

Rationale and Clinical Results of Inhibiting Interleukin-5 for the Treatment of Severe Asthma

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Abstract Severe asthma is responsible for considerable morbidity and a high proportion of the healthcare costs attributable to asthma. Management is not straightforward as the clinical, pathological and physiological features are heterogeneous and the relationships between these features are poorly understood. In recent years significant progress has been made in understanding this heterogeneity and eosinophilic asthma has emerged as a potentially clinically important phenotype because treatment with monoclonal antibodies against IL-5 is effective. This has required a change in our understanding of the role of eosinophilic airway inflammation in airways disease and the developments of reliable biomarkers of eosinophilic airway inflammation. We will review these developments and describe the clinical experience so far with treatment with monoclonal antibodies against IL-5.

Keywords Asthma · Eosinophils · Interleukin-5 · IL-5 · Inhibiting · Inhibition · Mepolizumab · Severe asthma · Treatment

List of Abbreviations

AHR	Airway hyperresponsiveness
BAL	Bronchoalveolar lavage
ECP	Eosinophil cationic protein
EDN	Eosinophil-derived neurotoxin
EPO	Eosinophil peroxidase
FeNO	Exhaled nitric oxide concentration

GM-CSF	Granulocyte-macrophage colony-stimulating factor
IgE	Immunoglobulin E
IL-5	Interleukin-5
MBP	Major basic protein
RCT	Randomised controlled trial
RNA	Ribonucleic acid
ROS	Reactive oxygen species
Th2	Type 2 T helper

Introduction

Asthma is a common chronic inflammatory disease of the bronchial airways affecting about 5–10 % of all adults and children. The disease is well controlled with inhaled therapy [1, 2] in most patients but around 10 % have severe asthma with significantly higher morbidity and mortality [3]. Severe asthma by definition is refractory to conventional therapy; it accounts for more than half of the asthma health and economic impact [4, 5]. Asthma exacerbations, defined as acute or sub-acute episodes of worsening of symptoms and lung function that are beyond normal day-to-day variability and are unresponsive to bronchodilator therapy, are responsible for a significant proportion of this increased disease burden [6]. New asthma therapies are desperately needed to address the huge unmet need of patients with severe asthma.

Inhaled and systemic corticosteroid treatment undoubtedly reduces airway inflammation as well as improving symptoms and lung function [7, 8]. However, corticosteroids are not fully effective in patients with severe asthma [9] and a proportion have evidence of persistent eosinophilic airway inflammation despite long-term use of corticosteroids even when given at doses and regimes that cause significant systemic side effects. Since the introduction of inhaled corticosteroid therapy in the early 1970s and inhaled long-acting β_2 -adrenergic receptor agonists in the 1980s, few new drugs have had significant

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impact on the management of these patients. One exception is omalizumab, a recombinant humanised monoclonal antibody directed against immunoglobulin E (IgE), licensed for the treatment of severe persistent allergic asthma. In these patients omalizumab has been shown to reduce exacerbations and corticosteroid requirements, and improve asthma-related quality of life [10], although in a population of patients who meet the manufacturer's criteria for treatment but are not selected in any other way, the benefits are modest and the cost significant [11]. This new therapeutic approach together with a better understanding of the immunopathological mechanisms underlying the disease has, however, set the scene for the development of other highly targeted biological therapies in the treatment of asthma.

Eosinophilic airway inflammation is an important pathological feature of allergic and eosinophilic asthma. Interleukin (IL)-5 is the key cytokine in eosinophil development and function and has recently been identified as a potential target for novel asthma therapies. In this review we will explore the biological significance of eosinophils and IL-5 in the pathogenesis of asthma and describe recent experience with different IL-5 inhibitors for the treatment of asthma.

Eosinophils and Asthma

Eosinophilic inflammation in asthma has been recognised for more than 120 years, but the role of eosinophils in the pathogenesis remains a matter of controversy and renewed debate [12]. Eosinophil leukocytes are terminally differentiated granulocytic white cells first discovered by Paul Ehrlich in 1879 [13]. They constitute 1–4 % of circulating white cells and are thought to have a natural role in the innate immunity against parasitic helminthic diseases [14]. After release from the bone marrow, eosinophils circulate for a brief period before entering the tissue. In the homeostatic state, tissue eosinophils form >99 % of the total eosinophil number [15]. The association of eosinophils with different allergic diseases, including asthma and anaphylaxis, has been known for a long time; however, the full nature of their contribution to the pathogenesis remains elusive. Eosinophils contain cytoplasmic secondary granules composed of four cytotoxic proteins: major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil peroxidase (EPO) and eosinophil-derived neurotoxin (EDN) [14]. Upon activation, eosinophils release their granules, leading to cellular damage mainly through the action of the aforementioned cytotoxic proteins on the cell membrane and ribonucleic acid (RNA). Activated eosinophils also achieve this cytotoxic function by producing reactive oxygen species (ROS). Furthermore, eosinophils also have an immunomodulatory function by secreting a wide range of cytokines, chemokines, peptide growth factors and lipid mediators. Most of these products are proinflammatory, only produced upon

eosinophil activation, and serve different roles in both type 1 T helper (Th1) and type 2 T helper (Th2) immune responses.

Eosinophilic asthma is characterised by increased eosinophil numbers in peripheral blood, bronchoalveolar lavage and airway tissue [16]. This is due to increased eosinophil production by the bone marrow, increased eosinophil infiltration of the airways and reduced eosinophil apoptosis. Airway eosinophils in asthma are more activated as evidenced by the increased degranulation and elevated levels of extracellular cytotoxic proteins [17]. In the airway tissue eosinophil cytotoxic proteins cause epithelial damage and have a direct spasmogenic effect on airway smooth muscle causing bronchoconstriction [18, 19]. The evidence for the significance of eosinophils in asthma is supported by the association of airway eosinophilia with airway remodelling, airway hyperresponsiveness (AHR), asthma severity and asthma exacerbations. Eosinophils have been shown to contribute to various aspects of airway remodelling, a collective term for all the microscopic structural changes seen in the airways of asthmatics. *In vitro*, ECP has been shown to have a profibrogenic influence on fibroblasts, an important effector cell in airway remodelling [20]. Furthermore, Humbles et al. demonstrated that depleting eosinophils in a murine model of asthma resulted in the prevention of some features of airway remodelling, namely increased extracellular collagen deposition and increased airway smooth muscle [21]. In a group of 20 severe eosinophilic asthmatics, Wenzel et al. showed an association between submucosal eosinophil numbers and thickness of the reticular basement membrane [22]. More importantly, in asthmatics, targeting eosinophils through using the IL-5 inhibitor mepolizumab resulted in reduction of subepithelial fibrosis [23].

Eosinophils have been linked to AHR, a primary characteristic feature of asthma, in animal models and in human asthmatics [24]. Also using an eosinophil-deficient murine model, Lee et al. showed the significance of eosinophils for AHR and mucus accumulation in asthma [25]. This has supported earlier work by Wardlaw et al. who identified a clear correlation between both eosinophil and MBP levels in bronchoalveolar lavage (BAL) and AHR in a group of mild atopic asthmatics [26]. Critically, eosinophilic inflammation was found to correlate with the two most clinically relevant aspects of asthma: clinical severity and exacerbations. Bousquet et al. clearly proved that the levels of eosinophils both in blood and BAL significantly correlate with asthma severity as measured by a scoring system based on symptom and medication use [17]. The involvement of eosinophils in loss of asthma control and asthma exacerbations has been well established. Eosinophilic inflammation increases prior to and during exacerbations and improves at the recovery phase [27]. In longitudinal studies of exacerbations, induced by reducing the dose of inhaled corticosteroids, the sputum eosinophil count, a validated biomarker of airway eosinophilic inflammation, was

found to correlate with loss of lung function and predict loss of asthma control [28, 29]. Indeed, asthma management strategies based on measuring eosinophilic inflammation to guide maintenance corticosteroid therapy were shown to significantly reduce severe exacerbations in a number of randomised trials [30, 31].

All the presented evidence suggests that eosinophils have a detrimental role in the pathogenesis of asthma, although fully establishing the nature of this role is far from being resolved.

Interleukin-5 and Its Role in Eosinophil Biology

Interleukin (IL)-5 is a haematopoietic cytokine produced by a range of leukocytes including CD4⁺ Th2 lymphocytes, eosinophils, basophils, mast cells and natural killer T cells [32]. IL-5 was first described in 1986 and is arguably the most significant cytokine modulating eosinophils [33]. The IL-5 gene is located on chromosome 5q close to a cluster of genes encoding a group cytokines co-expressed by Th2 cells including IL-3, IL-4 and granulocyte-macrophage colony-stimulating factor (GM-CSF) [34]. In humans, the IL-5 receptor (IL-5R) is expressed by both eosinophils and basophils and is composed of two subunits: an α -subunit (IL-5R α) specific for IL-5 and a β c-subunit (IL-5 β c) responsible for signal transduction and shared, hence the suffix c, with the specific/ α receptor subunits of IL-3 and GM-CSF receptors [35].

IL-5 plays an important role in almost all aspects of eosinophil biology in both the homeostatic and disease states [32]. Most of the evidence supporting the biological role of IL-5 was generated by either inhibiting or enhancing IL-5 in animal models and in humans. Modified mice with high IL-5 expression have high levels of bone marrow, blood and tissue eosinophils, while IL-5-deficient ones had lower levels [36]. In vitro, IL-5 has been shown to enhance adhesion of eosinophils to the vascular wall and also increase eosinophil degranulation [37, 38]. van Rensen et al. injected recombinant IL-5 in a group of mild asthmatics, which resulted in marked eosinophilia, although this did not affect AHR [39]. In another study on mild asthmatics, administration of recombinant IL-5 directly to the lungs resulted in increased numbers of activated airway eosinophils and high levels of ECP in the BAL. IL-5 administered through inhalation to a group of asthmatics resulted in a significant increase of AHR and sputum eosinophil count [40]. This shows that IL-5 affects eosinophil chemotaxis, activation and degranulation in asthma. Finally, incubation of human eosinophils with murine IL-5 leads to reduced apoptosis in and prolonged survival [41].

It is clear therefore that IL-5 is crucial in eosinophil haematopoiesis, maturation, migration, activation, function and survival. Due to the limited spectrum of cells that IL-5 acts on, selectively targeting IL-5 has been seen as a logical method for potentially treating conditions in which eosinophils

are thought to play a significant role including asthma, eosinophilic esophagitis, atopic dermatitis, hyper-eosinophilic syndrome and Churg-Strauss syndrome.

IL-5 Inhibition in the Treatment of Asthma

Different methods have been developed and trialled clinically on humans for inhibiting IL-5. These include using antagonists targeting either IL-5 or IL-5R. The early clues for the potential usefulness of inhibiting IL-5 in asthma was seen in animal models of the disease, where administering anti-IL-5, or using animal deficient in IL-5R α , resulted in the reduction of airway eosinophilic inflammation, AHR and amelioration of some features of remodelling [42, 43].

The first IL-5 antagonist used in a randomised controlled trial (RCT) in asthma is mepolizumab. Leckie et al. tested mepolizumab, a humanised monoclonal antibody against IL-5, in a small group of mild atopic asthmatics [44]. The main aim of this study was to assess tolerability of a single administration of the drug and its effect on eosinophils and AHR. Results showed significant reduction in both blood and sputum eosinophil levels in the treatment group but no effect on AHR or on the late asthmatic response. This study cast doubts on the role of eosinophils in asthma and seemed to suggest that IL-5 inhibition had no significant role in asthma therapy. The small number of patients and possible inclusion of non-eosinophilic asthmatics were the obvious major shortcomings of this study. Another RCT done with mepolizumab also on a group of non-selected mild asthmatics, with multiple doses over 20 weeks, showed an eosinophil-reducing effect on airway tissue, bone marrow and blood; however, no effect was seen on AHR or lung function [45]. In a larger study, focused mostly on clinical outcomes, mepolizumab was tested on patients with persistent asthma who were symptomatic despite treatment with moderate dose inhaled corticosteroids. The results were again disappointing as mepolizumab had no effect on most clinical endpoints including patient-reported symptom scores, lung function or rescue medications use. Interestingly, there was a reduction in the exacerbation rate in the treatment group, although not statistically significant [46].

The disappointing effects of these early clinical trials suggested to many that eosinophilic airway inflammation was not directly involved in the pathogenesis of 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 as great as that seen with corticosteroids. A third possibility was that eosinophilic airway inflammation contributes to exacerbations of asthma to a greater degree than day-to-day symptoms and disordered airway function. In keeping with this, studies have shown that exacerbations and symptom control are to some

extent disconnected within patients [47] and that their pharmacological control is different, with symptoms and lung function being more responsive to addition of a long-acting β_2 -agonist and exacerbations to a four-fold increased dose of inhaled corticosteroids [48]. Finally, studies that have examined the clinical effects of a management strategy that controls eosinophilic airway inflammation as well as symptoms have consistently shown that the sole benefit is a reduction in exacerbation numbers [30, 49].

A further key issue with the early clinical trials of anti-IL-5 is that patients were selected on the basis of symptoms and disordered airway function rather than the presence of eosinophilic airway inflammation. 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 [50] and are thus unlikely to respond to a treatment which targets this feature.

These findings provided a strong basis for a more rigorous evaluation of the effects of blocking IL-5 with two important modifications to the clinical trial design: (1) treating patients with evidence of active airway inflammation rather than an arbitrary and unrelated clinical measure such as a significant acute bronchodilator response and (2) assessing the effect of treatment on asthma exacerbations, an outcome measure that is directly and potentially causally related to eosinophilic airway inflammation.

Later Clinical Trial Experience with anti-IL-5 for Severe Asthma

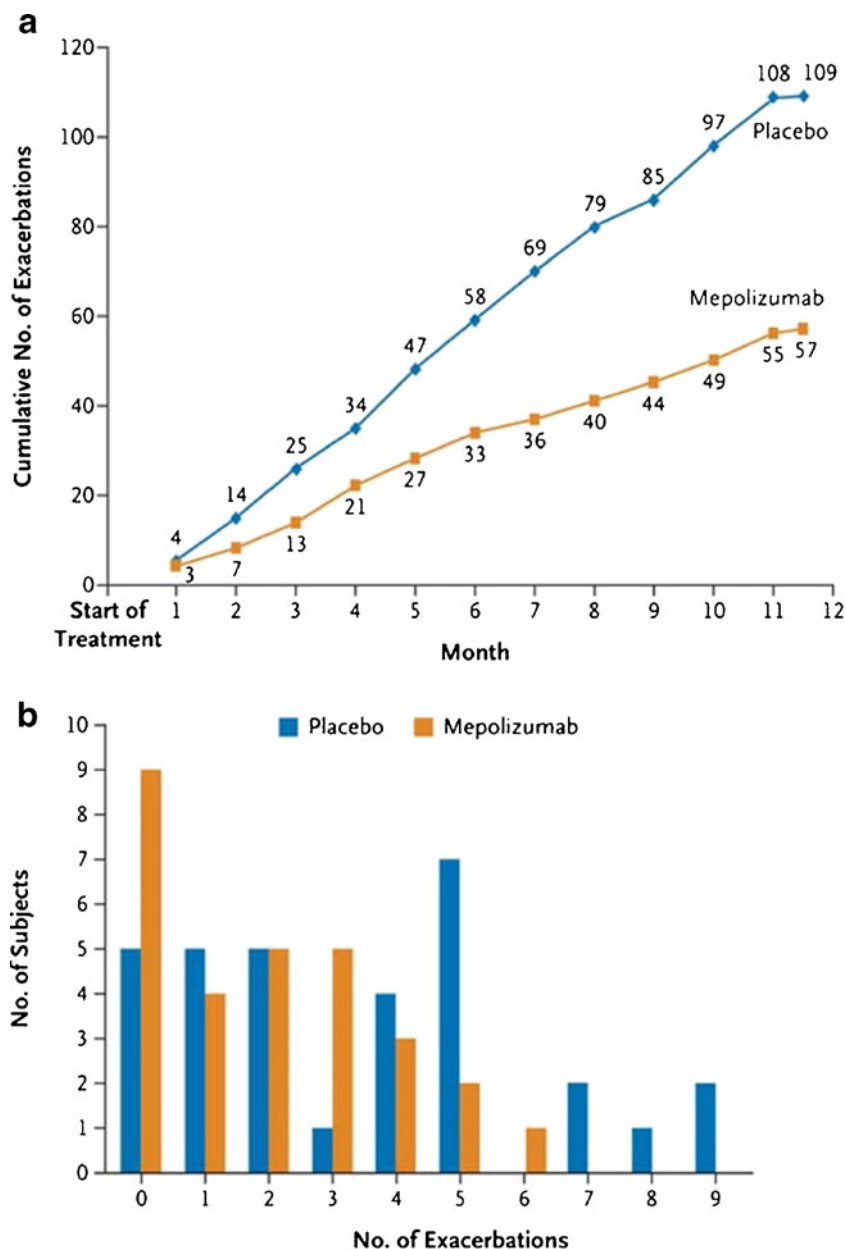
A beneficial effect of mepolizumab on asthma exacerbations was clearly shown in two further studies where the investigators were careful to evaluate the most appropriate outcome measure and minimise the effect of asthma heterogeneity by actively selecting patients with evidence of eosinophilic inflammation. Nair et al. examined the effect of mepolizumab treatment for 26 weeks on asthma exacerbations and steroid dose reduction in a RCT involving 20 subjects with severe eosinophilic asthma who had refractory symptoms despite treatment with oral and inhaled corticosteroid therapy [51]. There was a significant difference in the exacerbation frequency between the two trial arms with only 1 patient out of the 9 in the mepolizumab group having an asthma exacerbation, which was non-eosinophilic, compared to 10 patients out of the 11 in the placebo group. Furthermore, patients on mepolizumab were able to reduce their oral corticosteroid dose by 83 % compared to 47 % with placebo. Actively treated patients had a small improvement in the forced expiratory volume in 1 s (FEV1) and significant improvement in their patient-reported asthma symptoms scores as measured

by the Juniper Asthma Control Questionnaire. Haldar et al. enrolled 61 patients with recurrent exacerbations of asthma and evidence of eosinophilic airway inflammation, defined as a sputum eosinophil count >3 % on at least one occasion in the previous year [52]. The asthma-related quality of life scores were significantly better in the mepolizumab group, although no difference was seen in asthma symptoms, FEV1 or AHR. The main effect of treatment was a 48 % reduction in the number of severe asthma exacerbations requiring treatment with prednisolone (Fig. 1).

The multicentre GlaxoSmithKline-sponsored Dose Ranging Efficacy And safety with Mepolizumab in severe asthma (DREAM) trial was conducted as a phase IIb drug trial to specifically define the optimum dose of mepolizumab and to confirm efficacy and safety in patients with eosinophilic asthma [53•]. The primary endpoint was the frequency of clinically significant exacerbation over the 1-year duration of the treatment. Six hundred twenty-one patients were randomly assigned to either receive placebo or one of three different doses of mepolizumab, 75 mg, 250 mg or 750 mg, in a parallel-group 12-month study. Subjects were required to have evidence of eosinophilic airway inflammation as suggested by one of more of the following documented in the previous year: a sputum eosinophil count >3 %, an exhaled nitric oxide concentration (FeNO) of >50 ppb, an asthma-related peripheral blood eosinophil count of $>0.3 \times 10^9/l$ and/or a prompt deterioration in asthma control after a 25 % reduction in corticosteroid dose. Mepolizumab reduced severe exacerbation numbers by about 50 % in all the treatments groups compared to placebo, with no evidence of a dose-response effect. The blood and sputum eosinophil counts were reduced by treatment and there was evidence of a dose-related effect on sputum eosinophil counts. No improvement in asthma symptoms, quality of life, FeNO or lung function was seen. The drug was shown to be safe and well tolerated. Interestingly, testing the different baseline variables using multivariate methods established that only the degree of blood eosinophilia and the exacerbation frequency in the previous 12 months were associated with the response to the drug. To summarise the trials of mepolizumab in asthma, a meta-analysis on the subject was recently published. It included a total of 1,131 patients and confirmed that, in eosinophilic asthmatics, the drug reduces exacerbations and improves asthma-related quality of life [54•].

Other types of IL-5 inhibitors have been used in clinical trials for asthma. Reslizumab is another monoclonal antibody that neutralises IL-5 and like mepolizumab is administered intravenously. In a recent RCT in patients with poorly controlled eosinophilic asthma, reslizumab was shown to significantly improve FEV1 [55]. Additionally, patients in the reslizumab group had better asthma control, although the difference with the control group was not significant except in patients with concomitant nasal polyposis. Benralizumab is a monoclonal antibody that inhibits IL-5 by targeting IL-5R α

Fig. 1 Panel A shows the cumulative number of severe exacerbations that occurred in each study group over the course of 50 weeks. Panel B shows the distribution of the number of exacerbations among subjects in each study group during the treatment period of the study. The mean number of exacerbations per subject over the course of the 50-week treatment period was 2.0 in the mepolizumab group as compared with 3.4 in the placebo group (relative risk, 0.57; 95 % confidence interval, 0.32 to 0.92; $P=0.02$). (From Haldar et al. [52]. Mepolizumab and exacerbations of refractory eosinophilic asthma. *N Engl J Med* 360: 973–984. Copyright 2009 Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society)



and causing eosinophil cytotoxicity. Published clinical studies of benralizumab in asthma are limited but in a study with mild atopic asthmatics it has been shown to induce profound eosinopaenia [56]. Currently active phase II trials of benralizumab in asthmatics are ongoing [57]. TPI-ASM8 is a novel therapy that has anti-IL-5 action. It is composed of two antisense oligonucleotides that target IL5- β c and the chemokine receptor CCR3, which is expressed by a wide range of leukocytes including eosinophils, basophils, Th1 and Th2 cells [58]. In one study TPI-ASM8 reduced eosinophilic inflammation following allergen challenge in a small group of patients with atopic asthma [59]. The theoretical ability of benralizumab to inhibit eosinophils more profoundly by targeting the common part of the IL-5R, thus blocking IL-5, IL-3 and GM-CSF

signal transduction, and of TPI-ASM8 to inhibit a wider range of inflammatory cells suggests that these drugs might have more immunological impact than monoclonal IL-5 antagonists, although this remains to be proven. Small interfering RNA (siRNA), a class of double-stranded RNA that interferes with specific gene expression, have been used to inhibit IL-5 in animal models, although clinical studies are still awaited [60].

Potential Beneficial Effects of Anti-IL-5 in Other Eosinophilic Conditions

Since eosinophils have a central effector role in the pathogenesis of a range in other disease including atopic dermatitis,

hypereosinophilic syndrome (HES), Churg-Strauss syndrome, chronic obstructive lung disease (COPD), eosinophilic gastrointestinal disorders and eosinophilic nasal polyposis, anti-IL-5 treatment could potentially have a beneficial effect especially in patients with severe forms of these conditions. However, clinical trials of IL-5 inhibitors in eosinophilic diseases other than asthma are scarce. HES is a poorly understood heterogeneous disease characterised by eosinophil infiltration of various organs leading to tissue damage. Anti-IL-5 therapy with mepolizumab has shown promising results in patients with lymphocytic variant HES [61].

COPD is an inflammatory airway disease largely caused by smoking and similar to asthma is characterised by exacerbations that contribute significantly to the morbidity of the disease. Although the predominant inflammatory cellular component is neutrophils, airway eosinophils are elevated in more than a third of patients [62]. This eosinophilic group has more exacerbations and rapid disease progress and responds better to corticosteroid therapy than non-eosinophilic COPD sufferers. IL-5 levels are increased in the sputum of these patients and decreased with corticosteroid treatment [63]. This, together with the evidence from the aforementioned IL-5 inhibitors in eosinophilic asthma, suggests that IL-5 inhibition might have a role in the prevention of exacerbations in patients with eosinophilic COPD. Currently, an RCT using benralizumab in eosinophilic COPD patients is underway primarily to address this question.

Conclusions

Studying IL-5 inhibition in asthma has helped to redefine the role of eosinophils in the pathogenesis of the illness. The disappointing results of the early studies on IL-5 inhibition in asthma mainly resulted from the failure of these studies to select eosinophilic patients and chose an outcome measure that is causally linked to eosinophilic airway inflammation. Even the results of the largest multicentre international RCT on the subject, the DREAM study, might have been affected by this. In a large proportion of the randomised patients, the investigators of the DREAM study used FeNO to identify eosinophilic asthmatics, although FeNO is known to be a less reliable biomarker of eosinophilic inflammation and could result from other conditions not associated with eosinophilic inflammation [64]. IL-5 inhibition has been proven to reduce severe exacerbations in severe eosinophilic asthmatics and may have a steroid-sparing effect although further studies are needed to specifically confirm this. Traditional markers of asthma such as FEV1 and the acute bronchodilator response were largely not related to the efficacy of IL-5 inhibitors. These findings suggest that different definitions, ideas of disease and adoption of biomarkers that are not well known will be necessary in order to fully realise the potential of

anti-IL-5 treatment. The probable high cost of parenteral biological agents used for IL-5 inhibition in asthma will be a major limiting factor for their future use and the case for their cost-effectiveness will need to be made.

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Compliance with Ethics Guidelines

Conflict of Interest Ian D. Pavord has received speaker honoraria for speaking at sponsored meetings from AstraZeneca, Boehringer Ingelheim and GlaxoSmithKline; has served as a consultant for GlaxoSmithKline, AstraZeneca, Novartis, Merck, Boehringer Ingelheim and Aerocrine; has received honoraria for attending advisory panels with Almirall, Aerocrine, AstraZeneca, Boehringer Ingelheim, GlaxoSmithKline, Merck, Sharp & Dohme, Schering-Plough, Novartis, Dey and Napp; has received sponsorship to attend international scientific meetings from Boehringer Ingelheim, GlaxoSmithKline, AstraZeneca and Napp. He is also 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*.

Rachid Berair declares that he has no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with animal subjects performed by any of the authors. With regard to the author's research cited in this paper, all procedures were followed in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2000 and 2008.

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