]. Data from two independent pregnancy cohorts in Spain showed that DNA hypomethylation in the arachidonate lipoxygenase gene (ALOX12) measured in peripheral blood of children was associated with a higher risk of persistent wheezing at age 4 years [10▪].

Widespread changes in mRNA and miRNA expression in circulating CD81 but not CD41 T cells were associated with severe asthma [11]. Alterations of airway epithelial cell miRNA levels were described in both steroid-naive and more severe asthma, with inhaled corticosteroids (ICS) only modestly impacting miRNA levels. Interleukin 13 (IL-13) was linked to these alterations, especially to repression of miR-34/449 family members that have established roles in airway epithelial cell differentiation [12▪].

INFLAMMATORY PHENOTYPES

Phenotyping asthma based on the predominant inflammatory cell as assessed by induced sputum provides an insight into the underlying pathobiology of the disease. Treatment strategies based on sputum eosinophilic and noneosinophilic phenotypes have been successful and targeted treatment revealed new subclasses for eosinophilic asthma, such as IL-5 and IL-4/IL-13-driven inflammation. Moreover, eosinophilic or neutrophilic inflammation might persist in well controlled asthma patients, and therefore assessment of bronchial inflammation using induced sputum becomes necessary to define proper asthma control [13▪]. Flow cytometry analysis of leukocytes in induced sputum might improve the diagnostic accuracy of sputum cytology. A strong correlation between neutrophils, eosinophils and macrophages in microscopic examination and flow cytometry was reported [14▪].

Less is known on inflammatory phenotypes in the pediatric asthmatic population. Induced sputum was examined in children with severe and mild/moderate asthma using the same criteria as for adults to classify the inflammatory phenotypes.

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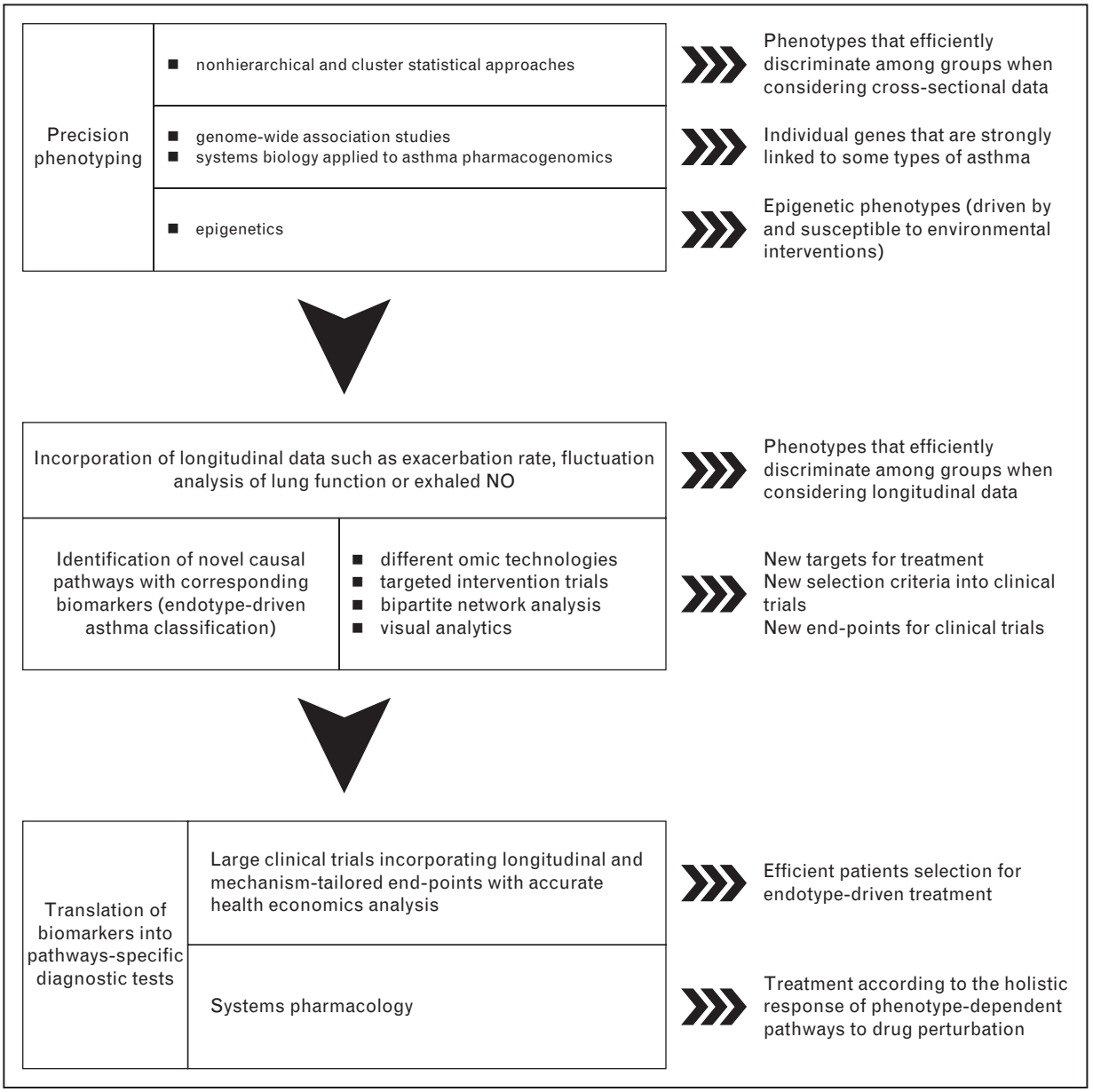

FIGURE 1. Stepwise approach to classify asthma.

Increased sputum inflammatory cells were recorded in 78% of children and noneosinophilic asthma was indentified in 41% cases. Sputum induction was repeated every 3 months in children with severe asthma over a 1-year period and twice in mild-to-moderate asthma over 3–6 months. Variability in sputum inflammatory phenotype was demonstrated in 63% of children, both in the severe and the mild-to-moderate asthma groups and was not related to change in ICS dose or asthma control, nor was it mirrored by a change in FeNO. There were no differences in severity, asthma control, atopy, ICS dose, or in the forced expiratory volume in the first second (FEV1) between children who were always noneosinophilic and those always eosinophilic [15▪].

Asthmatic granulomatosis

Larger-volume lung tissue biopsies were obtained through video-assisted thorascopic surgery in 19 patients with atypical difficult asthma requiring daily systemic corticosteroids. The abnormality of 10 cases was an unexpected finding of interstitial and airway centric nonnecrotizing granulomas associated with small airway changes such as eosinophilia and goblet cell hyperplasia. These patients were characterized by a disproportionately low forced expiratory flow (FEF 25–75%) predicted, peripheral blood eosinophilia and high FeNO persistent despite systemic corticosteroids, adult onset disease, female sex in 90% cases, and a family history of autoimmune disease in 60% cases. All 10 cases were

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treated with immunosupressants and all managed to reduce their daily dose of oral corticosteroids, while improving their lung function and decreasing their symptoms [16▪].

NEUROPHENOTYPES

Asthma symptoms and severity are increased during periods of increased stress or emotion. Functional MRI was used to measure neural activity in response to asthma specific emotional triggers, following allergen exposure. Asthmatics with a dual response to allergen challenge were compared with patients with only an immediate response and healthy controls. The anterior insular cortex was differentially sensitive to disease-relevant information, with greater activation related to increased late phase inflammation and lung function decline. This subset of asthmatics seems most vulnerable to the development of psychopathology and corresponding severe asthma and may benefit most from behavioral interventions aimed at reducing neural reactivity, such as meditation or neurofeedback [17▪].

ENDOTYPE-DRIVEN TREATMENT OF ASTHMA

Sputum eosinophils, FeNO, T helper 2 (Th2)-high, and genetic biomarkers can be used to predict response to ICS. Research is needed to find drugs complementary to the combination ICS/long-acting β2-agonist or leukotriene pathway antagonists in difficult asthma. A new class of drugs as a first-line treatment for mild-to-moderate noneosinophilic asthma is also needed. There are no major efforts in the profiling of the 'Th2 low' phenotype, representing half of adult and pediatric population. This approach could prove more cost-efficient than pursuing the pathway to antiIL-5, IL-4/IL-13 compounds that seem to target the same subpopulation of severe eosinophilic asthma representing less than 3% of the whole asthmatic population.

New and expensive biological therapies for asthma are emerging that are highly efficacious only for a selected group of patients. The response to an antiasthmatic drug may vary between individuals or for the same individual in relation to the outcome measures (dissociated effect). This is best exemplified by all biologicals used to treat severe asthma. For mepolizumab, for example, there is an overall effect on asthma exacerbations, but this is not met in all individuals and is not associated with improvements in lung function, symptoms, or asthma control [18▪▪]. Although biomarker-driven use of anti IL-5 and anti IL-13 compounds has markedly improved the response rate it still does not solve the huge interindividual and intraindividual response in terms of outcome measures. To further complicate the picture differences in efficacy at the target site in the airways depending on drug formulation/metabolism are also key players in the process (Fig. 2).

Selection of the target group of patients using an endotype-driven decision guided by a biomarker directly involved in asthma pathogenesis together with a clever selection of outcome measures (Table 1) should be acknowledged as an essential scaffold for the design of successful clinical trials. Several predictors of response were evaluated for Th2-driven inflammatory pathways (Table 2).

Blocking the Inteleukin-5 pathway

In the Dose Ranging Efficacy And safety with Mepolizumab (DREAM) in severe asthma trial selection based on the history of past severe exacerbations

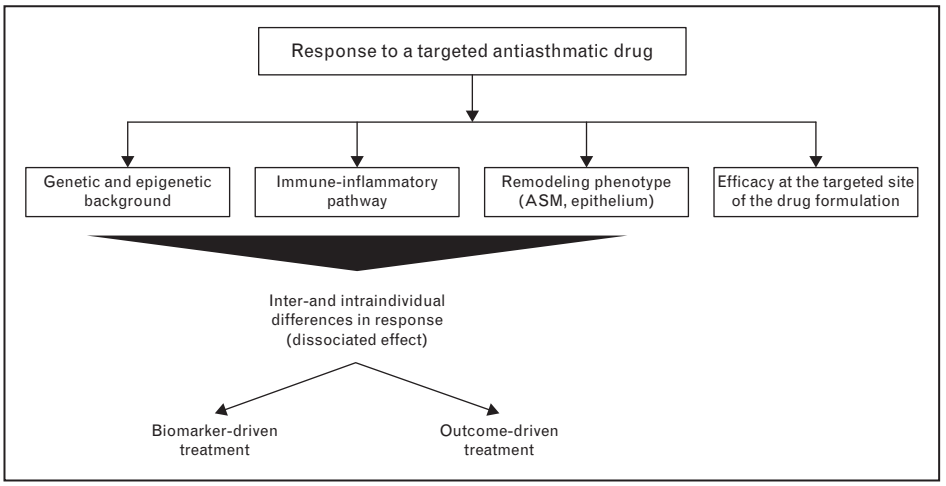

FIGURE 2. Response to a targeted antiasthmatic drug.

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Table 1. Judging targeted interventions in the asthma

Target	Type of intervention		Predictors of response	Outcome measures
Eosinophils	IL-5	Blocking antibodies (mepolizumab, reslizumab)	Sputum/serum Eos	Severe exacerbations
			FeNO	? lung function (reslizumab)
			Steroid resistance	? AHR
			History of exacerbations	
			Nasal polyps (reslizumab)	
	α-Chain of IL-5R	Blocking antibodies with additional ADCC activity	? blood eosinophils	
		Benralizumab (MEDI-563)		
	β-Chain of IL-5R	Antisense oligonucleotide (TPI ASM8)	Sputum eosinophils and ? progenitors	
Th2 inflammation	IL-4/IL-13 pathway	Anti IL-13 antibodies (lebrikizumab, IMA 638, tralokinumab, QAX576, CNTO 5825, GSK679586)	Serum periostin, FeNO, ? total or specific IgE, ?sputum/serum Eos, ? sputum IL-13, ? allergen induced EAR and LAR	Lung function, ? exacerbations, ?cough and sputum, ?airway obstruction and thickening (CT scan)
		Soluble form of the α subunit of the IL-4 receptor (altrakincept)		
		Anti-IL-4 receptor α (pitrakinra, AMG 317)	IL-4 receptor alpha gene SNPs (pitrakinra)	
	CRTH2 inhibitors	OC000459, ramatroban	? sputum eosinophils	Lung function
				Symptoms
				Respiratory infections
				QoL
	Potential future targets: IL-9, IL-25, IL-33, and TSLP		?	?
Neutrophilic inflammation	Altered microbiome	Long-term newer macrolides	Older age, longer duration of asthma	Lung function, exacerbations
			? PCR evidence of bacterial infection	
	Neutrophil acumulation	CXCR2 inhibitors	Increased sputum neutrophils	Lung function, exacerbations
			No infection	
			Low IgE	
			Low eosinophils	
	Potential future targets: IL-17		?	?
T cells	T-cell activation	daclizumab	Lack of eosinophilic or neutrophilic inflammation	Asthma control
				Exacerbations
				Lung function
				AHR
Airway smooth muscle cell	Phenotype switch into a quiescent cell	? miRNA	AHR	?
			Lung function fluctuation	
Airway epithelium	Increased antiviral properties	IFN β	Severe viral induced asthma exacerbations	Exacerbation rate

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Table 1 (*Continued*)

Target	Type of intervention		Predictors of response	Outcome measures
Remodeling	ASM	Bronchial termoplasty	Fixed airway obstruction	Airway obstruction and thickening (CT scan)
			Lack of airway inflammation	
	Small airways disease	Better inhaled formulations	Alveolar NO	Nocturnal symptoms
		Systemic treatment	Air trapping	AHR
				Exacerbations

?, unknown or small effect observed; AHR, airway hyperreactivity.

and evidence of eosinophilic inflammation (increased sputum or blood eosinophils; FeNO $\geq$50 ppb; prompt deterioration of asthma control after a 25% or less reduction in regular maintenance ICS or oral corticosteroids) proved successful as all three doses of mepolizumab significantly reduced the number of asthma exacerbations and delayed time to first exacerbation. Efficacy of mepolizumab increased with baseline eosinophil counts and number of exacerbations in the previous year, but not with atopic status, IgE, FEV1, or acute bronchodilator response. Baseline FeNO proved to be less closely associated with a response to mepolizumab than did blood eosinophil count [18▪▪].

In a small clinical trial selecting patients with persistent sputum eosinophilia and poor asthma control under ICS another anti IL-5 antibody, reslizumab showed a different efficacy profile with significant improvement in lung function in all patients. Asthma control questionnaire (ACQ) score improved only in patients with baseline scores greater than two and in those with nasal polyps. Possible confounding factors, such as the presence of aspirin-exacerbated airway disease or the use of leukotriene receptor antagonist in the nasal polyp group, were not considered. There was no effect on asthma exacerbations, probably due to the short duration of the study [19]. Two large phase III trials enrolling patients selected based on a blood eosinophil count of at least 400/μl will evaluate the impact of long-term reslizumab on lung function (ClinicalTrials.gov Identifiers: NCT01270464) and asthma exacerbations (ClinicalTrials.gov Identifiers: NCT01285323).

Blocking the interleukin-4/interleukin-13 pathway

The Study of Lebrikizumab (MILR1444A) in Adult Patients With Asthma Who Are Inadequately Controlled on Inhaled Corticosteroids (MILLY) trial was one of the first to validate a biomarker consistent with the therapeutic intervention. In a double-blind placebo-controlled 6-month trial in patients with inadequately controlled asthma lebrikizumab, an IL-13 blocking antibody, significantly improved FEV1 at 12 weeks [20▪▪]. In a follow-up study [21] severe exacerbation rate decreased significantly at 32 weeks. Two large Phase III in patients with uncontrolled asthma are underway to establish the safety and efficacy (asthma exacerbations as primary outcome) of lebrikizumab when administered over a 52-week period and to explore the value of serum periostin to select responders to treatment (ClinicalTrials.gov Identifiers: NCT01545440, LUTE trial and NCT01545453, VERSE trial).

The relation between FeNO and periostin as predictors of response to lebrikizumab needs further exploration. In the MILLY trial although both were separate predictors of response to lebrikizumab, they were moderately correlated, with an incomplete overlap, and the composite status did predict response only for both high periostin and FeNO [20▪▪]. In the Biomarkers in Corticosteroid-refractory Asthma (BOBCAT) trial serum periostin was strongly correlated with persistent sputum and tissue eosinophilia despite steroid treatment and regardless of sputum or tissue neutrophil counts. FeNO measurement detected fewer patients with tissue eosinophilia and exhibited greater overlap between eosinophil-low and eosinophil-high patients. In a logistic regression model incorporating age, sex, BMI, blood eosinophils, serum IgE, FeNO, and serum periostin, periostin level was the most significant single predictor of composite airway eosinophil status. While sputum and blood eosinophil counts and FeNO levels are subject to significant temporal variability, depending on allergen exposure, exacerbations, and steroid treatment in the BOBCAT there was relatively little intra-subject variability in serum periostin levels in three measurements over the course of up to 5 weeks [22▪▪].

Another antiIL-13 antibody, tralokinumab (CAT-354) administered over a 12-week period significantly improved lung function and decreased

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Table 2. Predictors of response to Th2 targeted treatment

Targeted intervention		Predictor of response	Strength of evidence
anti IL-5	Mepolizumab, reslizumab	History of asthma exacerbations	++
		Sputum eosinophils and steroid resistance	++
		Blood eosinophils	+
	benralizumab	Blood eosinophils	±
	reslizumab	Nasal polyps	+
		Uncontrolled asthma (ACQ)	+
anti IL-13/IL-4	Lebrikizumab	FeNO	+
		Serum periostin	+
	Tralokinumab	IL13 in sputum	±
	IMA-638, pitrakinra,TPI ASM8	Th2-driven inflammation and allergen induced EAR and LAR	±
	All	Th2-driven inflammation and prominent remodeling	?
anti IL-3, GM-CSF, CCL1, CCL5 and CCL11		Eosinophilic inflammation (sputum or serum)	?

EAR, early allergic reaction; LAR, late allergic reaction.

rescue medication use but did not improve the ACQ-6 score or the rate of asthma exacerbations. Patients were stratified prior to randomization to ensure at least 50% of randomized patients were atopic, defined as specific IgE to a panel of inhaled allergens. Neither atopy nor blood eosinophils were predictors for a better response. However, there was a trend for better response in FEV1 and ACQ-6 score in patients with increased sputum IL-13 [23▪]. A Phase IIb, 52-week study evaluating the effect of tralokinumab on asthma exacerbations in adults with uncontrolled, severe asthma is underway (NCT01402986).

The IL-4 receptor α amino acid variations might predict response to antiIL-4/IL-13 pathway inhibitors. Patients homozygous for the rs8832 common G allele responded to pitrakinra administration with decreased exacerbations, nocturnal awakenings, and limitation of activities by asthma. Both rs8832 and rs1029489 and several other intronic SNPs demonstrated a significant pitrakinra dose–response relationship in reducing asthma exacerbations. These SNPs were not associated with asthma exacerbations in the placebo group. However, the predictive power of SNPs was demonstrated only for the low-eosinophil-count group [24▪].

Chemoattractant homologous receptor expressed on Th2 cell inhibitors

There has recently been great interest in blocking prostaglandin PGD2, which is increased in patients with severe asthma who are not controlled on inhaled therapy. PGD2 is released from mast cells and attracts Th2 cells and eosinophils via the receptor chemoattractant homologous receptor expressed on Th2 cells (CRTH2). The effects of a potent and selective CRTH2 antagonist, OC000459, were evaluated versus placebo in a double-blind, parallel group small trial in steroid-free adults with moderate persistent asthma. There was a small effect on the FEV1 only in the per protocol population, which excluded non-compliant population. Quality of life improved and night-time asthma symptoms decreased significantly in both full analysis and per protocol populations. There was a trend in decreasing sputum eosinophil count. Adverse events were comparable to those on placebo and notably the respiratory infections were less common compared with placebo [25▪].

Targeting neutrophilic inflammation

Antineutrophil agents, like antiIL-8, have not met with much success in the past. Recently, a small molecule antagonist of CXC chemokine receptor 2 (SCH527123) administered orally has been tested in patients with severe asthma and sputum neutrophils more than 40%. SCH527123 proved successful in reducing sputum neutrophil numbers with suggestions toward improvement in clinical outcomes such as mild exacerbations and ACQ scores. There was no effect on lung function or sputum neutrophil activation markers (myeloperoxidase, IL8 or elastase) [26▪].

CONCLUSION

Recognition of asthma heterogeneity in relation to clinical significant outcomes is beyond any doubt. Several new methods to disentangle asthma complexity from mathematical and computational integrative methods to cellular and molecular

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endotypes are increasingly being validated as highly reproducible and clinically relevant. Further, deep phenotyping of airway smooth muscle and epithelial components of asthma, as well as of Th2 low inflammation is needed to move the field forward. Large endotype-driven clinical trials with clear outcomes will provide new insights into the mechanistic pathways of asthma, apart from improving treatment outcomes.

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Conflicts of interest

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There are no conflicts of interests in relation to this paper.

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