

**REVIEW PAPER**

# Biological treatment options for eosinophilic asthma, with consideration of markers of eosinophilic inflammation as predictors of treatment efficacy

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## ABSTRACT

Asthma is one of the most common allergic diseases affecting millions of people of all ages worldwide and influencing socioeconomic issues. Asthma, particularly uncontrolled asthma, not only reduces the quality of life but is also a major cause of disability for employment. The current pharmacologic strategy to therapy consists mostly of inhaled glucocorticoids and  $\beta$ -agonists, as well as leukotriene antagonists, long-acting muscarinic antagonists, and oral corticosteroids. However, the conventional therapy may provide insufficient control of severe asthma and may induce a number of adverse effects when used long-term. These limitations prompted the development of targeted biological drugs that inhibit the mediators involved in T helper 2-inflammation, which is linked to the pathogenesis of asthma, such as interleukin 4, 5, 9, 13 and immunoglobulin E. In many individuals, biological treatment provides significant relief and allows for the reduction or elimination of oral corticosteroids without compromising asthma control. However, some patients do not improve to their full potential. The cause of this phenomenon is unknown and requires further investigation.

## KEY WORDS

biological treatment, cytokines, asthma, eosinophils, type 2 inflammation.

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## INTRODUCTION

Asthma is a chronic inflammatory disease of the airways characterized by reversible airflow obstruction and easily triggered bronchospasms which affects more than 300 million people worldwide [1]. Symptoms include cough, particularly at night or early in the morning, wheezing and shortness of breath. The episodes are usually associated with allergen contact, irritants or air pollution and they can be suppressed spontaneously or with treatment. Factors e.g. viral infections, mold, cigarette smoke, exercise, cold air, some medications (aspirin,  $\beta$ -blockers) and, obviously, allergens may trigger exacerbations. Avoiding triggers mentioned above and effective pharmacotherapy may ensure good quality of life and proper asthma control [2].

It was thought for a long time that asthma is a homogenous disease. Nowadays, it is known that there are many mechanisms that could cause asthmatic symptoms. Several phenotypes and endotypes of asthma have been described. Initially, asthma phenotypes were defined by combinations of clinical features. In 2012, Wenzel sought to link biology to symptoms by describing six distinct kinds of asthma: early-onset allergic, late-onset eosinophilic, exercise-induced, obesity-related, neutrophilic, and paucigranulocytic asthma [3]. The term endotype refers to a subtype of a disease defined functionally and pathologically by a molecular mechanism or by treatment response according to GINA. The present-day classification divided to two basic groups T2-high and T2-low driven disease relies on the level of bronchial eosinophilia [4].

Type 2 immunity is induced by Th2 (T helper lymphocytes) and is associated with an increased production

of the cytokines related with Th2 including interleukin-4 (IL-4), IL-5, IL-9, and IL-13. Molecules with protease activity, e.g. allergens, can break cell tight junction proteins and get access to submucosal dendritic cells (DC). Damaged epithelial cells release cytokines such as TSLP, IL-25, and IL-33, which activate innate lymphoid cells (ILC) and DC. Mature DC move to nearby lymph nodes and they use MHC class II molecules to deliver allergen peptides to naïve T cells. Type 2 cytokines are produced by Th2 as well as ILC2 cells. IL-4 and IL-13 stimulate lymphocytes B to class-switch to IgE. In order to sensitize mast cells (MC), IgE binds to receptor FcRI (epsilon) on their surface. Mast cell-associated mediators such as tryptase, histamine, leukotrienes, prostaglandins, are released, causing smooth muscle contraction and hyperplasia of goblet cells. Then Th2 cytokines are produced and released [5, 6]. They play an important role in the inflammation process, e.g. IL-9 induces eosinophilic inflammation, mucus hypersecretion and hyperresponsiveness of the airway, whereas IL-5 play a crucial role in activation, recruitment and survival of eosinophils. On the other hand, T-regulatory (Treg) cells can decrease type 2 response by releasing immunoregulatory cytokines such as IL-10 and transforming growth factor- $\beta$  (TGF- $\beta$ ) [7, 8] (Table 1).

## ROLE OF EOSINOPHILS IN ASTHMA

Eosinophils are mature, definitely differentiated, acidophilic granulocytes that play a significant role in innate host defense against pathogens, especially parasites. Eosinophils have ability to damage pathogenic and also host cells through the release of toxic granule proteins and reactive oxygen species (ROS) [9].

Activated eosinophils contribute to inflammation of the airway and cause destruction of the bronchial mucosa by releasing multiple chemokines, cytokines, growth factors and lipid mediators. Increasing scientific evidence indicates that eosinophils play a significant role as effector cells involved in airway remodeling, a phenomenon characterized by subepithelial fibrosis, with fibroblast and myofibroblast accumulation beneath the subepithelial basement membrane, increased TGF- $\beta$ 1 expression, and excessive extracellular matrix (ECM) protein and metalloproteinase (MMP) deposition in the stroma underlying the bronchial epithelium [10]. Other changes of the airway structure observed in asthma include an increase in smooth muscle mass, goblet cell hyperplasia, and new blood vessel formation. The end result is increased thickness of the bronchial wall, leading to a reduction in the airway caliber, an exaggerated airway narrowing, and a progressive decline of respiratory function [11, 12]. Through effector mechanisms mentioned above, eosinophils have a great impact on tissue specific function [10].

**TABLE 1.** Comparison of inflammatory phenotypes of asthma [8]

Feature	Type 2 asthma	Non-type 2 asthma
Age of onset	Childhood, young age	Adulthood, later onset
Symptoms	May be significant	May be significant
Exacerbation rate	Higher	Lower
Obstruction	More	Less
SABA response	Better	Worse
Exhaled NO	Normal to elevated	Low to normal
Airway eosinophilia	Present	Absent
Allergic sensitization	Present	Absent
Response to corticosteroids	Better	Worse
Obesity	May be present	Frequent

Major basic protein (MBP), also known as proteoglycan 2, is an eosinophil granule protein. MBP's biological activities are mostly connected to its cytotoxic properties. It changes the charge of surface membranes, which results in disrupted permeability, disruption and harm to cell membranes. MBP plays a crucial role in eosinophil-mediated host defense against helminth infection. However, elevated levels of MBP have been also observed at eosinophil-rich sites of inflammation e.g. in bronchial epithelium in asthmatics. MBP activates histamine release by basophils and mast cells [13]. Eosinophil cationic protein (ECP) is another eosinophil granule protein. ECP plays an important role in eosinophil-related disorders, particularly in asthma. According to scientific studies, eosinophil overexpression corresponds with asthma severity and exacerbation frequency. In asthmatics, increased amounts of eosinophils are present in blood, in sputum, and bronchoalveolar fluid [14, 15].

Eosinophilic asthma typically presents in adulthood (40–50 years of age) and is characterized by T2-high eosinophilic inflammation of the respiratory tract [16]. Exacerbations are frequent and patients are often dependent on oral corticosteroids. Chronic rhinosinusitis and nasal polyposis could coexist with eosinophilic inflammation of the airways [17].

## EOSINOPHILS' HETEROGENEITY

Eosinophils' primary role in both health and sickness is to control local immunity and remodeling/repair [9]. Eosinophils play a role in host defense against various pathogens, tissue homeostasis and exhibit immunomodulatory activities. Eosinophils promote allergic inflammation through the release of pro-inflammatory mediators, proteins. The eosinophils' twin responsibilities in creating and suppressing inflammation imply that functional subtypes of eosinophils may exist. 'Resident eosinophils' (rEos) are present in healthy tissues in the absence of inflammation, in various locations e.g. in the airways, the uterus, the thymus and adipose tissue. However, they are most prevalent in the gastrointestinal tract. Eosinophils are found in almost every part of the gastrointestinal system, excluding the esophagus. Eosinophils in the intestine contribute to mucosal immunological homeostasis. For instance, the absence of eosinophils is related with decreased secretory IgA production at the intestinal mucosa, changes in the intestinal microbiome, and dysregulated mucosal barrier integrity [18]. Resident eosinophils are suggested to help maintain tissue homeostasis [19, 20]. The other type of eosinophils are inducible (iEos), which are IL-5 dependent cells and are associated with inflammatory conditions e.g. asthma, eosinophilic esophagitis (EoE). They are typically triggered by an allergic

reaction to dietary or environmental allergens via eosinophil-derived mediators (including eotaxin-3, IL-5, IL-13, TGF- $\beta$ , and periostin) [21].

Mesnil *et al.* conducted a mouse study to evaluate the role of rEo in the lungs. Firstly, it was proven that rEo and iEo differ from each other. They have dissimilar gene profile and expression of biomarkers [22]. iEos expressed a high level of various proinflammatory genes, including Slc3a2, Tlr4, C3ar1, Il13ra1, and Il6, consistent with their established function as proinflammatory effector cells. While, rEos expressed several genes involved in the negative regulation of immunological responses and tissue homeostasis, including Anxa1, Runx3, Nedd4, Ldlr and Serpinb1a [23, 24]. Flow cytometry was used to examine the expression of numerous surface molecules on rEos and iEos, e.g. Singlec-F, CD62L, C101L and CD125. CD62L, known also as L-selectin, is a cell adhesion molecule found on leukocytes including eosinophils. CD62L was shown to be expressed on the surface of rEos but not on the surface of iEos. On the other hand, rEos displayed modest amounts of CD101 (immunoglobulin superfamily, member 2), while CD101 was strongly expressed on the surface of iEos. CD62L and CD101 expression distinguishes between mouse lung rEos and iEos. Considering the above, CD101 could be a useful marker to discriminate between rEos (CD101lo) and iEos (CD101hi) [22].

## MARKERS OF EOSINOPHILIC INFLAMMATION

There are several markers used for the determination and monitoring of eosinophil-associated diseases. Concerning respiratory diseases we commonly use the following markers: the number of circulating eosinophils, organ-specific eosinophil levels, fractional exhaled nitric oxide (FeNO) [25]. Published studies have revealed eosinophil count as a promising marker for Th2-high asthma. We should remember that circulating eosinophils are present in the blood for around 8–18 h, in contrast to their prolonged life span of up to 14 days in tissue [26]. Eosinophils undergo migration from the bloodstream to the tissues. Typically, once eosinophils migrate into the tissues, the majority of them do not circulate again [26]. The number of circulating eosinophils in human peripheral blood ranges between 50 and 500/ $\mu$ l under normal conditions. Additionally, it is worth noting that blood eosinophils vary by around 20% during the day. The fluctuation in eosinophil levels frequently refers to the suppressive impact of cortisol hormones. The levels of cortisol are at their peak in the morning and decrease throughout the night, resulting in lower eosinophil counts in the morning and higher numbers at night [27].

FeNO concentration of 25 parts per billion (ppb) in adults indicates the presence of eosinophilic airway in-

flammation and serves as an indicator of the probability of responding to corticosteroid treatment [28]. Regarding spirometric measurements, there was a noticeable inverse association between the FeNO level and the FEV1% and a slightly positive relation between the FeNO level and bronchodilator reversibility [28]. Furthermore, a high FeNO level is regarded as a risk factor for poorly managed asthma and subsequent episodes of exacerbations [29].

Periostin is a matricellular protein, which plays a significant role in the remodeling and type-2 airway inflammation seen in asthma. Yuyama *et al.* originally showed that the expression of the POSTN gene, which encodes periostin, is upregulated by two essential type-2 cytokines in asthma: IL-4 and IL-13 [30]. Its production in airway epithelial cells is associated with the thickness of the airway basement membrane. Periostin may be reliably identified in blood, suggesting airway type-2 inflammation and remodeling [31]. Additionally, serum periostin was found to be a reliable indicator of the efficacy of lebrikizumab, an anti-IL-13 antibody, in treating patients with unstable asthma, as demonstrated in a phase 2b research. The group with elevated serum periostin levels exhibited a more significant improvement in FEV1 in response to lebrikizumab compared to the placebo group. However, this effect was not detected in the group with low serum periostin levels [32].

In fact, blood eosinophil counts, FeNO levels and serum periostin levels may represent markers of type-2 inflammation [33].

Moreover, density of eosinophils may play a significant role in asthma [34]. Two different cell types of eosinophiles have been discovered depending on cell density: normal and hypodense (< 1.085 g/l). The latter seem to be an activated phenotype. In healthy individuals peripheral blood show a low number of normodense and hypodense eosinophils, in contrast with the high amount of hypodense cells in patients suffering from allergies [35]. Higher cytotoxic eosinophil abilities were observed in the case of the hypodense cell population [36]. Lower density cells are characteristic of an increased number of receptors for complement and immunoglobulins, as well as an increased response to chemotaxis and intensified metabolic activity. Kuo *et al.* observed that the percentage of hypodense eosinophils is strongly related to severity of asthma. Patients diagnosed with mild asthma have a lower percentage of hypodense eosinophils in the blood than those with moderate asthma [37]. Furthermore, the distribution profiles of eosinophils changes to a prevalence of normodense cells after corticosteroid treatment, suggesting that corticosteroid drugs influence the eosinophil activation rather than simple decrease of the eosinophilic cell number [37, 38]. Experiments performed so far indicate that the clinical efficacy of corticosteroids might

be related to inhibition of the eosinophil conversion into hypodense subpopulations [37].

## AVAILABLE TREATMENT

Eosinophil-targeted biologics are divided according to their mechanisms of eosinophil reduction: IL-5 depletion (mepolizumab and reslizumab), antibody-dependent cytotoxicity (benralizumab) and interference with eosinophil transport to tissues (dupilumab). Mepolizumab binds soluble IL-5, preventing it from attaching to the IL-5 receptor on eosinophils and thereby suppressing eosinophil growth and activation [39]. Benralizumab is a recombinant humanized monoclonal antibody which possesses the particular feature of binding to a conformationally distinct epitope within domain 1 of the IL-5R-chain with high affinity. The absence of fucose (afucosylation) rockets its affinity for CD16a and triggers antibody-dependent cell-mediated cytotoxicity by natural killer (NK) cells [40]. In turn, dupilumab is a monoclonal antibody which targets the IL-4 receptor  $\alpha$  chain (IL-4R $\alpha$ ). There exist two types of receptors: type I receptor, composed of IL-4R $\alpha$ / $\gamma$ c heterodimers, which exclusively binds IL-4, and type II receptor, consisting of IL-4R $\alpha$ /IL-13R $\alpha$ 1, capable of binding both IL-4 and IL-13. Activation of these receptors initiates a cascade of signaling events, prominently involving JAK kinases (Janus kinase), such as JAK1/JAK3 (for type I receptors) and JAK1/Tyk2 (for type II receptors). Activated JAK kinases trigger further phosphorylation of specific tyrosine residues within the cytoplasmic domain of IL-4R $\alpha$ , leading to the activation of various signaling pathways, including STAT6, SHC/MAPK, IRS/PI3K/mTORC2/AKT and Shp-1. The IL-4/IL-13/IL-4R axis stimulates the differentiation of T helper cells type 2 (TH2), which mediates the pro-allergic adaptive immune response [41]. Dupilumab reduces type 2 inflammation indicators such as total IgE, periostin and plasma eotaxin-3. In 52-week clinical studies there was observed reduction in median percentage change in serum total IgE from -70.0% to -76.7%. In turn, the median percentage change in plasma eotaxin-3 in dupilumab-treated patients with asthma over placebo was -38.24% versus -0.16%. In a subset of patients with asthma and chronic rhinosinusitis with nasal polyps treated with dupilumab, temporary elevations in blood eosinophils were observed, followed by a return to near-baseline levels by the end of treatment [42].

Omalizumab is a humanized monoclonal anti-IgE antibody. It is dedicated for severe allergic asthma treatment, but also for other atopic disease – *chronic spontaneous urticaria*. Omalizumab inhibits free serum IgE binding to the Fc $\epsilon$ RI receptor on the surface of mast cells and basophils [43]. Additionally, omalizumab has

**TABLE 2.** Biological drugs available in Poland used in the treatment of severe asthma

Biological drug	Brand name	Mechanism of action	Dose [mg]	Frequency of administration [weeks]	Route of administration
Omalizumab	Xolair	Anti-IgE	75–600	4	Subcutaneous
Mepolizumab	Nucala	Anti-IL 5	100	4	Subcutaneous
Reslizumab	Cinqaero	Anti-IL-5	3 per 1 kg of bodyweight	4	Intravenous
Benralizumab	Fasenra	Anti-IL5 R	30	4 (first 3 times), then 8	Subcutaneous
Dupilumab	Dupixent	Anti-IL4 R	600 mg (1. dose); 300 mg	2	Subcutaneous

also been shown to diminish the expression of FcεRI on the surface of circulating mast cells and basophils, resulting in a reduction in the release of allergic mediators [44] (Table 2).

Additionally, there is a novel monoclonal antibody – tezepelumab, acting upstream, which is not yet available in Poland. Tezepelumab selectively inhibits the binding of thymic stromal lymphopoietin (TSLP) to its heterodimeric receptor. TSLP is secreted by the airway epithelium in response to many stimuli, including allergens, viruses and air pollutants, which initiates an inflammatory cascade [45]. Excessive production of TSLP can cause abnormal inflammation, which can result in worsening of asthma symptoms, hyperresponsiveness and structural changes in the airways [46]. The beneficial effects and safety of tezepelumab in individuals with severe, uncontrolled asthma were assessed in the randomized, placebo-controlled phase 2b PATHWAY and phase 3 NAVIGATOR trials. Tezepelumab treatment resulted in a decrease in the risk of asthma exacerbations per year when compared to a placebo in participants from both trials. This effect was observed in patients with either high or low baseline blood eosinophil count or FeNO levels, and was independent of the allergy status. Tezepelumab has shown enhancements in pulmonary function, disease control and quality of life [47, 48].

### COMPARISON OF ANTI-EOSINOPHIL BIOLOGICAL DRUGS

Generally, there are three biologics which are used for severe eosinophilic asthma treatment – mepolizumab, benralizumab and dupilumab. Despite their demonstrated efficacy, many questions remain unsolved, especially regarding the comparative effectiveness of the drugs. Clinical trials that have been performed were placebo controlled rather than providing head-to-head comparisons [49].

Akenroye *et al.* performed a meta-analysis on the efficacy and safety of mepolizumab, benralizumab and dupilumab in treatment of eosinophilic asthma. To account for clinical heterogeneity, as the eosinophil count is determinant of the efficacy of these treatments, researchers concentrated on groups of patients with eosinophil ranges of  $\geq 300$  cells/ml and 150–299 cells/ml. In the group of patients with eosinophils  $\geq 300$  cells/ml, all three biological drugs were substantially more effective than placebo in reducing exacerbations. In case of dupilumab (RR = 0.32; 95% CI) reduction of exacerbations was the most noticeable comparing to the other drugs: mepolizumab (RR = 0.37; 95% CI) and benralizumab (RR = 0.49; 95% CI). Improvement of spirometric parameter FEV1 was also the most explicit in the dupilumab group. Mean difference with dupilumab administration was 230 ml; benralizumab 150 ml and mepolizumab 150 ml. The best asthma control assessed by ACQ test was observed in the mepolizumab group. In patients with eosinophils 150–299 cells/ml, benralizumab (RR = 0.62; 95% CI) and dupilumab (RR = 0.60; 95% CI) were associated with lower exacerbation rates. The effect of both biologics was similar and greater than in the mepolizumab group. Improvement of FEV1 was comparable in mepolizumab and benralizumab groups, slightly better than in the dupilumab group. Mepolizumab caused the most serious side effects in both study subgroups [39]. The conclusion of the meta-analysis was that mepolizumab, dupilumab, and benralizumab are characterized by similar efficacy and safety in patients with severe eosinophilic asthma [49–53].

### REASONS OF TREATMENT INEFFECTIVENESS

In some patients, despite daily use of oral corticosteroids or management with biologics, persistent airway eosinophilia is observed. Emerging theories suggest the existence of localized mechanisms that are resistant to



maintenance corticosteroids, delaying inflammation resolution and causing recurrent exacerbations with distinctive clusters of free eosinophil granules (FEGs) in sputum. The elevated concentration of FEGs in the airways shows a robust correlation with the level of sputum eosinophil peroxidase (EPX) released during luminal eosinophil degranulation, signifying an “active” disease [54]. Mukherjee *et al.* observed the presence of pathogenic autoantibodies in the sputum of patients with persistent eosinophilia and severe asthma. Sputum autoantibodies to EPX (anti-EPX) and various antinuclear antigens, which are not detectable in circulation, imply that a polyclonal autoimmune process specific to the airways has occurred in these patients. The presence of anti-EPX shows a high correlation with the severity of asthma. Moreover, patients with prednisone-dependent eosinophilic asthma showed elevated levels of anti-EPX IgG [55].

Although patients with severe eosinophilic asthma have been properly qualified to biological therapy, they may not achieve expected asthma control and clinical improvement. Treatment with biotherapeutics reduce eosinophil count in the blood, but eosinophils may still be present in the airways [56]. Cytokines/chemokines generated by a variety of cells, including Th2 cells, play a key role in regulating transvascular eosinophil migration. The interaction of eosinophils with endothelial cells via the  $\alpha 4$  integrin/vascular cell adhesion molecule (VCAM)-1 pathway appears to be a significant step in selective eosinophil recruitment, according to accumulating data. Blood eosinophils spontaneously attach to VCAM-1 in response to the Th2 cytokines IL-4 and IL-13. The interaction of eosinophils with VCAM-1 increases granule protein release from eosinophils and may thus represent an initial step in the activation of these cells. Even in the absence of IL-5, eosinophilic airway inflammation may be maintained by the Th2 network, which includes a cascade of VCAM-1/CC chemokines/GM-CSF, may sustain eosinophilic infiltration and activation [57, 58].

Eosinophils from asthmatic patients have many phenotypic alterations, particularly in adhesive properties. Barthel *et al.* observed that human eosinophils recruited to the airway in response to segmental antigen exposure display an allosterically active version of  $\alpha M\beta 2$ , which is associated with greater  $\alpha M\beta 2$ -mediated adherence to various ligands and increased podosome formation. Integrins, including  $\alpha M\beta 2$  (CD11b/18),  $\alpha 4\beta 1$  (CD49d/29),  $\alpha L\beta 2$  (CD11a/18), are thought to mediate eosinophils rolling and arrest on endothelium, migration through endothelium and the basement membrane in response to chemotactic cues, and bronchial epithelial crossing into the airway lumen [59]. Experiments in humans using anti-IL-5 antibodies show that eosinophils play a role in the processes that lead to the deposition of certain matrix

proteins within the reticular basement membrane [60]. In asthmatics who were given three infusions of mepolizumab there was about a 90% reduction in blood and bronchial lavage eosinophils but only 55% of eosinophils in bronchial mucosal were diminished [61].

In fact, the number of eosinophils in the blood does not appear to be an adequate marker of the effectiveness of therapy. During dupilumab treatment, transient eosinophilia was observed. Also single cases of eosinophilic granulomatosis with polyangiitis have been described. Despite transiently elevated eosinophil counts, most patients show improvement in asthma control, exacerbation frequency and spirometric parameters [62].

### POSSIBLE NEW ANTI-EOSINOPHIL BIOLOGICAL DRUGS

New possible targeted anti-eosinophil drugs may block eosinophil receptors of potent eosinophil chemokines. CCR3 is the related receptor for major human eosinophil chemoattractant, expressed by eosinophils and crucial for their recruitment to the lung tissue through its binding to eotaxin [63]. Eosinophils move through the sinusoidal endothelium in the bone marrow and are released into the peripheral circulation under the effect of IL-5 and eotaxin [64]. The role of eotaxin was investigated in animal models. Aerosol administration of eotaxin to guinea pigs causes an influx of eosinophils into the airways. In turn, deletion of the eotaxin gene in mice reduces the early recruitment of eosinophils after allergen exposure [65].

Another possible future therapy could be anti-IL-33 biologics. In asthmatics, levels of IL-33 have been observed to be significantly higher in the peripheral blood, and IL-33 levels were negatively correlated to FEV1 and positively linked to clinical asthma severity [66, 67].

Sialic-acid-binding immunoglobulin-like lectin (Siglec)-8 is also being studied as a therapeutic target for the treatment of allergy and inflammatory diseases. It is a cell-surface inhibitory receptor, which is selectively expressed on blood and tissue eosinophils and mast cells, weakly on basophils [68]. Higher levels of soluble Siglec-8 were reported in the serum of severe asthmatics in comparison to healthy controls. Anti-Siglec-8 monoclonal antibodies have previously been demonstrated to directly cause apoptosis in isolated human eosinophils. A monoclonal antibody to Siglec-8 (lirentelimab) appears to induce apoptosis of cytokine-primed eosinophils via antibody-dependent cellular cytotoxicity [67, 69]. Youngblood *et al.* conducted an experiment to evaluate the effect of lirentelimab on tissue eosinophils. By using flow cytometry, eosinophils were phenotyped in order to compare the expression of surface markers in blood and tissue eosinophils. Two cell-surface markers, CD62L and IL-5 receptor, were shown to be

strongly expressed on peripheral blood eosinophils but considerably downregulated on human lung tissue eosinophils. In turn, the expression of Siglec-8 on tissue and blood eosinophils was similar. Lirentelimab can effectively reduce both tissue and blood eosinophils [69].

## POTENTIAL NOVEL NON-BIOLOGICAL ANTI-EOSINOPHIL DRUGS

The Janus kinase family is involved in the transduction of cytokine-mediated signals by interacting with and activating STAT (signal transducer and activator of transcription) proteins. Dysregulation of the JAK/STAT pathway is known to be involved in the pathogenesis of allergic inflammation, for instance in asthma. In eosinophils, IL-5 signals through the JAK2-STAT1/STAT5 and MAP kinase pathways control the expression of genes essential for cell survival and proliferation. Furthermore, through JAK-STAT, IL-31 has the ability to inhibit eosinophil apoptosis and promote the release of pro-inflammatory cytokines and chemokines [70]. Luschnig *et al.* investigated the efficacy of the oral JAK inhibitors: baricitinib (JAK1/2) and tofacitinib (JAK3) in reducing eosinophil effector function *in vitro* and *in vivo* in an animal model of allergic eosinophilic lung inflammation. It was shown that baricitinib inhibits eosinophil effector function more effectively than tofacitinib. Thus, targeting the JAK1/2 pathway seems to be a better therapeutic option for eosinophilic inflammation [71].

Additionally, Vernet *et al.* performed a cohort study that was based on gene mapping in asthmatic patients, their first-degree relatives and healthy controls. A couple of genes were evaluated e.g. RNASE2 and RNASE3, which encode eosinophil cationic protein (ECP) and eosinophil-derived neurotoxin (EDN), and also 4 other genes (JAK1) that are responsible for ECP and EDN levels [72]. ECP and EDN are eosinophil proteins which contribute to epithelium damages and airway remodeling. They have been associated with asthma exacerbations and severity of the disease. Higher levels of ECP and EDN have been observed in asthmatic patients than non-asthmatic individuals. Regarding the above mentioned, drugs inhibiting JAK1 have been identified as a potential therapeutic target for eosinophilic asthma [72, 73].

Dexpramipexole is an orally available synthetic aminobenzothiazole, which considerably reduces blood and tissue eosinophils with a good safety profile [74]. Siddiqui *et al.* performed a randomized controlled trial which assessed the safety and effectiveness of dexpramipexole in decreasing blood and airway eosinophilia in patients with asthma and blood eosinophil count greater than or equal to 300/ml. Available evidence indicates that dexpramipexole lowers eosinophils by preventing their maturation.

This means that the substance has no effect on mature cells. Eosinophil count reduction was seen starting at weeks 4 to 6. Nasal eosinophil peroxidase (EPX) is correlated to sputum eosinophil count in patients with severe asthma. An exploratory end point in the study was the nasal EPX week-12 ratio to baseline. The group which was administered 150-mg dexpramipexole twice a day had the highest decrease in nasal EPX. Additionally, nasal EPX ratio to baseline at week 12, was highly correlated with blood eosinophil count reduction [75].

## DISCUSSION

Eosinophils belong to a group of white blood cells, granulocytes, and are widely known for their potential to eliminate parasitic infections and their role in type 2 inflammation. Eosinophils produce factors of the type-2 pathway such as interleukin IL-4, IL-5, IL-13 and IL-25. This effect is responsible for developing inflammation and allergic reactions, observed e.g. in the airways of asthmatics [76].

IL-5 plays an important role in eosinophil maturation. It controls the development of eosinophils in the bone marrow, while they differentiate from myeloid precursor cells to the mature form. Humanized anti-IL-5 antibodies (mepolizumab and reslizumab) and also antibody against IL5-R (benralizumab) critically lower eosinophil levels in the blood by preventing eosinophil maturation in the bone marrow [77]. According to Moran *et al.* study, benralizumab caused an eosinophil depletion that was rapid and nearly complete, and its commencement of action was quite comparable to that of oral prednisolone [78]. The blood eosinophil count was considerably reduced after benralizumab was compared to mepolizumab 30 days following the first injection [78, 79]. However, mepolizumab and benralizumab have demonstrated comparable efficacy and safety in the long-term treatment of severe eosinophilic asthma, suggesting that the difference in onset and efficacy is not relevant in the chronic use of these drugs. Considering that benralizumab has a faster onset of action, it has the potential to be used as an alternate non-corticosteroid therapy for acute asthma exacerbations [78].

Several scientific studies attempted to clarify the response to anti-eosinophil treatments. These studies noted alterations in the expression of certain factors following drug administration. Specifically, it was observed that mepolizumab led to a decrease in the activation of peripheral blood eosinophils through  $\beta$ 2 integrin (CD 18), while  $\beta$ 1 integrin (CD 29) remained unaffected. The varying outcomes of anti-IL 5 therapy might stem from its selective inhibition of one aspect of eosinophil activation, as opposed to others [80].

Control of eosinophil apoptosis has become a therapeutic strategy for treating allergic diseases and other eosinophil-associated disorders [26]. However, in some cases peripheral blood eosinophils depletion does not ensure expected clinical improvement. Furthermore, dupilumab, monoclonal antibody anti-IL 4 R/anti-IL 13 approved for eosinophilic asthma treatment, may cause transient increase of blood eosinophils. Given this information, it appears that the blood eosinophil count may not be a reliable marker for assessing therapy effectiveness [81].

However, in a routine diagnostic process or qualification to the biotherapies only the total amount of eosinophils in peripheral blood is assessed [82]. Density of the cells or other makers of eosinophilic inflammation are not evaluated. There may be a relation between efficacy of biological treatment and some markers of eosinophilic inflammation. Further studies have to be performed in this field.

## CONCLUSIONS

Severe asthma is sometimes still difficult to determine appropriate treatment due to significant clinical heterogeneity. Eosinophils play a significant role in the pathogenesis of asthma. Markers of eosinophilic inflammation can be helpful in the determination of the asthma endotype, qualification for biological treatment and also in its monitoring.

However, availability of different biological drugs and assumedly proper qualification to the drug program do not always bring the success. Some patients still do not achieve expected improvement of the clinical state. The causes of side effects and lack of improvement after biological treatment are still unknown, and further observations and scientific studies are needed.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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