

EXPERT OPINION

1. Introduction
2. Anti-IgE tactics
3. Anti-IL-5 tactics
4. Tactics targeting the IL-4/IL-13 pathway
5. Anti-TNF- α tactics
6. Tactics targeting T cells
7. Additional tactics under study
8. Concluding remarks
9. Expert opinion

Disabling inflammatory pathways with biologics and resulting clinical outcomes in severe asthma

Massimo Caruso, Emanuele Crisafulli, Shirin Demma, Stephen Holgate & Riccardo Polosa[†]

[†]*University of Catania-AOU "Policlinico-V. Emanuele", Department of Clinical and Molecular Bio-Medicine – Institute of Internal Medicine and Clinical Immunology, Catania, Italy*

Introduction: Patients with severe asthma have a significant unmet need with persistent symptoms and/or frequent exacerbations despite high intensity treatment. These severe unrelenting symptoms have a huge impact on healthcare resources due to frequent hospital admissions and requirement for intensive and expensive medications. There is a compelling need for more effective and safer therapies to help severe asthma sufferers to achieve adequate control of their disease.

Areas covered: Expanding knowledge of innate and adaptive immune responses has led to development of new biologic approaches for severe asthma. Here, the authors will review the existing efficacy and safety data from clinical trials of some of the new biologic therapies that are in development for severe asthma. Their specific role in distinctively targeted subpopulations of severe asthmatics will be also discussed.

Expert opinion: Defining and phenotyping severe asthma patients will become increasingly important as some patients who were previously classified as having severe asthma may become well-controlled with a targeted phenotype-specific treatment. However, pharmacoeconomic concerns should also be taken into account given the elevated acquisition costs of recombinant human monoclonals and of the diagnostic screening procedures for the identification of potential responders.

Keywords: biologics, immunopharmacology, inflammatory pathways, monoclonal antibodies, severe asthma

Expert Opin. Biol. Ther. (2013) 13(3):393-402

1. Introduction

The recognition that asthma is an inflammatory disorder of the airways in the early 1950s led to the discovery of the beneficial effect of corticosteroids and the development of inhaled formulations. Today, optimal control of the disease can be achieved in the vast majority of asthma patients by inhaled formulations of corticosteroids and β_2 -adrenoceptor agonists. Yet, many asthma patients continue to have uncontrolled disease that requires either improved adherence to medications or high-intensity treatment [1]. These patients are at risk of developing significant side effects [2] and account for about 60% of asthma-related healthcare costs [3-5]. This is indication of the complexity of a disease characterised by marked heterogeneity in clinical phenotypes, inflammatory endotypes as well as therapeutic responses [6-8].

The evidence base for effective assessment, monitoring and management of asthmatics with severe disease is small and many unanswered questions remain [9]. Improvements in the clinical ability to phenotype patients with severe asthma to implement a more personalised therapeutic approach will likely lead to better outcomes, especially in those with treatable co-morbid conditions [10-12]. Nonetheless,

informa
healthcare

Article highlights.

- Asthma patients with uncontrolled disease require high-intensity treatment and are at risk significant side-effects. They are characterized by marked heterogeneity in clinical phenotypes, inflammatory endotypes as well as therapeutic responses.
- More effective and safer therapies to help severe asthma sufferers are required and the expanding knowledge of innate and adaptive immune responses is now leading to the development of new biologic approaches for severe asthma.
- Existing efficacy and safety data from clinical trials of new biologic therapies that are in development for severe asthma will be discussed with a specific focus on distinctively targeted subpopulations of severe asthmatics.
- The humanized IgG1 monoclonal antibody (mAb), omalizumab is the only biologic therapy recommended for patients with severe allergic asthma and increased levels of IgE, but its response is not uniform across all patients.
- Antagonising the eosinophilic mitogenic factor IL-5 with specific mAbs may have beneficial advantage, given that the eosinophil may also serve a pathogenetic role in the mixed Th2/Th1/Th17 endotype of severe asthma.
- Suppressing the IL-4/IL-13 pathway not only ablates Th2-mediated responses, but also modulates progression to more chronic severe form of the disease by preventing mucus hypersecretion, mucus metaplasia, smooth muscle proliferation and deposition of new matrix.
- Additional strategies targeting other mechanisms of asthma, include biological therapies interfering with TNF α , CD4, IL-9, OX40L, the IL-17 family members, and Toll-like receptors (TLRs) signalling.
- Defining and phenotyping severe asthma patients will become increasingly important as some patients who were previously classified as having severe asthma may become well-controlled with a targeted phenotype-specific treatment.

This box summarises key points contained in the article.

the current therapeutic arsenal for asthmatics with severe refractory disease is deficient and development of safer and more effective therapies remains a top priority.

The understanding of the disease has improved considerably over the last 20 years. The commonly accepted paradigm that Th2 cells are important in the pathogenesis of severe asthma [13] has been recently challenged and alternative T-cell subtypes (e.g., Th1 cells, regulatory T cells and Th17 cells) are now being taken into consideration [14,15]. The advent of recombinant DNA technology is enabling to translate this evolving knowledge base into the development of a growing number of biologic agents intended to disable several key targets associated with the complex inflammatory/remodelling phenotype of chronic severe asthma. The first biologic agent with an indication for severe asthma, the IgG1 monoclonal antibody (mAb) omalizumab, was approved by the Food and Drug Administration (FDA) in

2003. Since then a number of new biologics have entered clinical development as anti-asthma therapies.

In this narrative review article, the authors intend to appraise some of the new biologic agents that are in development for the severe asthma sufferers. Most of the agents discussed in the article may not directly relate to patients with severe asthma, because early trials often focus on proof-of-concept for a disease and not necessarily the intended target population.

2. Anti-IgE tactics

The humanised IgG1 monoclonal anti-IgE antibody (mAb), omalizumab (Xolair[®]) has been engineered to target a component of IgE (Fc ϵ 3 region) that usually binds to the high affinity receptor (Fc ϵ R1) on mast cells, basophils and dendritic cells (DCs) through formation of tri- and hexameric IgE containing immune complexes [16]. Omalizumab administration causes the free circulating IgE to fall rapidly, whereas complexed IgE initially increases before being effectively removed by the reticuloendothelial system [17]. This loss in free circulating IgE leads to progressive Fc ϵ R1 internalisation in mast cells, basophils and DCs thereby enhancing its therapeutic efficacy [18]. Loss of circulating IgE together with reduction in Fc ϵ R1 expression, markedly attenuates mast cells and basophils potential to release preformed and newly generated mediators and facilitates allergen uptake by DCs [19].

Thus, omalizumab is an attractive drug when attenuation in allergic inflammation is sought. But there is more than just allergy in most chronic severe asthma patients [6-8]. The role of IgE appears to extend beyond the allergic response as there is emerging evidence for a non-specific involvement 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 [20]. Thus, mAbs directed against IgE might inhibit not only allergic inflammation, but could also have a role in non-allergic persistent forms of inflammation as recently reported for other diseases (such as autoimmune chronic urticaria) [21].

Clinical trials in adults and children have confirmed the efficacy of omalizumab in severe allergic asthma with significant improvement in disease exacerbations, symptom control and overall use of inhaled corticosteroids (ICS) and β -agonists [22,23]. Recent expert panel guidelines now also recommend omalizumab in step V patients with severe allergic asthma and increased levels of IgE either as an alternative or in addition to oral corticosteroids [24]. However, there is little evidence about the ability of omalizumab to have significant steroid sparing effect, with the exception of a recent uncontrolled clinical trial in which a substantial dose reduction in oral corticosteroid requirements was shown [25].

A further limitation of omalizumab is that its response is not uniform across all patients: one-third of patients responded dramatically, with life-transforming experiences

that included being able to withdraw from corticosteroids, one-third with less clinically beneficial responses and one-third experienced no response at all [26]. Possible explanations for this discordance include a relative lack of correlation between free IgE levels and FcεR1 expression, difference in intrinsic cellular sensitivity and the importance of the ratio of antigen-specific IgE to total IgE [27]. Because of this variation in clinical responsiveness to omalizumab, it is advised that patients are assessed at 16 weeks after starting therapy at which stage a range of outcome measures is used to decide whether to continue or cease therapy [28].

A more potent form of anti-IgE that might have a broader spectrum of effects is in clinical 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 [29].

In vitro studies have demonstrated that cross-linking the low affinity IgE receptor (FcεRII, CD23) downregulates IgE synthesis. The primatised IgG1 anti-CD23 mAb, lumiliximab (IDEC-152; Biogen Idec Pharma) is well tolerated and reduces IgE concentrations in patients with mild asthma, but its clinical efficacy has not been reported [30], and no further clinical trials have been planned.

3. Anti-IL-5 tactics

Eosinophil infiltration and activation is known to be associated with the allergic asthma endotype and may serve a pathogenetic role [31]. However, accumulating evidence indicate that the mixed Th2/Th1/Th17 endotype of severe asthma can be characterised by a mixed granulocytic airway infiltrate in which eosinophils are uniformly present [32]. Moreover, on activation they degranulate and release lipid mediators, cytokines, cytotoxins, leukotrienes and PAF and pro-fibrogenic factors such as TGF-α, TGF-β, PDGF and MMP-9 that induce mucus hypersecretion, remodelling and airway hyperresponsiveness (AHR) [33,34].

IL-5, a Th2 cytokine intimately involved in the maturation, activation and survival of eosinophils, presented itself as an obvious candidate to target [35]. Studies in mice showed that ablation of the IL-5 gene or blockade of IL-5 or its receptor had dramatic effects in abolishing eosinophilic responses in the lungs with acute and repeated allergen challenge [36]. Antagonising IL-5 with mAbs may have beneficial advantage for asthma, and particularly for its eosinophilic predominant form.

A number of anti-IL-5 strategies are in clinical development for allergic diseases, including mepolizumab, reslizumab and benralizumab. Early clinical trials of mild-to-moderate asthmatic subjects with the IL-5 blocking monoclonal antibody mepolizumab (Bosatria; GSK) showed consistent substantial reductions in sputum and blood eosinophilia, but failed to demonstrate significant clinical or functional improvement [37-39]. 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 randomised controlled trials have reassessed the role of the eosinophil/IL-5 pathway in more severe forms of asthma by testing mepolizumab on asthma exacerbations in subgroups of patients with persistent airway eosinophilia despite systemic corticosteroid therapy [40,41]. In the study by Nair *et al.* [40], 20 patients with prednisone-dependent asthma and persistent sputum eosinophilia received intravenous infusion of either mepolizumab or placebo on a monthly basis. At 8-week follow-up, the authors reported a significant reduction in asthma exacerbations, clinically important dose reduction in prednisone use, small improvements in FEV₁ 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. In the study by Haldar *et al.* [41], patients with refractory eosinophilic asthma and recurrent severe exacerbations of their disease were recruited to receive 12 doses of i.v. mepolizumab (n = 29) or placebo (n = 32) on a monthly basis. 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 both studies. These preliminary findings have been recently confirmed in a large multicentre, double-blind, dose-ranging study of 616 patients with severe eosinophilic asthma [42]. Compared with placebo, i.v. infusion of mepolizumab reduced the rate of clinically significant asthma exacerbations by 48, 39 and 52%, respectively in the 75, 250 and 750 mg treatment group. Visits to emergency department and admissions also fell, although with variable results in the three arms of the study; 60% reduction with 75 mg, 42% with 250 mg and 48% with 750 mg. No significant adverse effects were reported.

However, it is important to recognise that not many patients with severe asthma have eosinophilic features and screening patients for sputum eosinophilia is not practical in most hospital settings. Therefore, the general applicability and commercial exploitation of anti-IL-5 strategies needs to be judiciously considered.

Perhaps, future studies should also establish the steroid sparing effect of mepolizumab in unselected patients with severe refractory asthma. Ideally, drugs targeting IL-5 signalling should not only reduce the rate of asthma exacerbations, but also facilitate tapering of oral corticosteroids.

Use of a second anti-IL-5 mAb, reslizumab (Cinquin or SCH55700; Ception Therapeutics), in severe persistent asthma in patients receiving oral or high doses of inhaled steroids did reveal a trend towards improvement in baseline lung function at the highest dose given, but not in other clinical

indices of disease activity [43]. In a larger randomised control trial of eosinophilic asthma that is poorly controlled by high-dose ICS [44], those on reslizumab showed significantly greater reductions in sputum eosinophils, improvement in lung function and a trend towards greater asthma control than placebo that was especially apparent in patients with concomitant polyposis.

Benralizumab (MEDI-563, Medimmune) is a humanised mAb directed against the α chain of the IL-5 receptor (anti-IL-5R α IgG1) 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 [45], and Phase II trials in asthma are currently underway.

4. Tactics targeting the IL-4/IL-13 pathway

IL-4 and IL-13 make good targets for severe asthma from an immunological, inflammatory and remodelling perspective. Blockade of these cytokines not only ablates Th2-mediated responses, but also modulates progression to more chronic severe form of the disease by preventing mucus hypersecretion, mucus metaplasia, smooth muscle proliferation and deposition of new matrix [46,47]. Both IL-4 and IL-13 induce their effects by signalling through the IL-4R α /IL-13R α 1 complex [48,49] and biological compounds targeting these molecules may provide 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 α (altrakinept; Nuvance) designed to block the interaction between IL-4 and its naturally occurring cellular receptors. Altrakinept given by inhalation to moderate persistent asthmatics prevented worsening in symptoms and reductions in FEV₁ on withdrawal of ICS [50]. No significant adverse events or development of anti-IL-4R antibodies were detected. However, the clinical development of altrakinept was halted when a large subsequent Phase II study in subjects with moderate persistent asthma failed to show substantial effects on pulmonary function or asthma symptoms and no significant effect on asthma exacerbation [51]. Likewise, attempts at blocking IL-4 directly with a mAb such as pascolizumab (SB 240683; PDL BioPharma) and similar mAbs made by PDL BioPharma also failed in clinical trials [52].

Next, a number of human IL-13 neutralising mAbs, including anrukinzumab, IMA-026, QAX576, CAT-354 and MILR 1444A have entered Phase I/II clinical trials of asthma. In a Phase IIa double-blind, placebo-controlled antigen challenge study, anrukinzumab (IMA-638; Wyeth, now Pfizer) reduced the severity of both early and late asthmatic responses with no safety concerns in patients with mild, atopic asthma [53]. A larger Phase II, randomised, double-blind, placebo-controlled, parallel-arm study of IMA-638 and IMA-026 in 56 patients with mild atopic asthma confirmed the beneficial effects of anrukinzumab

(IMA-638) on both early and late phase responses, whereas IMA-026 failed to show significant effects [54]. There was no effect of either antibody on allergen-induced AHR or sputum eosinophils [54]. Some subtleties exist in the way these two mAbs interact with IL-13. IMA-638 permits interaction of IL-13 with IL-13R α 1 or IL-13R α 2, but inhibits recruitment of IL-4R α to the IL-13-IL-13R α 1 complex, whereas IMA-026 competes with IL-13 interaction with IL-13R α 1 and IL-13R α 2 [55]. The potency and efficiency of this IL-13 clearance pathway indicates that cell surface IL-13R α 2 acts as an important scavenger for IL-13 with implications for the design of IL-13 antagonists. The effect of the anti-IL-13 mAb, QAX576 (Novartis), was studied on a clinical experimental model of allergic inflammation following nasal allergen challenges in patients with seasonal allergic rhinitis [56]. No apparent effects were detected in the QAX576 group on nasal lavage eosinophil levels or nasal symptom scores compared with placebo. A Phase II study of QAX576 in moderate-to-severe atopic asthmatics was withdrawn due to complexity of design and lack of enrolment. However, this molecule is still under development for asthma. Phase I studies with the humanised anti-IL-13 mAb, talokinumab (CAT-354; MedImmune), 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 [57]. A Phase II clinical trial has been completed, though the results have not yet been published [58]. Lebrikizumab (MILR 1444A; Genentech) is a humanised anti-IL-13 mAb, currently undergoing Phase II clinical trials in inadequately controlled asthmatics. Corren *et al.* [59] reported an improved FEV₁ (but not symptoms) in patients with moderate-to-severe asthma, including those on long-acting β -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.

Targeting IL-4 or IL-13 alone may be ineffective because of redundant mechanisms shared with the two cytokines and alternative approaches that suppress the activity of both IL-4 and IL-13 may be more beneficial. Using this concept, bioengineered versions (muteins) of IL-4 have also been developed, which potently inhibits the binding of both IL-4 and IL-13 to the shared IL-4R α /IL-13R α 1 complex [60]. A dry powder formulation of the IL-4 mutein pitrakinra (Aerovant; Aerovance Inc.) has entered Phase II clinical trials for the treatment of asthma and data from an initial Phase IIa trial showed

significant attenuation of the allergen-provoked late asthmatic response with no safety concerns [61]. A randomised controlled trial in patients with severe uncontrolled asthma has been completed, and showed good responses only in those subjects with high blood eosinophils [62]. AIR645, a dual inhibitor of IL-4 and IL-13, is a second-generation antisense drug targeting the mRNA encoding the IL-4R α subunit. Results from a Phase I study evaluating the safety, tolerability and pharmacokinetics of once-weekly treatment with inhaled AIR645 in healthy volunteers demonstrated very low systemic drug absorption and no serious adverse events [63]. AMG-317 is a fully human mAb currently under investigation for its ability to bind with high affinity to IL-4R α , 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 recently conducted to determine the safety and efficacy of subcutaneous AMG-317 (75, 150 or 300 mg) in patients with moderate-to-severe asthma [64]. Treatment did not have clinical efficacy with no improvements in the control of asthma, β -agonist use or lung function. However, AMG-317 did suppress exacerbations, particularly in patients on the higher dose of AMG-317 (300 mg) and with higher asthma symptom scores. Increasing the dose, treatment duration or application to specific asthma phenotypes may be more effective.

5. Anti-TNF- α tactics

There is now a significant body of evidence implicating Th1 inflammatory profile and overexpression of TNF- α also in the pathogenesis of chronic inflammatory disorders of the airways, including severe asthma and chronic obstructive pulmonary disease (COPD) [65,66]. TNF- α mediates the recruitment of neutrophils and eosinophils into the airways, uniquely suppresses glucocorticoid responsiveness in monocytes, promotes AHR and up-regulates the pathways involved in chronic airway remodelling and subepithelial fibrosis. Moreover, TNF- α suppresses glucocorticoid responsiveness in monocytes and interferes with glucocorticoid receptor signalling [67]. Thus, biological compounds targeting these molecules may be particularly useful in subpopulations of patients with chronic severe asthma and refractory disease.

The early clinical trials of TNF- α suppression in asthma employed etanercept (Enbrel), a fusion protein that mimics the inhibitory effects of naturally occurring soluble TNF receptors. Two initial small studies of etanercept showed that there were marked improvements in subjective (asthma-related control and QoL) as well as objective (spirometry, peak flows and AHR) measures of asthma and reduction in reliever medication use [68,69]. However, subsequent much larger trials of etanercept (25 mg twice weekly) in moderate-to-severe asthma were essentially negative [70,71].

Concurrently, also the human TNF- α neutralising mAbs, infliximab and golimumab were being trialled in asthma. In a small study of mild-to-moderate asthmatics, infliximab

treatment improved some lung function measures (diurnal variation in peak expiratory flow) and reduced exacerbations in moderate asthmatics [72], but the efficacy/safety of infliximab (and adalimumab) have never been specifically studied in patients with chronic severe asthma. In a large population of patients with severe persistent asthma randomised to receive either placebo or three increasing doses (50, 100 and 200 mg) of golimumab, no significant improvement in any of the efficacy measurements was reported [73]. No significant differences in any asthma outcomes were observed for the group as a whole, but when substratified into those with rhinosinusitis and > 12% bronchodilator reversibility, dose-related efficacy was observed. Unfortunately, the trial was terminated early due to a large number of serious infections in the golimumab group. In view of the conflicting efficacy findings and potential safety problems of the published studies, no further trials with anti-TNF- α agents for severe asthma have been planned.

6. Tactics targeting T cells

The important role of T cells in the pathogenesis of chronic severe asthma is being translated in intervention approaches targeted at these cells in human asthma. By reducing the number of circulating T cells, the chimeric anti-CD4 mAb keliximab [74] may be useful 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 use of this drug did not reach statistical significance in patients with corticosteroid refractory asthma and there was also a concern of possible adverse consequences [75]. Elevated CD25⁺ T cells, IL-2 and soluble (s) IL-2 receptor α chains (sCD25) have been demonstrated in the airways of patients with severe asthma [76]. With this in mind, Busse *et al.* [77] conducted a study to evaluate the efficacy and safety of a humanised mAb directed against the α (CD25) subunit of the high-affinity IL-2 receptor, daclizumab, 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 2 weeks for 12 weeks; the protocol design included an ICS tapering phase of 8 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 FEV₁, daytime asthma symptoms, increased time to exacerbation and short-acting β_2 -agonist use 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 reaction and viral meningitis. In an analysis of a subpopulation with more significant disease, findings were more impressive. This small study demonstrates that daclizumab may have a role in asthma, but

further studies are needed to identify subset of severe asthmatics in whom there may be a role for daclizumab as add-on therapy.

7. Additional tactics under study

Additional strategies targeting other mechanisms of asthma are currently being investigated. These include biological therapies interfering with IL-9, OX40L, the IL-17 family members IL-17A and IL-25 and Toll-like receptors (TLRs) signalling.

7.1 IL-9 and OX40L

Although IL-9 was originally described as a Th2 cytokine promoting mast cell proliferation and T-cell growth, it could also reproduce several features of chronic asthma including smooth muscle cell hyperplasia, eosinophilic airway inflammation, mucus production and AHR [78]. 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 [79]. A pilot study reported that MEDI528 could improve exercise-induced bronchospasm by reducing the maximum mean decrease in FEV₁ after exercise [80]. However, a recently completed study did not meet its end points and further development of this molecule for asthma has been stopped.

OX40 ligand (OX40L) is expressed on antigen-presenting cells (APCs) as well as B and T lymphocytes.

Activation of APCs by thymic stromal lymphopoietin (TSLP) leads to OX40L upregulation, which binds to OX40 and leads to Th2 cell differentiation and expansion of Th2 memory cells [81]. 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 [82]. 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 [83].

7.2 IL-17 family members

The IL-17 family members IL-17A and IL-25 (also known as IL-17E) have been reported to have a prominent role in inducing allergic responses, tissue remodelling, neutrophilic inflammation and Th17 cells activation [84]. These features are generally associated with the severe asthma endotype. Currently, two drugs in this category are in development for severe asthma: brodalumab (AMG-827) and secukinumab (AIN457).

Brodalumab (AMG-827; Amgen) is a human monoclonal antibody that selectively binds and blocks IL-17R signalling, thereby disabling the effect of several ligands of the IL-17 family, including IL-17A and IL-25.

A Phase II study to investigate safety and efficacy of AMG-827 in subjects with uncontrolled asthma is ongoing [85]. The primary objective is to determine if AMG-827 is effective compared with placebo as measured by change in Asthma Control Questionnaire (ACQ) composite scores.

Secukinumab (AIN457; Novartis Pharma) is a human monoclonal antibody targeting IL-17A that has recently completed Phase II clinical trials for plaque psoriasis. A Phase II study with AIN457 for severe asthma has been recently launched and it is currently recruiting patients for the evaluation of safety and efficacy [86].

7.3 Toll-like receptor

TLRs are cell surface or intracellular receptors that recognise pathogen-associated molecular patterns commonly conserved in bacteria, viruses and some fungi. Binding of these TLRs activates APCs, such as macrophages and DCs, to commence both innate and adaptive immune responses. Agents directed against TLRs may skew the cytokine balance from Th2 towards Th1, thereby affecting allergic diseases. To date, most of the work in allergic conditions has been focused on agonists for the TLR-9. An inhaled form of the TLR-9 agonist, Tolamba (Dynavax Technologies, Berkeley, CA, USA) has been studied in a Phase II clinical trial of atopic asthmatics [87]. Although treatment significantly increased IFN- γ and IFN- γ -inducing genes, it did not attenuate early or late phase asthmatic responses to allergen challenge or were there significant reductions in sputum eosinophils or Th2 gene expression. In another Phase II clinical trial, 63 persistent allergic asthmatics on ICS received either subcutaneous injections of the TLR-9 agonist, CYT003-QbG10 (Cytos Biotechnology, Zurich, Switzerland) or placebo. During the run-in period, all subjects were stabilised on beclomethasone and then 4 weeks into the study, the ICS was reduced by 50%, and if possible, reduced to zero in another 4 weeks. From 6 to 12 weeks, the treatment group had significantly better combined symptom and medication scores than placebo and, at 12 weeks, had stable FEV₁ compared with placebo. A subgroup analysis in subjects who were able to come off ICS after week 8 suggests that CYT003-QbG10 may be as effective as an ICS in asthma [88].

8. Concluding remarks

Several new biologic therapies which target specific inflammatory cells or receptors of inflammatory mediators are being investigated to help improve overall asthma control in patients with severe refractory asthma. However, 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, it is not surprising that the development of these new treatments might not be enough to suppress inflammation and control remodelling efficiently. Indeed, these highly specific therapies could be effective in some subsets of severe asthmatics but not others,

indicating a need to improve segregation of asthma patients into the subgroups most likely to respond to a particular therapy. Consequently, defining and phenotyping of patients with severe asthma will become increasingly important as some patients who were previously classified as having severe asthma because of poor asthma control despite maximal doses of ICS and long-acting β_2 -agonist may become well controlled with a targeted phenotype-specific treatment. However, pharmacoeconomic concerns should also be taken into account given the elevated acquisition costs of recombinant human monoclonals and of the diagnostic screening procedures for the identification of potential responders.

9. Expert opinion

Targeting specific inflammatory cells or receptors of inflammatory mediators relevant to asthma with new biologic therapies is an exciting area of therapeutic development, but the available evidence indicates that these highly specific tools can achieve only limited improvement in overall asthma control in patients with severe refractory disease.

Nonetheless, some patients appear to respond successfully to biologics. Therefore, the future of biologics for chronic severe asthma stands in the ability to exploit a more personalised therapeutic approach and to develop less specific therapies that could be more effective at suppressing inflammation and at controlling remodelling.

To circumvent the disadvantage of interfering with the action of individual cytokines, signalling of specific receptors and activation of single inflammatory cells, inhibition of kinase-based signalling cascades has been exploited and selective kinase inhibitors have been suggested to reduce expression and activation of several inflammatory mediators in severe asthma. Unfortunately, systemic administration of these inhibitors might

cause side effects and toxicity, and inhalatory formulations should then be engineered to reduce systemic exposure.

Future trials should not only look into the best method of administration, optimal dosage and duration of treatment, but also use appropriate outcome measures, patient selection and the inclusion of biomarkers to determine the efficacy, as well as to explore alternative mechanisms of action of biologic therapies currently approved for severe asthma.

Unfortunately, at the current stage of drug development, it is difficult to forecast the percentage of asthma patients that could benefit from these highly specific tools. Looking into the future, it is clear that more efforts should be devoted towards establishing the key characteristics of those who may respond to biologics and the cost-effectiveness of these interventions, but at the same time, a balance with valuable – but convenient and easy to implement – predictors of treatment response must be achieved. These should be the main priorities for academic researchers and pharmaceutical entrepreneurs around the world in order to achieve sound clinical impact for the severe asthma patients.

Declaration of interest

R Polosa has received grant support from CV Therapeutics, NeuroSearch A/S, Sandoz, MSD and Boehringer-Ingelheim; has served as a speaker for CV Therapeutics, Novartis, MSD and Roche; has served as a consultant for CV Therapeutics, Duska Therapeutics, Neuro-Search A/S, Boehringer-Ingelheim and Forest Laboratories and has received payment for developing educational presentations (including service on speakers' bureaus) from MSD and Pfizer. None of the remaining authors have any competing interests to declare and no funding was received in the preparation of this manuscript.

Bibliography

1. BTS/SIGN British Guideline on Management of Asthma. British Thoracic Society Scottish Intercollegiate Guidelines network. *Thorax* 2008;63(Suppl 4):iv1-121
2. Polosa R. An overview of chronic severe asthma. *Intern Med J* 2008;38(3):190-8
3. Smith DH, Malone DC, Lawson KA, et al. A national estimate of the economic costs of asthma. *Am J Respir Crit Care Med* 1997;156(3 Pt 1):787-93
4. Godard P, Chanez P, Siraudin L, et al. Costs of asthma are correlated with severity: a 1-yr prospective study. *Eur Respir J* 2002;19(1):61-7
5. Antoncelli L, Bucca C, Neri M, et al. Asthma severity and medical resource utilization. *Eur Respir J* 2004;23:723-9
6. Holgate ST, Polosa R. The mechanisms, diagnosis, and management of severe asthma in adults. *Lancet* 2006;Aug26368(9537):780-93
7. Moore WC, Meyers DA, Wenzel SE, et al. Identification of asthma phenotypes using cluster analysis in the severe asthma research program. *Am J Respir Crit Care Med* 2010;Feb15181(4):315-23
8. Lotvall J, Akdis CA, Bacharier LB, et al. Asthma endotypes: a new approach to classification of disease entities within the asthma syndrome. *J Allergy Clin Immunol* 2011;Feb127(2):355-60
9. Chanez P, Wenzel SE, Anderson GP, et al. Severe asthma in adults: what are the important questions? *J Allergy Clin Immunol* 2007;119(6):1337-48
10. Polosa R, Benfatto GT. Managing patients with chronic severe asthma: rise to the challenge. *Eur J Intern Med* 2009;Mar20(2):114-24
11. Currie GP, Douglas JG, Heaney LG. Difficult to treat asthma in adults. *BMJ* 2009;338:b494
12. Morjaria JB, Polosa R. Recommendation for optimal management of severe refractory asthma. *Journal of Asthma and Allergy* 2010;3:43-56
13. Robinson D, Hamid Q, Ying S, et al. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 1992;326(5):298-304
14. Cazzola M, Polosa R. Anti-TNF-alfa and Th1 cytokine-directed therapies for the treatment of asthma. *Curr Opin Allergy Clin Immunol* 2006;Feb6(1):43-50
15. Thorburn A, Hansbro P. Harnessing Regulatory T cells to Suppress Asthma: from Potential to Therapy. *Am J Respir Cell Mol Biol* 2010;43(5):511-19
16. Di Domenico M, Bisogno A, Polverino M, et al. Xolair in asthma therapy: an overview. *Inflamm Allergy Drug Targets* 2011;10:2-12
17. Demeule B, Shire SJ, Liu J, et al. A therapeutic antibody and its antigen form different complexes in serum than in phosphate-buffered saline: a study by analytical ultracentrifugation. *Anal Biochem* 2009;388:279-87
18. Beck LA, Marcotte GV, MacGlashan D, et al. Omalizumab-induced reductions in mast cell FcεR1 expression and function. *J Allergy Clin Immunol* 2004;114:527-30
19. Schroeder JT, Bieneman AP, Chichester KL, et al. Decreases in human dendritic cell-dependent T(H)2-like responses after acute in vivo IgE neutralization. *J Allergy Clin Immunol* 2010;125:896-901
20. Patou J, Gevaert P, Van Zele T, et al. Staphylococcus aureus enterotoxin B, protein A, and lipoteichoic acid stimulations in nasal polyps. *J Allergy Clin Immunol* 2008;121(1):110-15
21. Morjaria JB, Polosa R. Off-label use of omalizumab in non-asthma conditions: new opportunities. *Expert Rev Resp Med* 2009;3(3):299-308
22. Walker S, Monteil M, Phelan K, et al. Anti-IgE for chronic asthma in adults and children. *Cochrane Database Syst Rev* 2006(2):CD003559
23. Lanier B, Bridges T, Kulus M, et al. Omalizumab for the treatment of exacerbations in children with inadequately controlled allergic (IgE-mediated) asthma. *J Allergy Clin Immunol* 2009;124(6):1210-16
24. Global Initiative for Asthma (GINA). Global strategy for asthma management and prevention. 2009. Available from: <http://www.ginasthma.org>
25. Domingo C, Moreno A, Jose Amengual M, et al. Omalizumab in the management of oral corticosteroid-dependent IGE-mediated asthma patients. *Curr Med Res Opin* 2011;27(1):45-53
26. Bousquet J, Rabe K, Humbert M, et al. Predicting and evaluating response to omalizumab in patients with severe allergic asthma. *Respir Med* 2007;101:1483-92
27. Zaidi AK, Saini SS, Macglashan DW Jr. Regulation of Syk kinase and FcRβ expression in human basophils during treatment with omalizumab. *J Allergy Clin Immunol* 2010;125(4):902-8
28. Schumann C, Kropf C, Wibmer T, et al. Omalizumab in patients with severe asthma: the XCLUSIVE study. *Clin Respir J* 2011; Epub ahead of print
29. <http://www.gene.com/gene/pipeline/status/immunology/anti-m1/index.html>
30. Rosenwasser LJ, Busse WW, Lizambri RG, et al. Allergic asthma and an anti-CD23 mAb (IDEC-152): results of a phase I, single-dose, dose-escalating clinical trial. *J Allergy Clin Immunol* 2003;112(3):563-70
31. Rothenberg ME, Hogan SP. The eosinophil. *Annu Rev Immunol* 2006;24:147-74
32. Wenzel SE, Schwartz LB, Langmack EL, et al. Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. *Am J Respir Crit Care Med* 1999;160:1001-8
33. Flood-Page P, Menzies-Gow A, Phipps S, et al. Anti-IL-5 treatment reduces deposition of ECM proteins in the bronchial subepithelial basement membrane of mild atopic asthmatics. *J Clin Invest* 2003;112(7):1029-36
34. Tanaka H, Komai M, Nagao K, et al. Role of interleukin-5 and eosinophils in allergen-induced airway remodeling in mice. *Am J Respir Cell Mol Biol* 2004;31(1):62-8
35. Molino NA, Gossage D, Kolbeck R, et al. Molecular and clinical rationale for therapeutic targeting of interleukin-5 and its receptor. *Clin Exp Allergy* 2011; [Epub ahead of print]
36. Foster PS, Hogan SP, Yang M, et al. Interleukin-5 and eosinophils as therapeutic targets for asthma. *Trends Mol Med* 2002;8:162-7

37. Flood-Page P, Swenson C, Faierman I, et al. A study to evaluate safety and efficacy of mepolizumab in patients with moderate persistent asthma. *Am J Respir Crit Care Med* 2007;176(11):1062-71
38. 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(2):199-204
39. Leckie MJ, ten Brinke A, Khan J, et al. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 2000;356(9248):2144-8
40. Nair P, Pizzichini MM, Kjarsgaard M, et al. Mepolizumab for prednisone-dependent asthma with sputum eosinophilia. *N Engl J Med* 2009;360(10):985-93
41. Haldar P, Brightling CE, Hargadon B, et al. Mepolizumab and exacerbations of refractory eosinophilic asthma. *N Engl J Med* 2009;360(10):973-84
42. Pavord ID, Korn S, Howarth P, et al. Mepolizumab for severe eosinophilic asthma (DREAM): a multicentre, double-blind, placebo-controlled trial. *Lancet* 2012;380:651-9
43. Kips JC, O'Connor BJ, Langley SJ, et al. Effect of SCH55700, a humanized anti-human interleukin-5 antibody, in severe persistent asthma: a pilot study. *Am J Respir Crit Care Med* 2003;167:1655-9
44. Castro M, Mathur S, Hargreave F, et al. Reslizumab for poorly controlled, eosinophilic asthma: a randomized, placebo-controlled study. *Am J Respir Crit Care Med* 2011;184:1125-32
45. Busse WW, Katial R, Gossage D, 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(6):1237-44
46. Wills-Karp M, Luyimbazi J, Xu X, et al. Interleukin-13: central mediator of allergic asthma. *Science* 1998;282:2258-61
47. Oh CK, Geba GP, Molfino N. Investigational therapeutics targeting the IL-4/IL-13/STAT-6 pathway for the treatment of asthma. *Eur Respir Rev* 2010;19(115):46-54
48. Hart P, Bonder C, Balogh J, et al. Differential responses of human monocytes and macrophages to IL-4 and IL-13. *J Leukoc Biol* 1999;66(4):575-8
49. Hershey GK. IL-13 receptors and signaling pathways: an evolving web. *J Allergy Clin Immunol* 2003;111(4):677-90
50. Borish LC, Nelson HS, Lanz MJ, et al. Interleukin-4 receptor in moderate atopic asthma. A phase I/II randomized, placebo controlled trial. *Am J Respir Crit Care Med* 1999;160:1816-23
51. Borish LC, Nelson HS, Corren J, et al. Efficacy of soluble IL-4 receptor for the treatment of adults with asthma. *J Allergy Clin Immunol* 2001;107:963-70
52. Hart TK, Blackburn MN, Brigham-Burke M, et al. Preclinical efficacy and safety of pascalizumab (SB 240683): a humanized anti-interleukin-4 antibody with therapeutic potential in asthma. *Clin Exp Immunol* 2002;130:93-100
53. Gauvreau GM, Boulet LP, Fitzgerald JM, et al. The effects of IMA-638 on allergen induced airway responses in subjects with mild atopic asthma. *Eur Respir J* 2008;32(Suppl):52-827s
54. Gauvreau GM, Boulet L-P, Cockcroft DW, et al. Effects of Interleukin-13 Blockade on Allergen-induced Airway Responses in Mild Atopic Asthma. *Am J Respir Crit Care Med* 2011;183:1007-14
55. Kasaian MT, Raible D, Marquette K, et al. IL-13 antibodies influence IL-13 clearance in humans by modulating scavenger activity of IL-13Ralpha2. *J Immunol* 2011;187:561-9
56. Kariyawasam HH, Nicholson GC, Tan AJ, et al. Effects of anti-IL-13 (Novartis QAX576) on inflammatory responses following nasal allergen challenge (NAC). *Am J Respir Crit Care Med* 2009;179:A3642
57. Singh D, Kane B, Molfino NA, et al. A phase 1 study evaluating the pharmacokinetics, safety and tolerability of repeat dosing with a human IL-13 antibody (CAT-354) in subjects with asthma. *BMC Pulm Med* 2010;10:3
58. <http://clinicaltrials.gov/ct2/show/NCT00873860>
59. Corren J, Lemanske RF, Hanania NA, et al. Lebrikizumab treatment in adults with asthma. *N Engl J Med* 2011;365:1088-98
60. Fitch N, Morton M, Bowden A, et al. Preclinical evaluation of BAY 16-9996 a dual IL-4/IL-13 receptor antagonist. *J Allergy Clin Immunol* 2001;107(Suppl):S331-9
61. Wenzel S, Wilbraham D, Fuller R, et al. Effect of an interleukin-4 variant on late phase asthmatic response to allergen challenge in asthmatic patients: results of two phase 2a studies. *Lancet* 2007;370(9596):1422-31
62. <http://clinicaltrials.gov/ct2/show/NCT00801853?term=Aerovant+AND+asthma&rank=1>
63. Hodges MR, Castelloe E, Chen A, et al. Randomized, double-blind, placebo controlled first in human study of inhaled AIR645, an IL-4Ralpha oligonucleotide, in healthy volunteers. *Am J Respir Crit Care Med* 2009;179:A3640
64. Corren J, Busse W, Meltzer EO, et al. A randomized, controlled, phase 2 study of AMG317, an IL-4Ralpha antagonist, in patients with asthma. *Am J Respir Crit Care Med* 2010;181(8):788-96
65. Russo C, Polosa R. TNF-alpha as a promising therapeutic target in chronic asthma: a lesson from rheumatoid arthritis. *Clin Sci (Lond)* 2005;109(2):135-42
66. Morjaria JB, Malerba M, Polosa R. Biologic and pharmacologic therapies in clinical development for the inflammatory response in COPD. *Drug Discov Today* 2010;15:396-405
67. Van Bogaert T, De Bosscher K, Libert C, et al. Crosstalk between TNF and glucocorticoid receptor signaling pathways. *Cytokine & Growth Factor Reviews* 2010;21:275-86
68. Howarth PH, Babu KS, Arshad HS, et al. Tumour Necrosis Factor (TNF) alpha as a novel therapeutic target in symptomatic corticosteroid-dependent asthma. *Thorax* 2005;60:1012-18
69. Berry MA, Hargadon B, Shelley M, et al. Evidence of a role of tumor necrosis factor alpha in refractory asthma. *N Engl J Med* 2006;354(7):697-708

70. Morjaria JB, Polosa R, Holgate ST, et al. The role of a soluble TNF-alpha receptor fusion protein (Etanercept) in corticosteroid-refractory asthma: a double blind, randomised placebo controlled trial. *Thorax* 2008;63(7):584-91
71. Holgate ST, Noonan M, Chanez P, et al. Efficacy and safety of etanercept in moderate-to-severe asthma: a randomised, controlled trial. *Eur Respir J* 2011;37:1352-9
72. Erin E, Leaker B, Nicholson G, et al. The effects of a monoclonal antibody directed against tumor necrosis factor-alpha in asthma. *Am J Respir Crit Care Med* 2006;174(7):753-62
73. Wenzel SE, Barnes PJ, Bleecker ER, et al. A randomized, double-blind, placebo-controlled study of tumor necrosis factor alpha blockade in severe persistent asthma. *Am J Respir Crit Care Med* 2009;179(7):549-58
74. Kon OM, Sihra BS, Loh LC, et al. The effects of an anti-CD4 monoclonal antibody, keliximab, on peripheral blood CD4+ T cells in asthma. *Eur Respir J* 2001;18(1):45-52
75. Kon OM, Sihra BS, Compton CH, et al. Randomised, dose-ranging, placebo-controlled study of chimeric antibody to CD4 (keliximab) in chronic severe asthma. *Lancet* 1998;352(9134):1109-13
76. Azzawi M, Johnston PW, Majumdar S, et al. T lymphocytes and activated eosinophils in airway mucosa in fatal asthma and cystic fibrosis. *Am Rev Respir Dis* 1992;145(6):1477-82
77. Busse WW, Israel E, Nelson HS, et al. Daclizumab improves asthma control in patients with moderate to severe persistent asthma: a randomized, controlled trial. *Am J Respir Crit Care Med* 2008;178(10):1002-8
78. Oh CK, Raible D, Geba G, et al. Biology of IL-9 pathway and its therapeutic potential for the treatment of asthma. *Inflamm. Allergy Drug Targets* 2011;10:180-6
79. <http://clinicaltrials.gov/ct2/show/NCT00968669>
80. Parker J, Brazinsky S, Miller DS, et al. Randomized, double-blind, placebo-controlled, multicenter Phase 2A study to evaluate the effect of a humanized IL-9 monoclonal antibody (MEDI-528) on exercise-induced bronchospasm. *Am J Respir Crit Care Med* 2010;181:5394
81. Wang YH, Liu YJ. OX40-OX40L interactions: a promising therapeutic target for allergic diseases? *J Clin Invest* 2007;117:3655-7
82. Hoshino A, Tanaka Y, Akiba H, et al. Critical role for OX40 ligand in the development of pathogenic Th2 cells in a murine model of asthma. *Eur J Immunol* 2003;33:861-9
83. <http://clinicaltrials.gov/ct2/show/NCT00983658>
84. Aggarwal S, Gurney AL. IL-17: prototype member of an emerging cytokine family. *J Leukoc Biol* 2002;71(1):1-8
85. <http://clinicaltrials.gov/ct2/show/NCT01199289?term=AMG827+AND+asthma&rank=1>
86. <http://clinicaltrials.gov/ct2/show/NCT01478360?term=AIN457+AND+asthma&rank=1>
87. Gauvreau GM, Hessel EM, Boulet L, et al. Immunostimulatory sequences regulate interferon-inducible genes but not allergic airway responses. *Am J Respir Crit Care Med* 2006;174:15-20
88. Cytos. Placebo-controlled phase II study shows CYT003-QbG10 is safe and efficacious for the treatment of allergic asthma. 2010. Available from: http://www.cytos.com/userfiles/file/Cytos_Press_E_100521.pdf

Affiliation

Massimo Caruso¹, Emanuele Crisafulli¹, Shirin Demma¹, Stephen Holgate² & Riccardo Polosa^{†3}
[†]Author for correspondence
¹University of Catania-AOU "Policlinico-V. Emanuele", Department of Clinical and Molecular Bio-Medicine – Institute of Internal Medicine and Clinical Immunology, Catania, 95124, Italy
²Division of Infection, Inflammation and Repair, University of Southampton, Southampton, UK
³Professor, University of Catania-AOU "Policlinico-V. Emanuele", Department of Clinical and Molecular Bio-Medicine – Institute of Internal Medicine and Clinical Immunology, Catania, 95124, Italy
E-mail: polosa@unict.it