Mepolizumab for severe refractory eosinophilic asthma: evidence to date and clinical potential

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Abstract: Severe asthma is characterized by major impairment of quality of life, poor symptom control and frequent exacerbations. Inflammatory, clinical and causative factors identify different phenotypes and endotypes of asthma. In the last few years, new treatment options have allowed for targeted treatments according to the different phenotypes of the disease. To accurately select a specific treatment for each asthmatic variant, the identification of appropriate biomarkers is required. Eosinophilic asthma is a distinct phenotype characterized by thickening of the basement membrane and corticosteroid responsiveness. This review reports the latest evidence on an anti-IL-5 monoclonal antibody, mepolizumab, a new and promising biological agent recently approved by the FDA specifically for the treatment of severe eosinophilic refractory asthma.

Keywords: asthma, costs, effectiveness, IL-5, mepolizumab, phenotype

Introduction

According to recent data, the number of people with asthma around the world has increased from 235 to 334 million between 2011 and 2014, with about 180,000 deaths every year [Global Asthma Network, 2014]. Approximately 20% of these patients are affected by severe persistent asthma, of which one case out of five is inadequately controlled. Patients with uncontrolled severe asthma have a high risk of exacerbations, hospitalization and death [Peters *et al.* 2006].

Severe asthma is associated with high airway hyperresponsiveness and a variable, usually reversible airflow obstruction; from a clinical point of view, it is characterized by major impairment of quality of life, recurrent symptoms such as breathlessness, chest tightness, wheezing and cough. Airways inflammation is the most important pathophysiological mechanism of asthma and inflammatory, clinical and causative factors characterize different phenotypes and endotypes of asthma [Wenzel, 2012]. Many asthmatic subjects develop irreversible or partially reversible airflow obstruction and experience an accelerated

rate of lung function decline, mostly in absence of adequate therapy. In these patients, all the components of the bronchial wall undergo irreversible structural changes defined as 'airway remodeling', with an increased thickness compared with normal airways. Remodeling is present in subphenotypes with irreversible airflow obstruction and high airway hyperresponsiveness, which are often associated with increased disease severity [Al-Muhsen *et al.* 2011; Homer and Elias, 2005]. Recent studies have shown that an extended treatment with omalizumab significantly reduced airway wall and reticular basement membrane thickness, independently of eosinophilic infiltration [Hoshino and Ohtawa, 2012; Riccio *et al.* 2012].

Studies on large series of patients with well-characterized asthma have demonstrated that clinical and pathological features correlate either with eosinophilic or neutrophilic inflammation [Fahy, 2009]. On the basis of induced sputum, two other inflammatory phenotypes of asthma have been identified, that is, granulocytic and paucigranulocytic, most common in both adults and children

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with stable asthma [Simpson *et al.* 2006]. Eosinophilic asthma is a distinct phenotype characterized by thickening of the basement membrane in the airway mucosa and by corticosteroid responsiveness. In contrast, thickening of the basement membrane is not typical of noneosinophilic asthma, a relatively corticosteroid resistant condition frequently associated with severe disease [Pepe *et al.* 2005]. Some studies report that recurrent asthma exacerbations can predominate in a subgroup of patients with eosinophilic airway inflammation [Haldar *et al.* 2008]. Eosinophilic infiltration is a key feature of Th2-driven inflammation, and eosinophils can be a useful biomarker in guiding treatment [Holgate, 2008].

In this review we will discuss the results of mepolizumab treatment for severe refractory asthma on the basis of current evidence and the potential indications of this molecule for other eosinophilic-related disorders.

Eosinophilic inflammation, IL-5 and mepolizumab

Eosinophils have a role in acute and subacute processes involved in airway narrowing and in other important events, such as repair and remodeling associated with chronic eosinophilic inflammation. Eosinophil recruitment and survival in the airways and granule maturation are promoted by interleukin (IL)-3, granulocyte macrophage colony-stimulating factor (GM-CSF) and especially IL-5. IL-5 is a 134-amino acid protein that forms a 52-kDa homodimer related to both GM-CSF and IL-3 [Klein Wolterink *et al.* 2012; McKinnon *et al.* 1999; Menzies-Gow *et al.* 2003].

In addition to stimulating final differentiation of activated B cells to antibody-forming cells, IL-5 also enhances proliferation, differentiation and maturation of eosinophil precursors [Kouro and Takatsu, 2009]; in experimental murine models it seems to be involved in airway remodeling [Tanaka *et al.* 2004].

Mature eosinophils circulate in the blood for 6–10 h [Becchetti *et al.* 2011; Berek, 2016] to migrate then in connective tissues and end their life cycle after 8–12 days. Eosinophils have IgE receptors and are involved in the activation of allergic reactions and the defense against parasites and worms. They are able to internalize the antigen–antibody complex and release inflammatory mediators (such as major basic protein) killing microbial agents.

Their granules contain different mediators of allergic reactions, such as histaminase and arylsulfatase. A second important activity of eosinophils is the secretion of leukotrienes, which are involved in the pathophysiology of asthma by increasing the secretion of mucus and inducing bronchoconstriction [Becchetti *et al.* 2011; George and Brightling, 2016; Baptista-dos-Reis *et al.* 2015]. Eosinophilia is associated with a wide variety of conditions over asthma, such as atopic diseases, helminthic infections, hypersensitivity to drugs and cancer [Curtis and Ogbogu, 2015].

IL-5 is determinant in influencing stimulation, proliferation and differentiation of eosinophils, therefore this molecule and its receptor have been evaluated as a definite target in the treatment of eosinophilic disorders. This cytokine is largely produced by Th2 lymphocytes, Tc2 cells (type 2 cytotoxic T cells), eosinophils, mast cells and γδ-T cells [Klein Wolterink *et al.* 2012]. Type 2 innate lymphoid cells (ILC2s) have been shown to be a further source, while small amounts of IL-5 can be produced by epithelial cells, natural killer (NK) cells and NK T cells [McKinnon *et al.* 1999].

The type I receptor IL5Rα is expressed in particular by eosinophils. Consequently, these cells respond primarily to IL-5, whose gene is located on chromosome 5, close to the genes encoding IL-3, IL-4 and GM-CSF [Wells *et al.* 1994; Valent, 1994; Toba *et al.* 1999]. The IL-5 receptor includes an α and a βc chain; the first one is specific for the IL-5, while the βc subunit is also recognized by IL-3 and GM-CSF [Menzies-Gow *et al.* 2003; Kouro and Takatsu, 2009]. Antagonizing the IL-5 with specific monoclonal antibodies (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 [Caruso *et al.* 2013a].

The expression of IL-5 is regulated by several transcription factors including GATA3. Produced by TH2 lymphocytes and activated mast cells, IL-5 is an activator of eosinophils and represents a link between the T-cell activation and inflammatory responses mediated by eosinophils. The latter express on their surface specific receptors for the Fc fragment of IgE antibodies and are able to recognize and bind microorganisms (mainly helminths) opsonized by IgE.

Mepolizumab (SB-240563, GlaxoSmithKline) was the first biological anti IL-5 agent designed
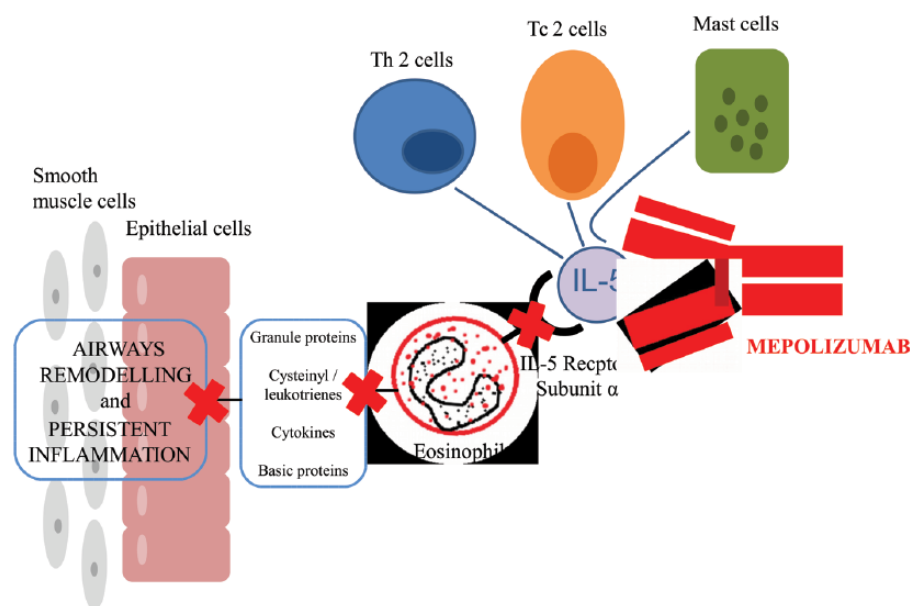
Figure 1. Mepolizumab prevents the binding to the alpha chain of the IL receptor on the eosinophil cell surface, with a reduction in inflammatory cascade.

and tested in randomized clinical trials (RCTs) on eosinophilic asthma and other eosinophilic diseases. Mepolizumab is a N-glycosylated IgG1 kappa humanized mAb formed by two light chains and two heavy chains bound by a disulphide bridge, with a molecular weight of 149.2 kDa including 3 kDa carbohydrate residues [Zia-Amirhosseini *et al.* 1999]. This drug binds IL-5 [Zia-Amirhosseini *et al.* 1999] with both high specificity ($IC_{50} < 1$ nM) and affinity ($K_d = 4.2$ pM), preventing binding to the alpha chain of the IL-5 receptor complex expressed on the eosinophil cell surface [Lopez *et al.* 1988; Ohnishi *et al.* 1993; Clutterbuck and Sanderson, 1990; Hart *et al.* 2001] (Figure 1). In November 2015 the US Food and Drug Administration (FDA) committee approved mepolizumab with the brand name Nucala® at the dose of 100 mg administered subcutaneously once every 4 weeks for the treatment of severe eosinophilic asthma [FDA, 2015].

Finally, in December 2015, the European Medicines Agency (EMA) approved a marketing authorization valid throughout the European Union as 'medicine under additional monitoring' [EMA, 2009].

Skepticism after the first clinical trials
The initial research studies on mepolizumab produced contradictory results in the clinical setting

and several authors questioned the efficacy of this new molecule in asthma treatment [Leckie *et al.* 2000; Flood-Page *et al.* 2003]. One of the studies showed nonsignificant data in terms of airway hyperreactivity (AHR), peak expiratory flow (PEF) and forced expiratory volume in 1 s (FEV_1) in spite of a decrease in airways and blood eosinophilia [Leckie *et al.* 2000; Flood-Page *et al.* 2003]. Another important question was the heterogeneity in the severity and clinical patterns of asthma in several studies, investigating from mild to moderate atopic asthma, persistent asthma and eosinophilic asthma with recurrent exacerbations. These doubts have been considered in a recent Cochrane systematic review [Powell *et al.* 2015]. A RCT had the aim of investigating the effect of three intravenous infusions of mepolizumab, 250 or 750 mg at monthly intervals, on clinical outcome measures in 362 patients with asthma experiencing persistent symptoms despite inhaled corticosteroid therapy [Flood-Page *et al.* 2007]. Mepolizumab was associated with a significant reduction in blood and sputum eosinophils in both treatment groups, but an incorrect sample selection (i.e. that did not take into account the level of eosinophilic inflammation in the airways) did not allow the study to reach statistically significant changes in any of the clinical endpoints measured. There was a nonsignificant trend for decrease in exacerbation rates in the mepolizumab 750 mg treatment group, therefore this treatment did not appear to add

Table 1. Key results of principal anti-interleukin (IL)-5 monoclonal antibodies studies in asthma.

Study	Population	Study design	Anti-IL-5 dosage	Results
Haldar *et al.* [2009]	Refractory eosinophilic asthma and a history of recurrent severe exacerbations	Randomized, double-blind, placebo-controlled, parallel-group study *n* = 61	750 mg (mepolizumab)	Reduction in rate of exacerbation, improving in AQLQ score, reduction of blood and sputum eosinophils
Nair *et al.* [2009]	Prednisone-dependent asthma with sputum eosinophilia	Randomized, double-blind, parallel-group trial *n* = 20	750 mg (mepolizumab)	Reduction in the number of blood and sputum eosinophils, prednisone sparing
Castro *et al.* [2011]	Eosinophilic asthma	Randomized, double-blind, placebo-controlled study *n* = 106	3.0 mg/kg (reslizumab)	Significant reduction in sputum eosinophils, improvements in airway function
Pavord *et al.* [2012]	Severe eosinophilic asthma	Multicenter, double-blind, placebo controlled trial (DREAM) *n* = 621	75 mg, 250 mg, or 750 mg (mepolizumab)	Effectiveness and well tolerated mepolizumab treatment, risk of asthma exacerbations reduction
Ortega *et al.* [2014] ortega [2014b]	Severe eosinophilic asthma	Randomized, double-blind, double-dummy trial (MENSA) *n* = 576	75 mg, 100 mg (mepolizumab)	Reduction in rate of exacerbations, improving in ACQ-5 and SGRQ-Q score
Bel *et al.* [2014]	Severe eosinophilic asthma	Randomized, double-blind, trial study (SIRIUS) *n* = 135	100 mg (mepolizumab)	Reduction in the glucocorticoid-dose stratum, reduction of the annualized rate of exacerbations
Bjermer *et al.* [2014]	Severe persistent asthma with elevated eosinophils	Multicenter, placebo-controlled, double-blind, 16-week study *n* = 311	0.3 or 3.0 mg/kg (reslizumab)	Significant improvement of overall FEV_1 and ACQ score
Castro *et al.* [2014]	Severe eosinophilic asthma	Randomized, controlled, double-blind, dose-ranging phase IIb study *n* = 324	2 mg, 20 mg, or 100 mg (benralizumab)	Reduce asthma exacerbations and blood eosinophils
Pham *et al.* [2016]	Eosinophilic asthma	Two independent study (phase I *n* = 14 and phase IIa *n* = 24) randomized, double-blind, placebo-controlled	100 or 200 mg (benralizumab)	Eosinophil depletion and significant reduction of eosinophil derived neurotoxin, eosinophil cationic protein
Gevaert *et al.* [2011]	Subjects with bilateral nasal polyps	Double-blind, placebo-controlled, randomized, two-center safety and pharmacokinetic study	3 mg/kg or 1 mg/kg	Reduction in blood eosinophil numbers and concentrations of eosinophil cationic protein and size reduction of nasal polyps

ACQ, Asthma Control Questionnaire; AQLQ, Asthma Quality of Life Questionnaire; FEV_1, forced expiratory volume in 1 s; SGRQ, St. George's Respiratory Questionnaire.

significant clinical benefit [Flood-Page *et al.* 2007]. As a matter of fact, early studies did not show a significant improvement in lung function, probably due to lack of efficacy in removing tissue eosinophils and pro-inflammatory cytokines [Powell *et al.* 2015; Simpson *et al.* 2006]. Only when an 'eosinophilic phenotype' was identified, a subgroup of potential mepolizumab responsive patients could be selected [Ortega *et al.* 2014a].

Another limitation is the possibility to define reliably the effectiveness and safety of mepolizumab because of a relatively small number of patients and a short duration of studies in literature. In the MENSA trial [Ortega *et al.* 2014b], 194 participants were treated with mepolizumab 100 mg SC for 32 weeks, while in the SIRIUS trial [Bel *et al.* 2014] 69 participants were treated with mepolizumab 100 mg SC for 20 weeks.

The majority of trials investigated intravenous mepolizumab, a formulation not licensed at the moment. The evidence for the currently licensed subcutaneous route is limited to two studies evaluating patients with severe eosinophilic asthma. Further research is actually needed on specific biomarkers to identify which subphenotypes of eosinophilic asthma can benefit from mepolizumab.

Another limitation is that reliable studies are lacking on patients of African descent or teenagers and children to obtain meaningful data about the net health benefits of mepolizumab in these subgroups. For this reason, the FDA required two post-marketing studies specifically in children aged 6–11 years. The optimal dosage and duration of treatment are still to be determined [Powell *et al.* 2015].

Clinical effectiveness data in asthma

Early studies failed their objectives probably because of improper selection of the study population. Leckie and colleagues performed the first randomized, double-blinded, placebo-controlled study on humans. There were nonsignificant improvements in term of AHR, PEF and FEV_1 in spite of decreased airways and blood eosinophilia after 4 and 16 weeks [Leckie *et al.* 2000]. A subsequent RCT in 362 patients with uncontrolled asthma despite inhaled corticosteroid therapy had the aim of evaluating the effect of three intravenous infusions of mepolizumab (250 or 750 mg every month), on clinical outcome measures [Flood-Page *et al.* 2007]. As already said, the study failed the clinical endpoints despite a significant reduction in blood and sputum eosinophils in both treatment groups [Flood-Page *et al.* 2007] (Table 1).

Two clinical trials demonstrated mepolizumab efficacy. The first was a RCT on 61 subjects who had refractory eosinophilic asthma and a history of recurrent severe exacerbations [Haldar *et al.* 2009]. Patients received infusions of either mepolizumab or placebo at monthly intervals for 1 year. Mepolizumab was associated with significantly fewer severe exacerbations than placebo, with a significant improvement in the score of the Asthma Quality of Life Questionnaire (AQLQ) and a decrease of eosinophil counts in the blood and sputum. However, there were no significant differences between the two groups with respect to symptoms, post-bronchodilator FEV_1 or airway hyperresponsiveness. The second report was

another RCT involving patients with persistent sputum eosinophilia and symptoms despite prednisone treatment [Nair *et al.* 2009]. Nine patients were assigned to receive mepolizumab, administered in five monthly infusions of 750 mg, and 11 patients to placebo. There were 12 asthma exacerbations in 10 patients who received placebo while only one patient who received mepolizumab had an asthma exacerbation. The use of mepolizumab was associated with a significant decrease in the number of sputum and blood eosinophils. Moreover, subjects who received mepolizumab were able to reduce their prednisone dose; improvements in eosinophil numbers, asthma control and FEV_1 were maintained for 8 weeks after the last infusion (Table 1).

A subsequent and conclusive study was the Dose Ranging Efficacy and Safety with Mepolizumab (DREAM) trial [Pavord *et al.* 2012]. It was a large multicenter, double-blind, placebo-controlled trial recruiting 621 subjects with a history of recurrent severe asthma exacerbations, and signs of eosinophilic inflammation. Patients were randomly assigned to receive one of three doses of intravenous mepolizumab (75, 250 or 750 mg) or matched placebo (100 ml 0.9% NaCl). Mepolizumab significantly reduced the number of asthma exacerbations in patients with severe eosinophilic asthma compared with placebo. In addition, treatment lowered blood and sputum eosinophil counts and was well tolerated for 12 months, despite a small effect on FEV_1, AQLQ and Asthma Control Questionnaire (ACQ) scores compared with the placebo group. These studies have represented an important advance for the selection and treatment of those patients affected by severe asthma with frequent exacerbations and persistent eosinophilia, accounting for about 40% of severe asthmatics [Rothenberg *et al.* 2008]. A post hoc analysis of the DREAM had the aim to assess the effect of mepolizumab treatment on reduction of exacerbations in atopic and non-atopic subjects, on seasonal patterns of response by subgroup and the changes in lung function and exhaled nitric oxide fraction (FeNO) according to subgroup [Simpson *et al.* 2006]. A reduction in exacerbations was observed, irrespective of atopy or IgE levels and more frequent in winter months, however treatment response was unaffected by season or atopy. A subsequent supervised cluster analysis was applied to data of the DREAM study to identify characteristics that maximized the differences among subgroups [Ortega *et al.* 2014c]. Three main parameters were identified in four

primary clusters: blood eosinophils, airway revers-ibility and body mass index (BMI). The reduc-tion in exacerbations and significant therapeutic benefit were higher in patients with eosinophilic inflammation who received mepolizumab, con-firming the eosinophilic phenotype as the one with the greatest response to therapy.

In the MENSA trial 576 patients were selected with recurrent asthma exacerbations and eosin-ophilic inflammation despite high doses of inhaled glucocorticoids to one of three study groups [Ortega *et al.* 2014b]. Patients were assigned to receive treatment with mepoli-zumab, administered as either a 75 mg intrave-nous dose or a 100 mg subcutaneous dose, or placebo every 4 weeks for 32 weeks. The pri-mary endpoint was the rate of exacerbations, other outcomes were FEV1, scores on the St. George's Respiratory Questionnaire (SGRQ) and the five-item Asthma Control Questionnaire (ACQ-5). The rate of exacerbations was reduced by 47% among patients receiving intravenous doses and by 53% among those receiving subcu-taneous administration. At the end of the study, the mean increase from baseline in FEV_1 was 100 ml greater in patients receiving intravenous mepolizumab than in those receiving placebo and 98 ml greater in patients receiving subcuta-neous mepolizumab than in the placebo group. There were also significant improvements in the SGRQ and ACQ-5 scores in the intravenous and subcutaneous mepolizumab than in the pla-cebo groups, with a safety profile of mepoli-zumab comparable with placebo. Also, this study confirmed the efficacy of mepolizumab administered either intravenously or subcutane-ously in terms of reduction of asthma exacerba-tions but, unlike other studies, improvements in markers of asthma control, including FEV_1, were demonstrated too.

Bel and colleagues performed a randomized, dou-ble-blind trial (SIRIUS study) involving 135 patients with severe eosinophilic asthma, with the aim to compare with placebo the glucocorticoid-sparing effect of mepolizumab 100 mg adminis-tered subcutaneously every 4 weeks for a duration of 20 weeks [Bel *et al.* 2014]. Patients in the mepolizumab group had a reduction in the gluco-corticoid dose 2.39 times greater than in the pla-cebo group (95% confidence interval [CI] 1.25–4.56; *p* = 0.008). Patients had also a relative reduction of 32% in the annualized rate of exacerbations (1.44 *versus* 2.12, *p* = 0.04) and a significant reduction of asthma symptoms (*p* = 0.004) (Table 1).

Another study examined a subpopulation of 188 oral corticosteroid (OCS)-dependent patients enrolled in the DREAM trial who continued to have frequent exacerbations. These subjects had received regular OCS (5–35 mg/day) for ⩾6 months and an additional controller without efficacy. Mepolizumab lowered the peripheral eosinophils and showed a significant glucocorti-coid-sparing effect in the non-OCS and OCS groups, reducing exacerbation rate in the 52-week treatment period, particularly in the OCS group [Prazma *et al.* 2014].

A multicenter, placebo-controlled, double-blind, parallel-group study is currently underway to eval-uate the effect of mepolizumab on health-related quality of life and other measures of asthma con-trol, primarily lung function, in patients with severe eosinophilic asthma [ClinicalTrials.gov identifier: NCT02281318].

Finally, but not least, in an interesting study on bronchial remodeling a partial eosinophil reduc-tion was obtained with mepolizumab treatment, associated with significant reductions in tenascin and lumican deposition in the reticular basement membrane [Phipps *et al.* 2004].

The other side of anti-IL-5 mAbs: benralizumab and reslizumab

In the last 15 years several clinical trials were conducted in asthma with anti-IL-5 mAbs other than mepolizumab, that is, reslizumab and benralizumab.

Reslizumab is a humanized anti-IL-5 monoclonal (IgG4/κ) antibody targeting circulating IL-5 with a high affinity, thus preventing its binding to spe-cific receptor [Kips *et al.* 2003]. An early study (Sch 55700) showed a long-term effect in reduc-ing both pulmonary eosinophilia and airway hyperresponsiveness in allergic mice, monkeys and rabbits [Egan *et al.* 1999].

Similarly to mepolizumab, early RCTs on patients with severe uncontrolled asthma despite standard treatment were not encouraging [Kips *et al.* 2003], because of the lack of improvement in FEV_1 or symptoms. However, also in this case a

significant reduction in circulating and sputum eosinophils was shown. Once identified the hypereosinophilic asthmatic phenotype (sputum eosinophils >3% or blood eosinophils >400/μl) clinical outcomes have shown an improving trend. In a phase II trial on asthmatic patients with nasal polyposis (NP) a significant amelioration of asthma symptoms was observed ($p = 0.012$) along with a slight improvement in asthma control measured by ACQ [Gevaert, 2006]. In subsequent phase III RCTs significant improvements in FEV_1 and ACQ score were found [Bjermer et al. 2014], particularly in the subgroup with NP [Castro et al. 2011].

Benralizumab (MEDI-563) is an IgG1, afucosylated anti-IL-5Ra mAb that binds to an epitope on IL-5Rα close to the IL-5 binding site. In a study on nonhuman primates, MEDI-563 depleted blood eosinophils and eosinophil precursors in the bone marrow, by induction of antibody-dependent cell-mediated cytotoxicity (ADCC) of eosinophils and basophils [Kolbeck et al. 2010]. In phase IIB RCTs on patients affected by severe hypereosinophilic asthma with a peripheral blood eosinophil counts >300 cells/μl, 20 and 100 mg benralizumab administered subcutaneously showed interesting clinical results, in particular a significant reduction in exacerbations compared with placebo [Ghazi et al. 2012; Castro et al. 2014].

A very recent study had the aim to evaluate the effects of benralizumab on eosinophil counts and activity. Sera were collected from asthma patients enrolled in two clinical phase I and phase IIa studies. A modulation and a significant reduction of blood eosinophils, IL-5, eosinophil-derived neurotoxin (EDN) and eosinophil cationic protein (ECP) were found, confirming a significant anti-inflammatory effect by acting on these mediators [Pham et al. 2016].

The search for predictive biomarkers
In clinical practice asthma management and follow up are often based on symptoms and lung function tests to assess airway obstruction and hyperresponsiveness. Unfortunately, these data do not correlate with the level of airway inflammation. Fiberoptic bronchoscopy with biopsies and bronchoalveolar lavage (BAL) have been considered until now the gold standard to evaluate airway inflammation, but they have the

disadvantage of being invasive and not easy to apply in real-life settings [Lommatzsch et al. 2013; Djukanović, 1996].

For these reasons, since the advent of biological therapies with omalizumab, the interest has further increased to identify new biomarkers potentially useful for patient selection and treatment guidance [Caruso et al. 2013b].

At present, serum IgE levels are the only applicable biomarker in clinical practice with good value, in predicting response to omalizumab for severe uncontrolled allergic asthma. Total serum IgE in adult patients with persistent allergic asthma showed almost always levels >150 IU/ml variable over time [Davila et al. 2015]. Some authors reported a significant association between total serum IgE and asthma or airflow limitation severity in a high percentage of patients with IgE >400 IU/ml, confirming the important role of IgE in this subgroup [Davila et al. 2015].

Omalizumab inhibits the binding of IgE to high affinity FcεRI receptors on mast cell and basophil surface, and reduces the free IgE levels in a short time [Djukanović et al. 2004; Gould and Sutton, 2008]. As a consequence, the expression of FcεRI is downregulated on inflammatory cells, such as basophils, mast cells and dendritic cells [Prussin and Metcalfe, 2006; MacGlashan et al. 1997; Lin et al. 2004] with subsequent inhibition of inflammatory mediator release. Another effect is the reduction of inflammatory cell recruitment, particularly eosinophils in sputum and tissues [Djukanović et al. 2004] and bronchial remodeling [Humbles et al. 2004].

Baseline IgE is the only parameter with a significant predictive value based on multivariate analysis from the INNOVATE study. A lower baseline IgE was associated with reduced clinical benefits [Bousquet et al. 2007] but analysis of data from patients enrolled in seven clinical trials indicated that omalizumab decreased asthma exacerbation rates across all IgE level quartiles but reached a statistical significance in the three upper IgE quartiles only ($p < 0.001$) [Bousquet et al. 2007]. In this group, total emergency visit rates declined and, the AQLQ score and the FEV_1 improved. In contrast, specific allergen IgE did not show any significant correlation with the clinical response to omalizumab [Wahn et al. 2009].

Unfortunately, to date we have no other reliable biomarkers in clinical practice, with a promising exception represented by periostin [Hanania *et al.* 2013] for the selection of suitable patients to therapy with omalizumab and lebrikizumab.

The main parameters assessed in human studies including RCTs to identify patients with eosinophilic asthma have been the level of eosinophils in induced sputum and blood, applied as a biomarker for selection and of response to treatment [Pavord *et al.* 2012; Castro *et al.* 2011; Wenzel *et al.* 2013]. Unfortunately, despite being considered a method of choice for establishing eosinophilic lung inflammation, induced sputum is not always practical to obtain and standardize.

In the DREAM study, blood eosinophil counts correlated with the response to mepolizumab, but the same did not happen with sputum eosinophilia [Pavord *et al.* 2012]. In a *post hoc* analysis, this important study showed that in patients with frequent exacerbations a single peripheral blood eosinophil count at screening ⩾150 cells/ml was a good predictor of response [Katz *et al.* 2014]. In contrast, those subjects with baseline eosinophil count <150 cells/ml had a limited reduction of asthma exacerbations. Since blood eosinophil levels can show spontaneous variations over time, a single measurement might not be sufficient to evaluate patients accurately, although this study has shown that a single analysis can be acceptable in RCTs.

In the MENSA trial [Ortega *et al.* 2014b] the authors enrolled subjects with a peripheral eosinophil count of at least 150 cells/µl at screening or at least 300 cells/µl sometime in the previous year. In this study a subanalysis was conducted on a group of patients with blood eosinophil counts >500 cells/µl, who had a better clinical response to mepolizumab.

Another and easier option is the expired FeNO. Levels of FeNO have a close correlation with eosinophil numbers in the airways as shown in several studies [Jatakanon *et al.* 1998]. Data and results are anyway controversial, because according to some authors [Schleich *et al.* 2010] FeNO has a good correlation with sputum eosinophils and can theoretically be used as a less-invasive biomarker supporting the use of IL-5 antagonizing agents [Westerhof *et al.* 2015]. Unfortunately, in another study FeNO reflected sputum eosinophilia in only 78% of patients [Wagener *et al.* 2015], increasing uncertainty on the use of this biomarker.

Serum periostin [or osteoblast-specific factor (OSF-2)] is an extracellular matrix protein originally identified in mesenchymal cells as osteoblasts, osteoblast-derived cells and periosteum. Periostin is implicated with many disease states and it is closely related with the biological activity of IL-13 [Izuhara *et al.* 2016]. So far, it has been studied mainly to predict responses and for the monitoring of treatment to lebrikizumab (an anti-IL-13 mAb) [Izuhara *et al.* 2016] and to omalizumab [Hanania *et al.* 2013; Tajiri *et al.* 2016]. Also in this case, however, the correlation with eosinophilic airway inflammation is not so clear, as well as the ability to accurately distinguish between eosinophilic and noneosinophilic asthma. Available data are contradictory, because in a study serum periostin levels were the best predictor of airway eosinophilia [Jia *et al.* 2012], while in another study periostin and total IgE were not able to discriminate between eosinophilic and noneosinophilic phenotypes of asthma [Westerhof *et al.* 2015; Wagener *et al.* 2015].

Treatment monitoring

The lack of long-term studies on mepolizumab does not allow to accurately define the appropriate timing and method of follow up. To the best of the authors' knowledge there are only few follow-up studies and none longer than 12 months [Haldar *et al.* 2014].

In our experience, evaluations useful for an effective and practical monitoring are represented by blood and sputum eosinophil counts, exacerbation rate and score of asthma control questionnaires, such as ACQ or Asthma Control Test (ACT). In clinical studies no significant differences in FEV_1 or FeNO have ever been reported. The ideal timing for clinical and laboratory monitoring could be initially after 4 weeks and then every 3 months, based on the pharmacokinetic and pharmacodynamic characteristics [Haldar *et al.* 2014; Ortega *et al.* 2014a].

When to stop treatment with mepolizumab or other biological agents is still a matter of debate. Despite a long experience with omalizumab a definite response has not been agreed yet, because

the relevant studies conducted so far are few and with small numbers of patients. The available data indicate that withdrawal of omalizumab may cause severe asthma exacerbations in most patients, with return to a pretreatment clinical state [Kupryś-Lipińska and Kuna, 2014]. The long-term efficacy of omalizumab was investigated after a treatment of 6 years in a group of 18 patients with severe IgE-mediated asthma and a withdrawal of 1 year [Nopp *et al.* 2007] and 3 years [Nopp *et al.* 2010]. In both cases a stabilization was shown of asthma-related symptoms, similar to that observed during the treatment period with omalizumab, associated with a down-regulation of basophil allergen sensitivity. Anyway, until these data will be confirmed on large case series, the general trend is to continue the treatment indefinitely.

To the best of the authors' knowledge, there is only one study that evaluated the effects of mepolizumab discontinuation [Haldar *et al.* 2014], that is, an unblinded, prospective, 1-year follow-up analysis in a series of 27 subjects on anti-IL-5 treatment in the previous 12 months. Withdrawal of mepolizumab was associated with a progressive increase of blood eosinophils to baseline counts over 6 months and with a significant rise in frequency of severe exacerbations after 12 months from cessation. A RCT is currently recruiting participants with the primary objective to evaluate whether patients with severe eosinophilic asthma who have received mepolizumab for at least 3 years need to continue this treatment to maintain benefit [ClinicalTrials.gov identifier: NCT02555371]. Secondary outcomes of the study are the ratio to baseline in blood eosinophil count, the time to a decrease in asthma control and, finally, the time to first exacerbation requiring hospitalization or emergency department (ED) visit.

Cost-effectiveness and comparative value

After the introduction of new treatment options for severe asthma such as omalizumab, bronchial thermoplasty and mepolizumab, the analysis of the economic impact needs greater attention because of the significant increase of direct costs. These could be partly offset by reduction in costs incurred by the health service (in particular, for hospitalizations), a reduction of indirect costs and improvement of quality of life as shown in some studies [Menzella *et al.* 2012, 2014]. Given the very recent introduction of mepolizumab in clinical practice, there are few data about pharmaco-economic aspects. Analysts and some reports estimate a cost from US$10,000 to US$15,000 per patient per year of treatment [NHS, 2015]. After FDA approval, the real price tag is US$32,500 a year per patient and approximately US$2700 for a single 4-week injection. A recent and very interesting cost-effectiveness analysis (CEA) conducted by the ICER Group was based on a simulation model of asthma outcomes and costs in a representative population of suitable patients to mepolizumab therapy [ICER, 2015]. The incremental cost-effectiveness of mepolizumab was evaluated using drug cost estimates derived from current prices and estimates of reductions in asthma exacerbations and OCS use from relevant clinical trial data. In a scenario analysis the price of mepolizumab was determined that would produce cost-effectiveness results at willingness-to-pay thresholds of US$50,000/quality-adjusted life year (QALY), US$100,000/QALY and US$150,000/QALY, respectively. Mepolizumab should have a value-based cost between US$7800 and US$12,000 a year, while the full list price per patient in the US is US$32,500 a year. Based on current acquisition prices the cost-effectiveness estimates are unfavorable, since they exceed commonly cited thresholds and other doubts arise from the lack of clinical trials evaluating benefits in the long term. To obtain a value correlated to the clinical benefit a discount of two-thirds to three-quarters from the current price list of mepolizumab would be necessary.

Another recent and very interesting study had the purpose to determine the cost-effectiveness of newest medical treatment strategies for severe refractory asthma, such as biological therapies (omalizumab and mepolizumab) and follow-on bronchial thermoplasty [Bogart *et al.* 2015]. The authors used a model based on US healthcare perspective, a hypothetical cohort of 10,000 adults affected by refractory asthma, an annual cycle and 10-year time horizon. In patient responders to biological treatment, the addition of bronchial thermoplasty was not cost-effective. Mepolizumab without bronchial thermoplasty was the most cost-effective option for biological responders, with a 10-year-per-patient cost of US$116,776 and 5.46 QALYs gained (ICER US$21,388). In the group of nonresponders to biological treatment, bronchial thermoplasty

proved to be a cost-effective treatment option (US$33,161/QALY).

Eosinophilic-related diseases other than asthma

Eosinophil-related diseases are very heterogeneous conditions such as hypereosinophilic syndromes (HES), atopic dermatitis (AD), eosinophilic granulomatosis with polyangiitis (EGBPA), eosinophilic esophagitis (EoE), severe NP and bronchial asthma.

Several studies and clinical trials have been performed in the last years, and many others are currently underway on monoclonal anti-IL-5 antibodies (Table 2). Most studies conducted until now have shown in general a reduction of eosinophil levels in the peripheral blood and sputum but clinical results seem often inconsistent [Leckie *et al.* 2000; Flood-Page *et al.* 2007; Haldar *et al.* 2009; Nair *et al.* 2009; Pavord *et al.* 2012].

The potential therapeutic effect of mepolizumab for HES (Table 2) has been investigated by various authors. An interesting study performed by Rothenberg and colleagues [Rothenberg *et al.* 2008] showed that treatment with mepolizumab had a corticosteroid-sparing effect in patients affected by HES and negative for FIP1-like 1-platelet-derived growth factor receptor α (FIP1L1-PDGFRA) fusion protein gene, confirming the effectiveness of the drug in this disease. An open-label multicenter study had the aim to evaluate long-term safety and efficacy of mepolizumab 750 mg i.v. every 4 weeks for 36 weeks. The anti-IL-5 molecule was safe and effective as a corticosteroid-sparing agent in treating adults with FIP1L1/PDGFRA negative HES [Roufosse *et al.* 2013].

It is well known that in AD, tissue and blood eosinophils are very activated, with delayed programmed cell death [Leiferman, 2001; Wedi *et al.* 1997], whereby mepolizumab was considered a potential treatment [Gnanakumaran and Babu, 2003]. A randomized double-blind, placebo-controlled, parallel group study (Table 2) enrolled 18 patients with AD to receive two single doses of 750 mg mepolizumab and 22 on placebo treatment. Peripheral blood eosinophils were significantly reduced in the treatment group compared with placebo but no clinical success was reached by the physician's global assessment (PGA), objective SCORAD and itch scoring [Oldhoff *et al.* 2005].

In EGBPA [also known as Churg–Strauss syndrome (CSS)], mepolizumab was used in an open-label study on 10 patients with refractory or relapsing EGBPA: the authors reported a remission in most subjects with a reduction of OCS [Moosig *et al.* 2011]. A later case-series studied seven patients with EGBPA, treated with only four doses of mepolizumab. A reduction of OCS dose was possible in all patients, associated with a stability of symptoms and an optimal safety profile during the entire course of the study [Kim *et al.* 2010]. As expected, after cessation of treatment, EGBPA symptoms reappeared with a progressive increase of eosinophils and the need to resume OCS, confirming that it is mandatory to continue mepolizumab for a long time, until new studies will concentrate on the optimal duration of therapy.

Furthermore, a new case study with refractory EGBPA patients showed how monthly doses of mepolizumab (750 mg i.v.) reduced asthma symptoms and eosinophilia in blood and airways [Kahn *et al.* 2010] (Table 2).

In another fascinating study, authors investigated whether mepolizumab treatment can reduce esophageal mast cell accumulation in 43 pediatric EoE biopsy specimens from a previous anti-IL-5 RCT. Response to anti-IL-5 (defined as <15 eosinophils per high power field following mepolizumab therapy) was found in the 40% of patients and 77% of all subjects had significantly fewer mast cells, IL-9+ cells and mast cell–eosinophil couplets in the esophageal epithelium following anti-IL-5 therapy [Otani *et al.* 2013]. A clinical correlation was also noted between the reduction of eosinophils and epithelial mast cells and symptoms, with a decrease in stomach and chest/throat pain, along with relief of swallowing discomfort.

In a larger study, 226 children and adolescents with EoE were treated with reslizumab. A median reduction from baseline to the end of therapy was shown in peak esophageal eosinophil counts (59%, 67% and 64% in the 1, 2 and 3 mg/kg reslizumab groups, respectively) *versus* placebo (24%, $p < 0.001$ for all comparisons). PGA scores improved in all treatment groups, but without statistically significant differences [Spergel *et al.* 2012].

In another study on adult patients [Straumann *et al.* 2010] a mild to moderate improvement was

Table 2. Main fields of application of mepolizumab in eosinophilic-related diseases.

Study	Disease	Study design	Dosage	Observations/results
Rothenberg *et al.* [2008]	HES	International, randomized, double-blind, placebo-controlled trial	750 mg i.v	Corticosteroid-sparing for patients negative for FIP1L1-PDGFRA who have the hypereosinophilic syndrome
Roufosse *et al.* [2013]		Open-label extension study	750 mg i.v	Mepolizumab was well tolerated and effective as a long-term corticosteroid-sparing agent in PDGFRA-negative HES
Oldhoff *et al.* [2005]	AD	Randomized double-blind, placebo-controlled, parallel group study	750 mg i.v	Two single doses of 750 mg mepolizumab did not result in clinical success in patients with AD, despite a significant decrease in peripheral blood eosinophils
Moosig *et al.* [2011]	EGBPA	Single-center, phase II, uncontrolled, investigator-initiated trial	750 mg i.v	Eight of ten patients had a remission and it was possible a reduction of OCS
Kim *et al.* [2010]		Open-label pilot study	750 mg i.v	Mepolizumab is a safe and well-tolerated therapy in patients with CSS, offering clinical benefit by enabling corticosteroid tapering while maintaining clinical stability
Kahn *et al.* [2010]		Case study	750 mg i.v	Mepolizumab reduced asthma symptoms and both blood and airway eosinophilia
Otani *et al.* [2013]	EoE	International, randomized, blinded, multicenter pediatric	750 mg i.v	Pediatric EoE patients had significantly fewer mast cells, IL-9+ cells, and mast cell–eosinophil couplets in the esophageal epithelium following anti-IL-5 therapy. eosinophils were one source of IL-9, they may support esophageal mastocytosis
Straumann *et al.* [2010]		Randomized, placebo-controlled, double-blind trial	750 mg i.v	Mepolizumab significantly reduced eosinophil numbers in esophageal tissues in adult patients with active EoO, and changes in the expression of molecules associated with esophageal remodeling were reversed. Minimal clinical improvement was achieved in a subgroup of patients with EoO. Mepolizumab had an acceptable safety profile, even at the high 1500 mg dose level
Gevaert *et al.* [2011]	NP	Randomized, double-blind, placebo-controlled	750 mg i.v	Mepolizumab produced a significant reduction in total polyp score in 12 of 20 patients, also confirmed by CT scan evaluations
ClinicalTrials.gov identifier: NCT02105961	COPD	Multi-centered, randomized, placebo-controlled, double-blind, parallel group, trial	100 mg i.v and 300 mg i.v	Ongoing
ClinicalTrials.gov identifier: NCT01463644		Multicenter, randomized, placebo-controlled, double-blind, parallel group, trial	750 mg i.v	Completed. Results still not available
ClinicalTrials.gov identifier: NCT02105948		Multicenter, randomized, placebo-controlled, double-blind, parallel group, trial	100 mg i.v	Ongoing

AD, atopic dermatitis; CSS, Churg–Strauss syndrome; COPD, chronic obstructive pulmonary disease; EGBPA, eosinophilic granulomatosis with polyangiitis; EoE, eosinophilic esophagitis; EoO, eosinophilic oesophagitis; FIP1L1-PDGFRA, FIP1-like 1-platelet-derived growth factor receptor α; HES, hypereosinophilic syndromes; IL, interleukin; OCS, oral corticosteroids; NP, nasal polyposis.

observed in eosinophil counts, but primary end-points (resolution of eosinophilia and symptoms at comparison between active treatment and placebo) were not met.

Finally, in severe NP, a significant study on 30 patients with 3 or 4 grade disease or recurrence after surgery refractory to corticosteroid therapy showed interesting results [Gevaert *et al.* 2011]. The subjects were randomized to receive either two single intravenous injections of 750 mg mepolizumab or placebo; the primary outcome was the reduction in NP score at 8 weeks after the first dosing. Secondary outcomes included changes in CT scan scores and functional assessments, such as nasal peak inspiratory flow (nPIF) or symptom score. Mepolizumab produced a significant reduction in total polyp score (TPS) in 12 among 20 patients, as confirmed by CT scan evaluations.

In the studies conducted so far, mepolizumab has been used in a broad range of eosinophilic related disorders, with a good safety profile and with no evidence of specific immune complex formation [Abonia and Putman, 2011].

Future perspectives
New potential areas of interest could extend the indications of anti-IL5 mAbs in the forthcoming years.

Chronic eosinophilic pneumonia (CEP) is an idiopathic condition defined by peripheral eosinophilia, eosinophilic infiltrates in the lung parenchyma occasionally associated with asthma. The pathophysiology of CEP remains unknown, but available data suggest that recruitment of eosinophils into the lung airspaces is a multifactorial process and eosinophil release of IL-5 and cytotoxic granular proteins are involved in the pathogenesis of tissue damage typical of the disorder [Akuthota and Weller, 2012]. On the grounds of this mechanism, an eosinophil-targeted therapy with mepolizumab deserves investigation in CEP patients [Akuthota and Weller, 2012].

In severe chronic obstructive pulmonary disease (COPD) patients, the eosinophil-predominant phenotype is associated with frequent exacerbations despite maximal standard therapy and high doses of OCS. The potential role of mepolizumab in COPD has not been determined yet (Table 1). Three phase III RCTs to evaluate the efficacy and safety of mepolizumab as an add-on treatment in COPD are ongoing and are enrolling patients [ClinicalTrials.gov identifiers: NCT02105961, NCT01463644, NCT02105948]. The primary endpoints of these studies are to evaluate the effect of mepolizumab in decreasing exacerbation rates, lowering sputum eosinophils from baseline and improving lung function, as documented for other eosinophilic-driven airway diseases.

A subgroup of COPD patients as potential candidates for mepolizumab treatment might be those with an eosinophil trait labeled as affected by the asthma–COPD overlap syndrome (ACOS) [Hizawa, 2016].

The possible overlap between omalizumab and mepolizumab
At present, omalizumab is the gold-standard treatment for severe allergic asthma; main clinical outcomes are represented by a reduction of exacerbations, an improvement of quality of life and a limited prescription of systemic corticosteroids [Normansell *et al.* 2014].

The effectiveness of omalizumab has been recently demonstrated not only in allergic asthmatic patients on long-term treatment but even in intrinsic asthma and chronic spontaneous orticaria-angioedema [Lommatzsch *et al.* 2014; Zhao *et al.* 2016]. These data support the hypothesis of a local production of IgE even without systemic sensitization [Forester and Calabria, 2010] and confirm that there is still much to understand about this biologic agent.

It is well known that the proportion of patients with severe asthma and IgE >400 UI/ml is significantly greater than in patients with moderate asthma [Davila *et al.* 2015]. Baseline IgE was the only predictor of efficacy of omalizumab in the INNOVATE trial and in other six studies on severe atopic asthmatics in which statistical significance was reached in the upper IgE quartile only ($p < 0.001$) [Humbert *et al.* 2005; Bousquet *et al.* 2007]. In the DREAM study, mepolizumab treatment allowed a significant reduction in severe asthma exacerbations irrespective of the baseline IgE levels or atopic status [Pavord *et al.* 2012]. In light of the partial overlap between the populations eligible for mepolizumab or omalizumab, a multicenter RCT has been planned with the aim of evaluating the effect of mepolizumab in patients with severe eosinophilic asthma not optimally controlled with omalizumab [ClinicalTrials.gov

identifier: NCT02654145]. Hopefully, the results will help clinicians to understand in which phenotype of severe allergic asthma anti-IgE or anti-IL-5 mAbs should be the first choice treatment. As reported previously, a recent study demonstrated that the anti-IL-5 agents can be effective even in patients who were nonresponders to omalizumab, with a steroid-sparing effect [Prazma *et al.* 2014].

In the EXTRA study, carried out on 850 patients, omalizumab was more effective in patients with higher blood eosinophils (>260 cells/μl), high FeNO and periostin levels [Hanania *et al.* 2013]. In this phenotype of patients anti-IL-5 drugs should be studied as the first choice treatment in nonresponders to anti-IgE molecules.

Conversely, the DREAM study [Pavord *et al.* 2012] demonstrated that a significant efficacy of mepolizumab was associated with two variables, that is, higher baseline peripheral blood eosinophils and frequent exacerbations. In patients properly selected according to these characteristics, mepolizumab should be considered as the first choice, also considering an important steroid-sparing effect allowing a consistent reduction of related side effects in the medium and long term.

Conclusion

The options provided to date for severe uncontrolled asthma (step 5 treatment of the GINA guidelines) are anti-IgE mAbs and OCS. OCS are associated with several adverse effects, especially in chronic therapy and should be used at the lowest possible dose [GINA, 2015]. A very recent study found that 93% of subjects with severe asthma had one or more pathologic conditions related to systemic corticosteroids, that is, type II diabetes, osteoporosis, dyspeptic disorders, including gastric/duodenal ulceration, cataracts, obesity, hypertension, etc. [Sweeney *et al.* 2016]. As expected, the relative risk was higher in corticosteroid-dependent asthma (CSD). Therefore, the search for new treatments must take into account as a priority the possibility to reduce the use of systemic steroids.

Advancing knowledge has led to a significant increase of therapeutic options, targeting different phenotypes of the disorder. However, it is not easy to select the right approach to the right patient due to the complexity of asthma pathogenesis and inflammatory pathways.

After the FDA approval of mepolizumab a new option is available for the treatment of severe asthma, after a decade of omalizumab as the only biologic agent. The positive but sometimes conflicting results from anti-IL-5 clinical trials make even more evident the need for careful phenotyping and endotyping of patients.

The potential responders to anti-IL-5 therapy are subjects with eosinophilia (>0.3×10^9/l in blood, >3% in sputum), possibly steroid-responsive, who manifest poor symptom control and frequent exacerbations. Anyway, persistent systemic and airway eosinophilia may not be sufficient to identify the appropriate candidates, particularly in systemic corticosteroid-dependent asthmatic patients, but a multimodal approach is necessary, based also on clinical experience. The development of specific reliable and useful biomarkers beyond eosinophilia is required to more accurately select specific asthma phenotypes and targeted treatments.

The minimum duration of treatment with anti-IL-5 mAbs is still questionable. Even for omalizumab, whose introduction into real-life clinical setting dates back to several years ago, there is no clear response yet. The limited data the minimum duration of treatment with anti-IL-5 mAbs is still questionable. Even for omalizumab, whose introduction into real-life clinical setting dates back to several years ago, there is no clear response yet. The limited data available at present would suggest to continue indefinitely. New studies underway or in the pipeline will probably allow a convincing answer to that topic and other open questions, such as the criteria for the selection of phenotypes with the best chance of response to treatment.

Severe asthma accounts for the largest part of the direct and indirect costs for the disease, mainly due to poor symptom control causing absence from school or work and serious psychological problems such as stress, anxiety and depression impacting heavily on quality of life [Accordini *et al.* 2013]. Despite an increasing evidence of clinical efficacy, monoclonal anti-IL-5 antibodies however imply a considerable increase of the asthma-related healthcare expenditure. Cost-effectiveness is therefore a fundamental issue of biological therapies to better define real-life utility of these agents and to establish their appropriate position in treatment guidelines.

Pharmacoeconomic studies are quite promising but it will be necessary to reduce the purchase costs to allow equity of access to biological treatments such as mepolizumab in our case.

Conflict of interest statement
Francesco Menzella and Luigi Zucchi participated in contracted research and clinical trials for Novartis, Sanofi and GlaxoSmithKline. The other authors report no conflicts of interest in this work.

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