



# Monoclonal antibodies for chronic refractory asthma and pipeline developments

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**Patients with severe asthma suffer persistent symptoms and/or frequent exacerbations despite high-intensity treatment. Their severe unrelenting symptoms have a huge impact on healthcare resources owing to frequent hospital admissions and requirement for intensive treatments. Consequently, there is an undeniable need for more-effective and safer medications. Expanding knowledge of innate and adaptive immune responses is leading to the development of novel therapies for severe asthma. Herein, we review efficacy and safety data from human clinical trials of monoclonal antibodies that are approved or under investigation for use in asthma. Future drug candidates directed at key targets and the specific role of monoclonal antibodies in distinctively targeted sub-populations of severe asthmatics will be also discussed.**

## Introduction

With well over 300 million asthma sufferers worldwide, asthma is a substantial health burden and expense. Asthma is now considered a heterogeneous inflammatory syndrome of the airways with several clinical phenotypes and inflammatory endotypes, but the pathogenic mechanisms important to these subtypes are incompletely understood [1–3]. Although there are no preventions or cures for asthma at present, the bulk of asthma patients can be easily treated by inhaled corticosteroids and  $\beta_2$ -adrenoceptor agonists. Nevertheless, for some patients asthma continues to be poorly controlled despite high-intensity treatment [4]. This group of patients are at risk of developing steroid-related side-effects [5] and account for ~60% of asthma-related healthcare costs [6–8].

The evidence base for the appropriate assessment and management of asthmatics with severe disease is small and many unanswered questions remain [9]. Many different terms have been used to improve clinical characterisation of patients requiring high-intensity treatment, including 'refractory asthma', 'difficult-to-treat asthma' or 'problematic asthma' [9]. Indeed, improved clinical characterisation of these patients will probably lead to better outcomes, especially in those with treatable co-morbid conditions [10–12]. However, the current therapeutic arsenal for

severe refractory asthma is deficient and development of safer and more-effective therapies remains a top priority.

In establishing a mechanistic rationale for drug development, it is crucial to pinpoint key targets associated with the complex cellular and molecular pathways responsible for the inflammatory/immune phenotype of chronic severe asthma. Induction, maintenance and progression of the inflammatory and remodeling responses of chronic severe asthma are driven by complex interactions of adaptive (i.e. dendritic cells, B cells and activated T cells) and innate immune responses (i.e. macrophages, neutrophils) with structural cells of the airways (i.e. epithelial cells, airway smooth muscle cells, myofibroblasts). These interactions lead to the secretion of preformed and/or newly synthesised mediators, immunoglobulin (Ig) E, cytokines, growth factors and chemokines, which results in distinct asthma phenotypes [13] (Fig. 1). The commonly accepted paradigm that Th2 cells are important in the pathogenesis of asthma [14] has been recently challenged. Emerging roles for chronic asthma have been proposed for other cells including Th1 cells [15] and, more recently, regulatory T cells and Th17 cells and their associated cytokines [16] resulting in a neutrophilic (rather than eosinophilic) or a mixed granulocytic airway infiltrate. Notably, patients with this asthma subtype are refractory to glucocorticoid treatment and bacterial, fungal and viral infections are implicated in the induction and

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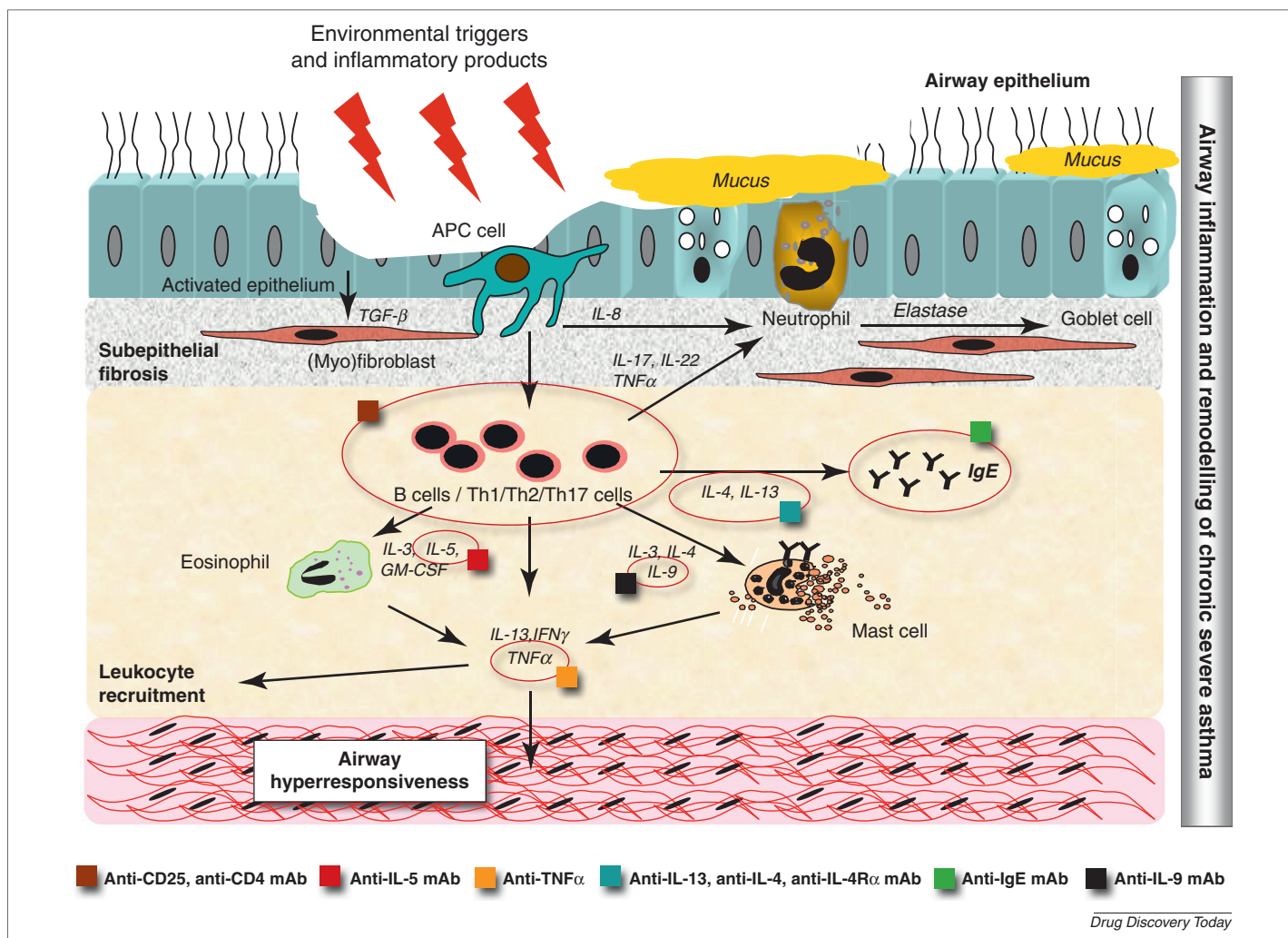


FIGURE 1

Cellular and molecular mechanisms promoting chronic asthma and related mechanism-based pharmacological rationale of mAbs for the treatment of severe asthma. An early step in the initiation of chronic airway inflammation is the activation and maturation of antigen-presenting dendritic cells (APCs) in response to exogenous (environmental triggers, infections) and endogenous (inflammatory products, hormones) triggers. Depending on the offending trigger, mature dendritic cells induce the differentiation of naive CD4<sup>+</sup> T cells into specific T-cell subsets, each producing their characteristic repertoire of cytokines, including IL-4, IL-5, IL-9, IL-13, as well as TNFα. In predisposed individuals, Th2 cell cytokines mediate eosinophil and mast-cell recruitment and activation. Th2-cell–B-cell interaction leads to IgE synthesis and IgE-sensitised mast cells release pro-inflammatory mediators upon allergenic triggering. These events can contribute to induction, maintenance and progression of the inflammatory and remodelling responses of chronic severe asthma. Besides, Th1 and Th17 cells might also be responsible for some of the pathogenic features in patients suffering from chronic forms of asthma, including epithelial apoptosis, smooth-muscle-cell activation, inflammatory neutrophilic response and mucous hypersecretion. On the basis of this model, various molecular targets can be investigated for biologics approaches in severe asthma (circles). Agents that inhibit IL-4/IL-13 (blue square), IL-5 (red square), TNFα (yellow square), IL-9 (black square) and IgE (green square) have been proposed and studied for their potential use. Furthermore, suppression of T-cell responses with a mAb directed against CD25 or CD4 (brown square) have been investigated.

progression of disease [17]. This expanding knowledge base is currently being translated into the development of a growing number of biologic agents that can interfere with several aspects of inflammatory signalling in chronic asthma (Fig. 1).

Monoclonal antibodies (mAbs) represent a form of immunotherapy using passive immunity where preformed antibodies against a target antigen are injected into the body. Because of their specificity, mAbs can efficiently target an antigen on a cell of interest or in the serum and block the binding of cytokines, immunoglobulins, hormones or proteins that promote certain unwanted functions including inflammatory and immune responses. Moreover, the advent of recombinant DNA technology has enabled the production of safer and more-effective mAbs [18]. The spectrum of disease states in which mAbs have been approved for therapeutic use includes

rheumatologic, dermatologic, autoimmune, oncologic, haematological, cardiovascular and gastrointestinal conditions [19]. In 2003, the FDA approved omalizumab, a recombinant humanised mAb that selectively binds to circulating human IgE, for use in severe asthma (available at: <http://www.fda.gov/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplications/TherapeuticBiologicApplications/ucm093373.htm>). Since then, a number of new biologic agents that interfere with several aspects of the inflammatory cascade in chronic asthma have entered clinical development.

We will provide an up-to-date overview of the currently available monoclonal antibodies that are approved or in development for use in asthma (Table 1). Many of the articles quoted do not involve patients with severe asthma because early studies often

TABLE 1

## Overview of monoclonal antibodies approved or in development for use in severe asthma

Drug type	Product name (company)	Drug type	Active ingredient	Dosage	Trial phase	Description
Anti-IgE mAb	Xolair <sup>®</sup> (Novartis)	Anti-IgE mAb	Omalizumab	150–375 mg (subcutaneously)	Approved	Reduces asthma exacerbations, improves symptom control and reduces need of ICS and $\beta$ -agonists in uncontrolled severe allergic asthma. In 2003, the FDA authorized omalizumab for use in asthma [27,28].
Anti-TNF $\alpha$ mAb	Remicade <sup>®</sup> (Centecor)	Anti-TNF $\alpha$ mAb	Infliximab	5.0 mg/kg (intravenous infusion)	Phase II	Some improvement in PEF diurnal variation and reduced exacerbations in moderate asthma. Efficacy/safety of infliximab not yet studied in chronic severe asthma [35].
Anti-TNF $\alpha$ mAb	Simponi <sup>®</sup> ; CNT0148 (Centecor)	Anti-TNF $\alpha$ mAb	Golimumab	50, 100 and 200 mg (subcutaneously)	Phase III	No significant improvement in any of the efficacy measurements and the trial was terminated early owing to a large number of adverse events. <i>Post hoc</i> analysis indicated less-severe asthma exacerbations in patients with a documented reversibility of FEV <sub>1</sub> with ICS and a history of sinusitis [36].
Anti-IL-5 mAb	Bosatria <sup>®</sup> (GlaxoSmithKline)	Anti-IL-5 mAb	Mepolizumab	750 mg (intravenous infusion)	Phase II	Some improvement in FEV <sub>1</sub> and asthma control together with clinically important dose reduction in prednisone use and reduced exacerbations in the eosinophilic refractory asthma subphenotype. No reported serious adverse events [48,49].
Anti-IL-4 mAb	SB240683 (PDL BioPharma)	Anti-IL-4 mAb	Pascalizumab	10 mg/kg (intravenous infusion)	Phase II	Disappointing results in asthma clinical trials. No further clinical development [57].
Anti-IL-13 mAb	IMA638 (Wyeth/Pfizer)	Anti-IL-13 mAb	Anrukizumab	200, 400 and 600 mg (subcutaneously)	Phase II	Encouraging efficacy and safety findings from early RCTs in mild atopic asthma, but not in persistent asthma, and its development was subsequently terminated. However, IMA026, another anti-IL-13 mAb, is currently investigated in persistent asthma [58,59].
Anti-IL-13 mAb	QAX576 (Novartis)	Anti-IL-13 mAb	–	1, 3 and 10 mg/kg (intravenous infusion)	Phase I/II	A study in moderate-to-severe atopic asthmatics was withdrawn owing to complexity of design and lack of enrolment. However, this molecule is still under development for asthma (available at: <a href="http://www.clinicaltrial.gov/ct2/show/NCT00598104">http://www.clinicaltrial.gov/ct2/show/NCT00598104</a> ).
Anti-IL-13 mAb	CAT354 (MedImmune)	Anti-IL-13 mAb	Tralokinumab	1, 5 and 10 mg/kg (intravenous infusion)	Phase I/II	Acceptable safety and tolerability profile in subjects with moderate asthma. A Phase II clinical trial has been completed, although the results have not yet been published [61] (available at: <a href="http://clinicaltrials.gov/ct2/show/NCT00873860">http://clinicaltrials.gov/ct2/show/NCT00873860</a> ).
Anti-IL-13 mAb	MILR1444A (Genentech)	Anti-IL-13 mAb	Lebrikizumab	250 mg (subcutaneously)	Phase II/III	Improved FEV <sub>1</sub> (but not symptoms) in moderate-to-severe asthmatics inadequately controlled on ICS. FeNO and periostin were predictive of the response. Currently undergoing Phase III clinical trials [62].
Anti-IL-4R $\alpha$ mAb	AMG317 (Amgen)	Anti-IL-4R $\alpha$ mAb	–	75, 150 and 300 mg (subcutaneously)	Phase II	No improvements in the control of asthma, $\beta$ -agonist use or lung function in patients with moderate-to-severe asthma. However, AMG317 did suppress exacerbations, particularly in patients on the higher dose of AMG317 (300 mg) and with higher asthma symptom scores [66].
Anti-IL-9 mAb	MEDI528 (MedImmune)	Anti-IL-9 mAb	–	30, 100 and 300 mg (subcutaneously)	Phase II	In a pilot study MEDI528 improved exercise-induced bronchospasm. Currently it is undergoing a Phase II clinical trial in adults with uncontrolled asthma (available at: <a href="http://clinicaltrials.gov/ct2/show/NCT00968669">http://clinicaltrials.gov/ct2/show/NCT00968669</a> ) [69].
Anti-CD4 mAb	IDECC9.1 (IDEC Pharma)	Anti-CD4 mAb	Keliximab	0.5, 1.5 and 3.0 mg/kg (intravenous infusion)	Phase II	Keliximab caused improvements in PEF and symptom scores, but failed to reach statistical significance in patients with corticosteroid refractory asthma. Furthermore, there is a concern of possible adverse consequences of such an approach [73].
Anti-IL2R $\alpha$ mAb	Zenapax <sup>®</sup> (PDL BioPharma)	Anti-IL2R $\alpha$ mAb	Daclizumab	1.0 mg/kg (intravenous infusion)	Phase II	In moderate-to-severe asthma, i.v. daclizumab caused small significant improvements in FEV <sub>1</sub> , asthma symptoms, and $\beta_2$ -agonist use and increased time to exacerbation. Findings were more impressive in patients with more-significant disease. There are no planned studies in asthma with daclizumab [75].

focus on proof-of-concept for a disease and not necessarily the intended ultimate population. Because of the cost involved in using mAbs and the potential risks for these agents, it is most appropriate that mAb use is restricted to patients unresponsive to traditional pharmacotherapy and/or those patients suffering undesirable side effects from pharmacotherapy.

### Anti-IgE strategies

The sentinel role of IgE in increasing allergen uptake by dendritic cells and activating mast cells and basophils for mediator release is central in the pathogenesis of allergic inflammation [20]. Consistent with this notion, IgG antibodies that are specific for the C3 domain of IgE and block IgE binding to FcεRI (and FcεRII, CD23) were shown to inhibit allergen-induced inflammatory responses [21].

However, there is now emerging evidence for a non-specific role of IgE in persistent forms of airway inflammation. Direct stimulation of T cells by superantigens – mainly of bacterial origin – is known to lead to local polyclonal IgE responses and cytokine release promoting and sustaining inflammation [22]. Thus, mAbs directed against IgE might inhibit not only allergic inflammation in asthma, rhinitis and atopic dermatitis but could also have a role in non-allergic persistent forms of inflammation as recently reported for other diseases (such as autoimmune chronic urticaria) [23,24].

Omalizumab (Xolair<sup>®</sup>) is a humanised IgG1κ non-anaphylactoid mAb that forms soluble immune complexes with free IgE at the same site (Cε3) that normally binds the high affinity IgE receptor, FcεRI, thus preventing FcεRI cross-linking and subsequent basophil and mast-cell activation [25,26]. Omalizumab can reduce serum-free IgE levels by 99% within two hours of administration, induce downregulation of FcεRI on basophils, dendritic cells and monocytes within seven days, and decreases serum, tissue and sputum eosinophilia.

Key criteria for omalizumab use include documented evidence of failure to achieve adequate control on medium- to high-dose inhaled corticosteroids (ICS) in conjunction with a long-acting β-agonist and/or frequent exacerbations, serum total IgE between 30 and 700 IU/ml and evidence of specific perennial allergen sensitivity (available at: <http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/Pulmonary-AllergyDrugsAdvisoryCommittee/UCM190449.pdf>).

Omalizumab has been shown to reduce asthma exacerbations, improve symptom control and reduce the need for ICS and β-agonists in Phase II and III trials of severe atopic asthmatics with symptoms despite being on maximal treatment [27]. The positive performance of omalizumab in early registrative trials has been replicated in several studies and now also in children 6–12 years of age [28]. Recent expert panel guidelines recommend considering omalizumab as an alternative or in addition to oral corticosteroids in step V and VI patients with severe allergic asthma [29]. However, there is little published data on the ability of omalizumab to have a significant systemic steroid-sparing effect. The recent uncontrolled clinical trial by Domingo and colleagues [30] reported a substantial, safe decrease in oral corticosteroid requirements after omalizumab therapy.

Published clinical trials with omalizumab indicate that the response to the drug is variable and no reliable predictors of

response have been identified. Although improvements correlate with IgE reduction, free IgE levels in non-responders are similar to those found in responders. A decrease in high-affinity IgE receptors and IgE bound to them are probably important to achieve a clinical response. Possible explanations for omalizumab non-responsiveness include a relative lack of correlation between free IgE levels and FcεRI expression, difference in intrinsic cellular sensitivity and the importance of the ratio of antigen-specific IgE to total IgE [31]. Injection site reactions are the most common adverse events observed among patients receiving omalizumab, occurring at a rate of ~45% [26]. Upper respiratory tract infections, sinusitis and headache have also been reported. Because omalizumab cannot bind to the mast-cell-bound IgE, it is unlikely to trigger mast cell degranulation and subsequent anaphylactic reactions. The frequency of anaphylaxis attributed to omalizumab has been estimated to be ~0.09%. Clinical data do not suggest a causal link between omalizumab and cancer.

More-potent anti-IgE antibodies that might have a broader spectrum of effects are in development. RG7449 is a novel, humanised mAb that binds to the M1 prime segment of membrane IgE and targets B lymphocytes before they produce IgE, rather than neutralising existing free IgE. Phase I/II clinical trials in asthma with RG7449 are being conducted by Genentech (available at: <http://www.gene.com/gene/pipeline/status/immunology/anti-m1/index.html>).

*In vitro* studies have demonstrated that cross-linking the low affinity IgE receptor (FcεRII, CD23) downregulates IgE synthesis. The primatized IgG1 anti-CD23 mAb, lumiliximab (IDEC152) is well tolerated and reduces IgE concentrations in patients with mild asthma, but its clinical efficacy has not been reported [32]. Currently, no additional clinical trials have been planned for lumiliximab in asthma.

### Monoclonal antibodies against tumour necrosis factor alpha (TNFα)

TNFα is a multifunctional pro-inflammatory cytokine produced by Th1 cells and macrophages. Although the role of TNFα in the pathogenesis of rheumatoid arthritis, Crohn's disease and psoriasis is well established, there is now a significant body of evidence implicating Th1 cells and TNFα in the pathogenesis of chronic inflammatory disorders of the airways, including severe asthma and chronic obstructive pulmonary disease (COPD) [33,34]. TNFα mediates the recruitment of neutrophils and eosinophils into the airways, uniquely suppresses glucocorticoid responsiveness in monocytes, promotes airway hyperresponsiveness and upregulates the pathways involved in chronic airway remodelling and subepithelial fibrosis. Thus, strategies targeting the effects of TNFα were studied in severe asthma patients.

Infliximab improved some lung function measures (diurnal variation in peak expiratory flow) and reduced exacerbations in moderate asthmatics [35], but the efficacy and safety of infliximab (and adalimumab) have never been studied in chronic severe asthma. In 309 patients with severe persistent asthma randomised to receive either placebo or three increasing doses (50 mg, 100 mg and 200 mg) of the humanised mAb golimumab, no significant improvement in each of the efficacy measurements was reported and the trial was terminated early owing to a large number of



adverse events in the golimumab groups [36]. Of note, *post hoc* analysis indicated that patients on golimumab (100 mg and 200 mg) with a  $\geq 12\%$  pre-study reversibility of FEV<sub>1</sub> with ICS and a history of sinusitis were less likely to experience severe asthma exacerbations. Improvements in subjective and objective asthma measures were observed after subcutaneous injection of etanercept (a soluble fusion protein that blocks TNF $\alpha$ ), but airway eosinophil or neutrophil numbers were not altered [37–39]. Clinical efficacy closely correlated with TNF $\alpha$  mRNA expression and receptor expression on circulating monocytes, but a concern was raised about safety [38]. A recent large, randomised, controlled, multicenter study assessing the efficacy and safety of etanercept (25 mg twice weekly) in patients with moderate-to-severe persistent asthma showed no improvements in any asthma measures [40]. However, there were also no reported safety concerns. In view of the conflicting efficacy findings and potential safety problems of the published studies no further trials with anti-TNF $\alpha$  agents for severe asthma have been planned.

### Monoclonal antibodies against interleukin (IL)-5

Infiltration and degranulation of leukocyte eosinophils in bronchial airways have been implicated in the pathogenesis of allergic asthma [41]. By releasing lipid mediators, cytokines, cytotoxins, platelet-activating factor (PAF) and pro-fibrogenic factors, such as transforming growth factor (TGF)- $\alpha$ , TGF- $\beta$  and platelet-derived growth factor (PDGF), that induce mucus hypersecretion, remodelling and airway hyperresponsiveness (AHR) [42,43], eosinophils could also have a role in more-persistent and/or severe forms of asthmatic inflammation. IL-5 is crucial for the differentiation and maturation of eosinophils in the bone marrow, and for eosinophil mobilisation, activation and survival [44]. Hence, antagonising IL-5 with mAbs might be a beneficial therapy for asthma, and particularly for eosinophilic predominant asthma. A number of anti-IL-5 mAbs are in clinical development for allergic diseases, including mepolizumab, reslizumab and benralizumab (MEDI563).

Early clinical trials of mild-to-moderate asthmatic subjects showed consistent substantial reductions in sputum and blood eosinophilia, but failed to demonstrate significant clinical or functional improvement [45–47]. There are several potential reasons for the lack of efficacy, but one of the reasons is that patients were selected on clinical and physiological parameters rather than eosinophilic inflammation. Recently, two small, randomised, controlled trials have reassessed the role of the eosinophil-IL-5 pathway in more-severe forms of asthma by testing mepolizumab (Bosatria<sup>®</sup>) on asthma exacerbations in subgroups of patients with persistent airway eosinophilia despite systemic corticosteroid therapy [48,49]. In the study by Nair and colleagues [48], 20 patients with prednisone-dependent asthma and persistent sputum eosinophilia received intravenous infusions of either mepolizumab or placebo on a monthly basis. At follow-up, the authors reported a significant reduction in asthma exacerbations, clinically important dose reduction in prednisone use, small improvements in FEV<sub>1</sub> and asthma control, and, as expected, reductions in blood and sputum eosinophil counts in the mepolizumab arm. The clinically important prednisone-sparing effect without the development of asthma exacerbations or deterioration of other physiological and clinical factors is perhaps the most important

finding. The size of the effect observed with mepolizumab is greater than those reported in many of the trials of immunomodulatory therapies [50].

In the study by Haldar and colleagues [49], patients with refractory eosinophilic asthma and recurrent severe exacerbations of their disease were recruited to receive intravenous (i.v.) mepolizumab ( $n = 29$ ) or placebo ( $n = 32$ ) on a monthly basis for one year. These authors reported a significant mean 12-month reduction in asthma exacerbations and quality-of-life scores after mepolizumab. They also showed a significant reduction in blood and sputum eosinophil counts in the patients receiving mepolizumab. No serious risks or adverse events associated with the administration of mepolizumab were reported in either study. However, it is important to recognise that few patients with severe asthma met inclusion criteria so, although positive effects were obtained, the general applicability and commercial use of anti-IL-5 strategies needs to be established. Screening patients for sputum eosinophilia is not practical in general and most specialty practices. Besides, even in patients with eosinophilic asthma, mepolizumab had no effect on other physiological and clinical factors. In these two trials, the only outcome that consistently improved was the exacerbation rate. The observed reduction in the number of severe exacerbations was similar to that reported with omalizumab in unselected patients with severe persistent asthma [26].

A Phase II clinical trial of the humanised IgG2 anti-IL-5 mAb by Ception Therapeutics, reslizumab (CINQUIL<sup>™</sup> or SCH55700), in subjects with eosinophilic asthma was recently completed, but no results have been reported yet. Benralizumab (MEDI563) is a humanised anti-IL-5R $\alpha$  IgG1 mAb that induces apoptosis of eosinophils and basophils by antibody-dependent cell-mediated cytotoxicity. A Phase I clinical trial of intravenous benralizumab demonstrated acceptable safety and tolerability [51], and Phase II trials in asthma are currently underway.

Antagonising IL-5 with mAbs might be beneficial only in the rare subgroups of patients with refractory asthma and evidence of hypereosinophilic airway inflammation, but not in severe asthma in general. Given that clinically important steroid tapering is highly desirable in this population, larger studies should also establish the steroid-sparing effect of mepolizumab in unselected patients with severe refractory asthma. A double-blind, placebo-controlled, dose-ranging efficacy and safety study with mepolizumab (75 mg, 250 mg and 750 mg by i.v. administration) administered every four weeks over a 52-week treatment period in patients with severe asthma is currently ongoing (available at: <http://clinicaltrials.gov/ct2/show/NCT01000506?term=mepolizumab+AND+asthma&rank=1>).

### Monoclonal antibodies targeting the IL-4/IL-13 pathway

IL-4 and IL-13 are produced by Th2 cells, activated mast cells, basophils, eosinophils and dendritic cells. They not only have a crucial role in allergic diseases by promoting IgE production, differentiation of naive T lymphocytes into Th2 cells (IL-4 only), growth and development of mast cells, eosinophil recruitment and AHR but also in the more chronic form of the disease [52]. IL-4 and IL-13 induce their effects by signalling through the IL-4R $\alpha$ /IL-13R $\alpha$ 1 complex [53,54]. However, it must be noted that IL-4

signalling is still possible through a heterodimeric complex formed by IL-4R and gamma-c. Biologic compounds targeting the IL-4/IL-13 signalling pathway have been developed as a new therapeutic modality for patients with uncontrolled severe asthma.

The first trial of IL-4 antagonism in asthma employed a soluble recombinant human IL-4R $\alpha$  (altrakincept), designed to block the interaction between IL-4 and its naturally occurring cellular receptors. Altrakincept given by inhalation has been trialled for efficacy in adults with moderate persistent asthma after corticosteroid withdrawal. The drug prevented increases in asthma symptoms and reductions in FEV<sub>1</sub> when inhaled corticosteroids were withdrawn [55]. No significant adverse events or development of anti-IL-4R antibodies were detected. However, a larger subsequent Phase II study of altrakincept in subjects with moderate persistent asthma failed to show substantial effects on pulmonary functions, asthma symptoms and asthma exacerbations [56]. This molecule is no longer under development for asthma. Clinical trials of pascolizumab (SB240683), a fully humanised anti-IL-4 mAb, and a similar mAb made by PDL BioPharma, had disappointing results in asthma clinical trials [57] and neither is being developed further.

A number of human IL-13-neutralising antibodies, including anrukinzumab, IMA026, QAX576, CAT354 and MILR1444A have entered Phase I/II clinical trials of asthma. Data from a Phase IIa double-blind, placebo-controlled, antigen challenge study of anrukinzumab (IMA638) have been reported in patients with mild, atopic asthma [58]. The drug reduced the early and late asthmatic responses by 46% and 49%, respectively, with no safety concerns. A larger Phase II, randomised, double-blind, placebo-controlled, parallel-arm study was completed in patients with persistent asthma. Results showed that anrukinzumab did not meet the clinical efficacy endpoint. Recently, 56 subjects with mild, atopic asthma were enrolled for two double-blind, randomised, placebo-controlled, parallel group trials by Wyeth/Pfizer to compare IMA638 and IMA026 IL-13 antibody treatments with placebo treatment on early- and late-phase allergen challenge responses. IMA638, but not IMA026, inhibited the early and late asthmatic responses [59]. There was no effect by either antibody on allergen-induced airway hyperresponsiveness or sputum eosinophils [60]. The effect of the anti-IL-13 mAb QAX576 (Novartis) was studied on inflammatory responses following nasal allergen challenges in patients with seasonal allergic rhinitis [60]. No apparent effects were detected in the QAX576 group on nasal lavage eosinophil levels or nasal symptom scores compared to placebo. A Phase II study of QAX576 in moderate-to-severe atopic asthmatics was withdrawn owing to complexity of design and lack of enrolment (available at: <http://www.clinicaltrial.gov/ct2/show/NCT00598104>). However, this molecule is still under development for asthma.

Phase I studies with the humanised anti-IL-13 mAb tralokinumab (CAT354) in subjects with moderate asthma have demonstrated that the drug administered intravenously has an acceptable safety and tolerability profile, with good bioavailability and linear pharmacokinetics [61]. A Phase II clinical trial has been completed, although the results have not yet been published (available at: <http://clinicaltrials.gov/ct2/show/NCT00873860>). Lebrikizumab (MILR1444A) is a humanised anti-IL-13 mAb, currently undergoing

Phase II clinical trials in inadequately controlled asthmatics. Corren *et al.* [62] reported an improved FEV<sub>1</sub> (but not symptoms) in patients with moderate-to-severe asthma, including those on long-acting  $\beta$ -agonists. Dividing the subjects into those with higher blood eosinophils and/or IgE did not improve the identification of responders. However, when a median split of serum periostin levels was used to divide the population those in the upper 50th percentile had a much more robust response to treatment than those in the lower half. Periostin was identified as a molecule highly expressed by airway epithelial cells (and probably other cells) in response to IL-13 and therefore was suggested to be a serum Th2 biomarker. Interestingly, FeNO was similarly predictive of response and, in fact, levels decreased with anti-IL-13 therapy. These data suggest the possibility of biomarker-driven therapy that might lead to better response rates.

Given the redundancy between IL-4 and IL-13, suppressing the activity of both would be more advantageous. Using this concept, bioengineered versions (muteins) of IL-4 have been developed, which potently inhibit the binding of IL-4 and IL-13 to the shared IL-4R $\alpha$ /IL-13R $\alpha$ 1 complex [63]. A recombinant variant of human IL-4, pitrakinra (AER 001), has entered Phase II clinical trials for the treatment of asthma. Encouraging preliminary proof-of-concept data from a Phase IIa trial have been reported from an antigen challenge study wherein a dry powder formulation of pitrakinra (60 mg, delivered via inhalation) reduced the severity of late asthmatic responses by 72% with no safety concerns [64]. A randomised controlled trial in patients with severe, uncontrolled asthma has been completed, and showed good responses only in those subjects with high levels of blood eosinophils (available at: <http://clinicaltrials.gov/ct2/show/NCT00801853?term=Aerovant+AND+asthma&rank=1>).

AIR645, a dual inhibitor of IL-4 and IL-13, is a second-generation antisense drug targeting the mRNA encoding the IL-4R $\alpha$  subunit. Results from a Phase I study evaluating the safety, tolerability and pharmacokinetics of once-weekly treatment with inhaled AIR645 in healthy volunteers demonstrated low systemic drug absorption and no serious adverse events [65]. AMG317 is a fully human mAb currently under investigation for the ability to bind with high affinity to IL-4R $\alpha$ , which functionally blocks the action of IL-4 and IL-13. A large Phase II, double-blind, placebo-controlled, multiple-dose clinical trial has been conducted recently to determine the safety and efficacy of subcutaneous AMG317 (75 mg, 150 mg or 300 mg) in patients with moderate-to-severe asthma [66]. Treatment did not have clinical efficacy with no improvements in the control of asthma,  $\beta$ -agonist use or lung function reported. However, AMG317 did suppress exacerbations, particularly in patients on the higher dose of AMG317 (300 mg) and with higher asthma symptom scores. It remains to be determined if alternative dosing strategies and/or targeting specific subgroups of asthma patients might lead to better results with AMG317.

### Monoclonal antibodies against IL-9

IL-9 was originally described as a Th2 cytokine promoting mast-cell proliferation, mastocytosis and T-cell growth [67]. In mouse models, IL-9 could also reproduce several features of chronic asthma by eliciting smooth-muscle-cell hyperplasia, eosinophilic airway inflammation, mucus production and AHR [68].

MEDI528 by Medimmune is a humanised IgG1 anti-IL-9 mAb currently undergoing a 24-week Phase IIb clinical trial of biweekly subcutaneous MEDI528 or placebo in adults with uncontrolled asthma (available at: <http://clinicaltrials.gov/ct2/show/NCT00968669>). A pilot study reported that MEDI528 could improve exercise-induced bronchospasm by reducing the maximum mean decrease in FEV<sub>1</sub> after exercise [69]. However, a recently completed study did not meet its endpoints and further development of this molecule for asthma has been stopped.

### Monoclonal antibodies against OX40 ligand

OX40 ligand (OX40L) is expressed on antigen-presenting cells such as macrophages, dendritic cells and endothelial cells, and also B and T lymphocytes. In the absence of IL-12, dendritic cells activated by thymic stromal lymphopoietin (TSLP) upregulate OX40L, which binds to OX40 and leads to Th2 cell differentiation and expansion of Th2 memory cells [70]. OX40L-deficient transgenic mice had attenuated asthmatic responses to allergen challenge compared with wild-type, and administration of an OX40L antibody to wild-type mice during sensitisation prevented asthmatic responses [71].

R0498991 (Roche Pharmaceuticals) is a humanised anti-OX40L mAb undergoing a Phase II clinical trial to assess safety of a subcutaneous formulation in subjects with allergic rhinitis. Efficacy of an intravenous formulation to attenuate allergen-induced responses in mild asthmatics is also under study (available at: <http://clinicaltrials.gov/ct2/show/NCT00983658>).

### Monoclonal antibodies against T cells

In view of the important role of CD4<sup>+</sup> T cells in the pathogenesis of chronic severe asthma, it is pertinent to consider intervention approaches targeted at these cells in human asthma.

The non-specific reduction in the number of circulating T cells achieved by keliximab, a chimeric mAb directed against the CD4 receptor [72], was postulated to have a beneficial effect in patients with severe corticosteroid refractory asthma. Keliximab caused a significant increase in morning and evening peak expiratory flow compared with placebo, and these changes were accompanied by a decrease in symptom scores, but these effects did not reach statistical significance in patients with corticosteroid refractory asthma [73]. Furthermore, there is a concern regarding possible adverse consequences of such an approach, because of the risk of immunodepression and of severe bacterial, fungal and viral infections.

The cell surface marker CD25, the alpha-chain of the IL-2 receptor, is widely reported as a marker of CD4<sup>+</sup> T-cell activation. Increased expression of CD25<sup>+</sup> T cells and elevated levels of soluble CD25 have been reported in the airways of patients with severe asthma [74]. With this in mind, Busse *et al.* [75] conducted a study to evaluate the efficacy and safety of a humanised mAb, daclizumab, directed against CD25 in patients with moderate-to-severe asthma. A total of 115 patients on medium-to-high dose ICS were switched to an equivalent dose of inhaled triamcinolone and then randomised to receive infusions of daclizumab ( $n = 88$ ) or placebo ( $n = 27$ ) every two weeks for 12 weeks. The protocol design included an ICS-tapering phase of eight weeks while on the study drug, and a follow-up phase of 16 weeks off the study drug. These authors reported small significant improvements in

FEV<sub>1</sub>, daytime asthma symptoms and short-acting  $\beta_2$ -agonist use and increased time to exacerbation in the daclizumab study group. Although there were no differences in mild and moderate adverse events between the two study groups (upper respiratory tract infection, nasopharyngitis, nasal congestion, rash and nausea), there were more patients with serious adverse events in the daclizumab group including anaphylactoid reactions and viral meningitis. In an analysis of a subpopulation with more-significant disease, findings were more impressive. This small study demonstrated that daclizumab could have a role in asthma. Currently, however, there are no planned studies in asthma with daclizumab.

### Concluding remarks

Although the understanding of asthma has improved considerably over the past 10–20 years, this has not translated into much advancement in the care of severe asthma patients. In fact, omalizumab is the only current monoclonal antibody available for the treatment of severe asthma, and it is restricted in its use and is not uniformly effective.

Owing to the complex nature of asthma, with various phenotypes and pathological mechanisms, and the fact that mAbs blocking the action of individual biological pathways might not be enough to suppress inflammation and control remodelling efficiently, it is not surprising that the development of new treatments is fraught with difficulties. Indeed, these highly specific therapies could be effective in some subsets of asthmatics but not others, indicating a need to improve segregation of asthma patients into the subgroups most likely to respond to a particular therapy. The challenges include the ability to define and phenotype patients better with severe asthma to implement a more personalised therapeutic approach that will afford a higher likelihood of a successful risk:benefit ratio. Nevertheless, the definitive importance of careful phenotypic classification of patients with severe asthma will be established only when detailed characterisation of hundreds of patients is completed and analysed, as proposed in the newly established pan-European consortium: The Unbiased Biomarkers for the Prediction of Respiratory Disease Outcome (U-BIOPRED; <http://www.fp7-consulting.be/en/ubiopred/>). Whatever the outcomes of these research programmes might be pharmacoeconomic concerns should also be taken into account because of the elevated acquisition costs of recombinant human mAbs and the diagnostic screening procedures for the identification of potential responders.

### Conflicts of interest

RP has received grant support from CV Therapeutics, NeuroSearch A/S, Sandoz and MSD; RP has served as a speaker for CV Therapeutics, Novartis, MSD and Roche; RP has served as a consultant for CV Therapeutics, Duska Therapeutics, Neuro-Search A/S, Boehringer-Ingelheim and Forest Laboratories; RP has received payment for developing educational presentations (including service on speakers' bureaus) from MSD and Pfizer.

TC has received consulting fees from: MedImmune, Kalobios, Actelion, Roche and Circassia. TC has been an investigator on grants to Creighton University from: Amgen, Merck, Novartis, Pfizer, Schering-Plough, Genentech, MedImmune, NIH and State of Nebraska. TC is Executive Vice President of AAAAI.

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