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Interleukin-5 pathway inhibition in the treatment of eosinophilic respiratory disorders: evidence and unmet needs

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Purpose of review
Human eosinophils were first identified and named by Paul Ehrlich in 1879 on the basis of the cell's granular uptake of eosin. Although eosinophils represent approximately 1% of peripheral blood leukocytes, they have the propensity to leave the blood stream and migrate into inflamed tissues. Eosinophils and their mediators are critical effectors to asthma and eosinophilic granulomatosis with polyangiitis (EGPA). Eosinophils are equipped with a large number of cell-surface receptors and produce specific cytokines and chemokines.

Recent findings
Eosinophils are the major source of interleukin-5 and highly express the interleukin-5Rα on their surface. Clinical trials evaluating monoclonal antibodies to interleukin-5 (mepolizumab and reslizumab) and its receptor interleukin-5Rα (benralizumab) have been or are underway in patients with eosinophilic asthma, EGPA and chronic obstructive pulmonary disease (COPD). Overall, targeting interleukin-5/interleukin-5Rα is associated with a marked decrease in blood and sputum eosinophilia, the number of exacerbations and improvement of some clinical parameters in adult patients with severe eosinophilic asthma. Pilot studies suggest that mepolizumab might be a glucocorticoid-sparing treatment in patients with EGPA. A preliminary study found that benralizumab did not reduce the exacerbations and did modify lung function in patients with eosinophilic COPD.

Summary
The review examines recent advances in the biology of eosinophils and how targeting the interleukin-5 pathway might offer benefit to some patients with severe asthma, EGPA, and COPD. Interleukin-5/interleukin-5Rα-targeted treatments offer promises to patients with eosinophilic respiratory disorders.

Keywords
asthma, benralizumab, chronic obstructive pulmonary disease, eosinophils, interleukin-5, mepolizumab, reslizumab

INTRODUCTION

Paul Ehrlich first named a bilobed nucleated cell as an 'eosin'-'ophil' in 1879 on the basis of the cell's granular uptake of eosin [1,2] which binds to cationic proteins present in specific granules. Eosinophils represent approximately 1% of peripheral blood leukocytes and their differentiation and activation are mainly regulated by interleukin-5 [3]. One of their characteristics is the capacity to adhere to activated blood endothelial cells, leave the blood stream to migrate into inflamed tissues, and concentrate at the site of certain types of inflammation [4–6] and tumors [7]. These cells were soon found in airway tissue and sputum of patients with asthma [8▪]. Over the years, eosinophils were identified as a prominent cell type in certain forms of asthma [8▪] and eosinophilic vasculitis [4,9].

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KEY POINTS

- Although eosinophils represent approximately 1% of peripheral blood leukocytes, they have the propensity to leave the blood stream and to migrate into inflamed tissues.
- Eosinophils and their mediators (e.g. interleukin-5) are critical effectors to asthma and EGPA.
- Anti-interleukin-5 (mepolizumab and reslizumab) and anti-interleukin-5 receptor α (benralizumab) are safe and efficacious in adult patients with eosinophilic asthma.
- Preliminary evidence suggests that mepolizumab is safe in patients with EGPA enabling glucocorticoid tapering without modifying lung function.
- Further studies should be focused on long-term safety and efficacy of anti-interleukin-5 antagonists in young patients with eosinophilic asthma, EGPA, and selected population of COPD, including ACOS.

Asthma is a chronic disease of the airways characterized by inflammatory, functional, and structural changes responsible for variable bronchial hyperresponsiveness and reversible expiratory airway limitation [10]. Airway inflammation is central to disease pathophysiology through the release of several proinflammatory mediators and remodeling of the airway wall [11]. Varying combinations of these complex processes explain the different asthma phenotypes [10]. Most asthmas are associated with T helper type 2 (T_h2) cell-dependent production of IgE and recruitment of eosinophils, mast cells, and basophils [11].

Eosinophilic granulomatosis with polyangiitis (EGPA) (formerly Churg–Strauss syndrome) is a systemic small-vessel vasculitis associated with asthma and eosinophilia [9]. EGPA commonly presents with an upper airway tract and lung involvement associated with persistent eosinophilia and upregulation of interleukin-5.

Chronic obstructive pulmonary disease (COPD), an inflammatory disease distinct from asthma, develops later in life, in smokers and is characterized by progressive irreversible airflow obstruction where a key role is played by CD8 T cells and neutrophils [12]. Acute exacerbations of COPD are usually associated with neutrophils, but can also present airway [13], sputum [14], or blood eosinophilia [15].

The review examines recent advances in the unique biology of the eosinophil, how dysregulated eosinophil functions promote different respiratory disorders, and how targeting the interleukin-5 pathway might offer clinical benefit to some patients with asthma, EGPA, and COPD.

THE UNIQUE BIOLOGY OF THE EOSINOPHIL

Human eosinophils are equipped with a large number of cell-surface receptors [16▪,17,18] (Fig. 1). Human eosinophils can be distinguished from other granulocytes by certain surface receptors selectively expressed on eosinophils [interleukin-5Rα, CC-chemokine receptor 3 (CCR3), cysteinyl leukotriene type 1, $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrins]. The epidermal growth factor-like module containing mucin-like hormone receptor 1 (EMR1) appears truly eosinophil specific [17,20]. A variety of inhibitory receptors that regulate eosinophil survival and activation have also been described including Siglec-8, CD300a, killer activating receptors, potassium inwardly rectifying channel, and FcγRIIb [19].

Eosinophils contain intracellularly the α splice variant of the glucocorticoid receptor (GR-A) in high copy number [21]. The proapoptotic GR-A isoform is five-fold higher in eosinophils than in neutrophils making eosinophils highly susceptible (and the neutrophil much less) to therapeutic effects of glucocorticoids, such as apoptosis [22].

Eosinophil specific granules contain four cationic proteins, major basic proteins (MBP), eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN), and eosinophil peroxidase (EPX) which exhibit cytotoxic activity and can cause significant tissue damage [23]. Lipid mediators produced by eosinophils include leukotriene C4 (LTC_4), platelet-activating factor (PAF), thromboxane B2 (TxB2), prostaglandin (PG)E1, and PGE_2. Human eosinophils are a major source of a wide spectrum of cytokines, including interleukin-5, interleukin-4, interleukin-13, TGFβ, and IFNγ [24–27] (Fig. 2). They also produce a wide spectrum of immunologically active factors, including chemokines [28,29]. Thus, it is likely to predict that eosinophils are equipped to perform different functions such as tissue repair and remodeling, angiogenesis [30], clearance of parasites [31], metabolic homeostasis [32], and immune cell activation [16▪,33]. During their transit in the bloodstream and at sites of inflammatory/immune reactions, eosinophils interact with and modulate the functions of several cells of the innate and adaptive immune system [16▪,34,35] (Fig. 3).

Eosinophils and their mediators participate in the pathophysiology of a variety of diseases, including allergic asthma [10,36▪], EGPA [4,9], and cancer rejection [7]. However, current data suggest that deficiency of eosinophils in animals and humans appears to have no ill effects on normal health [37].

INTERLEUKIN-5 AND EOSINOPHILS

Interleukin-5 is a cytokine that belongs to the β common chain family [together with interleukin-3

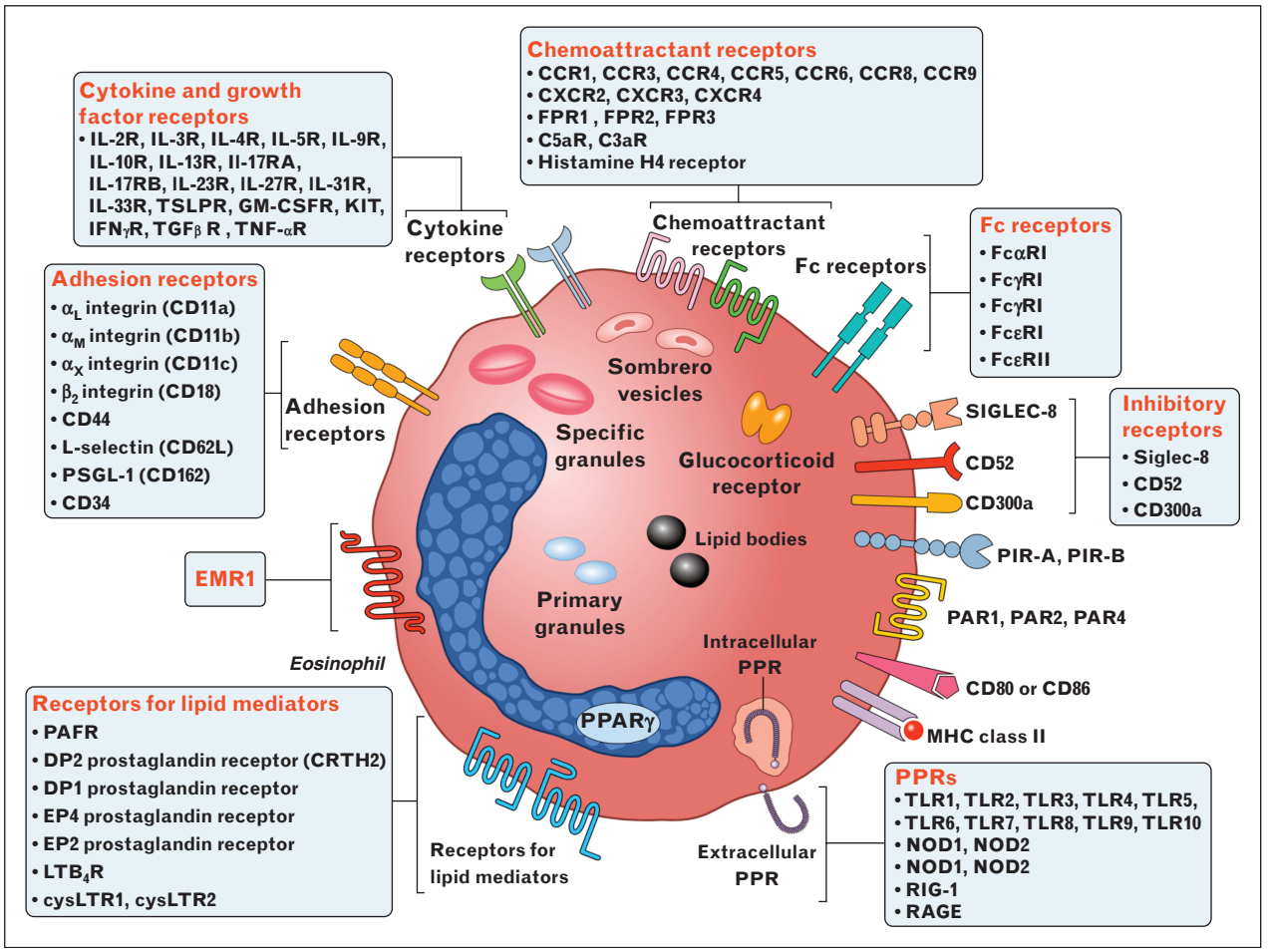

FIGURE 1. Human eosinophils display a wide spectrum of surface receptors that are important for their pleiotropic functions. Eosinophils express cell-surface receptors for cytokines and growth factors, chemokines, adhesion molecules, lipid mediators, chemoattractants, complement, immunoglobulins, Siglecs, histamine, PIRs, PARs, PPRs, CD40, CD80/CD86, and MHC class II. The epidermal growth factor-like module containing mucin-like hormone receptor 1 (EMR1) appears truly eosinophil specific [17]. Eosinophils contain the glucocorticoid receptor in high copy number [19]. The α variant of the glucocorticoid receptor is five-fold higher in eosinophils than in neutrophils making these cells highly susceptible to the therapeutic effects of glucocorticoids. Eosinophils contain specific granules containing several cationic proteins, primary granules, lipid bodies, and sombrero vesicles. CC, chemokine ligand; CCR, CC-chemokine receptor; CXCL, CXC-chemokine ligand; CXCR, CXC-chemokine receptor; PIRs, paired immunoglobulin-like receptors; PARs, proteinase-activated receptors; PPRs, pattern-recognition receptors.

and granulocyte-monocyte colony-stimulating factor (GM-CSF)] and binds an heterodimer receptor composed by the specific subunit interleukin-5Rα and common β subunit βc [3,38] (Fig. 4). Interleukin-5 plays a fundamental role in eosinophil differentiation in the bone marrow, recruitment and activation at sites of allergic inflammation [3]. Human eosinophils express about a three-fold higher level of interleukin-5Rα compared with basophils [39]. T_h2 cells, mast cells, $CD34^+$ progenitor cells, invariant natural killer T, group 2 innate lymphoid cells, and eosinophils themselves are major cellular source of interleukin-5 [40–42]. Group 2 ILCs are an important source of interleukin-5 contributing to tissue and blood eosinophilia [43]. Interestingly, blood eosinophils demonstrate circadian cycling and group 2 innate lymphoid cells control eosinophil number through the production of interleukin-5 [42]. Interleukin-5 modulates the differentiation and maturation of eosinophil in the bone marrow, their migration from blood to tissue sites [44], and the prevention of eosinophil apoptosis [45]. Interleukin-5 also appears to modulate the development and functions of human basophils and mast cells. Interleukin-5 enhances the release of mediators from human basophils [46] via the engagement of IL-5 receptor [42].

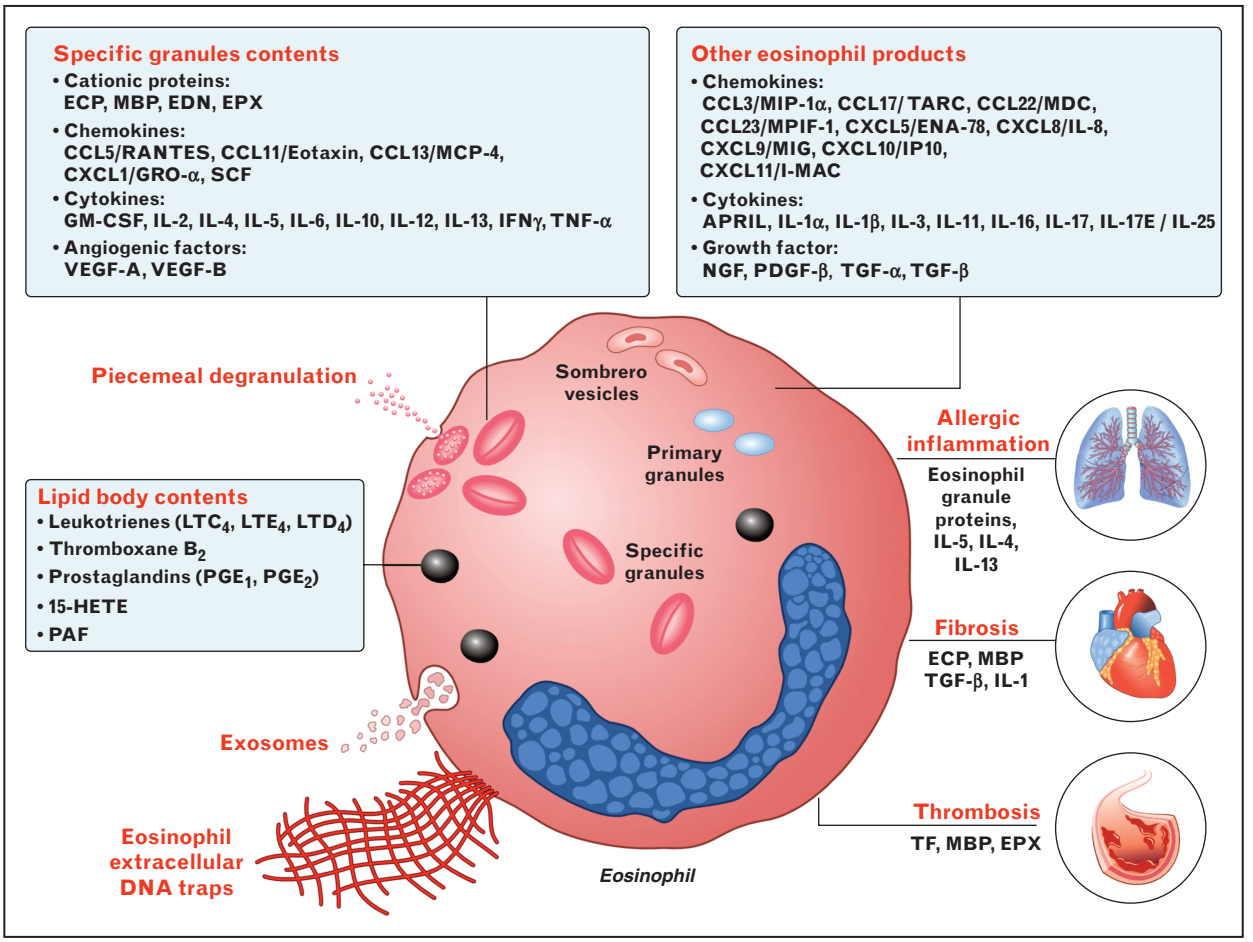

FIGURE 2. Eosinophils contain and/or release a wide array of preformed and *de novo* synthesized mediators important for their effector functions. Specific granules contain several cationic proteins, including MBP, ECP, EDN, and EPX. Eosinophils can degranulate by exocytosis or by piecemeal degranulation whereby individual granule contents are differentially secreted by activated eosinophils without disruption of the cell membrane. Sombrero vesicles are morphologically distinct vesicles that carry granules to the plasma membrane. Lipid bodies are structurally distinct sites within eosinophils that are responsible for synthesis of eicosanoid mediators of inflammation [26]. Eosinophils produce numerous chemokines, cytokines, growth and angiogenic factors that mediate allergic inflammation, fibrosis, and thrombosis. Eosinophils generate extracellular DNA traps [24] and secrete exosomes [27]. A nonexhaustive list of these products is shown in boxes. ECP, eosinophil cationic protein; EDN, eosinophil-derived neurotoxin; EPX, eosinophil peroxidase; MBP, major basic protein; PAF, platelet activating factor; PDGF, platelet-derived growth factor; SCF, stem cell factor; TF, tissue factor; TGF, transforming growth factor; VEGF, vascular endothelial growth factor.

EOSINOPHILS AND INTERLEUKIN-5 IN ASTHMA

There is increasing evidence that eosinophilic inflammation of the lungs is a hallmark of eosinophilic asthma and has been associated with elevated levels of interleukin-5 in bronchial biopsies from asthmatic patients [47]. Moreover, interleukin-5 mRNA is upregulated in the bronchial mucosa upon allergen challenge [48] and interleukin-5 concentrations correlate with clinical features of asthma [49]. Eosinophils play a critical role in the pathogenesis and severity of asthma through the action of interleukin-5. In the asthmatic lung, T lymphocytes and group 2 ILCs are main sources of interleukin-5 with eosinophils and mast cells contributing to the level of this cytokine [43,50]. Interleukin-25 stimulates T_h2 cells and group 2 ILCs to markedly increase the production of interleukin-5 [41,43]. The precise role of eosinophils as a prominent cell type in certain phenotypes of asthma was not firmly established until a number of clinical trials demonstrated that treatment with monoclonal antibodies against interleukin-5 significantly reduced the number of lung and blood eosinophils in patients with severe corticosteroid-resistant asthma [51▪▪,52–55,56▪▪,57]. Trials of therapeutics

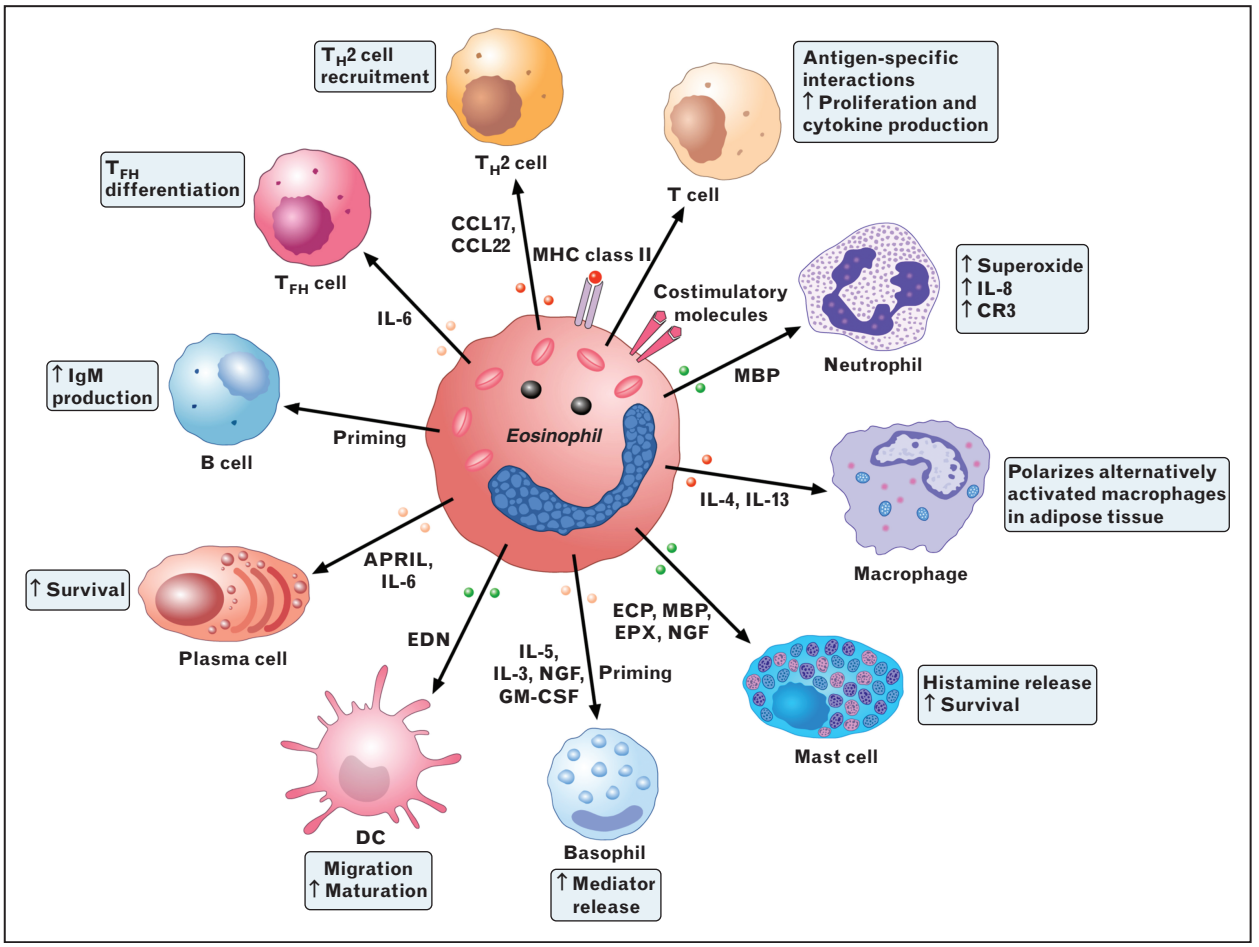

FIGURE 3. Eosinophils modulate the functions of a multitude of cells of the innate and adaptive immune system. Although not professional antigen-presenting cells (APC), eosinophils can express MHC class II and costimulatory molecules (CD80 or CD86), process antigens and stimulate T cells to proliferate and produce cytokines in an antigen-specific manner. Acting with dendritic cells (DCs), eosinophils regulate the recruitment of T helper 2 (T_h2) cells in response to allergen sensitization by producing CCL17 and CCL22. Eosinophil can also favor T follicular helper (T_{fh}) cell differentiation through the production of interleukin-6 [35]. Eosinophils also prime B cells and sustain long-lived plasma cells in bone marrow via the production of APRIL and interleukin-6. Eosinophils stimulated by CpG DNA and by EDN promotes the maturation and activation of DCs. MBP activates neutrophils causing the release of superoxide and interleukin-8 and increases their expression of the cell-surface integrin complement receptor 3 (CR3). Eosinophils also maintain alternatively activated macrophages (M2 macrophages) by producing interleukin-4 and interleukin-13. MBP, ECP, and EPX activate basophils and mast cells, resulting in the release of histamine. Eosinophil granule proteins also activate platelets. Eosinophil-derived NGF primes human basophils and modulates several functions of mast cells.

involving monoclonal antibodies to interleukin-5 and its receptor, interleukin-5Rα, and other approaches have been completed or are underway in patients with bronchial asthma, EGPA, and COPD.

CLINICAL TRIALS EVALUATING INTERLEUKIN-5 ANTAGONISM IN ASTHMA

Targeting interleukin-5 or interleukin-5Rα is an appealing approach to the treatment of patients with eosinophilic asthma. Anti-interleukin-5 monoclonal antibodies bind to interleukin-5 interfering with its ligation to interleukin-5-Rα expressed on the eosinophil and basophil membranes [3] (Fig. 4). Two different humanized anti-interleukin-5 monoclonal antibodies, mepolizumab (GlaxoSmithKline, Brentford, UK) and reslizumab (Teva Pharmaceuticals, Petha Tiqwa, Israel), have been developed and shown safety and efficacy in clinical trials for asthma.

Mepolizumab

Mepolizumab is a humanized monoclonal antibody (mAb) of the $IgG_{1/k}$ class which has been investigated for the treatment of asthma, atopic dermatitis,

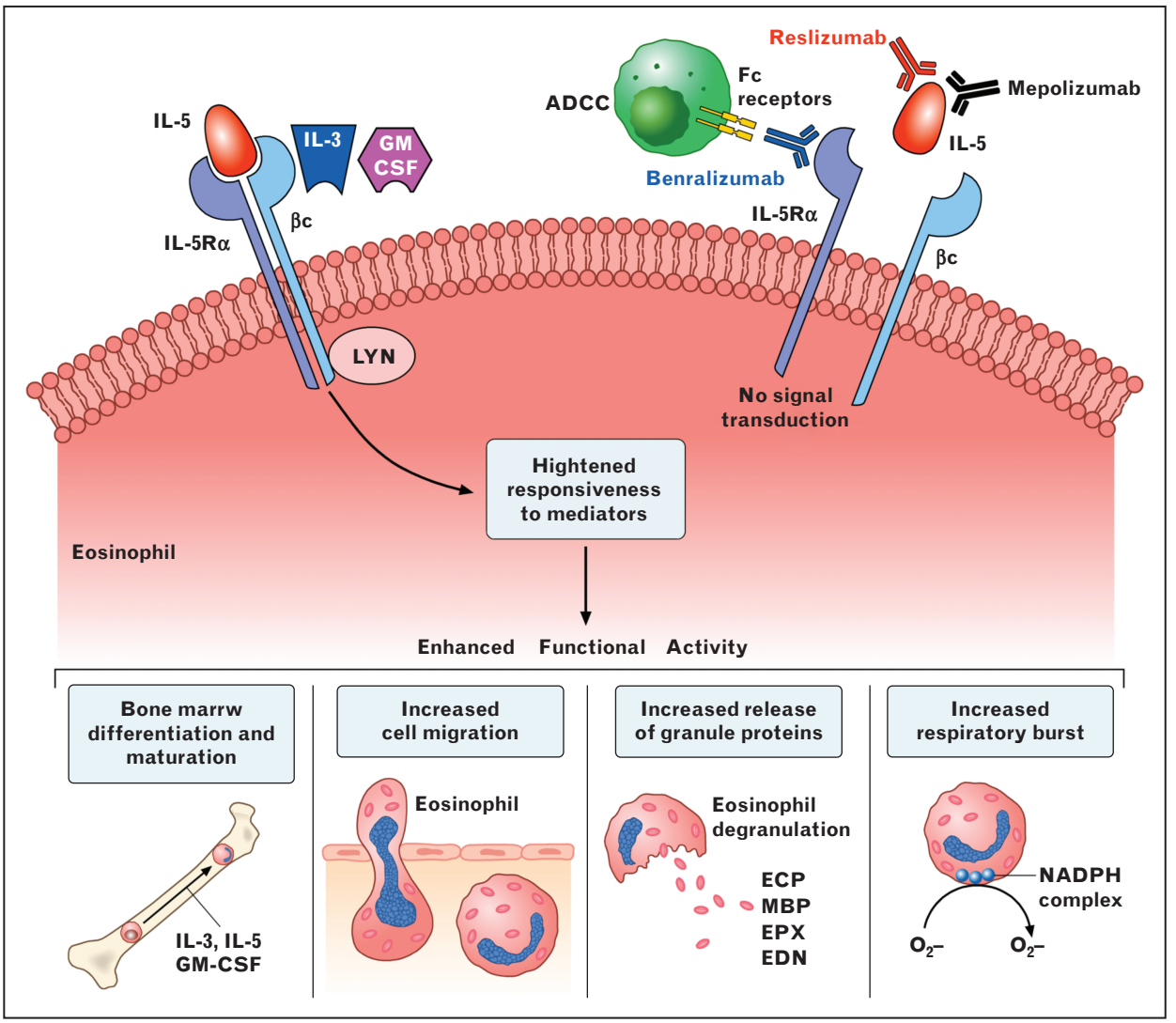

FIGURE 4. Interleukin-5 plays a fundamental role in the proliferation, maturation in the bone marrow, recruitment and activation at sites of allergic inflammation of eosinophils. The engagement of interleukin-5R through the interaction of interleukin-5 with interleukin-5αR and the βc subunit results in differentiation and maturation of eosinophils in the bone marrow, enhanced cell migration, release of granule proteins, and respiratory burst of eosinophils. Interleukin-3 and GM-CSF interact only with the βc subunit of interleukin-5R and enhance the functional response to stimuli of eosinophils (left side). Anti-interleukin-5 monoclonal antibodies (mepolizumab and reslizumab) bind to different epitopes of interleukin-5 blocking its ligation to interleukin-5Rα highly expressed on the human eosinophil membrane. Benralizumab is a humanized monoclonal antibody that binds to human interleukin-5Rα, resulting in inhibition of interleukin-5 activation. The latter approach also leads to antibody-dependent cellular cytotoxicity (ADDC) caused by Fc receptor binding on natural killer cells to the anti-interleukin-5Rα antibody on eosinophil (right side).

hypereosinophilic syndrome, eosinophilic esophagitis, nasal polyposis, and EGPA [51▪▪,53,55,56▪▪, 57–59]. Table 1 summarizes the clinical trials evaluating the effects of mepolizumab in asthma [51▪▪,53,55,56▪▪,57,58,60]. An initial study [58] on the safety and efficacy of mepolizumab (750 mg intravenously (i.v.) every 4 weeks for 3 months) in 11 patients with mild asthma showed that the antibody was well tolerated and induced a decrease of blood eosinophils, but did not deplete airway or bone marrow eosinophils. The authors found no difference between mepolizumab and placebo on peak expiratory flow rate, airway hyperresponsiveness, or forced expired volume in one second (FEV_1) in these patients with mild asthma. A subsequent larger study [53] in patients with refractory eosinophilic asthma confirmed that mepolizumab (750 mg i.v. every 4 weeks for 1 year) reduced blood and sputum eosinophils. This treatment reduced the number of severe exacerbations during the

Table 1. Clinical trials of mepolizumab in asthma (anti-interleukin-5, IgG_1 – Bosatria – GSK)

First author/ref/year	Disease severity	No. of patients treated	Dosage/delivery	Outcome summary
Flood-Page *et al.* [58], 2003	Mild asthma	11	750 mg i.v. every 4 weeks for 3 months	↓Blood Eos; ↓Airway Eos only by 50% = PEF, FEV_1, bronchial hyperresponsiveness
Haldar *et al.* [53], 2009	Eosinophilic asthma	61	750 mg i.v. every 4 weeks for 1 year	↓Blood + Sputum Eos; ↓Severe exacerbations; ↑QoL = FEVj, bronchial hyperreactivity
Nair *et al.* [55], 2009	Prednisone-dependent asthma	9	750 mg i.v. every 4 weeks for 5 months	↓Blood + Sputum Eos; ↓Exacerbations; Prednisone sparing effect
Pavord *et al.* [57], 2012	Severe eosinophilic asthma	462	75–250–750 mg i.v. every 4 weeks for 13 infusions	↓Blood + Sputum Eos; ↓Exacerbations = FEV_1, AQLQ, and ACQ scores
Bel *et al.* [51▪▪], 2014	Severe eosinophilic asthma	135	100 mg s.c. every week for 20 weeks	Glucocorticoid sparing effect; ↓Exacerbations; Improvement ACQ-5 score
Ortega *et al.* [56▪▪], 2014	Severe eosinophilic asthma	385	75 mg i.v. or 100 mg s.c. every 4 weeks for 32 weeks	↓Blood + Sputum Eos; ↓Exacerbations; ↑FEV_1; ↑ACQ-5 score
Basu *et al.* [60], 2015	Severe eosinophilic asthma			Healthcare resources and costs of mepolizumab versus placebo in a clinical trial (MENSA Study)

treatment and improved the quality of life score [Asthma Quality of Life Questionnaire (AQLQ)]. Also in this study, mepolizumab did not influence FEV_1 and bronchial hyperreactivity. In a similar study [55] performed on a limited number of nine patients with prednisone-dependent asthma, mepolizumab (750 mg i.v. every 4 weeks for 5 months) had similar effects on blood and sputum eosinophils and reduced exacerbations. This study showed that mepolizumab allowed prednisone sparing in patients who had asthma with sputum eosinophilia. In the largest study ever undertaken (462 patients) in severe eosinophilic asthma, mepolizumab (75, 250, or 750 mg i.v. every 4 weeks for 13 infusions) reduced blood and sputum eosinophilia and exacerbations. Also in this study [57], FEV_1, AQLQ, and Asthma Control Questionnaire (ACQ) scores were not modified by mepolizumab treatment.

Two recent studies have evaluated the efficacy and safety of mepolizumab administered subcutaneously (s.c.). In 135 patients with severe eosinophilic asthma, mepolizumab (100 mg s.c. every 4 weeks for 20 weeks) had a glucocorticoid-sparing effect and reduced exacerbations. The authors also reported a significant improvement in the ACQ-5 score [51▪▪]. In another study [56▪▪] in which 385 patients with severe eosinophilic asthma were treated with mepolizumab (75 mg i.v. or 100 mg s.c. every 4 weeks for 32 weeks), this treatment reduced blood eosinophils, the number of exacerbations, improved FEV_1 and ACQ-5 score. The latter study has been subjected to additional analysis showing that mepolizumab treatment significantly reduced the cost of the treatment of these patients [60]. An open-label study evaluating the pharmacokinetics and pharmacodynamics of mepolizumab administered s.c. in children from 6 to 11 years of age with severe eosinophilic asthma is underway (NCT02377427). First, on November 2015 the US FDA and, then, on December 2, 2015 the European EMA approved mepolizumab as an add-on maintenance treatment for adults with severe eosinophilic asthma.

Reslizumab

Reslizumab (formerly SCH55700, Cinquil; Teva Pharmaceuticals) is a humanized anti-interleukin-5 mAb of the $IgG_{4/k}$ class in clinical development for the treatment of eosinophilic inflammatory disorders. Reslizumab has been evaluated in randomized controlled clinical trials in patients with asthma [52,54] (Table 2) and nasal polyps [61]. In 18 patients with severe asthma reslizumab (0.03–1 mg/kg i.v. in single dose) was safe and decreased blood eosinophils for at least 4 weeks [62]. In a more recent study [52], reslizumab administered in 53 adult patients with severe eosinophilic asthma (3 mg/kg i.v. every

Table 2. Clinical trials of reslizumab in asthma (SCH55700 – anti-interleukin-5, IgG_4 – Cephalon Inc.)

First author/ref/year	Disease severity	No. of patients treated	Dosage/delivery	Outcome summary
Kips *et al.* [54], 2003	Severe asthmatics	18	0.03–1 mg/kg i.v. single dose	Safe; ↓Blood Eos
Castro *et al.* [52], 2011	Severe eosinophilic asthma	53	3 mg/kg i.v. every 4 weeks for 12 weeks	↓Blood Eos; ↑FEV_1; ↑ACQ-5 score; Particularly in patients with nasal polyps ±30% patients had nasal polyps

4 weeks for 12 weeks) reduced blood and sputum eosinophils, improved airway function (FEV_1), and increased ACQ score. The biological and clinical improvement was more marked in patients with nasal polyps that represented approximately 30% of patients with severe eosinophilic asthma. These findings have prompted multifaceted asthma studies that are currently underway. The US FDA Advisory Committee recommended approval for reslizumab on 11 December 2015 as an add-on maintenance treatment for adults with severe eosinophilic asthma.

Benralizumab

Benralizumab (formerly MEDI-563; MedImmune-AstraZeneca, London, UK) is a humanized mAb of the $IgG_{1/k}$ class that binds to human interleukin-5Rα, resulting in inhibition of interleukin-5 receptor activation. Benralizumab is not fucosylated and this enhances its binding to FcγRIIIa, leading to enhanced antibody-dependent cell-mediated cytotoxicity [3] (Fig. 4). Benralizumab binds with high affinity to the D1 domain of interleukin-5Rα on human eosinophils and basophils. Interestingly, human eosinophils express about a three-fold higher levels of interleukin-Rα compared with basophils [39]. In the latter study, it has been demonstrated that benralizumab induces eosinophil and basophil apoptosis mediated by antibody-dependent cell-mediated cytotoxicity.

Table 3 summarizes the clinical trials evaluating the effects of benralizumab in asthma [63,64▪▪,65,66]. The first study in 44 adult patients with mild atopic asthma a single dose of i.v. benralizumab (0.03–3– mg/kg) reduced blood eosinophils. Eosinopenia lasted 8–12 weeks. Benralizumab was associated with a transient, mild decrease of white

Table 3. Clinical trials of benralizumab in asthma (MEDI-563, Anti-interleukin-5a, IgG_1 – Medimmune)

First author/ref/year	Disease severity	No. of patients treated	Dosage/delivery	Outcome summary
Busse *et al.* [63], 2010	Mild atopic asthma	44	0.0003–3 mg/kg i.v. single dose	↓Blood Eos at dose 0.03–3 mg; Eosinopenia lasted 8–12 weeks Transient, mild decrease in WBC CRP increased ±5.5-fold Interleukin-6 increased CPK of peripheral muscular origin increased
Laviolette *et al.* [65], 2013	Eosinophilic asthma	26	1 mg/kg i.v.; 100 mg s.c. every month for 3 doses; 200 mg s.c. every month for 3 doses	↓Eos in blood, sputum and bronchial mucosa; ↓Basophils; Nasopharingitis 25%; Headache 25%; Nausea 22%
Castro *et al.* [64▪▪], 2014	Eosinophilic asthma	384	2–20–200 mg 2 s.c. every 4 weeks for the first 3 doses, then every 8 weeks for 1 year	20 mg and 100 mg↓ asthma; Exacerbation = FEV_1?
Nowak *et al.* [66], 2015	Asthma after acute attack	72	Single dose 0.3 mg/kg i.v. 1 mg/kg i.v. Evaluated up to 6 months	↓Blood Eos; ↓Exacerbations

blood cells and an increased of C-reactive protein (± 5.5-fold), interleukin-6 and creatine phosphokinase of peripheral muscular origin [63]. In another study of 26 adult patients with eosinophilic asthma, single-dose i.v. (1 mg/kg) and 3 s.c. doses (1 mg or 200 mg every month for 3 months) reduced eosinophil counts in blood sputum and airway mucosa/submucosa. Interestingly, also the number of basophils, which expressed interleukin-5Rα [39], markedly decreased [65].

In a phase 2b study, benralizumab was administered to 385 adult patients with eosinophilic, uncontrolled asthma. In asthmatics receiving benralizumab (20 and 100 mg two s.c. injections every 4 weeks for a total of 12 months) there were fewer exacerbations. The higher 100 mg dose of benralizumab also improved lung function, asthma control, and mean ACQ-6 score compared with placebo [64▪▪]. This study has provided useful information to design phase 3 studies underway in patients with moderate or severe asthma with peripheral eosinophil count of at least 300 cells/μl. In a recent study [66], a single dose of benralizumab (0.3 and 1 mg/kg i.v.) was administered to 72 adult patients with severe asthma resulting in emergency department visit with the assumption that these patients are at increased risk for exacerbations. A single dose of benralizumab reduced blood eosinophils and the exacerbations during the following 3 months.

CLINICAL TRIALS EVALUATING ANTISENSE OLIGONUCLEOTIDES IN ASTHMA

Antisense oligonucleotides can be used as a therapeutic strategy to down-regulate the transcription of specific proteins [67]. TPI ASM8 (Topigen Pharmaceuticals, Montreal, Canada) is a mixture of two modified phosphorothioate antisense oligonucleotides, one directed against the human βc chain shared by the interleukin-3, interleukin-5, and GM-CSF receptors, and the other directed against the chemokine receptor CCR3 present on human eosinophils, basophils [68], and mast cells [69]. With this broad spectrum of activity, it was hoped that TPI ASM8 may provide more complete inhibition of eosinophilic influx than agents targeting interleukin-5 alone. Inhalation of TPI ASM8 reduced eosinophils in sputum and attenuated the allergen-induced airway responses in subjects with mild asthma [70]. A subsequent study evaluated the dose-response effects of TPI ASM8 in mild asthmatics. It found that TPI ASM8 was safe and well tolerated at all doses and inhibited eosinophil influx in sputum and ECP after allergen challenge. Moreover, the oligonucleotides attenuated early and late responses to allergen and improved airway hyperresponsiveness to methacholine [71]. In another study [72], TPI ASM8 reduced allergen-induced sputum eosinophils, the early and late asthmatics responses and the number of eosinophil progenitors $CD34^+$ interleukin-$5\alpha R^+$ in mild asthmatics. Although therapy with this novel multitarget approach appears safe and promising, TPI ASM8 seems to have been discontinued.

CLINICAL TRIALS EVALUATING INTERLEUKIN-5 ANTAGONISM IN EOSINOPHILIC GRANULOMATOSIS WITH POLYANGIITIS

In 1951, J. Churg and L. Strauss [73] first described a form of systemic vasculitis occurring exclusively among patients with asthma and intense tissue eosinophils. This condition, called 'Churg–Strauss syndrome' for many years, has now been recognized as EGPA [9]. EGPA commonly presents with upper airway tract and lung involvement, cardiac and skin lesions. Although the pathogenesis of EGPA is multifactorial, the disease has a genetic background and can presumably be triggered by exposure to allergens or drugs [9]. The asthmatic and eosinophilic components suggest an activated T_h2 imbalance [74]. Interleukin-5 appears to be upregulated in active EGPA [74]. Eosinophils are increased both in peripheral blood and tissue lesions. Eotaxin-3, produced by epithelial and endothelial cells, might contribute to tissue influx of eosinophils [75]. Activated eosinophils release cationic proteins, thereby contributing to tissue damage. Moreover, eosinophils in EGPA produce interleukin-25, which induces T_h2 responses, thereby maintaining a vicious circle [76].

There is no consensus regarding the use of remission induction and remission maintenance therapeutic approach in patients with different forms of EGPA. Asthma is a well established and prominent clinical hallmark of EGPA. In a series of 383 patients, about 90% had asthma at EGPA diagnosis, with a mean onset of asthma to EGPA onset interval of 9 years [77]. In a series of 22 patients, the majority had severe or moderate asthma onset, and the condition was poorly controlled in 95% [78]. Cardiac involvement occurs in the majority of EGPA cases [77] and represents the major cause of early death and poor long-term prognosis. Eosinophil cationic proteins can activate human cardiac mast cells to induce the release of fibrogenic and vasoactive mediators [34,79].

A pilot study [80] tested the safety and efficacy of mepolizumab in seven steroid-dependent EGPA

patients unable to taper prednisone below 10 mg daily. The patients received 4 monthly infusions of mepolizumab (750 mg each) on top of their therapy. Most patients were able to taper their prednisone dose, achieved a better control of the disease and a reduction in peripheral eosinophil count. Interestingly, the lack of improvement in pulmonary function despite reduced peripheral blood eosinophil count suggests that other variables contribute to EGPA airway disease. Finally, relapses were the rule following treatment discontinuation. Another pilot study [81] in refractory/relapsing EGPA patients confirmed the glucocorticoid-sparing property of mepolizumab in a series of eight out 10 patients. These results suggest that adjunct therapy with mepolizumab might be a glucocorticoid-sparing treatment option in patients with EGPA and reiterates the role of eosinophil in this eosinophilic vasculitis. Several double-blind placebo-controlled clinical trials are underway (NCT00716651 and NCT02020889).

CLINICAL TRIAL EVALUATING INTERLEUKIN-5 ANTAGONISM IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE

COPD is a major cause of morbidity and mortality throughout the world that is primarily caused by tobacco smoking and indoor/outdoor pollution. It is characterized by irreversible, progressive airflow obstruction [82]. Chronic inflammation in the airways is mainly caused by $CD8^+$ T cells, macrophages, and neutrophils [12]. Approximately 20% of patients with COPD without asthma or atopy have persistent circulating and airway eosinophilia associated with an increased risk of exacerbations [14,83]. Importantly, asthma attacks with eosinophils predict mortality in COPD patients [15]. Inhaled glucocorticoids are the key drug to prevent exacerbations in severe COPD where eosinophilia is present [12].

Although asthma and COPD in their typical forms are distinct clinical entities, some patients have features of both diseases. Their condition is now called asthma-COPD overlap syndrome (ACOS) [84,85]. ACOS is still poorly understood and presumably includes several phenotypes necessitating different treatments [86,87].

A phase 2 trial tested the safety and efficacy of benralizumab in eosinophilic COPD (sputum eosinophils >3%) [88▪]. In patients with COPD benralizumab did not reduce the exacerbations and did not modify lung function. However, it was noticed a trend toward an improvement in FEV_1 and exacerbations in patients with a baseline blood eosinophils greater than 200 cells/μl and treated with benralizumab. Additional studies are needed to evaluate the safety and efficacy of interleukin-5-targeted therapy in different forms of COPD, including ACOS.

CONCLUSION

In several studies the administration of mepolizumab has been found to be well tolerated in adult patients with eosinophilic asthma [51▪▪,53,55,56▪▪,57] and EGPA [81,89] for periods of 3 months to approximately 1 year. Recent evidence demonstrates that eosinophils play a major role in cancer rejection [7] and several hematologic and tissue cancers can be associated with eosinophilia [90]. Moreover, it has been suggested that 'targeted antieosinophilic strategies may unmask or even accelerate progression' of certain tumors in few patients with hypereosinophilic syndrome [91]. Therefore, long-term studies should evaluate the safety of targeted antieosinophilic strategies.

The success of novel biological agents in general, and in particular for interleukin-5 pathway inhibition, in asthma largely depends on the ability to select the appropriate patients. Ideally, patients should be selected by an easily measurable biomarker. The blood and/or sputum eosinophil count appears to be closely associated with a clinical response to interleukin-5 pathway inhibition in adult patients with eosinophilic asthma [51▪▪,55, 56▪▪,57]. It is unclear whether eosinophilia is a useful biomarker to predict the efficacy of interleukin-5R targeting in patients with eosinophilic COPD. Other biomarkers in asthma might include activated eosinophil surface phenotype as detected by flow cytometry [92], elevated levels of blood and/or sputum interleukin-5 [93], soluble interleukin-5Rα [94], EMR1 [21], and soluble Siglec-8 [95]. We would like to suggest that the combined use of multiple biomarkers might be a better strategy to select asthmatic, COPD and ACOS patients responsive to interleukin-5 pathway inhibitors. The use of multiple inflammatory biomarkers has been already shown to improve the prediction of risk for cardiovascular disease [96].

It has been suggested that using supervised cluster analysis can help to select specific patients characteristics and therapeutic response to mepolizumab [97▪]. It is likely that in the future, biologic samples (e.g. blood cells, tissue biopsy, or sputum) from patients with eosinophilic respiratory disorders will be analyzed (e.g. biomarkers, transcriptomes, genes, microRNA, and others) for the purpose of phenotyping patients to tailor their treatment.

Severe eosinophilic asthma can occur in children [36▪]. Pharmacologic and biologic treatment of

Table 4. Examples of agents targeting directly or indirectly human eosinophils in preclinical or clinical development

Strategy	Target	Drug	Antieosinophil effects	References
Cell-surface protein	Siglec-8	Anti-Siglec-8 monoclonal antibody	Apoptosis	Nutku *et al.* [104]; Bochner *et al.* [105]
	CD172a		Inhibitor of signaling	Verjan Garcia *et al.* [106]
	CD300a		Activation of inhibitory receptor	Munitz *et al.* [19]
	Immunoglobulin -like receptor B			Munitz *et al.* [107]
	$\alpha_4\beta_1$, $\alpha_4\beta_7$	Natalizumab	Increase blood eosinophils and inhibits their tissue accumulation	Abbas *et al.* [108]
	$\alpha_4\beta_7$ integrin	Vedolizumab	No effect	Soler *et al.* [109]
	$\alpha_4\beta_7$, $\alpha_E\beta_7$	Etrolizumab	Unknown	
	CCR3	GW766944	Block chemokine-induced eosinophils *in vitro*; no effect *in vivo*	Neighbour *et al.* [110]
	CD52	Alemtuzumab	Deplete eosinophils *in vivo*	Wechsler *et al.* [25]
	CD131	CSL311	Unknown	
	CRT_H2	0C000459	Reduces tissue eosinophils	
		ACT-453859	CRT_H2 blockade	Gehin *et al.* [111]
	EMR1	Afucosylated anti EMR1 monoclonal antibody	Deplete primate eosinophils	Legrand *et al.* [20]
	Interleukin-4Rα	Dupilumab	Reduces airway eosinophils	Wenzel *et al.* [112]
	Interleukin-4Rα	AMG-317	Does not reduce airway eosinophils	Corren *et al.* [113]
	H4 Receptor	UR-63325 JNJ 28610244		Salcedo *et al.* [114]; Dib *et al.* [115]
Soluble mediator antagonist	Eotaxin-1	Bertilimumab	Inhibits Eotaxin-1 mediated eosinophil activation *in vitro*	Ding *et al.* [116]
	IgE	Omalizumab	Reduces eosinophils at sites of allergic inflammation and peripheral blood	Detoraki *et al.* [102]
	Interleukin-4	Altrakincept; Pascolizumab; Pitrakinra	Reduce eosinophils at sites of allergic inflammation	Borish *et al.* [117]; Hart *et al.* [118]
	Interleukin-13	Tralokinumab; Lebrikizumab; Anrukinzumab; RPC4046; QAX576	Reduce eosinophils in blood and at sites of allergic inflammation	Blanchard *et al.* [119]; Maselli *et al.* [120]
	TSLP	AMG157	Reduce eosinophils in blood and at sites of allergic inflammation	Gauvreau *et al.* [121]
Transcription factor	GATA3	SB010	Reduce interleukin-5 and late asthmatic response after allergen challenge	Krug *et al.* [103▪]

different forms of asthma can differ in children when compared with adults also because of distinct pathogenetic mechanisms [36▪], comorbidities (e.g. cardiovascular involvement) and pharmacokinetics/pharmacodynamics [98]. An open-label study is underway to characterize the pharmacokinetics/pharmacodynamics of mepolizumab administered s.c. in children from 6 to 11 years of age with severe eosinophilic asthma (NCT02377427). This study will also provide information whether eosinophilia is a useful biomarker to predict the efficacy of interleukin-5 targeting in children with eosinophilic asthma.

Omalizumab is approved in the treatment of adults and adolescents with severe asthma. A comparison of the efficacy and cost-effectiveness of omalizumab versus interleukin-5 pathway inhibitors in the treatment of adults and adolescents with severe asthma is needed in populations eligible to both the biologics.

Benralizumab binds with high affinity to the D1 domain of interleukin-5Rα present on both human eosinophils and basophils. Although eosinophils express about three-fold higher level of interleukin-5Rα compared with basophils, benralizumab induces apoptosis of both eosinophils and basophils) [39]. Thus, the possibility exists that benralizumab might have a more articulated effect compared with the monoclonal antibodies anti-interleukin-5. In addition, given the relevance of basophils and their mediators (e.g. interleukin-4, interleukin-3, leukotrienes, VEGFs, and others) in the pathogenesis of allergic disorders [99–101] it is possible that some of the clinical benefits of benralizumab in asthma might be because of its modulatory effect on these cells.

Asthma and COPD are distinct chronic inflammatory respiratory disease characterized by some similarities and many striking pathophysiological differences [84–86]. A total of 20–30% of patients with COPD without history of asthma have blood and tissue eosinophilia associated with increased risk of exacerbations [13,15,83]. In a phase 2 clinical trial, benralizumab did not reduce the exacerbation rate in eosinophilic COPD [88▪]. Further studies should focus on selected populations of COPD, including ACOS.

Asthma is a prominent feature of EGPA and there is no consensus on the use of remission induction and remission maintenance of EGPA. Preliminary evidence suggested that mepolizumab is safe in patients with EGPA enabling glucocorticoid tapering without modifying lung function [89]. Interestingly, in a pilot study [102] we have found that omalizumab has a glucocorticoid-sparing effect while decreasing blood eosinophils and improving lung function in EGPA patients.

In conclusion, targeted therapies with anti-interleukin-5 or anti-interleukin-5Rα seem safe and promising in short-term and medium-term treatment of selected adult patients with severe eosinophilic asthma. The long-term safety of these agents is an important issue that needs to be addressed also in the light of recent evidence of antitumor activity of eosinophils. Identification of novel biomarkers, in addition to sputum and blood eosinophilia, will allow a more selective identification of patients responsive to these treatments. Ongoing studies will provide information whether interleukin-5/interleukin-5Rα inhibition is safe and efficacious in children with eosinophilic asthma and selected patients with EGPA or COPD.

Several biologics, small molecules, and a GATA binding protein 3 (GATA3)-specific DNA enzyme [103▪] are advancing in clinical trials that would meet the criteria referred to as personalized or precision medicine treatment for patients with eosinophilic respiratory disorders (Table 4) [104–121]. Excitement is growing that within the next few years several biologics specifically targeting interleukin-5 pathway may become approved for clinical use in selected patients with eosinophilic inflammation. This will possibly happen when a wider range of specific biomarkers will lead us to a more precise identification of patients eligible for treatment with these biologic drugs [122▪].

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Conflicts of interest

There are no conflicts of interest.

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