# Eosinophils in the Field of Nasal Polyposis: Towards a Better Understanding of Biologic Therapies

Thibault Vanderhaegen[1,2] · Isabelle Gengler[3] · Arnaud Dendooven[2] · Cecile Chenivesse[4] · Guillaume Lefèvre[2,5] · Geoffrey Mortuaire[1,2,6]

## Abstract

Eosinophils are often considered as the pathologic landmark of chronic rhinosinusitis with nasal polyps (CRSwNP). Many studies emphasize their pivotal role in mucosal remodeling by their innate action via cytotoxic proteins degranulation. Eosinophil nasal recruitment from the bloodstream through endothelium diapedeses requires the intricate action between the nasal epithelium, epithelial cell-activated type 2 innate lymphoid cells, and adaptive immune cells secreting alarmins, cytokines, and specific chemokines. This immune pathway refers to a T-helper 2 (T2)-driven lymphocyte response, often considered as the main inflammatory process in CRSwNP in western countries. The release of T2 cytokines, among which interleukin (IL)-4, IL-5, and IL-13, fundamentally contributes to this immune response. New biologic agents capable of blocking T2 cytokines have been developed in the field of eosinophil-associated diseases, shifting the paradigm of treatment for patients with CRSwNP. The first part of this review describes each step of the eosinophil journey from hematopoietic stem cell maturation to nasal mucosa homing. The different eosinophil activation processes and their inflammatory functions are also described. This is followed by a discussion on currently available biologic therapies in CRSwNP with a specific focus on eosinophilic response. Beyond an eosinophil-blocking strategy, a cluster analysis of specific T2 biomarkers could be required to best predict the response to such biologic therapies in the future.

**Keywords** Biologic therapy · Interleukin · Chronic rhinosinusitis · Eosinophil · Nasal polyp

## Introduction

Chronic rhinosinusitis with nasal polyps (CRSwNP) is a major concern with a prevalence of 2–4% in western countries [1]. Symptoms of loss of smell, nasal congestion and/or nasal obstruction, and rhinorrhea have a significant impact on social and physical quality of life. They also cause a significant economic burden due to absenteeism and cost of treatment [2]. CRSwNP is associated with bronchial hyperreactivity or asthma in 31 to 42% of cases [3]. Non-steroidal anti-inflammatory drug-exacerbated respiratory disease (N-ERD) is also reported in 9.7% of patients [4].

Many pathophysiological mechanisms involving both innate and adaptive immune systems have been described to explain the persistent inflammation within the nasal mucosa [5]. It is believed that the disease originates at the epithelial barrier, where it is triggered by a specific sinonasal microbiotic environment, under certain genetic predisposition and inflammatory conditions [6, 7]. The immune response itself then promotes inadequate wound

✉ Geoffrey Mortuaire
geoffrey.mortuaire@chru-lille.fr

1 Otorhinolaryngology – Head and neck Department, Huriez Hospital, CHU Lille, Lille, France

2 Univ. Lille, Inserm, CHU Lille, U1286 - INFINITE - Institute for Translational Research in Inflammation, 59000 Lille, France

3 Department of Otolaryngology-Head and Neck Surgery, University of Cincinnati Medical Center, OH, Cincinnati, USA

4 Pneumology and Allergology Department, Calmette Hospital, CHU Lille, 59000 Lille, France

5 Medicine and Clinical Immunology Department, National Reference Center of Hyperesionophilic Syndromes, CHU Lille, Lille, France

6 Service d'ORL de chirurgie cervico-faciale, Hôpital Huriez, CHU Lille, 59037 Lille, France

healing of the epithelium, triggering abnormal tissue remodeling which leads to polyp formation [8, 9]. The intricate mechanistic hypotheses reported in the literature underline the important heterogeneity of CRS. The disease has multiple clinical presentations and biological features, with variable response to treatment (as demonstrated by new biologics targeting specific cytokines), shedding light on the probable inaccuracy of a single physiopathological model to explain chronic sinonasal inflammation [10].

Over the last few years, several studies have been conducted to delineate clinical and biological phenotypes of CRSwNP in relation with biomarker expression. Inflammatory endotypes have been described, with Caucasian population prone to lymphocytic T helper type 2 (Th2) inflammatory pathway (so called T2 inflammation) with an eosinophilic-skewed response and Asian population prone to Th1 or Th17 inflammatory pathways (T1 and T17 inflammation) [10]. The differences in biological patterns when considering ethnic origin may account for genetic factors, as US-second-generation Asians with CRS exhibit more non-eosinophilic patterns [11]. Environmental factors may also play a role in the inflammatory pattern, as seen in Thai patients with CRS symptoms who showed more eosinophilic patterns associated with more *staphylococcus aureus* colonization [12].

CRSwNP in the Caucasian population is biologically associated with overexpression of relevant T2 cytokines (Interleukin (IL)-4, IL-5, IL-13), leading to eosinophil (EO) recruitment and activation within nasal polyps. This profile is clinically associated with more frequent failure of steroid treatment, increase need for surgery to control the disease, and more frequent concomitant asthma compared with T1/T17 "neutrophilic-associated" CRSwNP [1]. The concept of "Eosinophilic Chronic Rhinosinusitis with or without Nasal Polyps" needs to be refined to account for suspected different underlying biological pathways. A minor part of Caucasian CRSwNP can exhibit a relatively EO deficient inflammatory

infiltrate with a predominant T2 cytokine profile. In that perspective we should also consider priming and activation status of EOs in the bloodstream and nasal mucosa to better describe CRSwNP endotypes beyond a given cytokine cluster of expression.

In western countries, the EO mediator is always regarded as a major actor of CRSwNP inflammation. EOs belong to the innate immune system. They account for a small proportion of blood leukocytes (< 5%) and are mostly tissue-resident cells. The two main roles of EOs have long been supposed to be host defense against helminths and allergic inflammation maintenance. Several studies have brought new insights in the field of EO-associated diseases and biology. They allow us to reconsider EOs as pluripotent actors in many inflammatory diseases and physiologic conditions [13] (Fig. 1).

Our review aims to describe EO involvement in CRSwNP when considering cell maturation, nasal homing, cytolytic properties. and interaction with the immune system. Each step leads to a better understanding of new treatment options offered by biologics in nasal polyposis.

## From Bone Marrow to Nasal Polyps

### EO Development and Maturation

EO lineage commitment has been reviewed by Mack et al. in 2020 [14]. Many available results come from mice studies and still need to be confirmed in human models. Herein, the review will focus on data obtained from human EO cell-line studies. Like other leukocytes, EOs are generated in the bone marrow from eosinophil progenitors (EoPs). These progenitors seem to derive from a common myeloid precursor (CMP), although EO might possibly derive from earlier progenitors [15]. The whole process depends on a precisely tuned combination,



**Fig. 1** Eosinophil effectors functions. Eosinophil activation is mediated by a wide variety of surface receptors that respond to diverse stimuli. Upon activation, eosinophils promote host protection via direct effects on pathogens or tumoral tissues, immune responses by modulation of lymphocyte and dendritic cell function, and airway inflammation via tissue damage, remodeling, and mucous hypersecretion. They are also involved in homeostatic functions (adipose, thymic and mammary gland development)

**Host protective**
Defense against mucosal pathogens
Anti-tumoral effect
- *Cytotoxicity by granule proteins (EPX, EDN, MBP, ECP)*
- *DNA traps (ETosis)*
- *Nitric oxide release*

**Proinflammatory**

Airway inflammation
- *Tissue damage and remodeling*
- *Fibrosis*
- *Airway hyperresponsiveness*
- *Mucus hypersecretion*
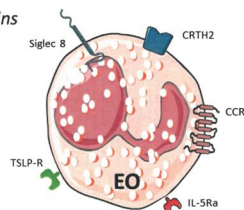
**Immunoregulatory**
T cell
- *Antigen presentation*
- *Th2 polarization*
B cell
- *Polyclonal IgE, IgM and secretory IgA production*
- *Plasma cells survival*
Dendritic cell
- *Activation*
- *Maturation*

**Homeostasis**
Metabolic homeostasis
- *Maintenance of adipose, protection against type 2 diabetes*
Plasma cells survival
Thymic and mammary gland development

in both time and quantity, of non-specific transcription factors, including GATA-binding protein transcription factors (whose main factor is GATA-1, C/EBP proteins, and PU.1) [14] (Fig. 2). According to McNagny et al.'s description of EO differentiation from other cell-lineages via subtle modifications in transcription factors, some authors speculate that different inflammatory environments could modify transcriptomic pathways, and consequently eosinophilopoiesis at the bone marrow level [16]. Along with their role in regulating EO differentiation, GATA-1, C/EBP, and PU.1 are involved in the transcription of several important EO proteins such as Major Basic Protein-1 (MBP-1), Eosinophil Peroxidase (EPO), or C-C Motif Chemokine Receptor 3 (CCR3) [17–19].

EoPs can be defined by a CD34+ IL-5 receptor alpha (IL-5Ra)+ CMP phenotype as these cells can only differentiate into EOs [15]. The interaction of IL-5Ra with its ligand on the EoPs membrane plays a pivotal role in EO maturation, proliferation, and survival properties. Activation processes through IL-5Ra is also essential to promote massive EO migration within tissues under inflammatory circumstances, such as CRSwNP [20].

IL-5 production is supported by Th2-cytokine secreting cells, mainly innate lymphoid cell type 2 (ILC2) and Th2 lymphocytes, but also at the mucosal level by mature EOs themselves leading to a self-stimulating survival loop under inflammatory circumstances [21, 22]. IL-5 independent activation pathways have been described in IL-5 deficient mice with almost normal
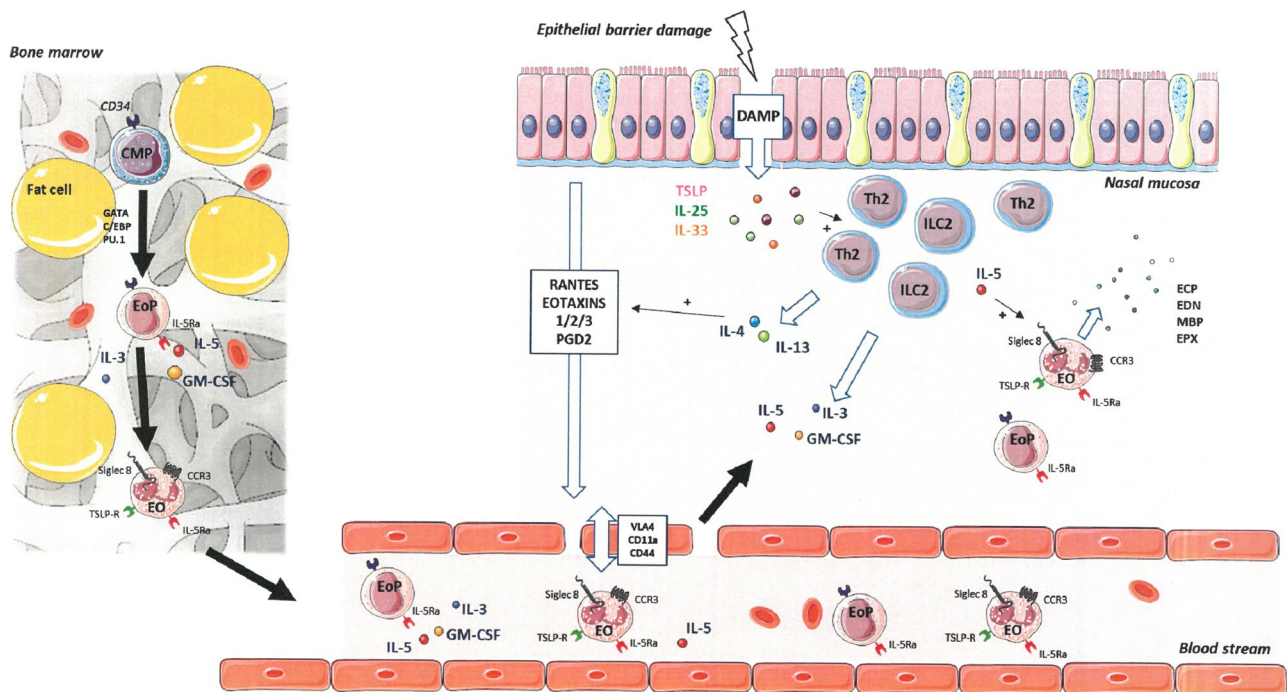


**Fig. 2** Eosinophil journey from bone marrow maturation to nasal migration. Eosinophil (EO) develops in the bone marrow. Transcription factor (such as GATA-1, C/EBP proteins and PU.1) and cytokines (such as interleukin (IL)-5, IL-3 and GM-CSF) are essential for its differentiation from CD34+ common myeloid precursor (CMP) to IL-5Ra+ eosinophil progenitors (EoP) and mature EO. Once mature, IL-5 controls the EO migration from the bone marrow to the blood. EO chemotaxis in nasal mucosa is mediated by the combination of the local production of cytokines and chemokines controlled by alarmins (thymic stromal lymphopoietin (TSLP), IL-25 and IL-33) secreted by epithelial cells after damage associated molecular pattern (DAMP) release under epithelial barrier dysfunction. The expression of homing molecules (Very Late Antigen (VLA)-4, CD11a) and of CD44 on the EO surface is required to allow interaction with their counter-ligands expressed on inflamed endothelium of the nasal mucosa. C-C Motif Chemokine Receptor 3 (CCR3)-binding eotaxins 1, 2, and 3 are also produced by epithelial cells and

fibroblasts at baseline and under lymphocytic T helper type 2 (Th2) stimulation. Regulated on Activation, Normal T Cell Expressed and Secreted (RANTES) and dysregulated prostaglandin D2 (PGD2) secretion are involved in the process of EO directional migration into the extravascular compartment. IL-4 and IL-13 released by Th2 cells and type 2 innate lymphoid cells (ILC2) contribute to EO chemotaxis. EO exhibit prolonged survival in nasal mucosa due to their protection from cell death by locally produced cytokines such as IL-5 and GM-CSF. Once activated, EO are able to express cytokines and cytotoxic granules proteins. These cytotoxic proteins (Major Basic Protein-1 (MBP), Eosinophil Peroxidase (EPX), Eosinophil Cationic Protein (ECP), Eosinophil Derived Neurotoxin (EDN)) are involved in nasal mucosa damage and tissue remodeling. Eosinophil progenitors (EoPs) have been identified in CRSwNP within nasal mucosa and might subsequently mature in the presence of a favorable local micro-environment

numbers of circulating and tissue-resident EOs, possibly via a Macrophage Inflammatory Protein-1 alpha (MIP-1α or CCL3)-CCR1 interaction on EoPs [23, 24]. Less specific cytokines are also important in promoting EO differentiation, proliferation, and activation, i.e., IL-3 and GM-CSF whose receptors share a common β chain subunit with IL-5 and IL-4, leading to EO activation in an IL-5-dependant manner [24, 25]. Interestingly, some cytokines produced by damaged nasal epithelium seem to be involved in EoPs differentiation even before IL-5 exposure, as for example IL-33 [26].

Both mature EOs and CD34+ IL-5Ra+ EoPs are able to migrate from the bone marrow to other extramedullary sites via the bloodstream. EoPs could play a direct role in eosinophilic inflammation and have been identified in CRSwNP within nasal mucosa [27]. EoPs might subsequently mature in the presence of a favorable local microenvironment, as demonstrated ex vivo in allergic nasal mucosa after exposure to IL-5 [28].

## Circulating Eosinophils in CRSwNP

Blood EO counts in CRSwNP patients with uncontrolled disease, or comorbid asthma stays most of the time within normal ranges, compared with healthy patients [29]. On the contrary, blood EO counts in some other eosinophilic disorders (helminth infection, hypereosinophilic syndrome) can easily ascend over 1.5 G/L, making eosinophil blood count a poor phenotyping marker in eosinophilic chronic rhinosinusitis diagnosis in comparison. However, some authors referred to absolute blood EO count as a possible prognostic factor of disease control, as they hypothesized that it would correlate to a lower number of tissue EO, which has yet to be demonstrated [30].

Beyond these considerations, other specific approaches to blood EO "activated status" could be more relevant to describe patient's inflammatory status. Circulating EOs can exhibit an activated phenotype as demonstrated by Dupuch and al., with a significant increase of EOs expressing activation markers CD49d (Very Late Antigen-4 (VLA-4)), CCR3, and CD25 (IL-2Rα) in patients with CRSwNP, and a higher oxidative metabolism compared with control patients [31]. Similarly, the activation status of blood EOs based on CD69 and CD44 membrane expression cytokines can be correlated to concomitant asthma in CRSwNP [29]. In a prospective study including patients with corticosteroid resistant CRSwNP, we showed that IL-5Ra was less expressed on EO membrane in nasal polyps of asthmatic patients. No difference was observed for IL-5Ra expression on EOs collected from blood samples of the two different patient populations [29].

## Eosinophil Tissue-Recruiting Modalities in CRSwNP

EOs are mainly tissue-resident cells with an estimated time spent in blood circulation of one day and a tissue lifetime believed to last up to several days [32].

After bone marrow maturation and their release into the blood stream, EOs are recruited at the level of the nasal mucosa where they start to exert their multiple functions (Fig. 2). Even though numerous studies tried to assess for a diagnostic mucosal EO threshold, there is no specific cut-off in quantitative tissue eosinophilia to reliably differentiate between eosinophilic CRS and non-eosinophilic CRS. Even if there is not yet an international consensus on the measure, a generally accepted 10% ratio of EO in nasal secretions seems to be in favor of eosinophilic CRS. A meta-analysis suggested that a count of 55 EOs or more per high-power field (× 400 magnification) on histologic specimens was specific enough to define eosinophilic CRS. This result still has to be reinforced by future studies as it appeared that included studies displayed a wide range of cut-off values (from 5 to 120) [33]. A higher tissue EO count could be associated with a more aggressive disease and a poorer disease outcome [34]. In a prospective study including 36 consecutive patients who underwent endoscopic sinus surgery for CRSwNP resistant to optimal medical treatment, we showed that mucosal eosinophilia was a marker of concomitant respiratory disease [35].

The expression of homing molecules (VLA-4, CD11a (Integrin αL), and of CD44 (Homing Cell Adhesion Molecule (H-CAM)) on the EO surface is required to allow for adherence onto endothelial cells and diapedesis into target tissues. This expression can be further modulated to maintain EOs within mucosal tissues after transendothelial migration [29]. We also showed that the expression of β integrins (VLA-4, CD11a) by EO purified from blood samples and nasal polyps of patients with steroid resistant CRSwNP was reduced both in asthmatic and non-asthmatic patients [29]. EO chemotaxis in nasal mucosa is mediated by a combination of locally produced cytokines and chemokines controlled by alarmins, released by triggered epithelial cells. Thymic Stromal Lymphopoietin (TSLP), IL-25, and IL-33 are produced after damage associated molecular pattern (DAMP) release [36, 37]. CCR3-binding eotaxins 1, 2, and 3 (C-C Motif Chemokine Ligand (CCL)11, CCL24, CCL26) are also produced by epithelial cells and fibroblasts at baseline and under Th2 inflammatory stimulation [38, 39]. Regulated on Activation, Normal T Cell Expressed and Secreted (RANTES/CCL5) and dysregulated prostaglandin secretion with increased chemotactic Prostaglandin D2 (PGD2) (via Chemoattractant-homologous Receptor expressed on TH2 cells (CRTH2) expressed by EOs) and

fewer anti-inflammatory Prostaglandin E2 (PGE2) are also involved in this process and contribute to upregulate the local inflammatory processes [40, 41]. Along with their own chemotactic abilities, these chemokines are able to promote a pro-Th2 inflammatory environment, through ILC2 activation and Th2 lymphocytes recruitment [36]. Subsequent production of IL-4, IL-13, and IL-5 contributes to EO attraction via the upregulation of endothelial adhesion markers [36, 42, 43].

EO tissue migration can be described as a stepwise procedure. Tethering and rolling are mediated by selectins, whereas firm adhesion and extravasation are mediated by integrins [44]. Only few studies described the role of adhesion molecules during human EO transendothelial migration in CRSwNP. Some authors showed that EO mucosal infiltration was related to the increased expression of Vascular Cell Adhesion Molecule-1 (VCAM-1) in nasal polyps [45, 46]. On the contrary Symon et al. showed no increase in the expression of VCAM-1 in nasal polyps but an overexpression of InterCellular Adhesion Molecule-1 (ICAM-1), P-selectin, and E-selectin [47]. Patel et al. found that EO tethering to IL-4 activated endothelial cells required P-selectin and VCAM-1 [48]. More recently, Eweiss et al. found that VCAM-1 expression was increased on nasal polyps vessels compared with the mucosal surface of healthy patients. This upregulated expression of VCAM-1 did not statistically correlate with the importance of EO mucosal infiltration and was comparable between nasal polyp tissue and inferior turbinate mucosa [49].

## Eosinophil Behavior in CRSwNP Mucosal Settings

EOs exhibit many roles in CRSwNP pathophysiology: cytotoxic function, immunoregulation, and chemotaxis (Fig. 1). Some are well-described, others are still in debate. Many hypotheses supporting EO pivotal role in CRSwNP are based on the analysis of biomarkers expression from total nasal polyp homogenates. Mechanistic assumptions may be incomplete and need to be considered with caution.

EOs can secrete multiple well-known cytotoxic cationic proteins (Major Basic Protein-1(MBP), Eosinophil Peroxidase (EPX), Eosinophil Cationic Protein (ECP), Eosinophil Derived Neurotoxin (EDN)) and reactive oxygen species (ROS) and more than 30 cytokines, chemokines, and growth factors involved in immunoregulation (Th1 cytokines (Interferon gamma, IL-12), Th2 (IL-5, IL-4, IL-13, IL-9) but also tumor necrosis factor alpha (TNFα), IL-6, IL-10, proliferation-inducing ligand (APRIL), tumor necrosis factor (TGF)-β, and vascular endothelial growth factor (VEGF)) [50].

All of these molecules are stored within large, dense granules called "specific granules," distinct from smaller "primary granules" containing Charcot-Leyden crystal protein (also known as Galectin-10), lipid bodies producing eicosanoids, including cysteinyl leukotrienes, prostaglandins, and thromboxanes [51]. Storages within granules, along with transcription of preformed cytoplasmic mRNA, enable pro or anti-inflammatory responses, with a fast release of preformed mediators after EO stimulation by either cytokines, immunoglobulins, platelet activation factor, or pattern recognition receptors [52–55]. Several ways of granule releasing have already been described [56] (Fig. 3):

- Compound exocytosis is a common cell mechanism leading to the fusion of secretory vesicles with the membrane and exteriorization of the granule contents.
- Cytolysis, with liberation of intact granules, able to release their cytotoxic activities inside the inflamed tissue long after the death of the EO.
- Piecemeal degranulation (PMD), an intriguing mechanism of specific-granule-content delivery, with differential secretion within EO granules, therefore leading to a precise cytokine-specific secretion despite heterogeneous granule contents.
- "ETosis," for extracellular DNA traps, is a recently described mechanism during which EOs release DNA "nets" associated with granule contents, either in an eosinophil-living mechanism involving mitochondrial DNA or a non-apoptotic cell-death mechanism involving nuclear DNA [57, 58]. ETosis can be triggered by lipopolysaccharide (LPS), complement factor 5a, eotaxin, or TSLP exposure and seem to be involved in the mechanism of antibacterial defense but can also promote inflammatory maintenance, acting as a DAMP [59]. ETosis could be compared with formerly described cytolytic EOs, found in tissue sections, and associated with free functional granules.

Degranulating patterns are mostly CD63-associated regulated mechanisms, as EO stimulation with eotaxin1/CCL11 seems to promote PMD, whereas stimulation with TNFα seems to induce preferentially exocytosis [60].

As EOs mainly exhibit secreting functions, degranulation was studied in several studies, on small populations of patients with CRS. Armengot et al. studied EO degranulation on 582 EOs from nasal polyps of 36 patients using electronic transmission microscopy [61]. They showed that 30% of EO were inactive; 42% exhibited signs of piecemeal degranulation. Cytolysis involved 27% of EOs and could eventually be related to ETosis phenomenon. In that study, apoptosis was negligible (0.3% of EOs). The degranulation process can be initiated in IL-3 or IL-5 primed EOs (EOs that have been in contact with IL-3 and IL-5) after interacting with IgG, IgA and IgE antibodies, CCR3-binding eotaxins, RANTES or Monocyte Chemoattractant Protein-4 (MCP-4), complement components, or fibrinogen
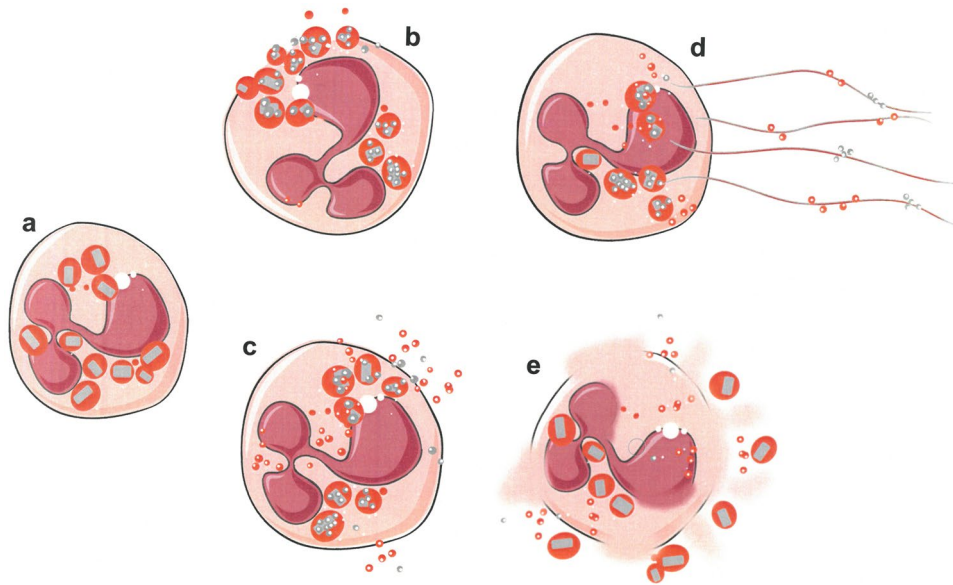
**Fig. 3** Processes of eosinophil granules secretions. Resting eosinophil shows the cytoplasm packed with granules full of contents with typical morphology. Each granule has a central dense core surrounded by a delimiting membrane **a**. Eosinophils may secrete their granule proteins by classic exocytosis (individual granule fusion with the plasma membrane and release of the total granule content) or compound exocytosis (intracellular granule–granule fusion before extracellular release) **b**, piecemeal degranulation (vesicular transport of small packets of materials from the secretory granules to the cell surface) **c**, ETosis (intact granules entrapped in web-like extracellular DNA traps) **d**, and/or cytolysis (extracellular deposition of intact granules upon cell lysis) **e**. More than one process can be involved in inflammatory responses. Storages of proteins (enzymes, cytokines, growth factors, and chemokines) within granules, along with transcription of preformed cytoplasmic mRNA, enable pro or anti-inflammatory responses, with a fast release of preformed mediators after EO stimulation

[53, 62–65]. However, as most of those results derive from in vitro models, they must be considered with caution as they do not reflect in vivo interactions within intricate inflammatory environments. So far, no study focused on degranulation patterns frequency and their relationship with disease severity.

Extracellular release of cytotoxic proteins is suspected to play a role in nasal mucosa remodeling, as blood and tissue overexpression of specific cytotoxic proteins from activated EO is associated with mucosal disease severity [66]. Tsuda et al. described that in vitro exposure of epithelial cells to EDN affected the epithelium to mesenchymal transition pathway with overexpression of Matrix MetalloProteinase-9 (MMP-9) mRNA. As EDN and MMP-9 are abundant in nasal polyps, they suggest that EDN could play a role in CRSwNP airway remodeling [67]. This hypothesis is concordant with a previous study analyzing in vitro impact of ECP on bronchial epithelial cell remodeling where overexpression of endothelin-1, TGF-alpha, TGF-beta1, platelet-derived growth factor (PDGF)-beta, epidermal growth factor (EGF) receptor, MMP-9, fibronectin, and tenascin were observed [68]. EDN also seems to act as an alarmin, attracting dendritic cells and maintaining a pro-Th2 environment [69].

Eosinophil extracellular trap formation or ETosis is a recently described EO function. It was observed in nasal secretions, partly explaining their viscosity in CRSwNP due to their high DNA and protein concentrations, and trapping pathogens to facilitate their clearance [57]. ETosis can be activated in primed EOs by TSLP, complement factor 5a, LPS, and eotaxins [70, 71]. ETosis association with *staphylococcus aureus* antigens is interesting as staphylococcal-associated IgE production has been widely described as a keystone for CRSwNP onset [72]. ETosis was observed in $8.8 \pm 4.8\%$ of nasal EOs and was associated with increased expression of both IL-5 and periostin along with higher levels of *staphylococcus aureus* colonization [73]. Ex vivo procedures with exposure to *staphylococcus aureus* at the epithelial barrier showed a significant increase in immediate ETosis. The same results were observed with *staphylococcus epidermidis* when incubated with TSLP [70]. As a consequence, ETosis seems to be associated with disease severity in both eosinophilic chronic rhinosinusitis and non-eosinophilic chronic rhinosinusitis [74].

Along with their secretory abilities, EOs play a role in chemotaxis [44]. They are able to locally attract other cells involved in the mucosal inflammatory response, including Th2 lymphocytes, neutrophils, dendritic cells or monocytes/macrophages via MBP-1, cationic proteins, or chemokines releasing [21, 69, 75, 76]. EO-produced CCL23 could also attract macrophages, monocytes, and dendritic cells locally

[21]. EOs can also play a minor antigen-presenting role, can contribute to prolonged B cell survival, and can support efficient IgA class-switching via APRIL secretion [77, 78].

EOs are also involved in tissue homeostasis and regeneration via regulatory and anti-inflammatory mechanisms [79]. They are consistently found in tissues with high epithelial cell turnover and major stem cell activity (small intestine, uterus, bone marrow, adipose tissue) [80]. The Local Immunity And/or Remodeling Repair (LIAR) hypothesis described by Lee et al. accounts for the nuanced EO role in both healthy and diseased patients. Instead of EOs exclusively mediating innate host defense, the authors suggested that accumulating tissue EOs become regulators of LIAR in both healthy and pathologic conditions [81]. Such hypotheses could be supported by variations in the local cytokine environment, differential effects on inflammatory and anti-inflammatory processes, or heterogeneous EO subpopulations [82, 83].

## From Pathophysiology to Biologic Therapies in CRSwNP

Over the last 15 years, biologic therapies targeting EOs markers and T2 inflammation pathways have been developed to target life-threatening eosinophilic disorders, like hypereosinophilic syndromes or severe asthma. Consisting of monoclonal antibodies (mAb), these molecules have also been thoroughly evaluated in other eosinophilic disorders such as atopic dermatitis, eosinophilic esophagitis, and subsequently in eosinophilic CRSwNP [84] (Fig. 4). Pro-T2 inflammatory profiles have been associated with medical and surgical failure in CRSwNP [85]. Biologics offer new therapeutic options when both steroids and endoscopic sinus surgery are insufficient to control disease severity and improve quality of life in the long term.

### Anti-immunoglobulin E

Anti-IgE mAb (omalizumab) was first used in asthmatic patients, but quickly assessed in CRSwNP as a high production of IgE can be found in nasal polyps, independently of associated allergy [86]. The therapeutic potential of omalizumab was reinforced after studies reported higher concentrations of *staphylococcus aureus* enterotoxins IgE in nasal polyps [87]. In small randomized, double-blinded, placebo-controlled studies, patients treated with anti-IgE mAb showed an improvement in CRS-related symptoms and endoscopic nasal polyp score (NPS), with decrease use of local steroid [88, 89]. Recently, 2 phase III trial studies including respectively 138 and 127 patients

showed that omalizumab significantly improved endoscopic findings, clinical symptoms, and patient-reported outcomes in severe CRSwNP with resistance to optimal medical treatment [90]. No change in blood EO count was reported in these studies (Table 1).

### Anti-Th2 Cytokines (IL-5, IL-4, IL-13)

Improvement in CRSwNP symptoms and/or radiologic score and/or nasal polyp size and/or surgery incidence has been described with anti-Th2 cytokines biotherapies [91–95]. The promising results of these double-blinded placebo controlled studies have been summed up in a review and a recent Cochrane analysis [96, 97].

Anti-IL-5 drugs have proved to be broadly effective with a decrease in blood EO count when patients are treated with rezlizumab and mepolizumab (Table 1). Immunomodulation of neutrophil-mediated inflammatory response was also suggested with decrease in serum IL-6, myeloperoxidase (MPO) and IL-1β levels (Table 1). The variable response to anti-IL-5 monotherapies in CRSwNP could be explained by a lower expression of IL-5R transmembrane protein in tissue eosinophils inside nasal polyps, possibly mediated by transcription modifications following GM-CSF and/or IL-3 and/or IL-5 stimulation [29, 98–100]. An increase in soluble IL-5Ra via a proteinase-mediated mechanism, or possibly via endocytic process of the whole receptor, has been advanced to explain these results [98, 101]. The findings of a multicentric randomized, double-blinded, placebo-controlled study with benralizumab (anti IL-5R) are still pending (OSTRO Clinical Trial Number: NCT03401229). Benralizumab is a humanized monoclonal antibody that targets the IL-5Ra chain. Unlike targeting soluble IL-5, this agent can induce antibody-dependent cellular cytotoxicity (ADCC) that can result in EO depletion.

Antibodies targeting the alpha subunit of IL-4 and IL-13 receptors show the most promising results in CRSwNP. Two multicentric randomized, double-blinded, placebo-controlled studies including respectively 276 and 447 patients demonstrated a significant improvement of CRS-related symptoms with a reduced polyp size and improved CT opacification [95]. A transient and non-significant increase in blood EO count was reported in both SINUS-24 and SINUS-52 studies. This is consistent with the hypothesis that dupilumab blocks EO nasal migration by inhibiting the production of EO-activating chemokines via a Th2 pathway (Table 1; Fig. 4).

### Ongoing Development of Targeted Therapies

Other targeted molecules are engineered to block EO functions at different levels (activation phase, homing stage, and degranulation) (Table 2):
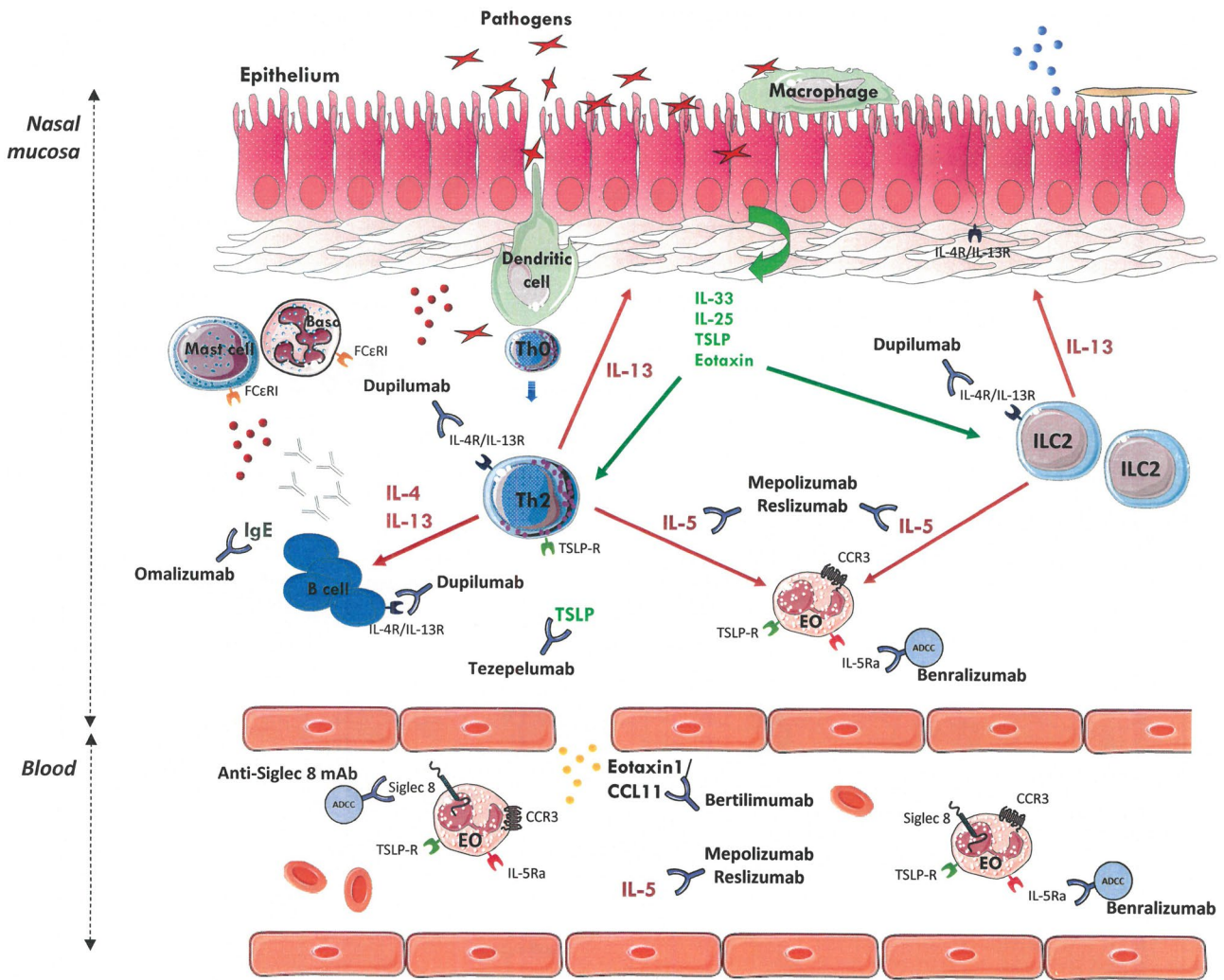
**Fig. 4** Mechanism of action of biologic therapies for treatment of CRSwNP. Some agents target immunoglobulins (Ig) (omalizumab), cytokines (mepolizumab, reslizumab), or chemokines (bertilimumab). Others target lymphocyte T helper type 2 (Th2), type 2 innate lymphoid cells (ILC2), or eosinophil (EO) surface receptors (respectively dupilumab, benralizumab, and anti-Siglec-8 mAb). Omalizumab binds to soluble IgE produced by B cell and blocks IgE interaction with mast cell and basophil via the high-affinity receptor for the Fc region of immunoglobulin E (FCεRI). Mepolizumab and reslizumab target soluble interleukin (IL)-5, whereas benralizumab targets the IL-5 receptor alpha chain (IL-5Ra) reducing EO activation and sur- vival. Dupilumab targets the IL-4 receptor alpha chain (IL-4R) and blocks the downstream signaling of IL-4 and IL-13 (i.e., release of eotaxin and thymic stromal lymphopoietin (TSLP) by the nasal epithelium, skewed-Th2 differentiation of naive helper T cell (Th0), activation and differentiation of B cells into plasma cells, tuning of ILC2). Anti-Siglec-8 mAb induce antibody-dependent cellular cytotoxicity (ADCC) leading to EO depletion. Tezepelumab binds to TSLP consequently reducing EO chemotaxis in nasal mucosa and type 2 cytokines release. Bertilimumab blocks eosinophil chemotaxis via eotaxin1/CCL11 pathway

A phase III clinical trial with oral anti-CCR3 treatment in patients with asthma or eosinophilic bronchitis demonstrated no clinical improvement despite high EO blood counts [102]. More recently, R321, a CCR3-binding inhibitory peptide scaffold with nanoparticles showed some efficiency in blocking EO recruitment in blood and airways, and decreasing airway hyperresponsiveness in a mouse eosinophilic asthma model [103].

Bertilimumab blocking Eotaxin1/CCL11 pathway has been evaluated in a phase IIa clinical trial on patients with pemphigus or pemphigoid auto-immune skin diseases associated with severe skin hypereosinophilia (Clinical Trial Number: NCT02226146). Results were promising with a substantial improvement in disease activity after only 3 doses, pushing for further studies to clarify the exact impact of bertilimumab.

Siglec-8 is a surface receptor expressed only on mature EOs, basophils, and mast-cells. Siglec-8 activation by its sialoglycan ligand induces EO apoptosis, thus making Siglec-8 an interesting target in eosinophil-mediated diseases. AK002/Antolimab, an anti-Siglec 8 antibody, is capable of

**Table 1** Biological findings of randomized, double-blinded, placebo-controlled studies with monoclonal antibody therapies in CRSwNP [88–95]

| Type of biologics | | Level of evidence | Subjects | Biological findings in the treatment group |
|---|---|---|---|---|
| Anti-IgE | Pinto et al. [88] | 2b | 14 patients with CRS received omalizumab ($n = 7$) or placebo ($n = 7$) | No change in EOs counts in nasal lavage |
| | Gevaert et al. [89] | 2b | 24 patients with CRSwNP + asthma received omalizumab ($n = 7$) or placebo ($n = 7$) | No change in BEC |
| | Gevaert et al. [90] | 1b | 138 CRSwNP patients received omalizumab ($n = 72$) or placebo ($n = 66$) (POLYP-1) 127 CRSwNP patients received omalizumab ($n = 62$) or placebo ($n = 65$) (POLYP-2) | NI |
| Anti-IL-5 | Gevaert et al. [91] | 2b | 24 CRSwNP patients received rezlizumab ($n = 16$) or placebo ($n = 18$) | Significant decrease of BEC, serum ECP, soluble IL-5Ra and nasal ECP |
| | Gevaert et al. [92] | 2b | 30 CRSwNP patients received mepolizumab ($n = 20$) or placebo ($n = 10$) | Significant decrease of BEC, serum soluble IL-5Ra and nasal IL6, MPO, IL1β |
| | Bachert et al. [93] | 1b | 105 CRSwNP patients received mepolizumab ($n = 54$) or placebo ($n = 51$) | Decrease of BEC without correlation to responder rate |
| Anti-IL-4/IL-13 | Bachert et al. [94] | 2b | 60 CRSwNP patients treated with dupilumab ($n = 30$) or placebo ($n = 30$) | Significant decrease of serum total IgE and eotaxin-3 Transient increase of BEC |
| | Bachert et al. [95] | 1b | 276 CRSwNP patients treated with dupilumab ($n = 143$) or placebo ($n = 133$) (SINUS-24) 448 CRSwNP patients treated with dupilumab ($n = 295$) or placebo ($n = 153$) (SINUS-52) | Constant decrease of serum total IgE, eotaxin-3, periostin and nasal ECP, IL-5, total IgE, eotaxin-3 Transient increase of BEC |

*BEC* blood eosinophil count, *ECP* eosinophil cationic protein, *EO* eosinophil, *MPO* myeloperoxidase, *NI* not investigated

inducing apoptosis and NK cell-dependent antibody-derived cytotoxicity on IL5-primed EOs in the bloodstream as well as on EOs collected from human lungs [104]. A multicenter phase II randomized, double-blinded, placebo-controlled trial evaluating this treatment in eosinophilic gastritis showed histologic and symptomatic improvements [105].

Anti-GM CSF monoclonal antibody KB003 was tested on patients with uncontrolled asthma. The primary outcome was Forced Expiratory Volume$_1$ (FEV$_1$) after 6 months of treatment, and secondary outcomes included exacerbation rate, general control of asthma, and safety of use. No statistical differences were found between the treatment group and the placebo group, except for patients with baseline blood EO count $\geq 0.3$ G/L in the KB003 group [106].

Several clinical trials using blocking monoclonal antibodies against alarmins are in being conducted in refractory asthma but also in CRSwNP. Tezepelumab, a monoclonal antibody targeting TSLP, showed a decrease in asthma exacerbations compared with placebo in patients with uncontrolled asthma in a phase II clinical trial [107].

## Perspectives

Describing the specific roles of EOs in CRSwNP is a challenging task, as nasal polyps are complex and heterogeneous inflammatory microenvironments harboring multiple inflammatory cells with intricate functions. Within the nasal mucosa, EOs can express either innate cytotoxic functions or immunoregulatory functions through different secretory pathways. The exact triggers of EO activation within the nasal mucosa and their impact on disease severity remain unclear. This may in part explain some heterogeneous results observed in recent clinical trials, as we can presume that EO role is incompletely understood. Another point of complexity is the intertwined inflammatory pathways involved in inflammatory mucosal diseases. EOs are one of the actors of a complex process but it would be a mistake to picture them as the major player of the equation. From a clinical standpoint, it seems that multitreatment approaches using biologics hold the most promising candidates to effectively target all aspects of the inflammatory response in patients with CRSwNP.

From a research and development perspective, a more functional approach to the behavior of EOs in the bloodstream and in tissues would be of great help to delineate specific target mechanisms. Many research teams now try to conduct large proteomic and transcriptomic analyses off nasal polyps in order to extrapolate EO functions in CRSwNP. Meanwhile, EO isolation and purification from tissues are still not easy to achieve. In vivo studies using animal models are only partially adequate, as EOs can exhibit functional differences between species. Finally, in vivo analysis of human EOs requires large blood samples and fresh nasal mucosal tissue, which could be considered invasive from an ethical standpoint.

Development of new in vivo study models, such as nasal mucosa organoids, could be important tools to better understand

**Table 2** Ongoing development of targeted therapies [102–107]

| Type of biologics | | Level of evidence | Subjects | Findings in the treatment group |
|---|---|---|---|---|
| Anti-CCR3 | Neighbour et al. [102] | 2b | 53 patients with eosinophilic asthma received CCR3 antagonists ($n = 25$) or placebo ($n = 28$) | No difference in sputum eosinophil %<br>No improvement in $FEV_1$ |
| | Grozdanovic et al. [103] | NA | Peptide nanoparticle-biased antagonist of CCR3 tested on a triple-allergen mouse asthma model | Effective blocking of EO recruitment into the blood, lungs, and airways<br>Prevention of airway hyperresponsiveness |
| Anti-Eotaxin1/CCL11 | Clinical Trial Number: NCT02226146 | NA | Open-label, proof-of-concept, single group study in 11 patients with newly diagnosed, moderate to extensive bullous pemphigoid who received bertilimumab for 4 weeks | Reduction of disease activity |
| Anti-Siglec-8 | Youngblood et al. [104] | NA | AK002 (antolimab) was tested in a passive systemic anaphylaxis humanized mouse model | Potent apoptotic and ADCC activity against EO<br>Prevention of systemic anaphylaxis through mast cell inhibition |
| | Dellon et al. [105] | 2b | 65 patients with eosinophilic gastritis and/or eosinophilic gastroenteritis received AK002 ($n = 43$) or placebo ($n = 22$) | Significant decrease of gastrointestinal EO count |
| Anti-GM CSF | Molfino et al. [106] | 2b | 160 patients with uncontrolled asthma received KB003 ($n = 78$) or placebo ($n = 82$) | Significant $FEV_1$ improvement in the KB003 group at week 24 in patients with baseline blood EO$\geq 0.3$ G/L |
| Anti-TSLP | Corren et al. [107] | 2b | 550 patients with uncontrolled asthma received tezepelumab at 3 doses ($n = 412$) or placebo ($n = 138$) | Persistent decrease in blood EO counts and total serum IgE levels in all tezepelumab groups |

*ADCC* antibody-dependent cellular cytotoxicity activity, *EO* eosinophil, *FEV₁* Forced Expiratory Volume₁, *NA* not applicable, *NI* not investigated

the role of EOs in CRSwNP. This review calls for collaborative thinking in the fields of proteomic analysis, cellular studies, and new in vivo research models to improve our understanding of this complex but fascinating disease.

## Declarations

## References

1. Fokkens WJ, Lund VJ, Hopkins C, Hellings PW, Kern R, Reitsma S, et al (2020) European Position Paper on Rhinosinusitis and Nasal Polyps. Rhinology 58(Suppl S29):1-464
2. Bhattacharyya N, Villeneuve S, Joish VN, Amand C, Mannent L, Amin N et al (2019) Cost burden and resource utilization in patients with chronic rhinosinusitis and nasal polyps. Laryngoscope 129(9):1969–1975
3. Klossek JM, Neukirch F, Pribil C, Jankowski R, Serrano E, Chanal I et al (2005) Prevalence of nasal polyposis in France: a cross-sectional, case-control study. Allergy 60(2):233–237
4. Rajan JP, Wineinger NE, Stevenson DD, White AA (2015) Prevalence of aspirin-exacerbated respiratory disease among asthmatic patients: a meta-analysis of the literature. J Allergy Clin Immunol 135(3):676-681.e1
5. Hulse KE, Stevens WW, Tan BK, Schleimer RP (2015) Pathogenesis of nasal polyposis. Clin Exp Allergy J Br Soc Allergy Clin Immunol 45(2):328–346
6. Chalermwatanachai T, Vilchez-Vargas R, Holtappels G, Lacoere T, Jáuregui R, Kerckhof FM et al (2018) Chronic rhinosinusitis with nasal polyps is characterized by dysbacteriosis of the nasal microbiota. Sci Rep 8(1):7926
7. Nakayama T, Hirota T, Asaka D, Sakashita M, Ninomiya T, Morikawa T et al (2020) A genetic variant near TSLP is associated with chronic rhinosinusitis with nasal polyps and aspirin-exacerbated respiratory disease in Japanese populations. Allergol Int Off J Jpn Soc Allergol 69(1):138–140
8. Lazard DS, Prulière-Escabasse V, Papon JF, Escudier E (1983) Coste A (2007) [Injury and epithelial wound healing: a pathophysiologic

hypothesis for nasal and sinus polyposis]. Presse Medicale Paris Fr 36(7–8):1104–1108

9. Radajewski K, Wierzchowska M, Grzanka D, Antosik P, Zdrenka M, Burduk P (2019) Tissue remodelling in chronic rhinosinusitis—review of literature. Otolaryngol Pol Pol Otolaryngol 73(5):1–4

10. Tomassen P, Vandeplas G, Van Zele T, Cardell LO, Arebro J, Olze H et al (2016) Inflammatory endotypes of chronic rhinosinusitis based on cluster analysis of biomarkers. J Allergy Clin Immunol 137(5):1449-1456.e4

11. Mahdavinia M, Suh LA, Carter RG, Stevens WW, Norton JE, Kato A et al (2015) Increased noneosinophilic nasal polyps in chronic rhinosinusitis in US second-generation Asians suggest genetic regulation of eosinophilia. J Allergy Clin Immunol 135(2):576–579

12. Katotomichelakis M, Tantilipikorn P, Holtappels G, De Ruyck N, Feng L, Van Zele T et al (2013) Inflammatory patterns in upper airway disease in the same geographical area may change over time. Am J Rhinol Allergy 27(5):354–360

13. Kanda A, Yasutaka Y, Van Bui D, Suzuki K, Sawada S, Kobayashi Y et al (2020) Multiple biological aspects of eosinophils in host defense, eosinophil-associated diseases, immunoregulation, and homeostasis: is their role beneficial, detrimental, regulator, or bystander? Biol Pharm Bull 43(1):20–30

14. Mack EA, Pear WS (2020) Transcription factor and cytokine regulation of eosinophil lineage commitment. Curr Opin Hematol 27(1):27–33

15. Mori Y, Iwasaki H, Kohno K, Yoshimoto G, Kikushige Y, Okeda A et al (2009) Identification of the human eosinophil lineage-committed progenitor: revision of phenotypic definition of the human common myeloid progenitor. J Exp Med 206(1):183–193

16. McNagny K, Graf T (2002) Making eosinophils through subtle shifts in transcription factor expression. J Exp Med 195(11):F43-47

17. Du J, Stankiewicz MJ, Liu Y, Xi Q, Schmitz JE, Lekstrom-Himes JA, et al (2002) Novel combinatorial interactions of GATA-1, PU.1, and C/EBPepsilon isoforms regulate transcription of the gene encoding eosinophil granule major basic protein. J Biol Chem 277(45):43481-94

18. Gombart AF, Kwok SH, Anderson KL, Yamaguchi Y, Torbett BE, Koeffler HP (2003) Regulation of neutrophil and eosinophil secondary granule gene expression by transcription factors C/EBP epsilon and PU.1. Blood 101(8):3265-73

19. Zimmermann N, Colyer JL, Koch LE, Rothenberg ME (2005) Analysis of the CCR3 promoter reveals a regulatory region in exon 1 that binds GATA-1. BMC 6:7

20. Takatsu K, Kouro T, Nagai Y (2009) Interleukin 5 in the link between the innate and acquired immune response. Adv Immunol 101:191–236

21. Poposki JA, Uzzaman A, Nagarkar DR, Chustz RT, Peters AT, Suh LA et al (2011) Increased expression of the chemokine CCL23 in eosinophilic chronic rhinosinusitis with nasal polyps. J Allergy Clin Immunol 128(1):73-81.e4

22. Spencer LA, Szela CT, Perez SAC, Kirchhoffer CL, Neves JS, Radke AL et al (2009) Human eosinophils constitutively express multiple Th1, Th2, and immunoregulatory cytokines that are secreted rapidly and differentially. J Leukoc Biol 85(1):117–123

23. Foster PS, Hogan SP, Ramsay AJ, Matthaei KI, Young IG (1996) Interleukin 5 deficiency abolishes eosinophilia, airways hyperreactivity, and lung damage in a mouse asthma model. J Exp Med 183(1):195–201

24. Fulkerson PC, Schollaert KL, Bouffi C (1950) Rothenberg ME (2014) IL-5 triggers a cooperative cytokine network that promotes eosinophil precursor maturation. J Immunol Baltim Md 193(8):4043–4052

25. Dougan M, Dranoff G, Dougan SK (2019) GM-CSF, IL-3, and IL-5 family of cytokines: regulators of inflammation. Immunity 50(4):796–811

26. Johnston LK, Hsu CL, Krier-Burris RA, Chhiba KD, Chien KB, McKenzie A et al (1950) (2016) IL-33 Precedes IL-5 in regulating

eosinophil commitment and is required for eosinophil homeostasis. J Immunol Baltim Md 197(9):3445–3453

27. Kim YK, Uno M, Hamilos DL, Beck L, Bochner B, Schleimer R et al (1999) Immunolocalization of CD34 in nasal polyposis. Effect of topical corticosteroids. Am J Respir Cell Mol Biol 20(3):388–397

28. Cameron L, Christodoulopoulos P, Lavigne F, Nakamura Y, Eidelman D, McEuen A et al (1950) (2000) Evidence for local eosinophil differentiation within allergic nasal mucosa: inhibition with soluble IL-5 receptor. J Immunol Baltim Md 164(3):1538–1545

29. Mortuaire G, Gengler I, Vandenhende-Szymanski C, Delbeke M, Gatault S, Chevalier D et al (2015) Immune profile modulation of blood and mucosal eosinophils in nasal polyposis with concomitant asthma. Ann Allergy Asthma Immunol 114(4):299-307.e2

30. Wang K, Deng J, Yang M, Chen Y, Chen F, Gao WX et al (2019) Concordant systemic and local eosinophilia relates to poorer disease control in patients with nasal polyps. World Allergy Organ J 12(8):100052

31. Dupuch V, Tridon A, Ughetto S, Walrand S, Bonnet B, Dubray C et al (2018) Activation state of circulating eosinophils in nasal polyposis. Int Forum Allergy Rhinol 8(5):584–591

32. Farahi N, Singh NR, Heard S, Loutsios C, Summers C, Solanki CK et al (2012) Use of 111-Indium- labeled autologous eosinophils to establish the in vivo kinetics of human eosinophils in healthy subjects. Blood 120(19):4068–4071

33. McHugh T, Snidvongs K, Xie M, Banglawala S, Sommer D (2018) High tissue eosinophilia as a marker to predict recurrence for eosinophilic chronic rhinosinusitis: a systematic review and meta-analysis. Int Forum Allergy Rhinol 8(12):1421–1429

34. Sharbel D, Li M, Unsal AA, Tadros SY, Lee J, Biddinger P et al (2020) Use of mucosal eosinophil count as a guide in the management of chronic rhinosinusitis. Int Forum Allergy Rhinol 10(4):474–480

35. Mortuaire G, Leroy X, Gengler I, Chevalier D, Prin L, Picry A (2015) Histopathological classification of refractory chronic rhinosinusitis with nasal polyps. Histol Histopathol 30(12):1447–1454

36. Patel NN, Kohanski MA, Maina IW, Workman AD, Herbert DR, Cohen NA (2019) Sentinels at the wall: epithelial-derived cytokines serve as triggers of upper airway type 2 inflammation. Int Forum Allergy Rhinol 9(1):93–99

37. Paris G, Pozharskaya T, Asempa T, Lane AP (2014) Damage-associated molecular patterns stimulate interleukin-33 expression in nasal polyp epithelial cells. Int Forum Allergy Rhinol 4(1):15–21

38. Yoshifuku K, Matsune S, Ohori J, Sagara Y, Fukuiwa T, Kurono Y (2007) IL-4 and TNF-alpha increased the secretion of eotaxin from cultured fibroblasts of nasal polyps with eosinophil infiltration. Rhinology 45(3):235–241

39. Saito H, Honda K, Asaka C, Ueki S, Ishikawa K (2016) Eosinophil chemotaxis assay in nasal polyps by using a novel optical device EZ-TAXIScan: role of CC-chemokine receptor 3. Allergol Int Off J Jpn Soc Allergol 65(3):280–285

40. Meyer JE, Bartels J, Görögh T, Sticherling M, Rudack C, Ross DA et al (2005) The role of RANTES in nasal polyposis. Am J Rhinol 19(1):15–20

41. Peinhaupt M, Sturm EM, Heinemann A (2017) Prostaglandins and their receptors in eosinophil function and as therapeutic targets. Front Med 4:104

42. Tojima I, Matsumoto K, Kikuoka H, Hara S, Yamamoto S, Shimizu S et al (2019) Evidence for the induction of Th2 inflammation by group 2 innate lymphoid cells in response to prostaglandin D2 and cysteinyl leukotrienes in allergic rhinitis. Allergy 74(12):2417–2426

43. Kotowicz K, Callard RE, Friedrich K, Matthews DJ, Klein N (1996) Biological activity of IL-4 and IL-13 on human endothelial cells: functional evidence that both cytokines act through the same receptor. Int Immunol 8(12):1915–1925

44. Rosenberg HF, Dyer KD, Foster PS (2013) Eosinophils: changing perspectives in health and disease. Nat Rev Immunol 13(1):9–22

45. Jahnsen FL, Haraldsen G, Aanesen JP, Haye R, Brandtzaeg P (1995) Eosinophil infiltration is related to increased expression of vascular cell adhesion molecule-1 in nasal polyps. Am J Respir Cell Mol Biol 12(6):624–632

46. Hamilos DL, Leung DY, Wood R, Bean DK, Song YL, Schotman E et al (1996) Eosinophil infiltration in nonallergic chronic hyperplastic sinusitis with nasal polyposis (CHS/NP) is associated with endothelial VCAM-1 upregulation and expression of TNF-alpha. Am J Respir Cell Mol Biol 15(4):443–450

47. Symon FA, Walsh GM, Watson SR, Wardlaw AJ (1994) Eosinophil adhesion to nasal polyp endothelium is P-selectin-dependent. J Exp Med 180(1):371–376

48. Patel KD (1998) Eosinophil tethering to interleukin-4-activated endothelial cells requires both P-selectin and vascular cell adhesion molecule-1. Blood 92(10):3904–3911

49. Eweiss A, Dogheim Y, Hassab M, Tayel H, Hammad Z (2009) VCAM-1 and eosinophilia in diffuse sino-nasal polyps. Eur Arch Oto-Rhino-Laryngol Off J Eur Fed Oto-Rhino-Laryngol Soc EUFOS Affil Ger Soc Oto-Rhino-Laryngol - Head Neck Surg 266(3):377–383

50. Davoine F, Lacy P (2014) Eosinophil cytokines, chemokines, and growth factors: emerging roles in immunity. Front Immunol 5:570

51. McBrien CN, Menzies-Gow A (2017) The biology of eosinophils and their role in asthma. Front Med (Lausanne) 4:93

52. Weller PF, Spencer LA (2017) Functions of tissue-resident eosinophils. Nat Rev Immunol 17(12):746–760

53. Suh K-S, Park HS, Nahm DH, Kim YK, Lee YM, Park K (2002) Role of IgG, IgA, and IgE antibodies in nasal polyp tissue: their relationships with eosinophilic infiltration and degranulation. J Korean Med Sci 17(3):375–380

54. Kato M, Kita H, Tachibana A, Hayashi Y, Tsuchida Y, Kimura H (2004) Dual signaling and effector pathways mediate human eosinophil activation by platelet-activating factor. Int Arch Allergy Immunol 134(Suppl 1):37–43

55. Kvarnhammar AM, Cardell LO (2012) Pattern-recognition receptors in human eosinophils. Immunology 136(1):11–20

56. Melo RCN, Weller PF (2018) Contemporary understanding of the secretory granules in human eosinophils. J Leukoc Biol 104(1):85–93

57. Yousefi S, Gold JA, Andina N, Lee JJ, Kelly AM, Kozlowski E et al (2008) Catapult-like release of mitochondrial DNA by eosinophils contributes to antibacterial defense. Nat Med 14(9):949–953

58. Ueki S, Melo RCN, Ghiran I, Spencer LA, Dvorak AM, Weller PF (2013) Eosinophil extracellular DNA trap cell death mediates lytic release of free secretion-competent eosinophil granules in humans. Blood 121(11):2074–2083

59. Ueki S, Tokunaga T, Fujieda S, Honda K, Hirokawa M, Spencer LA et al (2016) Eosinophil ETosis and DNA traps: a new look at eosinophilic inflammation. Curr Allergy Asthma Rep 16(8):54

60. Carmo LAS, Bonjour K, Ueki S, Neves JS, Liu L, Spencer LA et al (2016) CD63 is tightly associated with intracellular, secretory events chaperoning piecemeal degranulation and compound exocytosis in human eosinophils. J Leukoc Biol 100(2):391–401

61. Armengot M, Garín L, Carda C (2009) Eosinophil degranulation patterns in nasal polyposis: an ultrastructural study. Am J Rhinol Allergy 23(5):466–470

62. Fujisawa T, Kato Y, Nagase H, Atsuta J, Terada A, Iguchi K et al (2000) Chemokines induce eosinophil degranulation through CCR-3. J Allergy Clin Immunol 106(3):507–513

63. Badewa AP, Hudson CE, Heiman AS (2002) Regulatory effects of eotaxin, eotaxin-2, and eotaxin-3 on eosinophil degranulation and superoxide anion generation. Exp Biol Med Maywood NJ 227(8):645–651

64. Takafuji S, Tadokoro K, Ito K (1996) Effects of interleukin (IL)-3 and IL-5 on human eosinophil degranulation induced by complement components C3a and C5a. Allergy 51(8):563–568

65. Coden ME, Loffredo LF, Walker MT, Jeong BM, Nam K, Bochner BS et al (1950) (2020) Fibrinogen is a specific trigger for cytolytic eosinophil degranulation. J Immunol Baltim Md 204(2):438–448

66. Sun DI, Joo YH, Auo HJ, Kang JM (2009) Clinical significance of eosinophilic cationic protein levels in nasal secretions of patients with nasal polyposis. Eur Arch Oto-Rhino-Laryngol Off J Eur Fed Oto-Rhino-Laryngol Soc EUFOS Affil Ger Soc Oto-Rhino-Laryngol - Head Neck Surg 266(7):981–986

67. Tsuda T, Maeda Y, Nishide M, Koyama S, Hayama Y, Nojima S et al (2019) Eosinophil-derived neurotoxin enhances airway remodeling in eosinophilic chronic rhinosinusitis and correlates with disease severity. Int Immunol 31(1):33–40

68. Pégorier S, Wagner LA, Gleich GJ (1950) Pretolani M (2006) Eosinophil-derived cationic proteins activate the synthesis of remodeling factors by airway epithelial cells. J Immunol Baltim Md 177(7):4861–4869

69. Yang D, Rosenberg HF, Chen Q, Dyer KD, Kurosaka K, Oppenheim JJ (2003) Eosinophil-derived neurotoxin (EDN), an antimicrobial protein with chemotactic activities for dendritic cells. Blood 102(9):3396–3403

70. Morshed M, Yousefi S, Stöckle C, Simon HU, Simon D (2012) Thymic stromal lymphopoietin stimulates the formation of eosinophil extracellular traps. Allergy 67(9):1127–1137

71. Yousefi S, Simon D, Simon HU (2012) Eosinophil extracellular DNA traps: molecular mechanisms and potential roles in disease. Curr Opin Immunol 24(6):736–739

72. Gevaert E, Zhang N, Krysko O, Lan F, Holtappels G, De Ruyck N et al (2017) Extracellular eosinophilic traps in association with Staphylococcus aureus at the site of epithelial barrier defects in patients with severe airway inflammation. J Allergy Clin Immunol 139(6):1849-1860.e6

73. Lehmann AE, Scangas GA, Bergmark RW, El Rassi E, Stankovic KM, Metson R (2019) Periostin and inflammatory disease: implications for chronic rhinosinusitis. Otolaryngol-Head Neck Surg Off J Am Acad Otolaryngol-Head Neck Surg 160(6):965–973

74. Hwang CS, Park SC, Cho HJ, Park DJ, Yoon JH, Kim CH (2019) Eosinophil extracellular trap formation is closely associated with disease severity in chronic rhinosinusitis regardless of nasal polyp status. Sci Rep 9(1):8061

75. Jacobsen EA, Ochkur SI, Pero RS, Taranova AG, Protheroe CA, Colbert DC et al (2008) Allergic pulmonary inflammation in mice is dependent on eosinophil-induced recruitment of effector T cells. J Exp Med 205(3):699–710

76. Moy JN, Gleich GJ (1950) Thomas LL (1990) Noncytotoxic activation of neutrophils by eosinophil granule major basic protein. Effect on superoxide anion generation and lysosomal enzyme release. J Immunol Baltim Md 145(8):2626–2632

77. Schuijs MJ, Hammad H, Lambrecht BN (2019) Professional and 'amateur' antigen- presenting cells in type 2 immunity. Trends Immunol 40(1):22–34

78. Berek C (2016) Eosinophils: important players in humoral immunity. Clin Exp Immunol 183(1):57–64

79. Marichal T, Mesnil C, Bureau F (2017) Homeostatic eosinophils: characteristics and functions. Front Med 4:101

80. Abdala-Valencia H, Coden ME, Chiarella SE, Jacobsen EA, Bochner BS, Lee JJ et al (2018) Shaping eosinophil identity in the tissue contexts of development, homeostasis, and disease. J Leukoc Biol 104(1):95–108

81. Lee JJ, Jacobsen EA, McGarry MP, Schleimer RP, Lee NA (2010) Eosinophils in health and disease: the LIAR hypothesis. Clin Exp Allergy J Br Soc Allergy Clin Immunol 40(4):563–575

82. Mesnil C, Raulier S, Paulissen G, Xiao X, Birrell MA, Pirottin D et al (2016) Lung-resident eosinophils represent a distinct regulatory eosinophil subset. J Clin Invest 126(9):3279–3295

83. Xenakis JJ, Howard ED, Smith KM, Olbrich CL, Huang Y, Anketell D et al (2018) Resident intestinal eosinophils constitutively express antigen presentation markers and include two phenotypically distinct subsets of eosinophils. Immunology 154(2):298–308

84. Legrand F, Klion AD (2015) Biologic therapies targeting eosinophils: current status and future prospects. J Allergy Clin Immunol Pract 3(2):167–174

85. Mortuaire G, Gengler I, Carpentier C, Szymanski C, Chenivesse C, Lefevre G (2020) T helper 2 inflammatory markers are associated with recurrence in chronic rhinosinusitis with nasal polyps after endoscopic sinus surgery. Rhinology 58(5):444–450

86. Bachert C, Gevaert P, Holtappels G, Johansson SG, van Cauwenberge P (2001) Total and specific IgE in nasal polyps is related to local eosinophilic inflammation. J Allergy Clin Immunol 107(4):607–614

87. Gevaert P, Holtappels G, Johansson SGO, Cuvelier C, Cauwenberge P, Bachert C (2005) Organization of secondary lymphoid tissue and local IgE formation to Staphylococcus aureus enterotoxins in nasal polyp tissue. Allergy 60(1):71–79

88. Pinto JM, Mehta N, DiTineo M, Wang J, Baroody FM, Naclerio RM (2010) A randomized, double-blind, placebo-controlled trial of anti-IgE for chronic rhinosinusitis. Rhinology 48(3):318–324

89. Gevaert P, Calus L, Van Zele T, Blomme K, De Ruyck N, Bauters W et al (2013) Omalizumab is effective in allergic and nonallergic patients with nasal polyps and asthma. J Allergy Clin Immunol 131(1):110-116.e1

90. Gevaert P, Omachi TA, Corren J, Mullol J, Han J, Lee SE et al (2020) Efficacy and safety of omalizumab in nasal polyposis: 2 randomized phase 3 trials. J Allergy Clin Immunol 146(3):595–605

91. Gevaert P, Lang-Loidolt D, Lackner A, Stammberger H, Staudinger H, Van Zele T et al (2006) Nasal IL-5 levels determine the response to anti-IL-5 treatment in patients with nasal polyps. J Allergy Clin Immunol 118(5):1133–1141

92. Gevaert P, Van Bruaene N, Cattaert T, Van Steen K, Van Zele T, Acke F, et al (2011) Mepolizumab, a humanized anti-IL-5 mAb, as a treatment option for severe nasal polyposis. J Allergy Clin Immunol 128(5):989–995.e1–8

93. Bachert C, Sousa AR, Lund VJ, Scadding GK, Gevaert P, Nasser S et al (2017) Reduced need for surgery in severe nasal polyposis with mepolizumab: randomized trial. J Allergy Clin Immunol 140(4):1024-1031.e14

94. Bachert C, Mannent L, Naclerio RM, Mullol J, Ferguson BJ, Gevaert P et al (2016) Effect of subcutaneous dupilumab on nasal polyp burden in patients with chronic sinusitis and nasal polyposis: a randomized clinical trial. JAMA 315(5):469–479

95. Bachert C, Han JK, Desrosiers M, Hellings PW, Amin N, Lee SE et al (2019) Efficacy and safety of dupilumab in patients with severe chronic rhinosinusitis with nasal polyps (LIBERTY NP SINUS-24 and LIBERTY NP SINUS-52): results from two multicentre, randomised, double-blind, placebo-controlled, parallel-group phase 3 trials. Lancet Lond Engl 394(10209):1638–1650

96. Tsetsos N, Goudakos JK, Daskalakis D, Konstantinidis I, Markou K (2018) Monoclonal antibodies for the treatment of chronic rhinosinusitis with nasal polyposis: a systematic review. Rhinology 56(1):11–21

97. Chong LY, Piromchai P, Sharp S, Snidvongs K, Philpott C, Hopkins C, et al (2020) Biologics for chronic rhinosinusitis. Cochrane Database Syst Rev 2:CD013513

98. Gevaert P, Hellman C, Lundblad L, Lundahl J, Holtappels G, van Cauwenberge P et al (2009) Differential expression of the interleukin 5 receptor alpha isoforms in blood and tissue eosinophils of nasal polyp patients. Allergy 64(5):725–732

99. Wang P, Wu P, Cheewatrakoolpong B, Myers JG, Egan RW (1950) Billah MM (1998) Selective inhibition of IL-5 receptor alpha-chain gene transcription by IL-5, IL-3, and granulocyte-macrophage colony-stimulating factor in human blood eosinophils. J Immunol Baltim Md 160(9):4427–4432

100. Gregory B, Kirchem A, Phipps S, Gevaert P, Pridgeon C, Rankin SM et al (1950) (2003) Differential regulation of human eosinophil IL-3, IL-5, and GM-CSF receptor alpha-chain expression by cytokines: IL-3, IL-5, and GM-CSF down-regulate IL-5 receptor alpha expression with loss of IL-5 responsiveness, but up-regulate IL-3 receptor alpha expression. J Immunol Baltim Md 170(11):5359–5366

101. Liu LY, Sedgwick JB, Bates ME, Vrtis RF, Gern JE, Kita H et al (1950) (2002) Decreased expression of membrane IL-5 receptor alpha on human eosinophils: II. IL-5 down-modulates its receptor via a proteinase-mediated process. J Immunol Baltim Md 169(11):6459–6466

102. Neighbour H, Boulet LP, Lemiere C, Sehmi R, Leigh R, Sousa AR et al (2014) Safety and efficacy of an oral CCR3 antagonist in patients with asthma and eosinophilic bronchitis: a randomized, placebo-controlled clinical trial. Clin Exp Allergy J Br Soc Allergy Clin Immunol 44(4):508–516

103. Grozdanovic M, Laffey KG, Abdelkarim H, Hitchinson B, Harijith A, Moon HG et al (2019) Novel peptide nanoparticle-biased antagonist of CCR3 blocks eosinophil recruitment and airway hyperresponsiveness. J Allergy Clin Immunol 143(2):669-680.e12

104. Youngblood BA, Brock EC, Leung J, Falahati R, Bryce PJ, Bright J et al (2019) AK002, a humanized sialic acid-binding immunoglobulin-like lectin-8 antibody that induces antibody-dependent cell-mediated cytotoxicity against human eosinophils and inhibits mast cell-mediated anaphylaxis in mice. Int Arch Allergy Immunol 180(2):91–102

105. Dellon ES, Peterson KA, Murray JA, Falk GW, Gonsalves N, Chehade M et al (2020) Anti-Siglec-8 Antibody for eosinophilic gastritis and duodenitis. N Engl J Med 383(17):1624–1634

106. Molfino NA, Kuna P, Leff JA, Oh CK, Singh D, Chernow M et al (2016) Phase 2, randomised placebo-controlled trial to evaluate the efficacy and safety of an anti-GM-CSF antibody (KB003) in patients with inadequately controlled asthma. BMJ Open 6(1):e007709

107. Corren J, Parnes JR, Wang L, Mo M, Roseti SL, Griffiths JM et al (2017) Tezepelumab in adults with uncontrolled asthma. N Engl J Med 377(10):936–946