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WHAT GOES UP MUST COME DOWN: BIOMARKERS AND NOVEL BIOLOGICALS IN SEVERE ASTHMA

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Abstract

Asthma is a heterogeneous airway disease characterised by typical symptoms in combination with variable airway obstruction. Most patients with asthma have well controlled symptoms and a low risk of asthma attacks with inhaled corticosteroid treatment. However, a clinically important subgroup (~10%) remains symptomatic and/or at risk of asthma attacks despite maximum inhaled therapy. Patients with severe asthma are responsible for a significant proportion of healthcare costs attributable to asthma and have a large unmet need for better treatments. An important advance in recent years has been the recognition that severe asthma is heterogeneous with respect to clinical problems and the pattern of lower airway inflammation. Identification of eosinophilic inflammation in the airways has become an important priority as novel biologicals that target Th2 cytokines, such as anti-IL5, anti-IL-13 and combined anti-IL-4/13 are showing considerable promise as treatments for this subgroup. It has also become clear that anti-IgE (Omalizumab), the first monoclonal antibody registered for treatment of severe asthma, is only active in patients with active eosinophilic airway inflammation. The future will be identification of potentially responsive patients on the basis of raised biomarkers and, as suggested by the title of this review, targeted treatment with specific cytokine blockade that has a direct effect on the biomarkers. In this review we outline an approach to the clinical assessment of patients potentially suitable for biological treatment and describe in detail the likely clinical impact of established and new biological treatments.

List of abbreviations: AHR, airway hyperresponsiveness; BAL, bronchoalveolar lavage; IgE, Immunoglobulin E; IL-5, Interleukin-5; RCT, randomised controlled trial; Th2, type 2 T helper; FeNO, Fraction of Exhaled Nitric Oxide; PEF, peak expiratory flow; ILC-2, innate

lymphoid cell-2; ASM, airway smooth muscle; TSLP, thymic stromal lymphopoeitin; CXCR2, interleukin-8R-beta; ACQ, asthma control questionnaire; ICS, inhaled corticosteroids; OCS, oral corticosteroids; COPD, chronic obstructive pulmonary disease; FEV1, forced expiratory volume in 1 sec; RCT, randomised controlled trial.

Introduction

Asthma is a highly prevalent chronic inflammatory disease of the bronchial airways affecting 5-10% of all adults and children. An estimated 235 million people are diagnosed with asthma worldwide and the WHO estimates that a significant proportion is undertreated or undiagnosed. In developed countries the clinical manifestations of asthma are controlled with inhaled therapy in most patients[1,2]. However, around 10% of patients have severe asthma that is uncontrolled despite intense treatment regimens. This subgroup suffers from significantly higher morbidity and mortality[2], and accounts for more than half the asthma-related health and economic impact[3,4]. Asthma attacks, or acute or sub-acute episodes of worsening of symptoms and airflow obstruction make a significant contribution as they require immediate clinical attention and when severe, hospitalisation[5]. Patients with severe asthma therefore have a high unmet need and are in desperate need of alternative treatments. There has been intense interest from the pharmaceutical industry in finding better treatments for patients with severe asthma. Progress has been frustratingly slow and some treatments now known to be effective failed initial clinical trials. However, a better understanding of the heterogeneity of severe asthma and its lower airway pathology has resulted in more successful treatment outcomes lately. Most of the more promising treatment approaches specifically target eosinophilic airway inflammation and its identification has therefore become an important assessment of patients. In this review we outline an approach to the

clinical assessment of severe asthma, emphasize methods used to identify eosinophilic airway inflammation and overview some of the most promising new treatments targeting this process.

Clinical assessment

The diagnosis of asthma is based on recognition of typical symptoms (dyspnoea, cough and wheeze) in association with variable airflow obstruction[6]. The latter can be demonstrated by the response to a bronchodilator ($>12\%$ improve of FEV₁), by monitoring of peak expiratory flow (PEF) or by identification of airway hyperresponsiveness (i.e. a provocative concentration of Metacholine required to cause a 20% fall in FEV₁ of <8 mg/ml). Although asthma is the most common cause of episodic wheeze, cough and dyspnoea, there are other potential causes including dysfunctional breathing, vocal cord dysfunction, rhinitis, cough syndromes, and structural airway abnormalities like tracheomalacia and bronchomalacia[7]. These conditions are prevalent in patients presenting to a severe asthma clinic[8] as the accompanying symptoms do not respond to asthma treatments. The clinical assessment therefore has to be sufficiently rigorous to exclude these conditions with confidence[9]. This is not straightforward as abnormal PEF variability can occur independently of asthma[9] and all the conditions listed above can co-exist with asthma. Assessment of airway responsiveness and eosinophilic airway inflammation are generally more informative than more traditional tests[9]. Chronic obstructive pulmonary disease (COPD) can be difficult to distinguish from severe asthma with confidence and can be associated with eosinophilic airway inflammation. Our approach is to describe rather than categorise patients with shared features and focus the clinical assessment on identification of treatable aspects. Asthma can also be apparently severe and treatment resistant in patients who have not mastered the basics

of asthma management such as treatment adherence, correct inhaler technique and self-management. The clinical assessment should therefore include a thorough assessment of these aspects.

Phenotypes

After confirming the diagnosis of severe asthma the next step is to identify the inflammatory phenotype of asthma in order to direct treatment and risk reduction strategies. Severe asthma is characterised by a marked dissociation between symptoms and disordered airway function on one hand and lower airway pathology on the other so it is necessary to assess both to get the full picture (FIG 1). Distinguishing eosinophilic inflammation from non-eosinophilic inflammation is important for several reasons: 1) patients with eosinophilic airway inflammation are at high risk of severe attacks, including episodes requiring ventilation[10]; 2) a treatment model based on identification and treatment of eosinophilic airway inflammation has been proven to reduce attacks [11–13]; and 3) prednisolone, Omalizumab and a number of new biological agents are highly active in patients with this pattern of lower airway inflammations. Eosinophilic airway inflammation can be assessed directly by bronchoscopic techniques or by induced sputum or indirectly using the peripheral blood eosinophil count or fraction of exhaled nitric oxide (FeNO). The pros and cons and measurement characteristics of these methods are summarised in Table 1.

Th2-cytokines as potential treatment targets

Classically, the Th2 pathway is associated with eosinophilic inflammation triggered by allergens or parasites that come into contact with the epithelial barrier. As a first line of

defence, dendritic cells pick up the antigens and cause a response cascade after binding to T-helper cells at regional lymphoid sites. In case of a Th2 response, CD4⁺ lymphocytes become Th2-cells and start producing the cytokines IL-4, IL-5, IL-9 and IL-13 [14].

IL-4 produced by Th2-cells causes a general shift in Th0 cells to differentiate into Th2 cells and an immunoglobulin class switch, resulting in IgE production by B-cells. The produced IgE by altered B-cells (plasma cells) subsequently binds to mast cells and eosinophils that reside in the tissue enabling them to release their toxic granules upon antigen binding. Another Th2 cytokine, IL-5, is a very important systemic regulator of eosinophil dynamics in humans as it is a critical cytokine leading to maturation and activation of eosinophils. Locally it acts as a chemo-attractant and causes migration of eosinophils to sites of damage. IL-5 functions also in combination with IL-9 to recruit mast cells and eosinophils to an affected tissue site. IL-13 has various effector functions: it acts similarly to IL-4 in inducing IgE production by B-cells and it induces physiological changes in mucus production by epithelial cells and causes goblet cell metaplasia. However compared to IL-4, IL-5 and IL-9 it has a more extensive effect on airway smooth muscle and airway hyperresponsiveness in animal models[15]. Whether these effects are relevant to human asthma remains to be established.

Interestingly, a large subgroup of patients with severe asthma and eosinophilic inflammation is not atopic and has normal serum IgE. This counterintuitive finding complicates the understanding of the pathophysiology of eosinophilic inflammation, and implies that the pathology can be induced independently of exogenous allergens. A newly recognised class of cells might be the missing link between the ‘allergic’ Th2 response and persistent eosinophilic inflammation in patients without allergies. These cells were discovered in 2010 as nuocytes and eventually named innate lymphoid cells (ILC’s) [16].

ILC-2's, a subclass of ILC's, are able to produce large amounts of IL-5 and IL-13 but not IL-4, making them an attractive candidate for orchestrating the immune response in patients with non-atopic eosinophilic airway inflammation. The current paradigm for the role of ILC-2's is that disruption of the epithelial barrier by an external trigger, for example a virus, causes epithelial damage and enhances production of IL-25, IL-33 and thymic stromal lymphopoietin (TSLP) by epithelial cells[14]. These cytokines cause ILC2 activation in the tissue, thereby starting the production of Th2 cytokines. Production of prostaglandin D₂ and LTE₄ by recruited and activated eosinophils and mast cells might then enhance responsiveness of ILC2 cells leading to a perpetuating cycle[17].

Th2 cytokine blockers

Anti-IgE

Omalizumab is a recombinant humanized monoclonal antibody directed against Immunoglobulin E (IgE) [18] and registered for treatment of patients with severe persistent allergic asthma. In a recent Cochrane review[19], Normansell and colleagues reviewed 25 studies involving 6382 patients. The most important findings were that Omalizumab reduced exacerbations by about 40%, reduced steroid use, improved ACQ scores and improved health related quality of life scores. Side effects were similar in placebo groups and Omalizumab groups, except for some local skin reactions at the injection sites. An important comment by the authors is the fact that most studies included patients with moderate asthma, while the drug is registered for severe asthmatics. Therefore the effect of the drug has to be evaluated further in a group that might benefit more from the treatment than patients with moderate asthma.

A more practical difficulty is that most patients with severe asthma are not suitable for treatment as treatment is restricted based on the presence of a perennial allergy, serum IgE and weight. This is unfortunate as none of these characteristics has been shown to be associated with the response to Omalizumab and it is now clear that treatment efficacy is largely a function of the presence of eosinophilic airway inflammation and is accurately predicted by FeNO and serum periostin but not serum IgE [20,21].

Anti-IL-5 or Anti-IL-5R

Anti-IL-5 or Anti-IL-5R treatment influences eosinophil dynamics throughout the human immune system. Both basophils and eosinophils express the IL-5 receptor and can be targeted by blocking IL-5 or the IL-5 receptor. The first RCT with the IL-5 antagonist Mepolizumab in patients with asthma showed a strong biological effect[22]. A single dose of Mepolizumab(10 mg/kg) reduced blood eosinophil count for 16 weeks and sputum eosinophil count for up to 4 weeks. Moreover Mepolizumab was able to prevent blood eosinophilia during the late phase response after allergen challenge. Although the biological effect was strong, no effect was seen on AHR and the late asthmatic response.

Two larger RCT's [23,24] with Mepolizumab in patients with moderate asthma taking ICS confirmed the strong biological effect; however, both were disappointing in respect of the clinical effect on AHR, lung function, ACQ or rescue medication use. Interestingly this latter group did show a non-significant trend in the reduction of asthma exacerbations after Mepolizumab treatment.

The fact that these studies did not clearly show a clinical effect led many to question whether eosinophilic inflammation is of any importance for the key clinical outcomes in

asthma. The alternative possibility that treatment did not have a sufficiently large effect on eosinophilic airway inflammation was unlikely as the effects of treatment on sputum and airway biopsy eosinophil numbers was at least similar is observed with corticosteroid treatment.

A third possibility was that eosinophilic airway inflammation contributes to exacerbations of asthma to a greater degree than disordered airway function and symptom expression. In support to this, exacerbations and symptom control are to some extent disconnected within patients[25] and respond to treatment differently. Symptoms and lung function are more responsive to a long-acting beta2-agonist and exacerbations are more responsive to a 4-fold increased dose of inhaled corticosteroids[26].

In addition, studies which have examined the clinical effects of a management strategy which controls eosinophilic airway inflammation as well as symptoms have shown a large reduction in exacerbation numbers[11,12] but not much change in lung function and symptoms. It is now clear that asthma symptoms and traditional physiological tests of asthma are at best weakly associated with the presence of eosinophilic airway inflammation. As many as 80% of patients with asthma who remain symptomatic despite inhaled corticosteroids have no evidence of eosinophilic airway inflammation[27] and are thus unlikely to respond to a treatment which targets this feature.

These findings provided a strong basis for further evaluation of the effects of blocking IL-5 with two essential modifications to the clinical trial design: i) treating patients with evidence of active eosinophilic airway inflammation rather than an arbitrary and unrelated clinical measure such as a significant acute bronchodilator response; and ii) assessing the effect of treatment on asthma exacerbations, an outcome measure which is directly and potentially causally related to eosinophilic airway inflammation.

These studies showed that monthly IV injections of 750 mg Mepolizumab had a beneficial effect on exacerbations[28,29] and an oral steroid sparing effect [29]. In a large multi-center GSK sponsored study (DREAM), different doses of Mepolizumab were evaluated in a population with eosinophilic asthma identified with more permissive criteria including a raised blood eosinophil count and/or FeNO[30]. This study confirmed that treatment reduced asthma attacks by about 50% and showed that equivalent efficacy was apparent with a ten-fold lower dose of Mepolizumab. The study was large enough to begin investigating factors associated with treatment efficacy. Of the many variables assessed, only the blood eosinophil count and the prior frequency of asthma attacks were associated with the effect of treatment[30]. Neither the initial proof of concept study or DREAM showed much effect of Mepolizumab on asthma symptoms, quality of life, FeNO or lung function.

However, two recently published studies focused on severe asthma patients with a blood eosinophilia and >2 exacerbations in the past year found clear evidence of improvement in these measures as well as a significant OCS sparing effect (FIG2) [31] and a clear decrease in exacerbation rate (FIG3) [32] with monthly subcutaneous injections of 100 mg Mepolizumab. The more complete effect of Mepolizumab in these phase 3 trials may reflect better selection of a responsive population using the blood eosinophil count. No particular safety issues have been identified with long-term use of Mepolizumab and it is therefore a promising therapy for the patients who suffer from severe eosinophilic asthma.

Alternative anti-IL-5 treatments

Reslizumab is another type of IL-5 inhibitors that was used in RCT's for asthma. It was shown to significantly improve lung function, reduce sputum eosinophils and improve ACQ in a subgroup with nasal polyps[33]. Benralizumab, the anti-IL5-receptor alpha drug, causes

eosinophil cytotoxicity and has been shown to induce eosinopaenia in a study with mild atopic asthmatics[34] and it radically reduced tissue and sputum eosinophilia in another[35]. More recently, a Benralizumab Phase IIb dose-ranging RCT in patients with eosinophilic asthma and non-eosinophilic asthma showed a lowered exacerbation rate in the patients with eosinophilic asthma that received Benralizumab compared to placebo. Eosinophilic asthma was defined according to the ELEN-index, which predicts sputum eosinophilia based on non-sputum parameters. Interestingly, the post-hoc analysis revealed significant improvements in exacerbation rates, ACQ-6 and FEV₁ for patients with blood eosinophilia (>300 eosinophils/ μ L) [36]. Benralizumab did not reduce exacerbations in COPD patients with sputum eosinophilia and an exacerbation in the previous year[37]. However post hoc analysis of the data showed a similar pattern compared to its severe asthma equivalent, namely a trend towards lower acute exacerbations of COPD and improvement of the SGRQ-C (St. George Respiratory Questionnaire-C), CRQ-SAS (Chronic Respiratory Questionnaire-Self Assessed Standardised), and FEV₁ in the patients with higher blood eosinophil counts. In summary, the various ways of blocking IL-5 and its receptor are promising and might increase treatment effect in the future.

Anti-IL-13

IL-13 is closely linked to IL-4 and exerts similar functions by binding and activating the alpha subunit of the IL-4 receptor. This linkage is the subject of an ongoing debate about the relevance of singularly targeting either IL-4 or IL-13 in asthma. However, in the first trial in humans testing two anti-IL-13 compounds[38], using allergen challenge in mild atopic asthma, anti-IL-13 attenuated the drop in FEV₁ in the late phase.

A randomised controlled clinical trial of anti-IL-13 treatment (Lebrikizumab) in a cohort of moderate to severe asthma patients showed a small but significant improvement in FEV1[39]. The post-hoc analysis of this study was particularly noteworthy as patients with serum periostin concentrations above the median or a high FeNO had a greater FEV1 improvement and a strong trend to reduced exacerbations with treatment. This emphasizes again the importance of selecting the right endotype of asthma before starting specific treatment. Another IL-13 monoclonal antibody Tralokinumab, also showed an effect on FEV1 but no effect on clinical markers[40].

In a more recent study, the anti-IL-13 compound, GSK679586, showed no clinical effect in a well-defined cohort of 198 severe asthma patients, even though the authors retrospectively stratified the patients by periostin levels and by the presence of blood eosinophilia suggesting that anti-IL-13 might be less effective in more severe asthma[41].

IL4 receptor-alpha blockers

Blocking the alpha subunit of the IL-4 receptor affects both IL4 and IL-13 signalling. The first trial[42] with anti-IL-4-alpha blockers was a RCT that studied the effect of the fully human monoclonal antibody AMG 317 in patients with moderate to severe asthma and showed no beneficial effect on the main endpoint, which was reduction in the ACQ scores of the overall population. However it did show an effect in a subgroup that had high ACQ baseline scores, which therefore could be regarded as a more uncontrolled group. In the most recent trial by Wenzel et al. [43], a subgroup of patients with persistent moderate to severe asthma who had blood eosinophilia >300/ microliter received either placebo or anti-IL-4-alpha/IL-13 treatment (dupilumab). During the study, patients were withdrawn from LABA's first and secondly from ICS to observe differences in exacerbation rate (main endpoint).

There was a significant difference in exacerbation rate observed after withdrawal, in favour of the dupilumab group. Secondary endpoints, such as FEV1 improved significantly and ACQ also dropped in the treatment arm with evidence of a treatment effect on these measures before LABA and ICS were withdrawn. Treatment reduced FeNO, IgE levels, TARC and eotaxin-3 levels, providing evidence of a biological effect of the drug. One concern with the main endpoint is the definition of an exacerbation, also described by the authors. An exacerbation was defined as the need for systemic corticosteroids or doubling of the ICS dose, which differs from the current consensus statement in which >3 days of oral corticosteroids is used as a definition.

Prospects for Th-2 low disease

Prospects for modifying airway inflammation in Th-2 low disease is much more uncertain as the patterns of airway inflammation and its likely cause are poorly understood. Some encouragement is provided by the beneficial effects of long-term low dose macrolides in patients with non-eosinophilic asthma and by preliminary evidence of efficacy of a CXCR2 antagonist[44]. Neutrophilic airway inflammation might be driven by Th17 mediated processes[45].

In a first clinical trial anti-IL17, Brodalumab did not improve ACQ scores (primary endpoint) in a group of moderate to severe asthmatics[46]. However, treatment did have beneficial effects in a subgroup with high reversibility to albuterol. Patient selection was not optimal as the presence of neutrophilic airway inflammation was not confirmed and there was no marker of IL-17 involvement included for selection. More work is needed to understand the mechanisms and phenotypes of TH-2 low asthma.

Conclusion

The success of the introduction of novel biological agents in asthma largely depends on the ability to select the appropriate asthma patients. All 'successful' clinical studies that involved novel biological agents included a specific subgroup that was likely to be responsive to the treatment. Ideally, patients are selected by an easily measurable biomarker that is directly influenced by the treatment. Thus the everyday saying used in the title of this review very much applies for biological treatment of severe asthma.

It is interesting that existing data shows that FeNO and serum periostin are good biomarkers of treatment response to Omalizumab and biological agents targeting IL-13 and IL-4 whereas the blood eosinophil count is most closely associated with a response to anti-IL-5. Moreover, treatment with IL-5 reduces the blood eosinophil count but not FeNO whereas the reverse is true for Omalizumab, anti-IL-13 and anti-IL-4&13 (table 2). Whether these biomarkers can be used to identify sub-groups of patients within the TH-2 high population who are particularly suited to different cytokine blockade, will be an important research question for the future. Another important priority is to understand the pathophysiology of asthma in patients with no evidence of TH-2 mediated inflammation since patients with this phenotype of asthma have few treatment options.

Conflict of interest

BH declares that he has received sponsorship from Novartis and GSK to attend international scientific meetings. IDP has received speaker's honoraria for speaking at sponsored meetings from Astra Zeneca, Boehringer Ingelheim, GSK. He has received honoraria for attending advisory panels with Almirall, Astra Zeneca, Boehringer Ingelheim, GSK, MSD, Schering-

Plough, Novartis, Dey, Napp. He has received sponsorship to attend international scientific meetings from Boehringer Ingelheim, GSK, Astra Zeneca and Napp. He is Chief Medical Advisor to Asthma UK, a member of the UK Department of Health Asthma Strategy Group, a member of the BTS SIGN Asthma guideline group and joint editor in chief of *Thorax*. Neither IDP nor any member of his family has any shares in pharmaceutical companies.

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References

1. British Guideline on the Management of Asthma. *Thorax* 2008, 63 Suppl 4:iv1–121.
2. Holgate ST, Polosa R: The mechanisms, diagnosis, and management of severe asthma in adults. *Lancet* 2006, 368:780–93.
3. Bateman ED, Hurd SS, Barnes PJ, Bousquet J, Drazen JM, FitzGerald M, Gibson P, Ohta K, O'Byrne P, Pedersen SE, et al.: Global strategy for asthma management and prevention: GINA executive summary. *Eur. Respir. J.* 2008, 31:143–78.
4. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, Adcock IM, Bateman ED, Bel EH, Bleecker ER, et al.: International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur. Respir. J.* 2014, 43:343–73.
5. Jackson DJ, Sykes A, Mallia P, Johnston SL: Asthma exacerbations: origin, effect, and prevention. *J. Allergy Clin. Immunol.* 2011, 128:1165–74.

- Accepted Article
6. From the Global Strategy for Asthma Management and Prevention, Global Initiative for Asthma (GINA) 2014. Available from: <http://www.ginasthma.org/>.
 7. Weinberger M, Abu-Hasan M: Pseudo-asthma: when cough, wheezing, and dyspnea are not asthma. *Pediatrics* 2007, 120:855–64.
 8. Robinson DS, Campbell DA, Durham SR, Pfeffer J, Barnes PJ, Chung KF: Systematic assessment of difficult-to-treat asthma. *Eur. Respir. J.* 2003, 22:478–83.
 9. Hunter CJ, Brightling CE, Woltmann G, Wardlaw AJ, Pavord ID: A comparison of the validity of different diagnostic tests in adults with asthma. *Chest* 2002, 121:1051–7.
 10. Haldar P, Pavord ID, Shaw DE, Berry MA, Thomas M, Brightling CE, Wardlaw AJ, Green RH: Cluster analysis and clinical asthma phenotypes. *Am. J. Respir. Crit. Care Med.* 2008, 178:218–24.
 11. Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Bradding P, Wardlaw AJ, Pavord ID: Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. *Lancet* 2002, 360:1715–21.
 12. Jayaram L, Pizzichini MM, Cook RJ, Boulet L-PP, Lemièrè C, Pizzichini E, Cartier A, Hussack P, Goldsmith CH, Laviolette M, et al.: Determining asthma treatment by monitoring sputum cell counts: effect on exacerbations. [Internet]. *Eur. Respir. J.* 2006, 27:483–94.
 13. Chlumsky J, Striz I, Terl M, Vondracek J: Strategy Aimed at Reduction of Sputum Eosinophils Decreases Exacerbation Rate in Patients with Asthma. *Journal of International Medical Research* 2006, 34:129139.
 14. Licona-Limón P, Kim LK, Palm NW, Flavell RA: TH2, allergy and group 2 innate lymphoid cells. *Nat. Immunol.* 2013, 14:536–42.

- Accepted Article
15. Nath P, Leung SY, Williams AS, Noble A, Xie S, McKenzie AN, Chung KF: Complete inhibition of allergic airway inflammation and remodelling in quadruple IL-4/5/9/13-/- mice. *Clin. Exp. Allergy* 2007, 37:1427–35.
 16. Spits H, Artis D, Colonna M, Diefenbach A, Di Santo JP, Eberl G, Koyasu S, Locksley RM, McKenzie AN, Mebius RE, et al.: Innate lymphoid cells--a proposal for uniform nomenclature. *Nat. Rev. Immunol.* 2013, 13:145–9.
 17. Brusselle GG, Maes T, Bracke KR: Eosinophils in the spotlight: Eosinophilic airway inflammation in nonallergic asthma. *Nat. Med.* 2013, 19:977–9.
 18. Strunk RC, Bloomberg GR: Omalizumab for asthma. *N. Engl. J. Med.* 2006, 354:2689–95.
 19. Normansell R, Walker S, Milan SJ, Walters EH, Nair P: Omalizumab for asthma in adults and children. *Cochrane Database Syst Rev* 2014, 1:CD003559.
 20. Hanania NA, Wenzel S, Rosén K, Hsieh H-JJ, Mosesova S, Choy DF, Lal P, Arron JR, Harris JM, Busse W: 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.
 21. Humbert M, Busse W, Hanania NA, Lowe PJ, Canvin J, Erpenbeck VJ, Holgate S: Omalizumab in asthma: an update on recent developments. *J Allergy Clin Immunol Pract* 2014, 2:525–536.e1.
 22. Leckie MJ, ten Brinke A, Khan J, Diamant Z, O'Connor BJ, Walls CM, Mathur AK, Cowley HC, Chung KF, Djukanovic R, et al.: Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 2000, 356:2144–8.
 23. Flood-Page P, Swenson C, Faiferman I, Matthews J, Williams M, Brannick L, Robinson D, Wenzel S, Busse W, Hansel TT, 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.
24. Flood-Page PT, Menzies-Gow AN, Kay AB, Robinson DS: Eosinophil's role remains uncertain as anti-interleukin-5 only partially depletes numbers in asthmatic airway. *Am. J. Respir. Crit. Care Med.* 2003, 167:199–204.
25. Reddel H, Ware S, Marks G, Salome C, Jenkins C, Woolcock A: Differences between asthma exacerbations and poor asthma control. *Lancet* 1999, 353:364–9.
26. Pauwels RA, Löfdahl CG, Postma DS, Tattersfield AE, O'Byrne P, Barnes PJ, Ullman A: Effect of inhaled formoterol and budesonide on exacerbations of asthma. Formoterol and Corticosteroids Establishing Therapy (FACET) International Study Group. *N. Engl. J. Med.* 1997, 337:1405–11.
27. Green RH, Brightling CE, Pavord ID, Wardlaw AJ: Management of asthma in adults: current therapy and future directions. *Postgrad Med J* 2003, 79:259–67.
28. Haldar P, Brightling CE, Hargadon B, Gupta S, Monteiro W, Sousa A, Marshall RP, Bradding P, Green RH, Wardlaw AJ, et al.: Mepolizumab and exacerbations of refractory eosinophilic asthma. *N. Engl. J. Med.* 2009, 360:973–84.
29. Nair P, Pizzichini MMM, Kjarsgaard M, Inman MD, Efthimiadis A, Pizzichini E, Hargreave FE, O'Byrne PM: Mepolizumab for prednisone-dependent asthma with sputum eosinophilia. [Internet]. *N. Engl. J. Med.* 2009, 360:985–93.
30. Pavord ID, Korn S, Howarth P, Bleecker ER, Buhl R, Keene ON, Ortega H, Chanez P: Mepolizumab for severe eosinophilic asthma (DREAM): a multicentre, double-blind, placebo-controlled trial. [Internet]. *Lancet* 2012, 380:651–9.
31. Bel E, Wenzel S, Thompson P, Prazma C, Keene O, Yancey S, Ortega H, Pavord I: Oral Glucocorticoid-Sparing Effect of Mepolizumab in Eosinophilic Asthma. *The New England Journal of Medicine* 2014, doi:10.1056/NEJMoa1403291.

- Accepted Article
32. Ortega H, Liu M, Pavord I, Brusselle G, Chetta A, Humbert M, Katz L, Pharm.D. P., Keene O, Yancey S, et al.: Mepolizumab Treatment in Patients with Severe Eosinophilic Asthma. *The New England Journal of Medicine* 2014, doi:10.1056/NEJMoa1403290.
 33. Castro M, Mathur S, Hargreave F, Boulet L-PP, Xie F, Young J, Wilkins HJ, Henkel T, Nair P: Reslizumab for poorly controlled, eosinophilic asthma: a randomized, placebo-controlled study. *Am. J. Respir. Crit. Care Med.* 2011, 184:1125–32.
 34. Busse WW, Katial R, Gossage D, Sari S, Wang B, Kolbeck R, Coyle AJ, Koike M, Spitalny GL, Kiener PA, et al.: Safety profile, pharmacokinetics, and biologic activity of MEDI-563, an anti-IL-5 receptor alpha antibody, in a phase I study of subjects with mild asthma. *J. Allergy Clin. Immunol.* 2010, 125:1237–1244.e2.
 35. Laviolette M, Gossage DL, Gauvreau G, Leigh R, Olivenstein R, Katial R, Busse WW, Wenzel S, Wu Y, Datta V, et al.: Effects of benralizumab on airway eosinophils in asthmatic patients with sputum eosinophilia. *J. Allergy Clin. Immunol.* 2013, 132:1086–1096.e5.
 36. Castro M, Wenzel SE, Bleecker ER, Pizzichini E, Kuna P, Busse WW, Gossage DL, Ward CK, Wu Y, Wang B, 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.
 37. Brightling CE, Bleecker ER, Panettieri RA, Bafadhel M, She D, Ward CK, Xu X, Birrell C, van der Merwe R: Benralizumab for chronic obstructive pulmonary disease and sputum eosinophilia: a randomised, double-blind, placebo-controlled, phase 2a study. *Lancet Respir Med* 2014, 2:891–901.
 38. Gauvreau G, Boulet L-P, Cockcroft D, Fitzgerald J, Carlsten C, Davis B, Deschesnes F, Duong M, Durn B, Howie K, et al.: Effects of interleukin-13 blockade on allergen-

- induced airway responses in mild atopic asthma. *American journal of respiratory and critical care medicine* 2011, 183:1007–14.
39. Corren J, Lemanske RF, Hanania NA, Korenblat PE, Parsey MV, Arron JR, Harris JM, Scheerens H, Wu LC, Su Z, et al.: Lebrikizumab treatment in adults with asthma. [Internet]. *N. Engl. J. Med.* 2011, 365:1088–98.
40. Piper E, Brightling C, Niven R, Oh C, Faggioni R, Poon K, She D, Kell C, May R, Geba G, et al.: A phase II placebo-controlled study of tralokinumab in moderate-to-severe asthma. *The European Respiratory Journal* 2012, 41.
41. Boever E, Ashman C, Cahn A, Locantore N, Overend P, Pouliquen I, Serone A, Wright T, Jenkins M, Panesar I, et al.: Efficacy and safety of an anti-IL-13 mAb in patients with severe asthma: A randomized trial. *Journal of Allergy and Clinical Immunology* 2014, 133:989996.e4.
42. Corren J, Busse W, Meltzer E, Mansfield L, Bensch G, Fahrenholz J, Wenzel S, Chon Y, Dunn M, Weng H, et al.: A randomized, controlled, phase 2 study of AMG 317, an IL-4Ralpha antagonist, in patients with asthma. *American journal of respiratory and critical care medicine* 2010, 181:788–96.
43. Wenzel S, Ford L, Pearlman D, Spector S, Sher L, Skobieranda F, Wang L, Kirkesseli S, Rocklin R, Bock B, et al.: Dupilumab in Persistent Asthma with Elevated Eosinophil Levels. *The New England Journal of Medicine* 2013, doi:10.1056/NEJMoa1304048.
44. Nair P, Gaga M, Zervas E, Alagha K, Hargreave FE, O’Byrne PM, Stryszak P, Gann L, Sadeh J, Chanez P: 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.

45. Chesné J, Braza F, Mahay G, Brouard S, Aronica M, Magnan A: IL-17 in Severe Asthma: Where Do We Stand? *Am. J. Respir. Crit. Care Med.* 2014, doi:10.1164/rccm.201405-0859PP.
46. Busse WW, Holgate S, Kerwin E, Chon Y, Feng J, Lin J, Lin S-LL: 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.

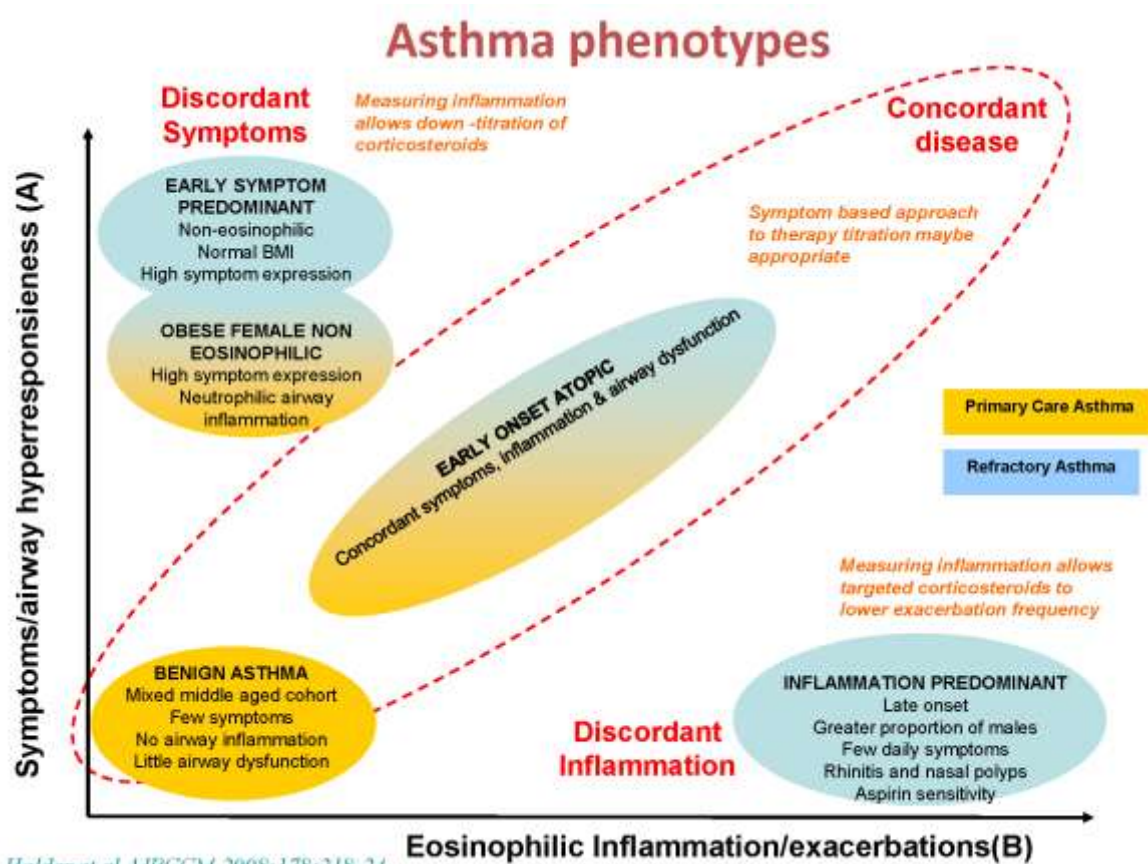


FIG 1. The existence of a variety of disease subtypes has been identified and graphically displayed by using unbiased clustering approaches on large cohorts of asthma patients. Haldar et al. described these by means of eosinophilic inflammation/exacerbation frequency and symptom expression/airway hyperresponsiveness. Notably, there are two subtypes of

disease with discordant symptoms and one with discordant inflammation. These subtypes are missed if symptom expression only is taken into account.

Reprinted with permission of the American Thoracic Society. Copyright © 2014 American Thoracic Society. Haldar P, Pavord ID, Shaw DE, Berry MA, Thomas M, Brightling CE, Wardlaw AJ, Green RH: Cluster analysis and clinical asthma phenotypes. American journal of respiratory and critical care medicine 2008, 178:218–24. Official Journal of the American Thoracic Society.

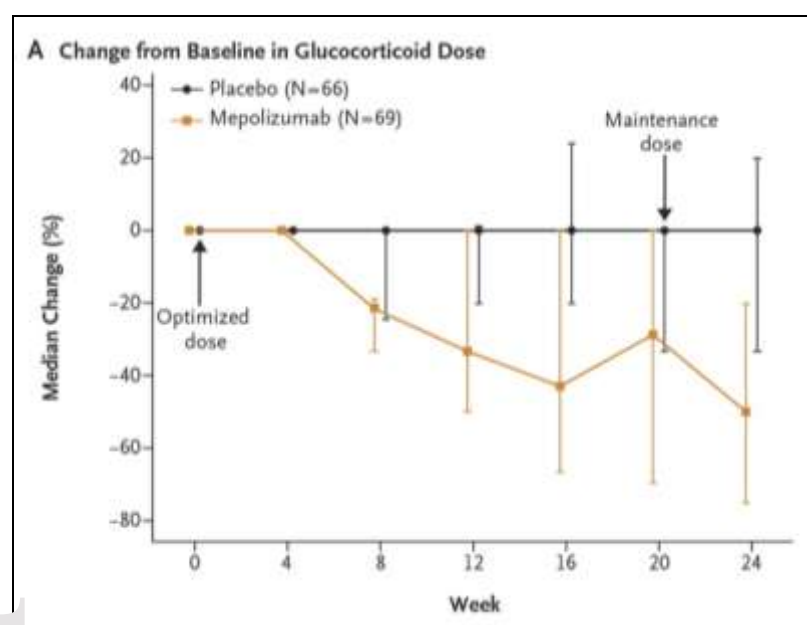


FIG 2. Bel EH et al. N Engl J Med 2014;371:1189-1197. Median percentage reduction from baseline in the daily glucocorticoid dose in the two study groups. At 24 weeks, the median percentage reduction was 50% in the mepolizumab group, and there was no reduction in the placebo group ($P=0.007$). The I bars represent 95% confidence intervals.

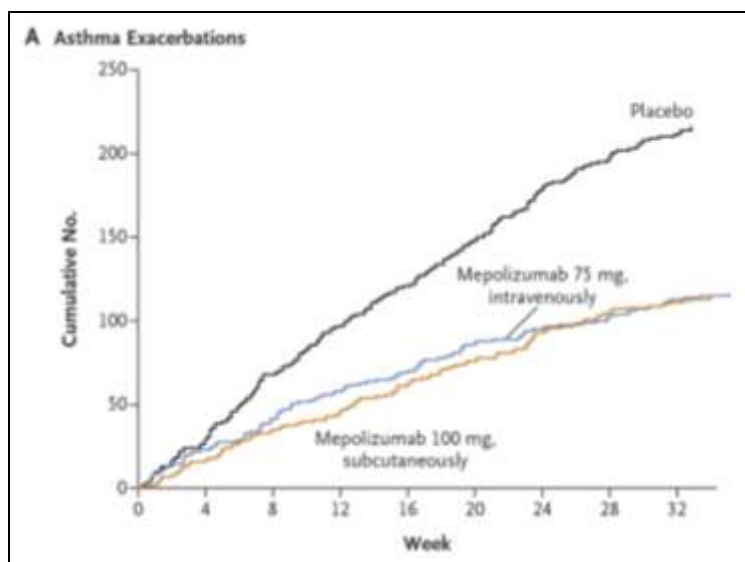


FIG3. Numbers of asthma exacerbations in patients receiving either intravenous or subcutaneous mepolizumab or placebo. The rate of exacerbations was reduced by 47% (95% confidence interval [CI], 29 to 61) among patients receiving intravenous mepolizumab and by 53% (95% CI, 37 to 65) among those receiving subcutaneous mepolizumab, as compared with those receiving placebo ($P < 0.001$ for both comparisons).

Table 1. Biomarkers of eosinophilic inflammation used in RCT's with monoclonal antibodies to preselect patients in adult asthma

Biomarker	Association with treatment response	Invasiveness	Comments
FeNO	Corticosteroids, anti-IL-13, anti-IL-4&13, anti-IgE	Non-invasive	Easy, quick, not specific, cheap, generally available
Serum IgE	Not associated	minimal	Although recommended to measure, there is no clear association between IgE as a biomarker and treatment responses or clinical outcome
Serum Periostin	Anti-IL-13*	minimal	Effect shown with Anti-IL-13, high costs
Blood eosinophil count	Anti-IL-5*, anti-IL4/13 (?)	minimal	Generally available, high clinical impact, predicts anti-IL-5 response. Not a clear predictor in anti-IL4/13 treatment
Sputum eosinophil count	Corticosteroids, Anti-IL-5	moderate	Specialist centers, tissue specific, time-consuming.

*Proven clinical efficacy in combination with this treatment.

Table 2. Effect of anti-inflammatory associated monoclonal antibodies on clinical markers and biomarkers in adult asthma

Mono-clonal antibody	Biomarker used for selection	FEV ₁	AHR	ACQ	Exacerbations	OCS - sparing effect	QLQ	Blood eos.	Sputum eos.	FeNO	Serum IgE	Comments
Effect on clinical endpoints								Effect on biomarkers				
Anti-IL-5	PB eosinophil count, exacerbation rate	0/+	0	0/+	++	++	+	↓↓	↓	0	0	Clinical effects in specific subgroup of severe asthma
Anti-IgE	Blood IgE*, spec. IgE level, sputum eos, SPT*, FeNO, per.	+	0	+	++	unclear	+	Stable or small ↓	↓	↓↓	↓↓	Most RCT's focused on moderate asthma, sparse evidence in severe asthma
Anti-IL-13	Periostin level, FeNO	+	unclear	0/+	unclear	N/A	0	↑	unclear	↓	↓	Partially based on subgroup analysis
Anti-IL-4/IL-13	Periostin and sputum eos, IgE, FeNO	+	Exp.	unclear	unclear	unclear	N/A	Stable or ↑	unclear	↓	↓	Promising combination, one RCT

+ = clinically important; 0 = measured and no effect observed; N/A = not attributable/not measured; unclear = measured, not enough data points for conclusion. Exp., expected; FEV₁, Forced Expiratory Volume in 1sec.; AHR, airway hyperresponsiveness; ACQ, asthma control questionnaire; OCS, oral corticosteroid; ICS, inhaled corticosteroid; QLQ, quality of life questionnaire; eos., eosinophils; FeNO, fraction of exhaled nitric oxide; IgE, Immunoglobulin E; RCT, randomised controlled trial; IL, Interleukin; *Blood IgE does not correlate with treatment response to anti-IgE