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Personalized medicine with biologics for severe type 2 asthma: current status and future prospects

Marie Godar [a,b,c], Christophe Blanchetot[a], Hans de Haard[a], Bart N. Lambrecht [b,c,d,f,*], and Guy Brusselle [e,f,*]

[a]argenx BVBA, Zwijnaarde, Belgium ; [b]VIB-UGent Center for Inflammation Research, Ghent, Belgium ; [c]Department of Internal Medicine, Ghent University, Ghent, Belgium ; [d]Department of Pulmonary Medicine, ErasmusMC, Rotterdam, The Netherlands; [e]Department of Respiratory Medicine, Ghent University Hospital, Ghent, Belgium; [f]Department of Epidemiology and Respiratory Medicine, ErasmusMC, Rotterdam, The Netherlands

ABSTRACT

Asthma affects more than 300 million people worldwide and poses a large socioeconomic burden, particularly in the 5% to 10% of severe asthmatics. So far, each entry of new biologics in clinical trials has led to high expectations for treating all severe asthma forms, but the outcome has only been successful if the biologic, as add-on treatment, targeted specific patient subgroups. Indeed, we now realize that asthma is a heterogeneous disease with multiple phenotypes, based on distinct pathophysiological mechanisms, called endotypes. Thus, asthma therapy is gradually moving to a personalized medicine approach, tailored to individual's asthma endotypes identified through biomarkers. Here, we review the clinical efficacy of antibody-related therapeutics undergoing clinical trials, or those already approved, for the treatment of severe type 2 asthma. Biologics targeting type 2 cytokines have shown consistent efficacy, especially in patients with evidence of type 2 inflammation, suggesting that the future of asthma biologics is promising.

Introduction

Asthma is described in the 2016 Global Initiative for Asthma report as "a heterogeneous disease, usually characterized by chronic airway inflammation. It is defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough that vary over time and in their occurrence, frequency and intensity, together with variable expiratory airflow limitation".[1] To better understand its heterogeneity, which is highlighted by the variety of clinical presentations, physiologic characteristics, pathogenic pathways and outcomes, the concept of asthma phenotyping has emerged.

An asthma phenotype is defined by the International European Respiratory Society/American Thoracic Society guidelines, "as the composite, observable characteristics of an organism, resulting from interaction between its genetic make-up and environmental influences, which are relatively stable – but not invariable – with time".[2] Asthma phenotypes were initially focused on combinations of clinical, physiologic and hereditary characteristics, but they have evolved to link biology to phenotype.[3] Interestingly, these phenotypes are now evolving into asthma endotypes, wherein a specific biological pathway is identified that explains the observable properties of a phenotype, with the goal to improve therapy.[3] Endotypes would differ in terms of genetic susceptibility, environmental risk factors, age of onset, airway inflammation, clinical presentation, prognosis and response to standard and new therapies,

but definitive clustering of these characteristics or their relation to pathobiology remains uncertain (Supplementary Figure 1).[4]

With almost 1 in 8 children and 1 in 12 adults affected, asthma is one of the most common chronic diseases, resulting in up to 300 million people affected worldwide.[4] In many patients, the disease can be controlled by a combination of non-specific drugs, an inhaled corticosteroid (ICS) and a short- or long-acting β_2-adrenergic agonist (LABA). Nevertheless, in 5 to 10% of patients, the disease runs a severe course. In such cases, the patient will require treatment with high-dose ICS plus a second controller medication (such as LABAs or leukotriene receptor antagonists) or systemic steroids to prevent the disease from becoming 'uncontrolled', or the disease may remain 'uncontrolled' despite these treatments.[2] This loss of control manifests as frequent severe exacerbations that require systemic steroids or hospitalization. However, responses to these treatments can vary and do not modify the course of the disease, requiring an urgent need for new and more effective drugs to prevent the occurrence of potentially life-threatening episodes.

Biologics (i.e., drugs produced by living cells through biological processes, and mimic natural biological substances such as antibody-related therapeutics[5]) targeting specific inflammatory pathways have emerged as promising personalized medicines in the treatment of severe asthma. Their use is explained by the fact that the disease is characterized by inflammatory responses

involving multiple pathways and triggers. Increased levels of inflammatory molecules, cytokines in particular, have been identified in clinical samples and their role in disease pathogenesis and pathophysiology have been demonstrated in preclinical studies using asthma mouse models, leading to their extensive investigation as potential targets.[4] Consequently, there are currently a dozen biologics, mainly antibody-related therapeutics, in clinical studies for patients with moderate-to-severe asthma.

The economic and healthcare benefits of treating asthma are considerable. Most costs for asthma come from the severe asthmatics that require frequent hospital admissions due to exacerbations, which are often caused by virus infection. More specifically, the total cost of asthma has been estimated to be approximatively €17.7 billion in Europe, of which €9.8 billion is accounted for by the indirect costs of loss of productivity.[6] In the US, the annual costs amount to $18 billion.[7] Although the costs of biologics are expensive compared to current treatment options, effective biologics for a well-defined endotype may be cost effective in the long-term, by preventing hospital admissions due to severe asthma exacerbations and by reducing systemic side effects of ICS.[8]

In this review, we focus on the importance of a personalized medicine using a biomarker-driven approach, the immunological basis of type 2 and non-type 2 inflammations in asthma, the development of biologics that interrupt specific pathways involved in type 2 inflammation, the evidence of efficacy of these personalized treatments in recent clinical trials, their limitations, and the emergence of novel approaches.

Personalized medicine to treat severe asthma using a biomarker-driven approach

The increased use of biologics in clinical trials has facilitated the understanding of asthma heterogeneity and the subsequent development of asthma endotypes (Supplementary Figure 1). It underscored the importance of selecting the appropriate patient subsets with the correct target, dosing and mode of delivery during the clinical trials of these new biologics, by using the adapted outcome measures to the biological pathway(s) being targeted. From this comes the need for a personalized medicine using a biomarker-driven approach for development of biologics in severe asthma treatments (Supplementary Table 1).

One important tool in the development of personalized medicine is the application of biomarkers to stratify patients.[9] A biomarker is defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes or pharmacologic responses to a therapeutic intervention".[10] Targeted therapy benefits mostly from a biomarker that encompasses both high diagnostic, theragnostic (i.e., the ability to predict treatment effect) and prognostic capacities. Ideally, the biomarker might be the pathophysiological therapeutic target itself (i.e., the 'maker' of disease in a specific endotype).[11] Importantly, biomarkers are critical in the design and implementation of efficient and cost-effective clinical trials.[12]

An early asthma biomarker was the induced sputum cell count (eosinophils and neutrophils). Indeed, the presence of high sputum eosinophil count was found to be predictive of response to corticosteroid therapy.[13] Since then, asthma

biomarkers have expanded to include mainly blood eosinophil counts, total serum immunoglobulin E (IgE) levels, fraction of exhaled nitric oxide (FE_{NO}) in the exhaled air and serum periostin (Table 1).[14–17]

The technique of induced sputum cell count (eosinophils and neutrophils) has been pivotal in the emergence of the concept of asthma endotyping. Although it is technically demanding and time-consuming, several centers have applied this technique to characterize airway inflammation.[18] Based on sputum cell count analysis, in addition to clinical phenotyping (including allergen skin-prick tests and/or allergen-specific serum IgE) and type 2 biomarkers (Table 1), two groups of airway inflammations in asthma have been described: type 2 (allergic eosinophilic and nonallergic eosinophilic asthma) and non-type 2 (neutrophilic, paucigranulocytic and mixed granulocytic asthma).

Type 2 and non-type 2 airway inflammations in asthma

Type 2: allergic and non-allergic eosinophilic asthma

Most children and roughly 50% of adults have allergic eosinophilic asthma, in which the disease coincides with allergic sensitization (atopy) defined by the presence of serum IgE antibodies and/or a positive skin-prick test to the (lipo)proteins of common inhaled allergens such as Derp 1 from the house dust mite *Dermatophagoides pteronissinus*.[4,19] In contrast, nonallergic eosinophilic asthma often develops later in life (i.e., late onset asthma) and, per its definition, has neither IgE reactivity to allergens in the serum nor any obvious involvement of the adaptive immune system such as T-helper type 2 cells (T_H2) cells.[4] This form of the disease is often associated with chronic rhinosinusitis and nasal polyps, and is difficult to treat, often requiring long-term treatment with systemic steroids (Table 2).[4]

T_H2 cells and type 2 innate lymphoid cells (ILC2) are master drivers of type 2 immunity by expressing the transcription factor GATA3, which controls type 2 cytokine production (Figure 1).[4] Interleukin (IL)-4 and IL-13 are central cytokines to the type 2 inflammation induced by T_H2 cells and ILC2s, but also by other type 2 inflammatory cells such as eosinophils, alternatively activated macrophages, basophils and mast cells.[20] In chronic severe asthmatics, basophils are also a prominent source of IL-13 and IL-5, and might sustain the disease process in an IL-33-dependent manner (Figure 1).[21] IL-4 receptor alpha (IL-4Rα) is the common receptor subchain for IL-4 and IL-13, which can be dimerized with γc (expressed on cells restricted to hematopoietic cell lineages) or with IL-13Rα1 (expressed fairly ubiquitously, e.g., airway epithelial cells). IL-4 activates both type of receptors, whereas IL-13 only activates the receptor dimerized with IL-13Rα1. Thus, whilst both interleukins can promote IgE isotype switching in B cells, only IL-4 activates T_H2 effector cells (Figure 1).[22–24]

IL-13 signalling leads to the differentiation of bronchial epithelial cells into mucus-producing goblet cells and may potentiate airway smooth muscle cell contraction (Figure 1).[20] IL-4 is required for T_H2 priming and maturation, whereas T_H2 differentiation, promoted by the dendritic cells, is enhanced by cytokines made by epithelial cells, such as thymic stromal lymphoietin (TSLP), IL-33, IL-25 and granulocyte-macrophage colony-stimulating factor

Table 1. Asthma biomarkers used in clinical trials to predict the response to biologics directed at mediators of type 2 asthma.

Biomarker	Strengths	Weaknesses	Companion phenotyping for biologics
Induced sputum cell (eosinophils and neutrophils) analysis	• Correlates with: 　o Airway inflammation 　o Decreased FEV_1 　o Increased bronchial hyperresponsiveness 　o Exacerbation risk • Treatment responses (increased sputum eosinophil count significantly correlates with asthma severity)	• Difficult to obtain • Expensive • Technically demanding • Time consuming • Not widely available technique	• Good biomarker to adjust treatment with inhaled corticosteroids • Has been used to predict the response to anti-IL-5 (e.g., mepolizumab) in specific centers
Blood eosinophil count	• Correlates with airway inflammation • Inexpensive • Easy to obtain (in contrast to induced sputum eosinophil count) • Predictor of response to multiple type 2 targeting therapies	Reduced blood eosinophil counts in patients treated with oral corticosteroids (chronically or oral corticosteroids burst)	• Best predictive and responsive biomarker for anti-IL-5 (e.g., mepolizumab and reslizumab) and anti-IL-5Rα (e.g., benralizumab) • Readily available in clinical practice worldwide • Has been shown to predict the response to anti-IgE
Total serum IgE	• Correlates with airway inflammation • Inexpensive • Easy to obtain • Sensitive	Not specific for allergic asthma	Predictive biomarker for anti-IgE (e.g., omalizumab)
Exhaled nitric oxide	• Correlates with airway inflammation (higher levels of nitric oxide are released from epithelial cells of the bronchial wall) • Easy to obtain • Noninvasive measurement • Indicator of airway IL-13 activity: strongly correlated with the expression of NOS2 in asthmatic airway epithelial brushings (NOS2 is strongly induced in epithelial cells by IL-13)	• Expensive • Not widely available • Influenced by allergy, gender, smoking and inhaled corticosteroids	• Predictive biomarker for anti-IgE (e.g., omalizumab) • Predictive and responsive biomarker for anti-IL-13 (e.g., tralokinumab) and anti-IL-4Rα (e.g., dupilumab)
Serum periostin	• Correlates with airway inflammation (accelerates allergen-induced eosinophil recruitment in the lung and esophagus) • Accurate measurement in serum	• Expensive • Not readily available • Weak association with airway periostin level	Has been used as: • predictive biomarker for anti-IgE (e.g., omalizumab) • predictive and responsive biomarker for anti-IL-13 (e.g., tralokinumab) and anti-IL-4Rα (e.g., dupilumab)

FEV_1: forced expiratory volume in 1 second; IL: interleukin; IgE: immunoglobulin E; IL-4Rα: interleukin-4 receptor alpha; IL-5Rα: interleukin-5 receptor alpha; NOS2: nitric oxide synthase.

Table 2. Characteristics of type 2 and non-type 2 airway inflammations in asthma.

Asthma	Atopy	Airway inflammation	Sputum-based cellular analysis	Natural history	Clinical and physiological features	Pathobiology and biomarkers	Response to therapy	Comorbidities
Type 2	Atopic	Early-onset allergic eosinophilic asthma	≥3% sputum eosinophils and <76% sputum neutrophils	• Early age of onset • Most patients have mild-to-moderate asthma; rarely severe since childhood or deterioration in adulthood	• Allergic symptoms/recurrent exacerbations/sensitization/atopy • Increased blood eosinophil count, sputum eosinophils, IgE and high or normal FE$_{NO}$ • Strong family history (related genetic factors)	• Specific IgE • T$_H$2 cytokines • FE$_{NO}$ • T$_H$2-related genes (17q12) • Subbasement membrane thickness • Sputum and blood eosinophil counts (≥300 cells per μL)	Responsive to inhaled corticosteroids and to anti-IgE	• Allergic rhinitis • Allergic dermatitis
	Nonatopic	Late-onset nonallergic eosinophilic asthma	<3% sputum eosinophils and <76% sputum neutrophils	• Adult age of onset • Severe from onset	• Sinusitis • Less allergic • Recurrent exacerbations • High FE$_{NO}$ and serum IgE • Reduced pulmonary function despite shorter disease duration	• Corticosteroid-refractory eosinophilia • IL-5 • FE$_{NO}$ • Sputum and blood eosinophil counts (≥300 cells per μL)	• Relatively corticosteroid-refractory or requires higher doses/oral corticosteroids • Responsive to anti-IL-4/IL-13 and anti-IL-4Rα	• Chronic rhinosinusitis • Nasal polyps
Non-type 2	Nonatopic	Neutrophilic	<3% sputum eosinophils and ≥76% sputum neutrophils	Adult age of onset	• Low FEV$_1$ • More air trapping • Bacterial infection • Increased sputum neutrophil count • Severe airway obstruction	• T$_H$17 pathways • IL-8 • Sputum neutrophil count	• Corticosteroid insensitive (steroids can enhance airway neutrophilia by inhibiting neutrophil apoptosis and by promoting neutrophil activation) • Responsive to anti-LTB4 (adults and children) and macrolides (adults and children)	• Respiratory infections • Obesity • Smoking • Air pollution
	Nonatopic	Paucigranulocytic	<3% sputum eosinophils and <76% sputum neutrophils	Adult age of onset	?	?	?	
	Nonatopic	Mixed granulocytic	≥3% sputum eosinophils and ≥76% sputum neutrophils	• Adult age of onset • Severe from onset	• High FE$_{NO}$ • Granulomas • Increased sputum eosinophils and neutrophils	• FE$_{NO}$ • Sputum neutrophil and eosinophil counts	?	

Type 2 airway inflammation in asthma consists of both early- and later-onset diseases over a range of severities. It is likely that most of early-onset allergic asthma is mild, but that an increasing complexity of immune processes leads to greater severity. Additionally, later-onset nonallergic eosinophilic asthma without traditional allergic elements is more likely to be severe. Non-type 2 airway inflammation in asthma consists of later-onset diseases including neutrophilic, paucigranulocytic and mixed granulocytic asthma. Note that the sputum-based cellular analysis presented here was defined by Demarche et al., 2016.[18] FE$_{NO}$: fractional exhaled nitric oxide; FEV$_1$: forced expiratory volume in 1 second; IL: interleukin; IgE: immunoglobulin E; LTB4: leukotriene B4; T$_H$2: T-helper type 2 cell; T$_H$17: T-helper type 17 cell.
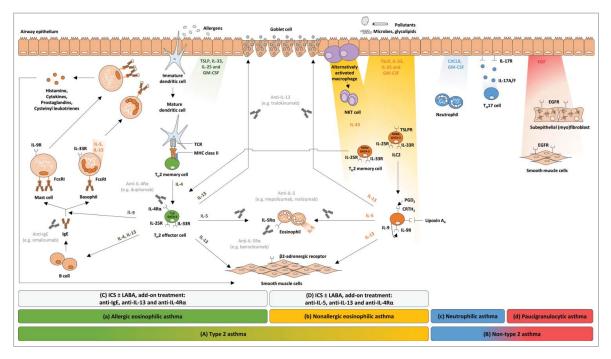
Figure 1. Simplified schematic representation of four different types of airway inflammation in asthmatic patients. (A) Type 2 consists of allergic and nonallergic eosinophilic asthma. (a) In allergic eosinophilic asthma, T-helper type 2 (T_H2) cell lymphocytes and mast cells drive eosinophilic airway inflammation in an allergen-specific, immunoglobulin E (IgE)-dependent manner. (b) In nonallergic eosinophilic asthma, innate lymphocytes such as natural killer T cells (NKT cells) and innate lymphoid cells type 2 (ILC2) cells might contribute to airway eosinophilia via the production of interleukin (IL)-5, in response to pollutants or infectious agents. (B) Non-type 2 consists of neutrophilic and paucigranulocytic asthma. (c) The mechanisms underlying neutrophilic asthma need to be elucidated, but the IL-17 pathway and CXCL8 have been associated with the airway neutrophilia. More precisely, IL-17A and IL-17F play important roles in host responses to extracellular pathogens via the upregulation of antimicrobial proteins and induction of cytokines and chemokines involved in neutrophil expansion (e.g., GM-CSF) and recruitment (e.g., CXCR ligands). (d) Paucigranulocytic asthma has been poorly studied. It is thought to be not inflammatory and is characterized by the absence of increased numbers of inflammatory cells, suggesting the involvement of non-inflammatory mechanisms mediated by airway remodeling responses that lead to extensive airway narrowing. The biologics being evaluated in clinical trials or already approved as add-on treatment, on top of high-doses inhaled corticosteroid (ICS) and a short- or long-acting β_2-adrenergic agonist (LABA), for (C) allergic and (D) nonallergic eosinophilic asthma are depicted in light grey. $CRTH_2$: prostaglandin D2 receptor 2; CXCL8: C-X-C motif chemokine ligand 8; CXCR: C-X-C chemokine receptor; EGF: epidermal growth factor; EGFR: epidermal growth factor receptor; FcεRI: Fc epsilon receptor I; GM-CSF: granulocyte-macrophage colony-stimulating factor; ICS: inhaled corticosteroid; IgE: immunoglobulin epsilon; ILC2: innate lymphoid cell type 2; IL: interleukin; IL-4Rα: interleukin-4 receptor alpha; IL-5Rα: interleukin-5 receptor alpha; IL-9R: interleukin-9 receptor; IL-25R: interleukin-25 receptor; IL-33R: interleukin-33 receptor; LABA: long-acting β_2-adrenergic; MHC: major histocompatibility complex; NKT: natural killer T; PGD_2: prostaglandin D2; TCR: T-cell receptor; T_H2: T-helper type 2 cell; T_H17: T-helper type 17 cell; TSLP: thymic stromal lymphoietin; TSLPR: thymic stromal lymphoietin receptor.

(GM-CSF).[25] Those cytokines also exert an important tissue checkpoint for full activation of primed T_H2 cells and ILC2s that enter target organs such as the lungs (Figure 1).[26] It is also now clear that T_H2 memory cells can secrete cytokines even in the absence of T-cell receptor ligation in nonallergic eosinophilic asthma, and could react to non-specific stimuli imposed on the airway epithelium (Figure 1).[26,27]

IgE and IL-5 are two other important proteins in type 2 asthma. IgE, produced by B cells, binds to the type I high affinity IgE receptor (FcεRI), which activates mast cells and basophils associated with IgE-mediated hypersensitivity when cross-linked by allergen (Figure 1).[4] IL-5 is produced, like IL-13, by multiple cell types implicated in severe asthma, including T_H2 cells, ILC2s, eosinophils and basophils. IL-5 binds to IL-5 receptor complex principally expressed on eosinophils, and is involved in the differentiation and maturation of eosinophils in the bone marrow and in their survival and migration to tissues (Figure 1).[4]

Non-type 2: noneosinophilic asthma

Although asthma is classically associated with eosinophilia and type 2 cytokines, some asthmatic patients show a neutrophil-predominant disease with an absence of T_H2 cytokines and their downstream signatures.[28–30] These non-type 2 asthma patients have generally adult-onset disease, and are less likely to be atopic (Table 2).[31–34] The underlying causes and triggers are not well understood, but are heterogeneous and might encompass obesity, respiratory infections, smoking and air pollution. Some patients with non-type 2 asthma seem to have neutrophilic inflammation with less severe reversible airway obstruction and a T_H17 cytokine milieu (Figure 1).[35–37] The cytokine production by T_H17 cells and other IL-17-producing cells is resistant to inhibition by corticosteroids, which explains why neutrophil-rich inflammation driven by IL-17 is the pathological correlate of a subgroup of patients with steroid-resistant asthma (Table 2).[4] Additionally, other patients have mixed granulocytic asthma when both eosinophils and neutrophils are increased or paucigranulocytic asthma when both these inflammatory cells are below the thresholds (Table 2 and Figure 1).[18,38] These non-type 2 asthma groups remain poorly defined, clinically heterogeneous and without specific biomarkers, making molecular endotyping and targeted therapy approaches difficult.

Importantly, the identification of the type 2 and non-type 2 airway inflammations has fostered the concept of targeted biologics and patient's stratification, introducing personalized medicine in severe asthma treatment.

Type 2 biologic approaches

Immunoglobulin E

Omalizumab is a monoclonal IgG1κ antibody with a human framework and complementarity-determining regions from a humanized anti-IgE murine antibody (MAE11), produced with hybridoma technology (Table 3 and Figure 1).[39] Omalizumab inhibits IgE effector functions by blocking IgE binding FcεRI on mast cells, but does not cause mast cell activation because it cannot bind to IgE on cell surfaces where the FcεRI receptor already masks the anti-IgE epitope (Figure 1).[40] With prolonged treatment, omalizumab also reduces the expression of FcεRI on mast cells and basophils.[41] Omalizumab became the first monoclonal antibody approved by the Food and Drug Administration (FDA, 2003) and European Medicines Agency (EMA, 2005) to treat asthma patients of 12 years and older (Table 3).

Nevertheless, the use of omalizumab has been limited by its expense (US wholesale prices for omalizumab average almost $1300 per patient-month for an asthmatic patient who weighs less than 90 kg[42]), the need for multiple injections that may then lead to injection-site reactions, a black box warning on anaphylaxis, and new warnings on cardiovascular risk.[43] Thus, an improved ability to predict responsive patients with high certainty was important, requiring new clinical trials or prospective observational cohort studies (e.g., registries). Although omalizumab has not been prospectively studied in patients identified based on other type 2 biomarkers, a retrospective analysis of the Phase 3 study by Hanania et al. (EXTRA trial)[44] divided patients into those with and without type 2 inflammation based on median splits of blood eosinophil counts, serum periostin and FE$_{NO}$ levels (Supplementary Table 2). Patients with biomarker levels greater than the median had greater reductions in asthma exacerbations with omalizumab therapy compared with those with levels lower than the median (Supplementary Table 2).[45] Although no other outcomes were affected, this approach should be prospectively validated.

Continuation of omalizumab after 5-year treatment resulted in continued benefit, as evidenced by improved symptom control and reduced exacerbation risk in the XPORT trial (Supplementary Table 2).[46] Approximately half of the patients remained free of exacerbations during the one-year study period despite withdrawal of omalizumab. Although this study suggested that omalizumab might have beneficial disease-modifying effects, this positive outcome could also be due to the natural history of the disease (e.g., spontaneous evolution from severe asthma towards mild-moderate asthma in a subgroup of patients after about 5 years or improvement in asthma control thanks to removal from persistent allergen exposure such as domestic animals, e.g., cats and dogs).

In 2016, FDA and EMA approved an expanded age range for omalizumab to include children six to 11 years of age with moderate-to-severe persistent asthma, having a positive skin test or in vitro reactivity to an airborne allergen and symptoms that are inadequately controlled with ICS (Table 3 and Supplementary Table 2). This approval followed successful pediatric clinical trials such as one conducted by Lanier et al. 2009 (Supplementary Table 2).[47] Nevertheless, to date, strong biomarkers to identify responders are still lacking. These issues, as well as further cost-effectiveness analyses, are still open and need to be investigated in future pediatric studies.

Interleukin-13

Lebrikizumab is a humanized IgG4κ antibody that binds IL-13 with high affinity at an epitope that strongly overlaps with the binding site of IL-4Rα and inhibits its activity (Table 3 and Figure 1).[48] In previous studies, lebrikizumab treatment was associated with significantly and substantially decreased serum periostin, FE$_{NO}$ and serum IgE, and modestly increased peripheral blood eosinophil count.[17,49] Notably, the extent of the pharmacodynamic effect was greater in subjects who had high periostin levels at baseline.[17] Nevertheless, lebrikizumab did not consistently show significant reduction in asthma exacerbations in biomarker-high patients (periostin $\geq$50 ng/mL or blood eosinophils $\geq$300 cells per μL) in more recent replicate Phase 3 studies (LAVOLTA I and II),[50] leading to the discontinuation of this biologic in asthma (Supplementary Table 2). The discontinuation could be due to the non-optimal patient selection through the use of serum periostin, which has only a weak association with airway periostin level and is therefore not the best biomarker for IL-13 activity in the airways. In contrast, FE$_{NO}$ is a good biomarker of IL-13 activity in the airway and is responsive to treatment with anti-IL-13 antibodies (Table 1).

Fourteen weeks of treatment with tralokinumab, another human monoclonal IgG4λ antibody targeting IL-13 (Table 3 and Figure 1), was associated with only modest improvement in FEV$_1$ and some decrease in β2-agonist use (Supplementary Table 2).[51] In a large Phase 2 study, tralokinumab did not significantly reduce asthma exacerbation rates in patients with severe uncontrolled asthma. Improvement in FEV$_1$ with tralokinumab given every 2 weeks and results of post-hoc subgroup analyses suggested a possible treatment benefit in a defined population of patients with severe uncontrolled asthma (Supplementary Table 2). This effect is being further investigated in ongoing Phase 3 trials, along with the potential utility of periostin and dipeptidyl peptidase-4 (DPP-4, a gene whose expression is induced by IL-13) as biomarkers of interleukin-13 pathway activation.[52] Nevertheless, tralokinumab did not meet the primary endpoint of a significant reduction in the annual asthma exacerbation rate in the overall population of severe, uncontrolled asthma patients, compared with placebo in STRATOS I, the first of two pivotal Phase 3 trials (Supplementary Table 2). Nevertheless, in a planned analysis, a clinically-relevant reduction in annual asthma exacerbation rate was observed in a subpopulation of patients with an elevated biomarker associated with increased IL-13 activity.[53] Thus, this sub-group of patients will now be the focus for the future analysis of STRATOS II, the second ongoing pivotal Phase 3 trial. Indeed, STRATOS I explored the potential use of biomarkers to identify patients with an enhanced response to tralokinumab, whereas STRATOS II is designed to validate the biomarker population identified in STRATOS I. Overall, these recently published clinical trials on therapeutic antibodies targeting IL-13, have highlighted relevant limitations with partial effects that could be due to overlapping biological actions of IL-4 and IL-13. The combination approach inhibiting both IL-4 and IL-13 is likely to be more effective.

Table 3. Biologics evaluated for the treatment of moderate-to-severe type 2 asthma.

Target	Cell(s) targeted	International non-proprietary, proprietary and common name	Format, species and development technology	Company	Mechanism	Developmental and regulatory approval status
IgE	• Mast cells • Basophils	• Omalizumab • XOLAIR® • rhuMab-E25	• IgG1κ • Humanized • Hybridoma technology	• Genentech • Novartis	Targets the Cε3 domain of IgE	Phase 4: • FDA (June 20, 2003) and EMA (25 October 2005) approvals as add-on therapy to treat moderate-to-severe persistent allergic asthma, having a positive skin test or *in vitro* reactivity to an airborne allergen and symptoms that are inadequately controlled with inhaled corticosteroids, in patients 12 years and older. • EMA (January 23, 2014) and FDA (July 07, 2016) approvals in children six to 11 years of age.
IL–13	• Structural cells • Macrophages • B cells	• Lebrikizumab • MILR1444A/RG 3637	• IgG4κ • Humanized	• Chugai Pharmaceutical • Genentech • Roche • Tanox	Targets specifically IL-13	Phase 3, discontinued
		• Tralokinumab • CAT-354	• IgG4λ • Homo sapiens • Cambridge Antibody Technology	• Astrazeneca • LEO Pharma • MedImmune	Targets specifically IL-13	Phase 3
IL–4Rα/ IL–4	• Structural cells • T cells • Macrophages • B cells	• Dupilumab • DUPIXENT® • REGN668/SAR23 1893	• IgG4κ • Homo sapiens • VelocImmune®	• Regeneron Pharmaceuticals • Sanofi	Targets specifically IL-4Rα, inhibiting IL-4 and IL-13 signaling pathways	Phase 3
		• Pitrakinra • AEROVANT® • AER 001	15-kDa recombinant human IL-4 variant	• Aerovance • Bayer	Inhibits binding IL-4 and/or IL-13 to IL-4Rα	Phase 3, discontinued
		• Altrakincept • NUVANCE®	54-kDa soluble recombinant extracellular portion of the human IL-4Rα	• Amgen	Targets specifically and inactivates IL-4 without mediating cellular activation	Phase 2, discontinued
		• AMG 317	• IgG2κ • Homo sapiens	• Amgen	Targets specifically IL-4Rα, inhibiting IL-4 and IL-13 signaling pathways	Phase 2, discontinued
IL–5	Eosinophils	• Mepolizumab • NUCALA® • SB-240563	• IgG1κ • Humanized	• GlaxoSmithKline	Targets specifically IL-5	Phase 4: FDA (November 04, 2015) and EMA (02 December 2015) approvals as an add-on maintenance treatment of patients with severe asthma aged 12 years and older, with an eosinophilic phenotype
		• Reslizumab • CINQAIR® (US)/ CINQAERO® (EU) • JES1-39D10	• IgG4κ • Humanized	• UCB Celltech • Schering-Plough • Teva Pharmaceuticals	Targets specifically IL-5	Phase 4: FDA (CINQAIR®, 23 March 2016) and EMA (CINQARO®, 23 June 2016) approvals for use with other asthma medicines for the maintenance treatment of severe eosinophilic asthma in patients aged 18 years and older
IL–5Rα	• Eosinophils • Basophils	• Benralizumab • KHK4563, MEDI-563	• Afucosylated IgG1κ • Humanized • POTELLIGENT®	• Astrazeneca • MedImmune • Kyowa Hakko Kirin	Binds and induces depletion of IL-5Rα-expressing target cells by antibody-mediated cellular toxicity	Phase 3

The hybridoma technology corresponds to the process of fusion between different somatic cells to produce hybrid cells in which there is often one fused nucleus. The core focus of the Cambridge Antibody Technology was partly on phage and ribosome display technologies, allowing the discovery of tralokinumab. VelocImmune® is the technology developed by Regeneron Pharmaceuticals, for producing fully human monoclonal antibodies from immunized humanized mice. BioWa, Inc., a member of the Kyowa Hakko Kirin Group, is the exclusive licensor of the POTELLIGENT® technology, which enables monoclonal antibodies to be manufactured in 100% fucose-free form, resulting in significant enhancement of antibody-dependent cellular cytotoxicity and tumor cell-killing activity. EMA: European medicines agency; FDA: food drug administration; IgE: immunoglobulin epsilon; IgG: immunoglobulin gamma; IL: interleukin; IL-4Rα: interleukin-4 receptor alpha; IL-5Rα: interleukin-5 receptor alpha.

Interleukin-4 receptor alpha

Dupilumab, a human monoclonal IgG4κ antibody targeting IL-4Rα and inhibiting both IL-4 and IL-13 signalling pathways, increased lung function and reduced severe exacerbations in patients with uncontrolled persistent asthma irrespective of baseline eosinophil count (Table 3, Figure 1 and Supplementary Table 2).[54] Dupilumab is currently being studied in Phase 3 studies and the results are expected end of 2017 (Supplementary Table 2). Dupilumab is FDA-approved for atopic dermatitis and a proof-of-concept study in nasal polyposis was clearly positive.[55] Altogether, these results implicate an added benefit for treatment with dupilumab in patients with severe asthma and co-morbidities such as atopic dermatitis (e.g., in younger patients) or nasal polyposis (e.g., in late-onset asthmatics). Notably, three other approaches (a recombinant human IL-4 variant, a recombinant extracellular portion of the human IL-4Rα, and an antibody) targeting IL-4Rα were discontinued due to lack of efficacy (Table 3).

Interleukin-5

The importance of targeting the appropriate patient subsets was highlighted by the clinical development of treatments targeting IL-5. These treatments did not significantly improve lung function in an unselected population whilst they improved asthma control and reduced exacerbations in selected patients with severe asthma exhibiting an eosinophilic phenotype.[56,57] The importance of IL-5 in driving the persistent systemic and airway eosinophilic inflammation has been demonstrated by the efficacy of mepolizumab, a humanized monoclonal IgG1κ anti-IL-5 antibody approved by the FDA and EMA (Table 3 and Figure 1), to further decrease eosinophilic inflammation in those patients with refractory asthma despite high dose of inhaled or oral corticosteroids.[58,59] The clinical relevance of the persistent eosinophilic inflammation was demonstrated by the reduction in exacerbation rate and the improvement in quality of life observed in severe eosinophilic asthma patients receiving mepolizumab, even if only a modest effect was observed on FEV_1.[58] The DREAM study, a large intravenous mepolizumab trial in patients with severe asthma receiving high-dose ICS/LABA treatment with evidence of eosinophilic/type 2-high inflammation (≥1 in the prior year: sputum eosinophils >3%, >300 eosinophils per μL peripheral blood, or FE_{NO} >50 ppb) identified blood eosinophil counts of 300 cells per μL or greater as a highly predictive biomarker of treatment response (Supplementary Table 2).[56] Three different doses of mepolizumab (75, 250 or 750 mg at 4-week intervals) were equally effective in decreasing clinically significant asthma exacerbations compared with placebo, with the greatest reductions seen in those with the highest blood eosinophil counts and greatest prior exacerbation history. No effect on other asthma outcomes were observed, including symptoms and FEV_1 due to impressive placebo effects on patient-reported outcomes.[56] Similarly, in the MENSA trial, a large Phase 3 study of patients with eosinophilic asthma (based on peripheral blood eosinophilia) with recurrent exacerbations despite high-dose ICS (monthly 75 mg-intravenous or 100 mg-subcutaneous), mepolizumab significantly decreased exacerbations by 47% to 53%, increased

FEV_1 and modestly affected symptoms and asthma control scores compared with placebo (Supplementary Table 2).[60]

Like mepolizumab, reslizumab is another humanized monoclonal IgG4κ anti-IL-5 antibody approved by the FDA and EMA (Table 3 and Figure 1). Two recent duplicate trials compared reslizumab (3 mg/kg dose, intravenous administration) to placebo in patients with poorly controlled asthma using medium-to-high doses ICS, a blood eosinophil count of ≥400 cells per μL, and at least one severe asthma exacerbation in the previous year.[61] In both Phase 3 studies, patients receiving reslizumab had a significant reduction in the frequency of asthma exacerbations compared with those receiving placebo[61] (Supplementary Table 2), reinforcing the role of eosinophils in several asthma outcomes.

Interleukin-5 receptor alpha

Benralizumab, a humanized afucosylated monoclonal IgG1κ antibody that binds to IL-5Rα, blocks IL-5 receptor signalling and induces antibody-directed cell-mediated cytotoxicity leading to depletion of IL-5Rα-expressing target cells (eosinophils and basophils, Table 3 and Figure 1).[62] In the recent Phase 3 SIROCCO trial, the results confirm the efficacy and safety of benralizumab for patients with severe asthma and elevated blood eosinophils, which are uncontrolled by high-dosage ICS plus LABA, and provide support for benralizumab as an additional option to treat this disease in this patient population (Supplementary Table 2).[63] In another recent Phase 3 study (CALIMA trial, Supplementary Table 2), benralizumab significantly reduced annual exacerbation rates and was generally well tolerated in patients with severe, uncontrolled asthma with blood eosinophils 300 cells per μL or greater.[64]

Overall, approaches targeting the IL-5 pathway have been efficacious, with prominent effects on exacerbations in adult patients with uncontrolled severe eosinophilic asthma. Nevertheless, it remains unclear whether approaches to block the receptor IL-5Rα and opsonize eosinophils, will differ in efficacy, safety or target endotype from those targeting the ligand IL-5.[43] However, the optimal dosing interval between injections appears to be different and advantageous for benralizumab (8 weeks) versus for mepolizumab and reslizumab (4 weeks) (Supplementary Table 2).

Limitations of monotherapies: emergence of combination therapies for severe asthma?

Translation of discoveries into treatments is challenging, time-consuming and expensive as illustrated by the 10-year gap between the development of omalizumab, the first biologic for the treatment of severe allergic asthma, and its approval by FDA in 2003.[12] Generally, only about 1 in 10 therapeutic candidates that enter Phase 1 trials becomes a marketed product, and this high failure rate contributes substantially to the high costs of drug development.[65]

Implicit in this observation is the fact that preclinical models do not easily or perfectly simulate the vast heterogeneity of the human disease, and that correlation of pathophysiology with clinically measurable and meaningful outcomes such as symptoms, asthma control and exacerbations, is difficult.[66] Indeed,

although the testing of biologics might begin in animal models, the immunologic response in human is more complex and heterogeneous, encompassing multiple endotypes and regulated by distinct biological pathways, implicating that asthma is a syndrome rather than a single disease (Supplementary Figure 1).[32,67] Moreover, it is known that endotypes may somewhat vary over time[29,68] and also overlap, because there is currently no clear demarcation between these groupings. Therefore, patients may exhibit clinical or pathologic features of multiple groups, emphasizing the limitations in the current understanding of endotypes and the difficulty to use them routinely in clinical practice at this stage.[13] Consequently, the concept of treating severe asthma by targeting a single molecule has had limited success.

ICS, which is currently the mainstay therapy, is thought to act by suppressing a range of pro-inflammatory pathways.[69,70] However, long-term use of high-dose ICS therapy has potential to cause systemic side effects, such as impaired growth in children, decreased bone mineral density, skin thinning and bruising, and cataracts.[71] The emergence of the heterogeneity and the different endotypes of the disease processes where many different inflammatory mediators, cytokines and cells are dysregulated further highlights that new approaches are required to treat asthma effectively. Many studies show that airway inflammation, remodelling and airway hyperresponsiveness are dissociated and may be mediated by different mechanisms under different conditions.[72] Thus, combination therapies, targeting reciprocally regulated inflammatory and potentiating pathways in asthma, may be a more effective therapeutic approach for severe asthma.

Interestingly, in a cross-sectional study of asthmatics of varying severity (n = 51), gene expression of endobronchial tissue analysis revealed three 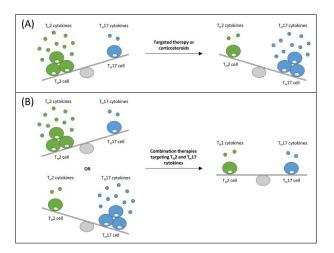major patient clusters: type 2, type 17, and type 2/type 17-low.[73] Type 2 and type 17 patterns were mutually exclusive in individual patient samples, and their gene signatures were inversely correlated and differentially regulated by IL-13 and IL-17A. The dichotomous pattern of T_H2 and T_H17 signatures was explored in a preclinical model of allergen-induced asthma, which showed that type 2 cytokine suppression promoted T_H17 responses. Neutralization of IL-4 or IL-13 resulted in increased T_H17 cells and neutrophilic inflammation in the lung. However, neutralization of IL-13 and IL-17 protected mice from eosinophilia, mucus hyperplasia, airway hyperreactivity and abolished the neutrophilic inflammation, which suggests that combination therapies targeting both pathways may maximize therapeutic efficacy across a patient population comprising both type 2 and type 17 endotypes (Figure 2).[73]

Conclusion

The treatment of severe asthma in both adults and children still relies heavily on the use of high-dose ICS plus a second controller medication (such as LABAs or leukotriene receptor antagonists), or systemic steroids.[4] However, responses to these treatments can vary and do not modify the course of the disease, requiring an urgent need for new and more effective drugs to prevent the occurrence of potentially life-threatening episodes.

The addition of omalizumab as the first targeted biologic approved for asthma treatment has led to renewed optimism of improvements in outcomes in patients with type 2 severe asthma. Biologic approaches targeting type 2 inflammation have since emerged as promising new personalized medicines in patients with evidence of type 2 inflammation based on specific biomarker profiles (Table 1 and Figure 1). Indeed, whereas the monoclonal anti-IL-5 antibodies mepolizumab and reslizumab were not beneficial in unselected adult patients with moderate asthma, they decreased asthma exacerbations and improved symptoms and lung function in severe asthma patients with persistent sputum eosinophil counts or increased blood eosinophil levels (Supplementary Table 2). The first clinical trials using type 2-targeting biologics have highlighted the importance of determining the optimal biomarkers necessary to identify and understand which endotypes are responsive to specific biologics to identify which patients will benefit from which biologics at which optimal dose (Supplementary Table 1), thereby allowing a better prediction of responses to biologics. Progress in this field will not only allow better diagnosis and targeted treatment, but will also provide feedback on the fundamental research questions that need to be addressed. Interestingly, there is a potential for other biologics to provide benefit in treatment of severe asthma, such as anti-TSLP or anti-IL-33 monoclonal antibodies,[4] especially if the key elements for successful biologic development are applied (Supplementary Table 1), such as the identification of the endotypes who will respond to these biologics identified through biomarkers (Supplementary Figure 1).

Nevertheless, predicting response to therapy remains problematic. Because the results of the clinical trials summarized in Supplementary Table 2 varied even for biologics directed toward the same signalling pathway, it is appreciated that the

Figure 2. Simplified schematic representation of targeted T_H2 and T_H17 cytokines therapies that can lead to amplification of activity of the opposing pathway in a murine house dust mite model of asthma.[73] (A) With suppression of T_H2 activity by targeted therapy or corticosteroids, a T_H17-permissive environment exists. A direct relationship between T_H17 and T_H2 disease exists, whereby, through mutual cross regulation, T_H17 asthma may represent a transition or switch away from T_H2-mediated disease. Thus, by treating T_H2 patients with corticosteroids, T_H17 asthma may have been promoted. (B) Combination therapy targeting T_H2 cytokine, such as interleukin-13, and T_H17 cytokine, such as interleukin-17, in patients expressing either a T_H2 or T_H17 signature may provide additional efficacy over single T_H2 or T_H17 inhibition. T_H2: T-helper type 2 cell; T_H17: T-helper type 17 cell.

response to a biologic can be confounded by multiple factors, including treatment duration, dose, asthma severity and endotype and differing outcome measures assessed.[43] Moreover, much remains to be understood regarding their long-term efficacy and safety, their comparative therapeutic efficacy, and, finally, their cost-effectiveness. Additionally, with only omalizumab approved for children of six to 11 years of age (Table 3), accurate biomarkers to identify responders are still currently lacking and cost-utility analyses need to be investigated for pediatric studies. Otherwise, development of biologics in severe asthma patients lacking type 2 biomarkers remains in its infancy and will require greatly improved molecular understanding of their underlying pathologies.

It is known that endotypes may somewhat vary over time[29,68] and also overlap, because there is currently no clear demarcation between these groupings. Consequently, the concept of treating severe asthma by targeting a single molecule has been successful only in highly selected patient subgroups. Combination therapies, targeting reciprocally regulated inflammatory and potentiating pathways in asthma, may be a more effective therapeutic approach for a broader population of patients with severe asthma.

Disclosure of potential conflicts of interest

C.B and H.D.H. are employees at argenx. G.B. has, within the last 5 years, received honoraria for lectures from AstraZeneca, Boehringer-Ingelheim, Chiesi, GlaxoSmithKline, Novartis, Pfizer, Teva, UCB and Zambon; he is a member of advisory boards for AstraZeneca, Boehringer-Ingelheim, GlaxoSmithKline, Novartis, Sanofi/Regeneron and Teva. The other authors disclosed no potential conflicts of interest.

Author contributions

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Funding

ORCID

Marie Godar http://orcid.org/0000-0002-5477-7726
Bart N. Lambrecht http://orcid.org/0000-0003-4376-6834
Guy Brusselle http://orcid.org/0000-0001-7021-8505

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