Received: 10 July 2023 | Revised: 21 August 2023 | Accepted: 27 August 2023

DOI: 10.1111/all.15884

Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY EAACI WILEY

EAACI POSITION PAPER

Eosinophils—from cradle to grave

An EAACI task force paper on new molecular insights and clinical functions of eosinophils and the clinical effects of targeted eosinophil depletion

Milos Jesenak[1,2,3] | Zuzana Diamant[4,5,6] | Dagmar Simon[7] | Ellen Tufvesson[4] | Sven F. Seys[8] | Manali Mukherjee[9,10] | Paige Lacy[11] | Susanne Vijverberg[12] | Tomas Slisz[6] | Anna Sediva[13] | Hans-Uwe Simon[14,15] | Ilja Striz[16] | Jana Plevkova[17] | Jurgen Schwarze[18] | Radovan Kosturiak[2,19] | Neil E. Alexis[20] | Eva Untersmayr[21] | Martina Koziar Vasakova[6] | Edward Knol[22,23] | Leo Koenderman[22,24]

[1]Department of Clinical Immunology and Allergology, University Teaching Hospital in Martin, Martin, Slovak Republic

[2]Department of Paediatrics, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, University Teaching Hospital in Martin, Martin, Slovak Republic

[3]Department of Pulmonology and Phthisiology, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, University Teaching Hospital in Martin, Martin, Slovak Republic

[4]Department of Clinical Sciences Lund, Respiratory Medicine, Allergology and Palliative Medicine, Lund University, Lund, Sweden

[5]Department Microbiology Immunology & Transplantation, KU Leuven, Catholic University of Leuven, Leuven, Belgium

[6]Department of Respiratory Medicine, First Faculty of Medicine, Charles University and Thomayer Hospital, Prague, Czech Republic

[7]Department of Dermatology, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland

[8]Laboratory of Clinical Immunology, Department of Microbiology and Immunology, KU Leuven, Leuven, Belgium

[9]Department of Medicine, McMaster University, Hamilton, Ontario, Canada

[10]The Firestone Institute for Respiratory Health, Research Institute of St. Joe's Hamilton, Hamilton, Ontario, Canada

[11]Department of Medicine, University of Alberta, Edmonton, Alberta, Canada

[12]Amsterdam UMC Location University of Amsterdam, Pulmonary Diseases, Amsterdam, The Netherlands

[13]Department of Immunology, 2nd Faculty of Medicine, Charles University, Motol University Hospital, Prague, Czech Republic

[14]Institute of Pharmacology, University of Bern, Bern, Switzerland

[15]Institute of Biochemistry, Brandenburg Medical School, Neuruppin, Germany

[16]Department of Clinical and Transplant Immunology, Institute for Clinical and Experimental Medicine, Prague, Czech Republic

[17]Department of Pathophysiology, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, Slovak Republic

[18]Child Life and Health and Centre for Inflammation Research, The Queen's Medical Research Institute, University of Edinburgh, Edinburgh, UK

[19]Outpatient Clinic for Clinical Immunology and Allergology, Nitra, Slovak Republic

[20]Center for Environmental Medicine, Asthma and Lung Biology, Department of Paediatrics, University of North Carolina, Chapel Hill, North Carolina, USA

[21]Institute of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria

[22]Department Center of Translational Immunology, University Medical Center Utrecht, Utrecht, The Netherlands

[23]Department Dermatology/Allergology, University Medical Center Utrecht, Utrecht, The Netherlands

[24]Department Pulmonary Diseases, University Medical Center Utrecht, Utrecht, The Netherlands

Correspondence
Milos Jesenak, Department of Clinical Immunology and Allergology, University Teaching Hospital in Martin, Martin, Slovak Republic.
Email: jesenak@gmail.com

Abstract

Over the past years, eosinophils have become a focus of scientific interest, especially in the context of their recently uncovered functions (e.g. antiviral, anti-inflammatory, regulatory). These versatile cells display both beneficial and detrimental activities

Zuzana Diamant, Department of Clinical Sciences Lund, Respiratory Medicine, Allergology and Palliative Medicine, Lund University, Lund, Sweden.
Email: z.diamant@4gems.nl

under various physiological and pathological conditions. Eosinophils are involved in the pathogenesis of many diseases which can be classified into primary (clonal) and secondary (reactive) disorders and idiopathic (hyper)eosinophilic syndromes. Depending on the biological specimen, the eosinophil count in different body compartments may serve as a biomarker reflecting the underlying pathophysiology and/or activity of distinct diseases and as a therapy-driving (predictive) and monitoring tool. Personalized selection of an appropriate therapeutic strategy directly or indirectly targeting the increased number and/or activity of eosinophils should be based on the understanding of eosinophil homeostasis including their interactions with other immune and non-immune cells within different body compartments. Hence, restoring as well as maintaining homeostasis within an individual's eosinophil pool is a goal of both specific and non-specific eosinophil-targeting therapies. Despite the overall favourable safety profile of the currently available anti-eosinophil biologics, the effect of eosinophil depletion should be monitored from the perspective of possible unwanted consequences.

KEYWORDS
allergic diseases, biologics, biomarker, COVID-19, eosinophils, non-allergic diseases

1 | EOSINOPHILS AND EOSINOPHILIA—INTRODUCTION

Eosinophils display both beneficial and detrimental activities in immunity which balance between maintaining health and homeostasis on one hand and causing disease on the other hand. Their role in the pathophysiology of various allergic and non-allergic conditions and diseases has been recognized for decades.[1,2] Consequently, this has led to the development of a broad spectrum of therapies targeting eosinophils, either non-specifically by the inhibition of several upstream or downstream immune pathways or specifically by eosinophil-targeted treatments with biologics.[3,4]

More recently, several so far unknown physiological functions of this cell population have been identified. In the context of these recent insights, eosinophils appear to behave as a double-edged sword with important regulatory (immunomodulatory), anti-inflammatory, anti-parasitic and anti-viral properties to maintain the homeostasis in the body.[5,6] Alternatively, the involvement of pro-inflammatory eosinophils in the initiation, progression and persistence of inflammation with tissue remodelling is well-known and has been documented for many decades. Therefore, the eosinophil counts in biological specimens from different body compartments may serve as a biomarker that reflects the underlying pathophysiology of specific diseases, predict treatment success and monitor therapeutic progress.[3,4,7] The precise definition of eosinophilia and the discrimination between a truly pathological condition and hypereosinophilia as an epiphenomenon is crucial for a correct interpretation and application of eosinophils as a biomarker in clinical practice.

In the context of these novel insights, the EAACI taskforce on eosinophils, which includes both basic scientists and clinicians, aimed to shed more light on the differentiated functions of eosinophils to be considered in clinical practice as well as to evaluate the potential consequences of eosinophil depletion with targeted therapies. For clinically applicable algorithms aimed at guiding (biologic) treatments, we would like to refer to fairly recent reviews, including an EAACI task force paper.[7-10]

2 | EOSINOPHILS IN HEALTH, HOMEOSTASIS AND PROTECTIVE RESPONSES

2.1 | Origin and life cycle of human eosinophils

Eosinophils are innate immune cells and members of the family of white blood cells (WBC).[11] These cells were first described by Paul Ehrlich in the 19th century.[12] Eosinophils have a characteristic bilobed nucleus and large granules that stain intensely with the dye eosin, giving the cells their name. The granules contain several enzymes and cationic proteins, including peroxidases, lysosomal enzyme and major basic protein (MBP). Eosinophils originate from the bone marrow where they are produced from a myeloid progenitor shared with basophils.[13] At the myelocyte stage, the progenitors stop dividing and enter into a maturation phase of approximately 4 days during which the cells mature into functional granulocytes.[14] This process is under the control of cytokine receptors (e.g. βc/CD131 containing receptors: CD116/CD131, CD123/CD131 and CD125/CD131, binding to GM-CSF, IL-3 and IL-5, respectively[15]), alarmin receptors (e.g. ST2 binding to IL-33)[16] and specific transcription factors (e.g. GATA1/2 and C/EBPα).[17] Subsequently, mature eosinophils

are released from the bone marrow and can be detected at low numbers in the peripheral blood (approximately 50–150 cells/μL of blood/1%–3% of total WBC) in homeostasis/health.[18] The possibility of in situ eosinophilopoiesis has been also described.[19] In homeostasis, the half-life of eosinophils in the peripheral blood is unknown but is estimated between 11 and 63 h.[20-22] Hereafter, little is known of the fate of eosinophils.

In health, eosinophils can be detected in several tissues such as the gut and adipose tissue with various homeostatic functions (Figure 1). In several diseases, particularly those associated with allergies, increased numbers of pre-activated or primed eosinophils are found in peripheral blood and in inflamed target tissues.[25] Besides classical allergic diseases associated with eosinophilic infiltration of the target organs, a broad spectrum of non-allergic conditions (e.g. non-allergic eosinophilic asthma and eosinophilic bronchitis) associated with high eosinophil counts both in blood and tissue exists, for example eosinophilic granulomatosis with polyangiitis (EGPA) and chronic rhinosinusitis with nasal polyps (CRSwNP). Interleukin-5 (IL-5) is a pivotal cytokine for the life cycle of eosinophils as it (i) is a growth factor for eosinophil progenitors, (ii) is involved in the mobilization of eosinophils from the bone marrow and (iii) plays an important role in their activation and homing into target tissues.[26] Nonetheless, the presence of IL-5 does not seem to be solely essential for eosinophil development as IL-5 knockout mice still have eosinophils[27] and a trial with mepolizumab (anti-IL-5 monoclonal antibody (mAb)) in patients with eosinophilic esophagitis (EoE) showed marked decreases in eosinophils in peripheral blood and inflamed tissue, but did not affect eosinophil numbers in the duodenum.[28] Similarly, mepolizumab treatment significantly decreased eosinophils in the peripheral blood and sputum[29] but failed to substantially reduce airway tissue eosinophils as well as their degranulation products (major basic protein, MBP).[30] This may be due to a lack of IL-5 responsiveness of a putative subset of resident lung eosinophils.[31] It is important to emphasize that the concept of resident lung eosinophils in humans still awaits confirmation. Alternatively, the low IL-5 responsiveness can by caused by downregulation of the IL-5R alpha after homing of eosinophils from the blood to the lung in segmental allergen-challenged allergic patients.[32] There is growing consensus that IL-5 is important in reactive eosinophilia, while it seems less important for homeostatic eosinophils within tissues. Detailed and comprehensive overview of all the migration and activation factors of eosinophils as well their mediators and receptors are summarized in Gigon et al. (2023).[33]

2.2 | Functions

Traditionally, eosinophils have been described as important cells of the innate immune defence against multicellular parasites, particularly helminths. This is largely based on observations of eosinophilia associated with parasitic diseases and of parasite killing by eosinophils and their toxic granules in vitro.[34] However, the situation may not be as clear cut and may also differ across species, as for instance, in mouse models, eosinophils only showed variable contribution to parasite killing.[35] The location of eosinophils in human and mice are similar, suggesting roles for these cells as identified in mice to have similar functions in humans, but this needs to be established in future studies. Yet, one of the most important roles

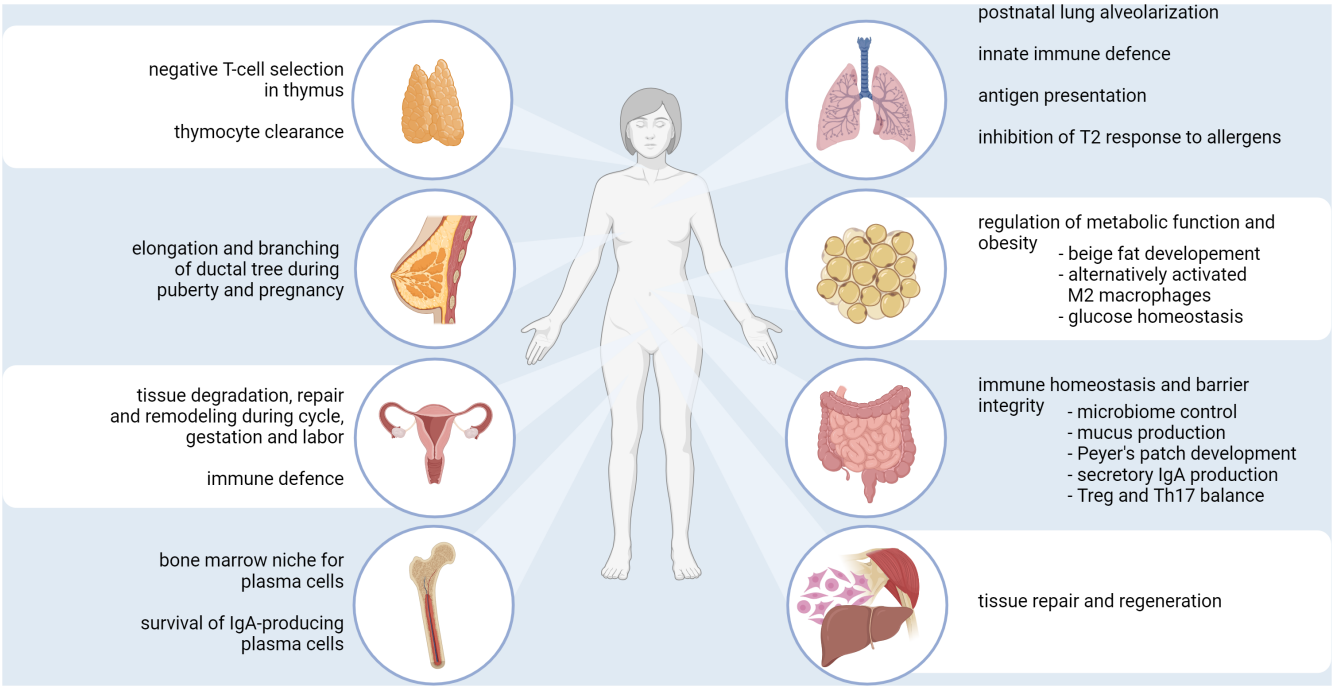

FIGURE 1 Eosinophils in health – overview of known functions and biological effects (adapted and modified according to Rodrigo-Munoz et al. 2021; Klion et al. 2020; Shah et al. 2020).[11,23,24] Created with BioRender.com

of eosinophils is maintaining tissue homeostasis in different organs including the lung, gastrointestinal tract, thymus, adipose tissue and uterus. They support normal function of the immune system (immunotolerance), support fertility and prevent obesity and bronchial hyperreactivity (see Figure 1).[24,36,37] This is mainly based on the fact that these eosinophils are found in multiple healthy human tissues (see for review)[5] and in mouse models of metabolism,[38,39] endometriosis[40] and other tissue functions.[41] The mechanisms underlying these functions will be discussed in more detail below.

2.3 | Surface receptors

Eosinophils express a multitude of receptors which have been extensively detailed in a recent review by Klion et al. (Figure 2).[11] Several receptors are important for the specific therapeutic targeting of eosinophils in diseases. Eosinophils express three receptors with a common β-chain (GM-CSF-, IL-3- and IL-5 receptor) that are all involved in the control of the life cycle of the eosinophil, including survival. In addition, Siglec-8 expressed by eosinophils is also associated with their survival. Several eosinophil receptors are involved in adhesion to the endothelium (e.g. L-selectin, Mac-1/CD11b/CD18, VLA-4/CD49d) and chemotaxis (e.g. CCR3/CD193, C5aR/CD88, platelet-activation receptor).[42] Furthermore, several receptors are associated with the activation of eosinophils (e.g. FcγRII/CD32A, FcαR/CD89, CR3 – CD11b, glucan receptors).[33,43,44,45,46]

2.4 | The concept of priming

Eosinophils are highly cytotoxic because of their intracellular components, which may become a potential risk for host tissues if not properly controlled. A crucial mechanism herein is pre-activation or priming. Under homeostatic conditions, eosinophils are rather refractory cells that may only respond to high concentrations of activators, such as opsonized particles. However, when eosinophils encounter low concentrations of cytokines or other immune mediators in vitro, they can quickly (within 1–15 min depending on the

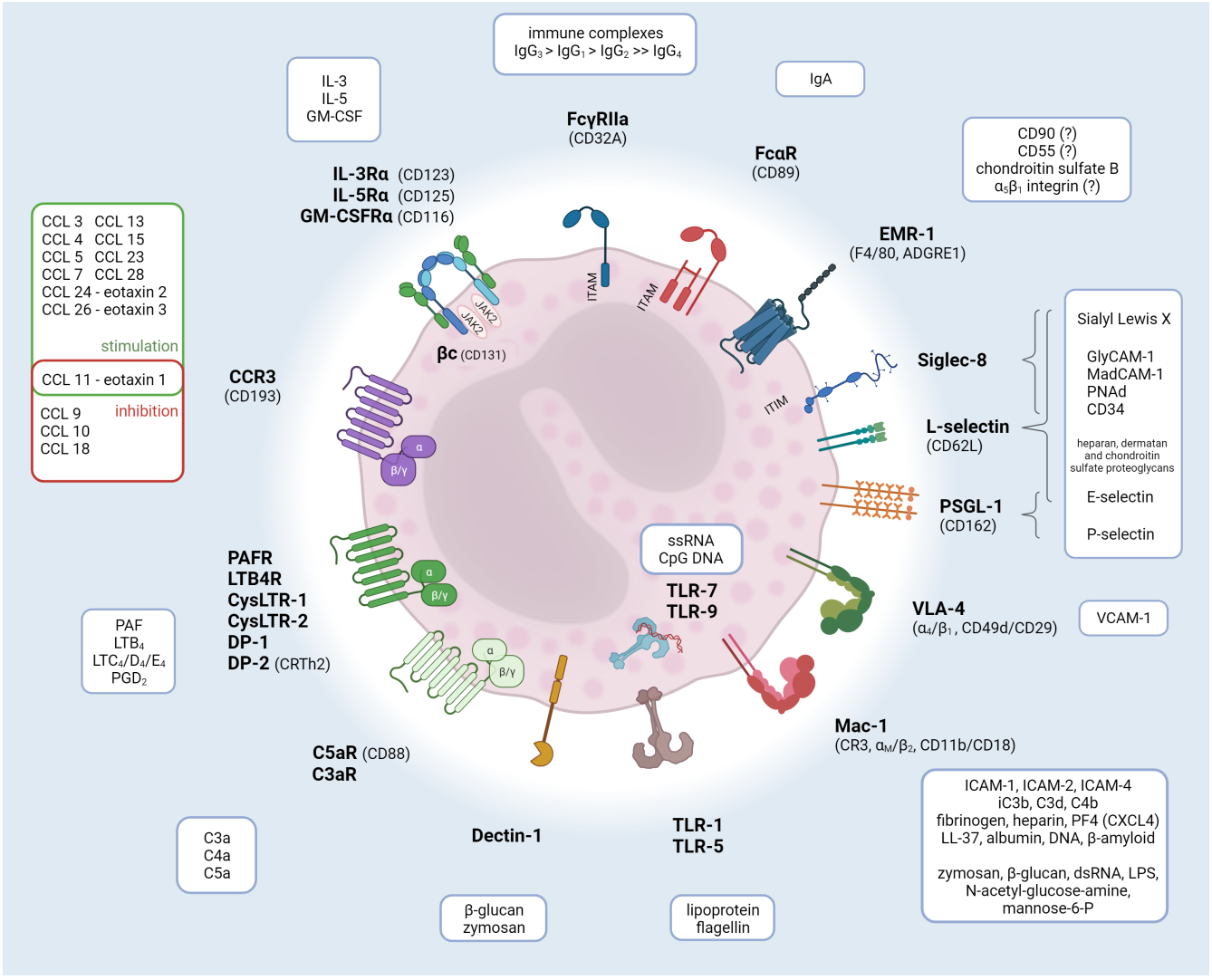

FIGURE 2 Summary of the crucial receptors on surface of the eosinophils and their ligands.[11,33] Created with BioRender.com

priming agent) change to a pre-activated phenotype making them highly responsive to these targets.[25,47] Pre-activation may also occur in patients with eosinophil-mediated disease.[48]

2.5 | Effector mechanisms

Eosinophils possess an arsenal of cytotoxic functions that are particularly exerted extracellularly: that is, within the synapse between the cell and its large (i.e. parasite) targets. These functions include the abundant production of reactive oxygen species (ROS) by a membrane-bound NADPH oxidase (NOX-2),[49] degranulation of highly cytotoxic granular proteins (e.g. major basic protein [MBP], eosinophil peroxidase [EPX] and human-specific eosinophil cationic protein [ECP], or eosinophil-derived neurotoxin [EDN])[50] or peptides (e.g. polycationic peptides) into the synapse and killing of extracellular targets by eosinophil extracellular trap (EET) formation.[51] In addition, eosinophils are a rich source of a multitude of cytokines (e.g. IL-4 and IL-13), chemokines and bio-active lipid mediators (e.g. leukotriene C4 and platelet-activating factor) that are released upon activation.[52-54]

2.6 | Degranulation and EET formation

With eosinophils being relatively inert or refractory while in circulation or in tissues, they must undergo receptor-mediated activation to release their cytotoxic contents and cause tissue damage. Eosinophils are home to a highly unique secretory organelle known as the crystalloid granule, which contains MBP at high concentrations leading to the formation of a crystalline core. Contents of crystalloid granules can only be released from eosinophils through degranulation. Several modes of degranulation occur in eosinophils, most falling under the category of classical exocytosis involving SNARE-mediated membrane fusion (including compound exocytosis and piecemeal degranulation, the latter mostly seen in allergic inflammation).[53,55,56,57,58,59] In addition, free eosinophil granules may be released as intact, membrane-bound organelles by a form of necrotic release known also as cytolysis, which has recently been shown to use molecular components of the necroptotic pathway.[60] Eosinophils also release DNA into the extracellular space during EET formation. The molecular mechanism of this process is still poorly understood.[51,61] EET formation occurs independently of degranulation although granule proteins have been detected on DNA strands.[62] The association of granule products with DNA has been suggested both prior[51,63] and after its release.[64] It has been shown that EETs add to the viscosity of mucus in the nasal exudates of chronic rhinosinusitis (CRS) patients.[65] Moreover, EETs have also been associated in humans with Charcot-Leyden crystals that have been historically associated with eosinophilia.[66] In addition, the eosinophil-derived Charcot-Leyden crystals in mucus in asthma patients play a role in allergic inflammation, goblet cell metaplasia, IgE synthesis, and bronchial hyperreactivity.[67]

2.7 | Eosinophils as part of innate immunity

Apart from being involved in the defence against parasites, eosinophils are also involved in other aspects of immunity. These novel functions are currently emerging, and more research is essential to confirm their relevance in humans in vivo.

- *Anti-viral functions*: eosinophils are capable of inactivating viruses. For years, it was known that granular proteins, such as eosinophil cationic protein (ECP) and eosinophil-derived neurotoxin (EDN), clearly possess RNAse activity that can antagonize viral replication in situ. A recent study implied that this anti-viral effect is lost in patients with allergic asthma.[68] This may explain why viral infections notoriously precede exacerbations of allergic asthma. Eosinophils possess several pathogen-related receptors capable of recognition of viral antigens (e.g. Toll-like receptors 3, 7, 9 and RIG-I receptor), they produce several cytokines with anti-viral effect (e.g. IL-2, IL-12 and IFN-γ), express co-stimulatory molecules (e.g. CD80, CD86, CD28 and CD40) and actively participate on viral antigen presentation to CD8 T lymphocytes.[23,69,70,71] Another interesting area of possible role of eosinophils is the global pandemic of COVID-19 caused by novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Several possible defence mechanisms of eosinophils in COVID-19 were suggested and described, for example direct effect of certain granular proteins and antigen-presenting functions.[69] While eosinopenia was identified as a prospective screening, diagnostic and prognostic tool, the true role of eosinophils in lung pathology pursuant this infection is unclear.[72] Eosinopenia was shown to be an early convenient diagnostic and screening tool for COVID-19 infection[73,74] and as a prognostic marker of disease severity and unfavourable outcome in patients with COVID-19 pneumonia.[75,76] Interestingly, eosinophilia (especially in asthmatic patients treated with inhaled corticosteroids) was associated with improved COVID-19 outcome.[77] However, studies analysing the outcome of COVID-19 in severe asthmatic patients treated with biologics showed inconsistent results.[78,79]
- *Other anti-infectious effects of eosinophils*: The prominent role of eosinophils in parasitic infections has been well-established. These effects include antigen presentation and modulation of T-cell responses. They modulate the production of IgE and mucus production from goblet cells. Moreover, their granular proteins are directly involved in parasites killing and neutralization[35,80] On the contrary, eosinophils can also possess detrimental effect in certain parasitic infections which can contribute to tissue damage.[81] Eosinophils also play a role in the complex defence against selected bacteria. Although their phagocytic activity and bacterial killing is lower compared with neutrophils, they contribute to the clearance of selected bacteria while their granular proteins and enzymes help to neutralize bacterial proteins.[82,83] Formation of eosinophil extracellular traps (stimulated by several mediators, for example thymic stromal lymphopoietin in humans) is an important

phenomenon in bacterial killing.[84] Finally, eosinophils exert also anti-fungal activities. They use their versatile CD11b surface receptor for recognition of β-glucan—a major cell wall component of fungi.[46] Proteases released from fungi activates protease-activated receptors in eosinophils followed by the release of various cytokines. Moreover, eosinophils can probably ingest fungal spores.[85]

- *Modulation of inflammation and fibrosis*: often overlooked are the regulatory or even anti-inflammatory properties of eosinophils. Even in the context of mast cell-induced inflammation, a concept postulated by Austen in 1978, eosinophils can modulate the detrimental effects of mast cell activation, for example by oxidatively deaminating histamine or enzymatically inactivating other mast cell inflammatory mediators.[86] In addition, eosinophils have been found to be able to suppress T cells and hence the name "regulatory eosinophils" was established.[87] In addition, eosinophils play a pathophysiological role in fibrogenesis by the release of TGF-β to stimulate collagen production by parenchymal cells.[88] The role of eosinophils in tissue remodelling has recently been excellently reviewed by Siddiqui and colleagues.[89]
- *Tissue homeostasis*: some years ago, Lee and colleagues put forward the so-called "Local Immunity and/or Remodelling/Repair (LIAR) hypothesis," implying that eosinophils are an integral part of maintaining tissue functions at the sites they reside under homeostatic conditions: for example within the gut,[24] adipose tissue,[38,39] cervix and endometrium.[40,41] Their homeostatic functions depend on or are associated with the function of the tissue where residential eosinophils are found: (human) reproduction in the uterus or placenta, glycaemic control in adipose tissue, gut function in intestines and adipose tissue remodelling.[39,90] Recently, an intriguing new study even implies eosinophils in sustaining physical and immunological fitness during ageing.[91] Unfortunately, the majority of homeostatic functions of eosinophils has been described in murine models. It is, therefore, imperative to study which of these murine data can be translated into the human situation.
- *Other regulatory functions*: Another area of growing interest involves the role of eosinophils in the defence against certain tumours, particularly those of the gut.[92] Although it is too early to define such a role, preliminary evidence in gut tumours showed that tissue eosinophilia is associated with a favourable outcome (*see also the part 5.2*).[93-95]

3 | CLASSIFICATION OF EOSINOPHILIC SYNDROMES

Eosinophilia is associated with a wide range of diseases with a variety of underlying causes which may affect different organs. The diagnostic approach to a wide range of eosinophilic syndromes is facilitated by the well-established division into *primary* and *secondary (reactive) eosinophilic states* (Figure 3)[94] which have been further refined according to updated classifications.[96,97] Recently, new refined diagnostic criteria and classification of primary eosinophil disorders was proposed[98]:

- *Familial (hereditary) hypereosinophilia*—frequently detected in childhood and sometimes associated with immunodeficiencies;
- *Hypereosinophilia of unknown significance*—without familial clustering, underlying pathology, related molecular(genetic) abnormalities or hypereosinophilia-driven organ damage;
- *Secondary (reactive) hypereosinophilia*—non-clonal eosinophilia driven by overproduced cytokines and
- *Primary (clonal, neoplastic) hypereosinophilia*—driven by neoplastic eosinophils.

The classification of secondary eosinophilia is more challenging as many clinical situations are associated with eosinophilia that can be both part of the pathogenesis of the disease or a bystander phenomenon. As the discrimination between the two is often unknown examples are mentioned rather than clear classification criteria. The basic classification of eosinophilia based on the international consensus is provided in Table 1. In the current review, we will focus on eosinophilia mainly in the context of respiratory conditions and related pathologies.

4 | EOSINOPHILS AS A BIOMARKER TO AID DIAGNOSIS AND PREDICT AND/OR MONITOR TREATMENT RESPONSE

4.1 | Sampling techniques for eosinophils and related biomarkers from different compartments

Eosinophils can be detected in several body compartments, which include both fluids and tissues. Across these compartments, the presence of eosinophils may vary within individuals, depending on factors such as age and different sampling techniques reflect the inflammation in defined locations.[103] For the assessment and quantification of eosinophils in eosinophilic pulmonary syndromes, for example certain asthma phenotypes, bronchial biopsies have been traditionally considered the 'gold' standard as they provide information on the inflammatory and structural components of eosinophils and their spatial relationship within the lung tissue. Other endoscopically retrieved lung specimens include bronchoalveolar lavage (BAL) fluid, bronchial wash (BW) and bronchial brushings (BB).[104] These techniques allow qualification and quantification of the cellular components (including gene expression analysis) often combined with soluble fractions. However, their invasiveness and other drawbacks, such as the substantial dilution, negatively affect reproducibility (esp. BAL). Other biases including site selection (biopsies) or the risk of a pneumothorax (esp. BB), have driven the focus toward less invasive sampling methods including sputum analysis, sinonasal samplings, peripheral blood sampling and exhaled air analysis.[7,105] However, within individual patients, blood eosinophils show substantial variability over time,[106] while

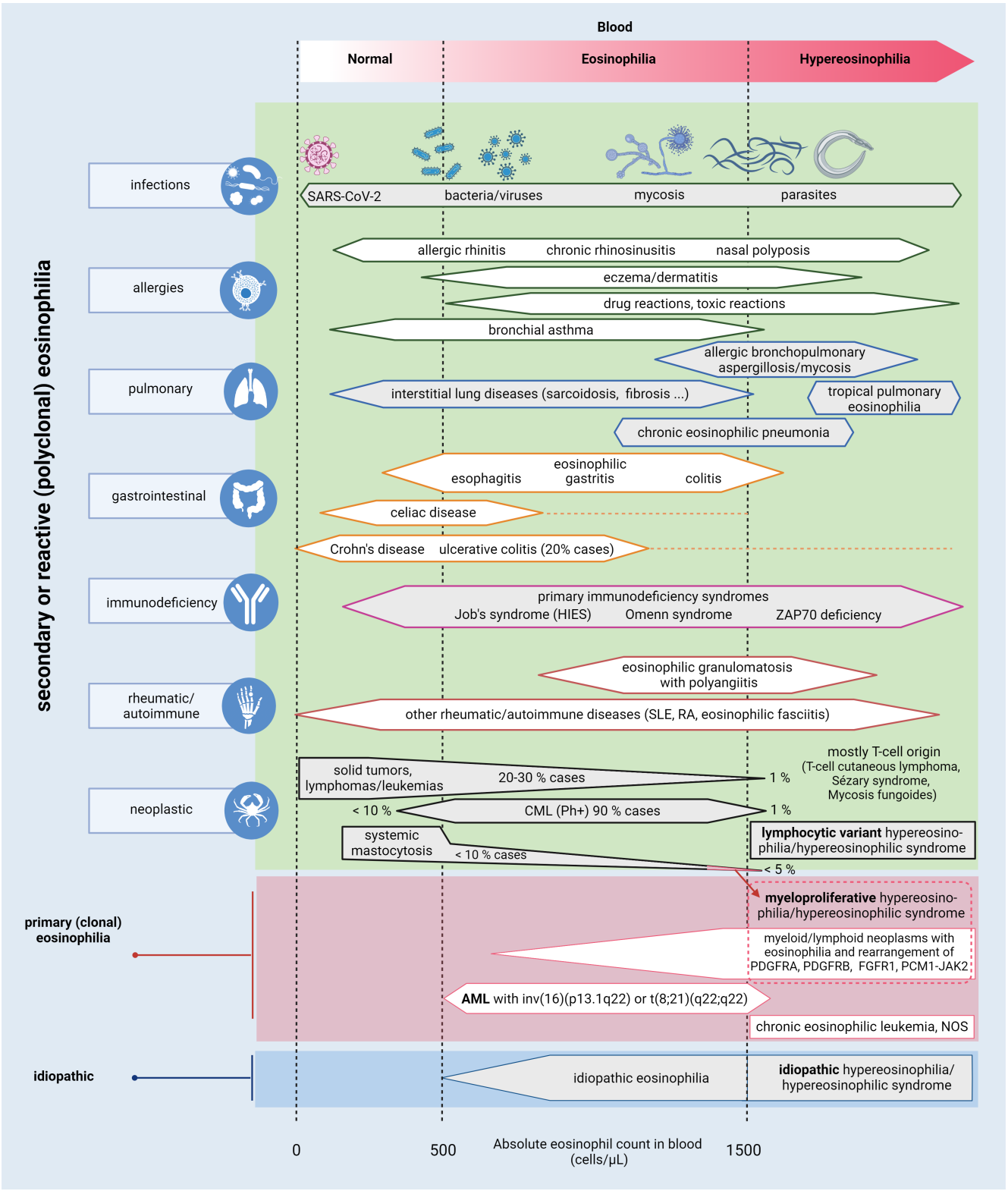

FIGURE 3 Schematic classification of eosinophilia. Created with BioRender.com

eosinophil numbers may vary across different lung specimen[107] as well as across different body compartments.[30] Hence, data from sampling sites should be interpreted cautiously and in the context of treatment.[108,109]

4.1.1 | Blood eosinophils

The determination of eosinophil counts in peripheral blood is fast and inexpensive allowing assessment of the activity of allergic

TABLE 1 Modified schematic classification of eosinophilic syndromes and associated conditions.[96-102]

	Secondary (reactive) eosinophilia[a]	
Primary (clonal) eosinophilia Group	**Group**	**Examples**
Myeloid and lymphoid neoplasms with gene rearrangement of *PDGFRA, PDGFRB or FGFR1 or with PCM1-JAK2, ETV6-JAK or BCR-JAK2*	Allergic disorders	Bronchial asthma, atopic dermatitis, contact dermatitis, chronic allergic rhinosinusitis with/without nasal polyposis, allergic acute and chronic urticaria
Chronic eosinophilic leukaemia not otherwise specified including cases with *ETV6-ABL1*, *ETV6-FLT3* or *BCR-JAK2*	Infectious diseases	Parasitic, bacterial, viral and fungal infections
Atypical chronic myeloid leukaemia with eosinophilia	Dermatoses (non-allergic)	Wells syndrome (eosinophilic cellulitis), pemphigus vulgaris, Gleich syndrome (episodic angioedema with eosinophilia), chronic spontaneous urticaria
Chronic myelomonocytic leukaemia with eosinophilia	Gastrointestinal disorders	Primary gastrointestinal eosinophilic disorders (esophagitis, gastritis, enterocolitis), chronic pancreatitis, inflammatory bowel diseases, coeliac disease
Chronic myeloid leukaemia in accelerated phase or transformation	Vasculitis	Polyarteritis nodosa, eosinophilic granulomatosis with polyangiitis (Churg-Strauss syndrome)
Acute myeloid leukaemia with eosinophilia	Rheumatic diseases	Systemic lupus erythematosus, rheumatoid arthritis, eosinophilic fasciitis (Shulman diseases)
Acute lymphoblastic leukaemia (only if eosinophils demonstrated to be a part of the neoplastic clone)	Respiratory non-allergic diseases	Acute and chronic eosinophilic pneumonia (incl. Löffler syndrome), allergic bronchopulmonary aspergillosis/mycosis, sarcoidosis, eosinophilic bronchitis
Systemic mastocytosis	Neoplastic disorders[e]	Solid tumours, systemic mastocytosis, lymphomas and acute lymphoblastic leukaemia, Langerhans cell histiocytosis
Hereditary (familial) hypereosinophilia	Drug induced eosinophilia	Antibiotics, anticonvulsants, antimalarial, ACE-inhibitors, non-steroidal anti-inflammatory drugs
Idiopathic hypereosinophilic syndrome[b]	Primary immunodeficiencies	Hyper-IgE syndromes, DOCK8 deficiency, Omenn syndrome, Commel-Netherton syndrome
Idiopathic (hyper) eosinophilia[c]	Miscellaneous	Chronic graft-versus-host disease, atheroembolic disease, IgG_4-related diseases
Overlap hypereosinophilic syndrome[d]		

[a]In most cases, eosinophilia may be triggered by eosinopoietic cytokines.

[b]Persisting eosinophilia (≥1.5 × 10^9/L) for at least 6 months associated with tissue damage. If tissue damage is absent, preferred term is idiopathic hypereosinophilia.

[c]Exclusion of the following: reactive eosinophilia, lymphocyte-variant hypereosinophilia, chronic eosinophilic leukaemia not otherwise specified, myeloid malignancies associated with eosinophilia, eosinophilia-associated myeloproliferative neoplasms or acute myeloid/lymphoid leukaemia.

[d]Eosinophilic disease restricted to a single organ system accompanied by peripheral eosinophilia ≥1.5 × 10^9/L.

[e]Disorders in which the eosinophils are not part of neoplastic clone.

TABLE 2 Levels of eosinophils in different body compartments.[18,98,110,111]

	Cut off
Blood	[cells/μL]
Normal in healthy males	<120
Normal in healthy females	<100
Eosinophilic asthma	>150
Induced sputum	[%]
Normal in healthy individuals	<2–3
Cerebrospinal fluid	[cells/μL]
Normal in healthy individuals	<10

diseases, parasitic infections and other eosinophilic disorders. The commonly used 'normal' values for eosinophil counts in blood are less than 0.30–0.45 × 10^9/L (i.e. 300–450 cells/μL) when including atopic subjects; however, ranges may vary among different laboratories (usually between 50 and 500/μL (Table 2).[98,110] Infants and toddlers have physiologically higher upper threshold.[18,111] Moreover, certain studies with biologics defined blood eosinophilia even at a lower threshold, for example ≥150 cells/μL or ≥250 cells/μL. A recent large study in the general population showed that male sex, younger age, current smoking, obesity and the presence of metabolic syndrome were associated with higher blood eosinophil counts; when combined with the diagnosis of asthma, COPD and atopy, these factors were additive.[18] When excluding these factors, blood eosinophil median values were 120 cells/μL in healthy males and 100 cells/μL in healthy females, respectively. Numbers of blood eosinophils are known to be affected by diurnal variations and tend to be higher late at night.[112] Circadian regulation of eosinophils seems to be at least partially controlled by type 2 innate lymphoid cells (ILC2) cells.[113] On the contrary, physical exercise may reduce the numbers of circulating eosinophils.[114] Although blood eosinophil counts do not completely reflect airway eosinophilic inflammation, particularly in children with

severe asthma, in patients with high dose systemic corticosteroids or other immunosuppressants,[109,115] blood eosinophilia may be helpful for identification of eosinophilic asthma phenotype at a threshold of ≥150 cells/μL.[116,117] Published cut-off values predicting clinical response to anti-IL-5 biologics are >150 cells/μL for mepolizumab, >300 cells/μL for benralizumab and >400 cells/μL for reslizumab,[118] respectively. It needs to be emphasized that eosinophil numbers do not tell the whole story as the cells can exhibit different stages of (pre)-activation and/or different phenotypes. Combining eosinophil numbers and their phenotypes might provide better information on the role of eosinophils in disease. This has recently been reviewed.[119,120]

4.1.2 | Sputum analysis

Assessment of airway inflammation is a pivotal part of diagnosis, phenotyping and clinical management of patients with complex airways disease.[121] Changes in sputum cellular indices are reproducible, reliable[122] and responsive to anti-inflammatory treatments.[123-126] Using sputum as a strategy to guide treatment in asthma has been shown to lower the risk ratio of exacerbations and the total number of exacerbations requiring prednisone burst, when compared to just clinical guidelines.[123,127] Since then, sputum measurements have been extended beyond cell counts.[128] Reports from different research laboratories globally have successfully and reproducibly measured a number of fluid-phase mediators, type 2 cytokines, activation markers, gene signatures, miRNA gene network,[129-134] flow cytometric-based cell surface receptor expression and phagocytosis[135,136] and microbiome, that are associated with different disease populations, indices of disease severity as well as treatment effects.[137,138]

Eosinophilia can be detected in pulmonary diseases like asthma, eosinophilic pneumonias and hypersensitivity pneumonitis and idiopathic pulmonary fibrosis by means of induced sputum, or bronchoscopic methods like bronchoalveolar lavage, bronchial and transbronchial biopsies, cryobiopsy and, if needed, also by surgical lung biopsy.[139-143]

Gene expression patterns related to eosinophils have been quantified from sputum of asthma patients and in some studies were well-correlated with blood eosinophils.[144-146] Although spontaneously coughed up sputum can be obtained easily and can provide useful information (esp. in COPD),[147] induced sputum generally yields better quality, higher cell yield and more reproducible samples.[148,149] Presently, there are two widely used standardized sputum protocols with different processing/analysis techniques, that is the entire/whole sample method and the plug selection method. Both methods yield reproducible inflammatory cell counts (eosinophils, neutrophils), the former, however, typically has greater proportions of squamous epithelial cells and lower cell viability placing some limitations on differential cell count interpretation.[150,151] Sputum eosinophilia is usually defined as >2% or ≥3% of inflammatory cell counts[7,124,152] and in patients with asthma (across severities) has been found to be usually well-correlated with baseline blood eosinophils.[117,153]

4.1.3 | Sinonasal sampling techniques

Several studies showed the usefulness of eosinophil measurements in sinonasal samples for evaluating the presence of an allergic or type 2 inflammatory component in rhinitis and chronic rhinosinusitis (CRS).[154-156] To this end, nasal lavage (NAL), nasal secretion sampling with sponges, nasal brushes (NAB), nasal swabs and nasal biopsies (NB) are the most commonly applied techniques with varying ease of sampling, processing and analysis.[105,157,158]

In patients with asthma, nasal eosinophilia has been used as an indicator of eosinophilic asthma[159] and appeared to better predict airway (sputum) eosinophilia than blood eosinophil counts.[160]

4.1.4 | Other tissue samplings

Eosinophilic infiltration in cutaneous tissue obtained by skin biopsy can be found in numerous pathologic conditions, for example atopic dermatitis, eosinophilic cellulitis, granuloma faciale, eosinophilic pustular folliculitis, recurrent cutaneous eosinophilic vasculitis, chronic spontaneous urticaria and other diseases even in the absence of blood eosinophilia.[100,161,162] In atopic dermatitis, intact eosinophils in skin are rare, but significant deposits of eosinophil-derived proteins are indicative of their local activation.[163] Although rarely a major diagnostic criterion, the presence and the number of eosinophils in skin biopsies is often used in the differential diagnosis of drug-induced skin eruptions versus acute graft versus host disease (GvHD), despite some conflicting evidence.[164-166]

Eosinophilia in cerebrospinal fluid (CSF) has been reported (i.e. ≥10 eosinophils/μL or ≥10% of total leukocyte count) in a number of conditions including eosinophilic meningitis—a rare condition caused by helminthic infections,[167] bacterial or fungal meningitis, hypereosinophilic syndrome (HES) and in children with CSF shunts. In the latter condition, CSF eosinophilia appeared a risk factor for shunt malfunction.[168,169]

High eosinophil counts in umbilical cord have been associated with intra-amniotic infections. While in healthy state the foetal white cell pool is relatively small, in severe infections, immature neutrophils and even eosinophils may be recruited to umbilical and chorionic vessels causing umbilical vasculitis.[170]

4.2 | Activation markers and surrogate biomarkers of eosinophilia

Eosinophil cationic protein (ECP) is the most commonly used clinical biomarker for eosinophil activity, and can be quantified in, for example plasma, serum, saliva, BALF, sputum and nasal lavage.[171,172] It

is a useful tool in assessing asthma severity and in monitoring anti-inflammatory asthma therapy.

When using ECP as a biomarker, one should be aware that ECP levels are affected by age, smoking, circadian rhythm and seasonal variation. Serum ECP has been successfully used in guiding anti-inflammatory therapy in childhood asthma.[173] Furthermore, polymorphisms have been identified in genes coding for ECP and some of them have been shown to be associated with asthma[174] and allergic symptoms.[175] Other polymorphisms cause lower ECP levels, and specific genotyping could therefore be recommended in future asthma studies which include ECP measurements.[176]

Measurement of *eosinophil peroxidase (EPX) and eosinophil-derived neurotoxin (EDN)* in either blood or urine may be an alternative to ECP measurements in blood as a reflection of eosinophil turnover and activity.[177,178] In asthma, increased EDN levels have been observed in both blood and urine with further increases in symptomatic patients, while levels were reduced in response to ICS.[179] Furthermore, EDN is a promising candidate particularly in children: serum levels have been shown to correlate with disease severity[180] and urine levels can predict the development of asthma in wheezing children.[181] EPX in sputum, nasal and pharyngeal samples was reported to be a specific marker of eosinophil activity comparable to ECP[182] and associated with asthma severity.[177,183] In addition, some studies imply that both granule proteins are expressed also by neutrophils, although in much lower amount.[182,184]

Although currently not used clinically and requiring flow cytometric measurements, upregulated expression of many cell surface receptors and cell surface integrins on blood, sputum and BAL eosinophils are markers of eosinophil activation as is a decrease in side scatter activity upon eosinophil degranulation (Figure 2).[185]

Validating markers of eosinophilia in relevant biological fluids is essential given the advancement of phenotype/endotype-driven precision medicine. These biomarkers are not only essential for choosing relevant treatment but also for monitoring treatment response.[186]

4.3 | Fractional exhaled nitric oxide and its correlation with eosinophils

Fractional exhaled nitric oxide (FeNO) is a point-of-care biomarker of type 2 inflammation which can be simply and non-invasively measured in exhaled air from both adults and children (>4 years).[187] Given its correlation with blood eosinophils[188] and responsiveness to corticosteroids,[189,190] FeNO has been considered a surrogate marker of eosinophilic airway inflammation for many years.[191] Despite a modest relationship with sputum eosinophils,[116] both biomarkers reflect different, partly overlapping, inflammatory pathways underlying several chronic respiratory disorders including asthma. In line with their different origins, biologics targeting eosinophils (i.e. anti-IL-5 monoclonal antibodies) failed to show a decrease in FeNO levels despite a substantial reduction in blood and/or airway eosinophils.[192,193] The discrepancy between FeNO, sputum and blood eosinophilia was described by many authors.[194,195] This discrepancy could be explained by the differences between allergic and non-allergic eosinophils asthmatic phenotypes and different sources of FeNO in classic allergic and T2-high phenotype. Different sources and the fact that all three biomarkers reflect different underlying mechanisms (or in the case of sputum vs. peripheral blood different locations) is the most important point, especially while, for example anti-IL5 strategies decrease significantly peripheral eosinophils and not FeNO and, for example dupilumab decreases FeNO and not so much peripheral blood eosinophils.[194]

Unsurprisingly, recent analyses showed an overall superior sensitivity and specificity for blood eosinophils compared with FeNO in identifying airway eosinophilia (defined as sputum eosinophils ≥3%).[196] However, for overall asthma management including the prediction of asthma exacerbations, both blood eosinophils and FeNO appeared to have additive prognostic value.[197] Moreover, in patients with severe asthma, FeNO could predict the responsiveness and clinical effect of selected biologics, especially dupilumab.[198]

4.4 | Eosinophilia as readout for immune responses associated with cancer

Eosinophilia has also been observed in some cancers, including breast, ovarian, cervical, prostate, colo-rectal, oral squamous and some haematological cancers (e.g. Hodgkin's lymphoma). The origin and the role of increased eosinophil numbers seem to differ across different cancers and vary from tumour-stimulating to anti-tumour activity.[199] The tumoricidal function of eosinophils is mainly in solid tumours and can be mediated by α-defensins, TNF-α, granzymes A and IL-18,[200,201] while promoting regulatory T cells treatment is primarily directed at the main pathology.[202] Despite these data, it is still unclear whether this cancer-associated eosinophilia is an innocent bystander process or whether eosinophils play a causative role in the pathogenesis of these tumours. It is also possible that at least in part eosinophilia can be caused by the treatment of the tumour rather than the tumour itself.[203] It is, however, important to emphasize that eosinophils might play a positive role in immune therapy at least in the treatment of certain cancers. This topic has been addressed in a recent review by Grisaru-Tal et al.[92]

5 | EOSINOPHILIA AS THERAPY-GUIDING TOOL FOR TARGETED ANTI-EOSINOPHILIC TREATMENTS

The treatment of eosinophil-associated diseases depends on the underlying pathomechanism that is whether eosinophilia is due to (i) a primary or clonal process or (ii) a secondary, reactive one.[100,204] Eosinophil-targeted therapies are aimed to reduce the eosinophil-associated inflammation and consequently, to alleviate clinical signs

and symptoms and to allow tapering off oral corticosteroids. Additionally, clinical trials with eosinophil-targeted treatment helped to provide novel information on the role of eosinophils and mediators either acting on/or produced by eosinophils in human diseases and homeostasis.

5.1 | Treatment of clonal eosinophilic disorders

The most common molecular defect identified in patients with clonal eosinophilic disorders is the *FIP1L1-PDGFRA* gene fusion that results in constitutive, ligand-independent PDGFRA tyrosine kinase activity.[205] For patients with PDGFR-associated disease, the tyrosine kinase inhibitor (TKI) *imatinib* is first-line therapy and produces rapid and dramatic clinical and haematological responses with molecular remission (no detectable FIP1L1-PDGFRA) typically observed within 2–3 months of treatment.[206-208] In order to overcome imatinib resistance, second- and third-generation TKIs have been developed. Of those, *midostaurin* and *ponatinib* proved to be effective against D816V, the most common *KIT* mutation in patients with systemic mastocytosis who may also present with eosinophilia.[209] Additional therapeutic strategies have been outlined by Radonjic-Hoesli et al. (2015).[204]

5.2 | Treatment of reactive eosinophilic disorders

The therapeutic approaches for reactive eosinophil disorders (e.g. eosinophilic asthma, rhinosinusitis) are either to directly target eosinophils or to inhibit cells and mediators stimulating eosinophilia and eosinophil activation. So far, corticosteroids (CS) via topical, inhalant or systemic route have widely been used as first-line therapy and may control eosinophilic inflammation in many cases. CS exert direct effects on eosinophils, for example by inducing eosinophil apoptosis or indirect ones by affecting inflammatory and tissue cells interacting with eosinophils resulting in a decreased production, recruitment and activation of eosinophils. Long-term use of especially systemic steroids causes harmful side effects.[210,211] This underscores the benefits of eosinophil-targeted therapies in these conditions.[212-214]

5.2.1 | Direct anti-eosinophil-targeted therapies

The past two decades have witnessed a tremendous boost in the development of anti-cytokine and anti-cytokine receptor monoclonal antibody therapies for the treatment and management of eosinophilic diseases.

Anti-IL-5 monoclonal antibodies—mepolizumab, reslizumab

The mounting popularity has remained with targeting the IL-5 pathway given its prime role in orchestrating eosinophil biology from maturation to mobilisation to degranulation.[61,215] Anti-IL-5 monoclonal antibody therapy with *mepolizumab* or *reslizumab* resulted in a significant improvement of clinical signs and symptoms in the eosinophilic subtype of asthma,[216-218] chronic rhinosinusitis with nasal polyps (CRSwNP)[219] and hypereosinophilic syndrome,[220-223] whereas trials in atopic dermatitis,[224] eosinophilic esophagitis[225] and bullous pemphigoid[226] revealed missing or moderate effects.

Effects reported on anti-IL-5 therapy with mepolizumab or reslizumab in patients with severe eosinophilic asthma, consist of reduced numbers of exacerbations, improved severity and quality of life scores, decreased numbers of blood and sputum eosinophils, systemic corticosteroid sparing effects and improvement in lung function.[217,218,227,228] In initial studies, mepolizumab failed to significantly improve clinical features of asthma (allergen-induced late response, airway hyperresponsiveness, FEV_1 and peak flow recordings) as patients had not been selected for eosinophilic asthma.[29,229] Following a paradigm shift, Nair et al. (2009)[218] and several other investigators confirmed clinical efficacy in patients with eosinophilic asthma.[229] Mepolizumab is currently indicated as add-on therapy for adults and children (age ≥6 yrs.) with severe uncontrolled eosinophilic asthma, in two dosing regimens.[230] It should be pointed out that the children with severe asthma have been underreported in clinical trials with biologics (e.g. 1%–6% of the study populations with mepolizumab) and available efficacy and safety data for the paediatric population are scarce.[231] In patients with CRSwNP, another chronic type 2 respiratory condition often coinciding with severe asthma, increased IL-5 levels in nasal secretions at baseline predicted clinical response to anti-IL-5 treatment with reslizumab.[232] However, clinical efficacy of anti-IL-5 targeting treatment has so far only been established for mepolizumab[233,234] in large number of patients with recurrent refractory CRSwNP with or without concomitant asthma, and consequently, this biologic has been implemented into concurrent treatment algorithms.[235-238] Clinical efficacy in the treatment of CRSwNP was also confirmed for other biologics: that is omalizumab[239] and dupilumab.[237,240,241]

The absent or moderate clinical efficacy of anti-IL-5 therapy in other eosinophil-associated diseases (e.g. atopic dermatitis, eosinophilic esophagitis, bullous pemphigoid) appeared to be related to the incomplete reduction in eosinophil numbers within the target tissues.[30,225,226] In line with the initial studies in asthma,[29,30] a phenotype selection toward a more eosinophil-driven disease might be required to reach clinically relevant effects using these targeted treatment modalities. Independently of efficacy, in all clinical studies a significant reduction and even full depletion of blood eosinophils has been observed.[29,217,226] In addition to blood eosinophils, mepolizumab decreased the numbers of mature eosinophils within the bone marrow by 70%, as well as myelocytes and metamyelocytes by 37% and 44%, respectively, without affecting the numbers of blood and bone marrow $CD34^+$, $CD34^+$/IL-5R alpha$^+$ cells (progenitors of eosinophils) and/or eosinophil/basophil colony-forming units.[242] To note, mepolizumab did not alter the physiological infiltration of eosinophils in the duodenal mucosa of patients with eosinophilic esophagitis.[28] Of similar interest, despite decreasing eosinophil (cells expressing IL-5Rα) numbers following a segmental allergen challenge in allergic asthmatics, mepolizumab (750 mg subcutaneously) had only a limited effect on airway activation markers.[243] In line with these

observations, mepolizumab at the currently recommended dose (100 mg subcutaneously q4wk) does not completely abolish sputum eosinophils or any other cellular source of type 2 cytokines such as the innate lymphoid cells type 2 (ILC2) despite a significant reduction in blood eosinophils.[244] Recent evidence from real-life studies suggests similar findings where both mepolizumab and reslizumab can normalize blood eosinophil levels, and yet 43% of patients respond suboptimally.[245] Approximately 78% of these suboptimal responders show sputum eosinophilia despite (at least) 4 months of therapy.[108] In the MEX study, asthma exacerbations while on mepolizumab were eosinophilic in nature, as evident by sputum eosinophilia >2% and high FENO > 50 ppb. Such persisting eosinophilia may well account for the lack of disease-modifying effects of (subcutaneous) anti-IL5 strategy.[246]

Other studies, however, showed, that sputum eosinophil count may not represent a more useful biomarker than blood eosinophils for predicting treatment response to mepolizumab.[247] Of note, recent data showed that IL-5 may influence airway epithelium cells with negative impact on barrier function and immune capability.[248] Interestingly, in patients with eosinophilic asthma and nasal polyposis with AERD, mepolizumab 100 mg s.c. was able to induce epithelial tight junction-related genes. Biological treatment effects not exclusively due to anti-eosinophil activity may thus be contributing to mechanisms of treatment response in asthma.[249] As disease modifying asthma therapies should target fundamental pathobiological mechanisms involved in asthma,[250] for example immune epithelial barrier disruption,[251] it remains to be seen how targeting of eosinophils—important players in Th2 immunity—truly contributes to achieve disease modification (keeping in mind the LIAR hypothesis and emerging concept of different eosinophilic subsets, see below).

According to published data on anti-IL-5 strategies so far, there does not seem to be an increased risk of neoplasms, infections and/or autoimmunity in humans.[252-258] In long-term studies, both mepolizumab and reslizumab showed a positive benefit–risk profile without evidence for specific adverse event patterns in neither paediatric nor adult patients.[253,254,255,259] Respiratory tract infection, headache and bronchitis were the most frequently reported adverse events based on an open-label long-term extension safety study in patients with severe eosinophilic asthma (COLUMBA).[260] Whereas this was an open-label study, it could not be determined if the respiratory tract infections were increased due to treatment or typical of the disease. Anti-drug antibody (ADA) responses, which mainly were transient in adults, but no neutralizing antibodies have been observed in adults or children.[259,260] Nevertheless, the immune system of children is still under development and long-term effects of IL-5 inhibition remain unclear, this warrants further investigation and long-term monitoring.

Anti-IL5R monoclonal antibody—benralizumab

Benralizumab exerts dual function by interfering with IL-5 binding to the IL-5 receptor alpha chain and promoting antibody-dependent cell-mediated cytotoxicity (ADCC) with consequent enhanced eosinophil apoptosis.[261] In adult patients with severe, uncontrolled eosinophilic asthma, benralizumab as add-on therapy decreased the annual exacerbation rates, improved lung function and asthma symptom scores, as well as reduced oral CS use.[199,262,263] However, in patients with mild to moderate, persistent asthma, no clear relationship between blood eosinophil counts and FEV_1 was observed following benralizumab therapy.[264] Benralizumab was reported to significantly reduce both mature eosinophils and eosinophil progenitor cell numbers in peripheral blood, airway mucosa/submucosa, sputum and bone marrow (as well as peripheral blood basophils) in patients with (severe) eosinophilic and/or corticosteroid-dependent asthma.[265-267] Based on two smaller early phase studies in asthma, in parallel with reduced blood eosinophil numbers, serum eosinophil-derived neurotoxin (EDN) and eosinophil cationic protein (ECP) levels decreased upon benralizumab.[268] Interestingly, while no changes in TNF-α or IFN-γ levels were observed, serum IL-5, eotaxin/CCL11 and eotaxin-2/CCL24 levels increased after benralizumab administration.[268] Despite an overall similar reduction in peripheral eosinophils across participating patients with OCS-dependent, severe eosinophilic asthma, in a phase III clinical trial with add-on benralizumab, 20% of patients were unable to reduce their corticosteroid dose without losing asthma control.[263] In a real-life setting, suboptimal response to benralizumab was observed in 27% out of 74 severe asthmatics who were clinically prescribed this biologic. The majority of exacerbations were non-eosinophilic, associated with airway infections and reduced NK cells.[269] Of note, add-on benralizumab compared with placebo failed to significantly lower the annualized rate of COPD exacerbations in two large studies (GALATHEA and TERRANOVA) in patients with moderate to severe COPD with blood eosinophilia,[270] while only a subgroup of responders could be characterized by pooled data analysis.[271] In this context, it should be noted that the role of eosinophils may differ between COPD and asthma.[272]

In patients with *PDGFRA*-negative hypereosinophilic syndrome (HES), benralizumab treatment resulted in a significant clinical improvement with suppression of bone marrow and tissue eosinophilia with the possibility of withdrawal of background therapies.[221]

Long-term observation of patients on benralizumab treatment revealed no differences in the rate and pattern of adverse events and in particular severe adverse events associated with infections as compared to the placebo groups.[273] However, recent analysis of the exacerbations in patients treated with benralizumab showed that a sub-optimal response (SR) to therapy was associated with the presence of various respiratory infections (e.g. by *Moraxella catarrhalis*, *Streptococcus pneumoniae*, *Staphylococcus epidermidis*, *Haemophilus influenzae*, *Pseudomonas aeruginosa* and metapneumovirus). Patients treated with mepolizumab or reslizumab showed lower frequency of infections compared with benralizumab-treated patients. Sub-analysis of the patients with zero eosinophils in sputum during the benralizumab treatment showed still higher incidence of respiratory infections[82,273] Moreover, the use of the IL-4 receptor-α blocking antibody dupilumab was associated with less respiratory infections (and hence less use of anti-infective medication) in patients with moderate-to-severe asthma or severe CRSwNP.[274] Therefore, it is key to consider

all potential consequences of manipulating the pool of eosinophils, which likely contains inflammatory, regulatory (homeostatic) and residential cells with distinct functions and activities. Whether dramatic depletion of eosinophils would impose long-term consequences on the organism in terms of side effects (especially infections) should be followed up and monitored (*see also chapter 5.3*).

5.2.2 | Other biologics affecting eosinophilic diseases

The first biologic used to treat allergic diseases (including asthma) was anti-IgE (see below). Later evidence from among others the U-BIOPRED (Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes) program applying sputum transcriptomics strongly suggests that in addition to IL-5 (and IgE), other cytokines such IL-33, thymic stromal lymphopoietin (TSLP) and IL-13 may be mediators of/contributors to eosinophilia.[275] In addition, there are additional markers associated with eosinophilic conditions because of certain pathomechanisms which may directly or indirectly contribute to the development of eosinophilia and associated diseases.

Anti-IgE monoclonal antibody—omalizumab

Omalizumab forms complexes with free IgE blocking its binding to receptors on mast cells and basophils. Since eosinophils do not express functional high-affinity IgE receptor (FcεRI), omalizumab seems to exhibit indirect effects in eosinophilic diseases. Omalizumab has been proven effective in allergic/IgE-mediated diseases including asthma as it reduces the frequency of exacerbations and decreases the use of CS in paediatric and adult patients.[276-278] Approximately 60% of asthmatic patients respond to treatment.[279] In addition to a reduction in serum IgE and IgE^+ cells within the airway mucosa, a decrease of overall type 2 inflammation including eosinophils, $CD3^+$, $CD4^+$ and $CD8^+$ T lymphocytes; B lymphocytes, cells positive for the high-affinity Fc receptor for IgE in the airway mucosa has been reported following omalizumab treatment.[280] An additional treatment response has been observed on the IgE^+ antigen-presenting cells, that is monocytes, plasmacytoid DCs, limiting the facilitated antigen presentation and activation of T cells.[281] In allergic and non-allergic patients with CRSwNP and comorbid asthma, anti-IgE therapy decreased the size of nasal polyps and yielded beneficial effects on airway symptoms (nasal congestion, anterior rhinorrhoea, loss of sense of smell, wheezing and dyspnoea).[238] A comprehensive review of 25 RCTs reports that patients with severe uncontrolled allergic asthma with high blood eosinophil counts and high FeNO, indicative of ongoing type 2/eosinophilic airway inflammation had greater reduction in asthma exacerbations upon treatment with omalizumab.[282] Therefore, prescription of omalizumab to allergic (atopic) and clearly eosinophilic asthmatics seems justified when targeted eosinophil-depleting treatment options are unavailable. However, omalizumab was unable to curb airway eosinophilia in more severe asthma, irrespective of blood eosinophil counts or atopy status.[283] Indeed, severe asthmatics who exacerbated on omalizumab, when switched to mepolizumab (targeting IL5) showed significant clinical improvement.[284] When applied in eosinophilic esophagitis or atopic dermatitis, omalizumab failed to improve the clinical course despite depletion of IgE.[285-287] Omalizumab was shown to moderately reduce tissue eosinophils in the duodenum and gastric antrum but not in oesophagus, while FcεRI expression on basophil and dendritic cell as well as free IgE levels were all significantly decreased in patients with eosinophilic gastrointestinal diseases.[288] In patients at risk for geohelminth infections, omalizumab therapy was not associated with increased morbidity attributable to intestinal helminths.[289] Overall, omalizumab has a favourable safety profile.[290]

More recently, another high-affinity monoclonal anti-IgE antibody, *ligelizumab*, has been developed to overcome some of the limitations of omalizumab. Although ligelizumab showed superiority in inhibition of IgE binding to FcεRI, basophil activation and IgE production by B lymphocytes, it was less potent than omalizumab in inhibiting the interaction of IgE with CD23. However, its effect on eosinophilia was not studied.[291] In a phase II trial, ligelizumab failed to demonstrate superiority on the Asthma Control Questionnaire 7 (ACQ-7) over placebo or omalizumab in severe asthmatics,[292] but showed potentially promising results in the treatment of chronic spontaneous urticaria (CSU) in another clinical study.[293] However, its final position in the management of CSU in relation to omalizumab needs to be established.

Anti-IL-4/IL-13Rα monoclonal antibody—dupilumab

Dupilumab blocks the shared IL-4/IL-13 receptor α-chain and thus the activity of IL-13 and IL-4 resulting in an inhibition of type 2 inflammatory responses. Dupilumab was shown to significantly improve clinical outcomes in several type 2 diseases including moderate-to-severe eosinophilic asthma,[294,295] atopic dermatitis,[296,297] CRSwNP,[239,298,299] perennial allergic rhinitis with comorbid asthma[300] and eosinophilic esophagitis (EoE).[301]

Although dupilumab is effective in controlling type 2/eosinophilic diseases, transient blood eosinophilia has been reported with dupilumab treatment.[295,299] This phenomenon can be explained by the reduced expression of IL-4/IL-13-induced VCAM-1 on endothelial cells restricting eosinophil adhesion and tissue extravasation,[302] as well as by the inhibition of the direct effects of IL-4 on eosinophils reducing their chemotactic response.[303] Moreover, reduction of the chemotactic agent eotaxin-3, VCAM-1 and thymus and activation-regulated cytokine (TARC) after dupilumab without simultaneous inhibition of eosinophilopoiesis in bone marrow might also reduce eosinophil extravasation.[304] Additional mechanisms potentially underlying dupilumab-induced eosinophilia have been recently described in a review by Olaguibel et al.[305] Blood eosinophilia has been reported in 4.1% of asthma patients on dupilumab treatment which in 4 out of 52 patients was associated with worsening of blood eosinophilia and the development of chronic eosinophilic pneumonia[304] as well as eosinophilic pleural effusions and a cardiovascular accident associated with atrial fibrillation and (cutaneous) vasculitis in two respective case reports of uncontrolled asthma.[306] Another study reported dupilumab-associated eosinophilia in <1% of AD patients which was

mainly transient.[307,308] In the pathogenesis of dupilumab-associated conjunctivitis, reported in 8.6%–22.1% of atopic dermatitis patients, a prominent eosinophil influx was demonstrated in the conjunctiva.[309] In a recent analysis of 11 dupilumab clinical studies, transient eosinophilia was reported in 0–13.6% of the treated patients with various diagnosis, it did not affect the efficacy of the treatment and was rarely of clinical consequence. Clinical symptoms of associated with eosinophilia were rare (7 patients in 4666 dupilumab-treated patients) and occurred only in patients with asthma or CRSwNP.[310] Treating physicians should be aware of this side phenomenon and the patient should be closely monitor regarding the potential eosinophil-related morbidity.[311] Current update of GINA 2023 suggests not to use of dupilumab in patients with current or historic blood eosinophilia >1500 cells/μL.[312]

Anti-TSLP monoclonal antibody—tezepelumab

Tezepelumab was studied in atopic dermatitis[313] and asthma[314] showing significant reduction of atopic dermatitis severity scores and asthma exacerbation rates, respectively, compared to placebo irrespective of baseline blood eosinophil counts or total IgE levels in asthma patients. In the phase II randomized double blind (CASCADE) study, blocking TSLP (tezepelumab) reduced airway submucosal inflammatory cells (eosinophils, neutrophils, T cells and mast cells) retrieved from bronchial biopsies.[315] In the phase III (NAVIGATOR) trial, tezepelumab reduced asthma exacerbation rates, improved asthma control and lung function especially in patients with eosinophils ≥300 cells/μL. Furthermore, a significant decline in annual asthma exacerbations was also observed in patients with eosinophils <300 cells/μL.[316] However, in another phase III (SOURCE) asthma trial, tezepelumab failed to allow a significant OCS dose reduction versus placebo, while an improvement was observed in patients with higher baseline eosinophil numbers (≥150 cells/μL).[295] Tezepelumab is now registered both in the United States and in Europe.

Novel targeted therapies under investigation

Eosinophils express various surface molecules and receptors, for example CD52, receptors for TSLP, IL-33, prostaglandin D2 (DP2 or previously CRTh2) and Siglec-8, while also releasing cytokines which may serve as drug targets in eosinophilic diseases.

Alemtuzumab is a monoclonal antibody targeting CD52 currently registered for the treatment of relapsing–remitting multiple sclerosis and certain type of leukaemia. CD52 is expressed amongst other cells also on eosinophils and the treatment with alemtuzumab led to complete haematological response in 10/11 patients with idiopathic hypereosinophilic syndrome (I-HES) and chronic eosinophilic leukaemia-not otherwise specified (CEL-NOS).[317] Repeated bone marrow analysis showed a normalized eosinophil percentage (complete remission) in 3, and more than 50% reduction in eosinophil percentage (partial remission) in another 3 out of 8 patients. However, adverse events were common and related to infusion reactions and lymphopenia-related viral infections.[318]

Targeting DP2 (CRTh2), the prostaglandin D2 receptor, by several DP2 antagonists including setipiprant, fevipiprant and timapiprant, showed some protection against the allergen-induced late response[319,320] and significant reduced sputum eosinophils along with improvements in lung function in patients with (allergic) eosinophilic asthma,[321,322] as well as improved nasal and ocular symptoms in allergic subjects exposed to grass pollen[323] and decreased the oesophageal eosinophil load associated with reduced disease activity in patients with eosinophilic esophagitis.[324] Two phase III trials of fevipiprant (LUSTER-1 and LUSTER-2) only showed very modest effects on exacerbations in patients with severe asthma, thus leading to discontinuation of further development of the drug for this indication.[325]

Another potential therapeutic target is Siglec-8, expressed on eosinophils. Chimeric antibodies directed against Siglec-8 were shown to reduce IL-5-induced eosinophilia in healthy and eosinophilic donors.[326] A single dose of *AK002* (*lirentelimab*), an anti-Siglec-8 antibody, led to a complete depletion of blood eosinophils in healthy individuals already 1-h post-dosing and persisted up to 84 days. However, the ENIGMA-2 phase 3 trial in patients with eosinophilic gastrointestinal disease missed the symptomatic co-primary endpoint (press release by manufacturer).[327] However, as the long-term consequences of complete depletion of eosinophils are unclear, further studies are needed.[328]

Dexpramipexole, a synthetic aminobenzothiazole, is an orally bioavailable small molecule originally developed for treating amyotrophic lateral sclerosis (ALS), which was coincidentally shown to reduce eosinophils both in peripheral blood and in target tissues. Therefore, it has been subsequently tested in eosinophilic diseases such as hyper-eosinophilic syndrome[329] and CRSwNP with blood eosinophilia.[330] In CRSwNP patients, dexpramipexole (for 6 months) had a favourable safety profile and effectively reduced eosinophils both in peripheral blood and in NP-tissue in the majority of patients but failed to reduce the nasal polyps' size and to improve upper respiratory symptom scores.[330] A recent safety and efficacy study ('EXHALE') clearly showed a marked reduction of peripheral eosinophils in eosinophilic asthma patients.[158] Interestingly, the study showed a favourable effect on lung function albeit being underpowered for this endpoint.

A proof-of concept study investigating an *anti-IL-33 antibody* (etokimab) in atopic dermatitis, reported a marked improvement of disease severity associated with a significant decrease of blood eosinophils upon a single administration.[331]

IL-13 is a key cytokine in type 2 diseases, and eosinophils were shown to express the IL-13 receptor and release functional IL-13.[61,332] Several antibodies blocking IL-13 have been/are under clinical investigation for asthma—for example *tralokinumab* or *lebrikizumab*[333,334] and atopic dermatitis.[335] Stratification of asthma patients revealed best effects on lung function in those with more pronounced type 2 profile/blood eosinophilia.[304] But overall, neutralizing IL-13 alone seems to have limited effects on eosinophilic airway inflammation and clinical outcomes in asthma while the eosinophil-lowering

effects are most likely indirect.[336] In contrast, more promising results were shown in atopic dermatitis.[337]

5.3 | To completely block or not completely block eosinophils—*that is the question*

Presently, an expanding armamentarium of new drugs targeting the eosinophils have been introduced into drug development while some of them have entered clinical practice. These targeted drugs range from relatively specific for inflammatory eosinophils (Mepolizumab/anti-IL-5) to targeting all IL-5R positive cells including (at least to a certain degree) resident eosinophils (Benralizumab/anti-IL-5R). Associated with their application in chronic diseases, it is essential to understand the 'cost' of losing resident (i.e. homeostatic) eosinophils from healthy tissues during long-term treatment of type 2/eosinophilic inflammation (Figure 4).[108,256,257,258,338]

Historically, eosinophils have been associated with helminthic infections and allergic diseases. As mentioned before, recent evidence revealed their important involvement in innate immune responses displaying regulatory/dampening effects.[5,86] The Local Immunity and/or Remodelling/Repair (LIAR) hypothesis suggests that resident tissue eosinophils secure local homeostasis, prevent remodelling and promote tissue repair.[41] This was supported by a mouse model showing the presence of homeostatic resident eosinophils.[31] So far, the anti-IL-5 trials and anti-IL-5R trials recorded limited adverse reactions to eosinophil depletion, but studies were mainly focussed on adult patients. Even the longitudinal follow-up studies showed that all the drugs were well-tolerated, and no adverse effects of eosinophil depletion were reported.[344] However, with benralizumab, it has come to notice that there is increased incidence of recorded respiratory infections which are not apparent with anti-IL-5 neutralising mAb therapies (mepolizumab and reslizumab). Moreover, the increase in infections may not be only ascribed to depleted eosinophils, but may also be the effect of the depletion of other IL-5R$^+$ cells, such as basophils, involved in host defence.[82,269] Despite the fact that both anti-IL5 and anti-IL5R showed favourable safety profiles with overall similar adverse events (in kind and number), future studies should provide further insight how the potential gatekeeper role of eosinophils based on their innate immunity involvement as well as their role in tissue homeostasis will be affected by long-term deep depletion.

5.4 | Off-target effects: lessons to be learned?

A worrying finding is that treatment with different immunomodulatory drugs can induce rare unforeseen side effects depending on the (immune) status of the patient. This rather cryptic issue is best illustrated by the effect of anti-IL-5 treatment in patients who suffer from rheumatoid arthritis combined with asthma. Andreev et al. have recently described an immune suppressive function of eosinophils in joint tissue. In a mouse model of serum-induced arthritis the authors describe compelling evidence that eosinophils are involved in dampening the inflammatory response in arthritis lesions.[345] Importantly, the authors also describe a flare-up in the joints of RA patients who were treated with anti-IL-5 therapy. This finding is

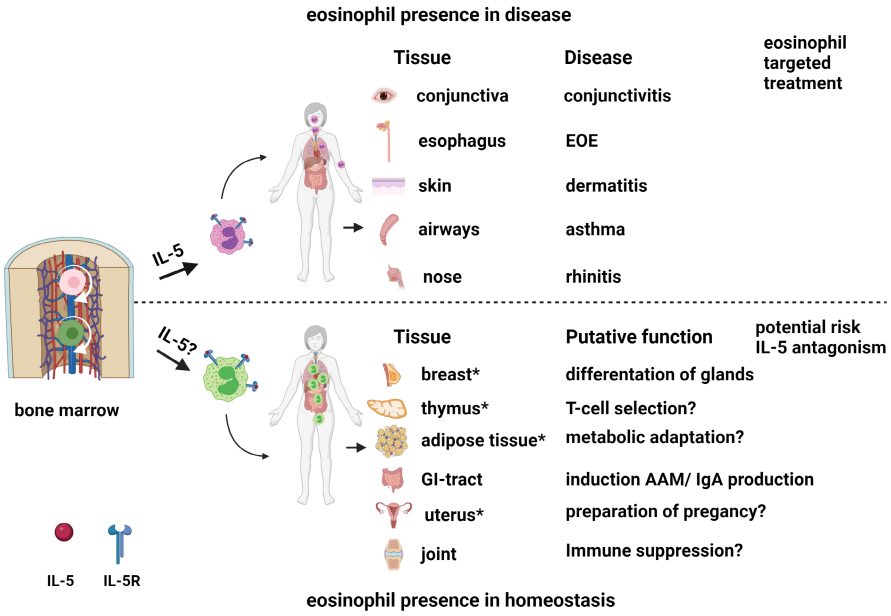

FIGURE 4 Balancing the potency of eosinophil inhibition in disease and tissue homeostasis. Inhibition of eosinophils, mostly via blocking IL-5 and/or its receptor has beneficial in many, predominantly allergic diseases. This IL-5 is produced by several cell types including Th2 cells[339], type 2 innate lymphoid cells[340], bone marrow stromal cells[341], mast cells[342] and even eosinophils.[343] The recent appreciation of the potential role of eosinophils in tissue homeostasis, outlined in the LIAR hypothesis, indicate a potential risk of the antagonism of resident non-inflammatory eosinophils. These cells are responsive to IL-5, but their differentiation in the bone marrow seems independent of this cytokine. *only implicated in the mouse. Created with BioRender.com

TABLE 3 Possible effect of anti-eosinophil biologics on eosinophil counts in different specimen.

Molecule	Mepolizumab	Reslizumab	Benralizumab	Dupilumab	Omalizumab	Tezepelumab
References	[218,219,222,225,228,229,242,243,247]	[227,254]	[192,261,262,265,268]	[241,265,296,299,301,307]	[239,276,279,283,287]	[315,348,349,350,351]
Target	IL-5	IL-5	IL5R	IL4/13Rα	IgE	TSLP
Inhibition of eosinophils	Direct	Direct	Direct	Indirect	Indirect	Direct
Eosinophils in						
Peripheral blood	↓↓	↓↓	↓↓↓	↓ (↑[a])	↓	↓↓
Airway/lung tissue	±	↓	↓↓↓	↓	±	↓↓
Sputum	↓	↓	↓↓	↓	↓	↓
BALF	↓	↓	↓↓	?	↓	↓↓
Oesophagus	↓	↓	↓[b]	↓↓	ø	?
Duodenum	ø	?	↓↓	?	↓	?
Bone marrow	↓	↓	↓↓	ø	?	?
Residential lung eosinophils	ø	ø	Possible ↓	ø	ø	?

[a]Transient increase followed by normalizing/decline in the majority of the subjects.

[b]Under investigation, available case reports.

supportive of the hypothesis that targeting type 2 inflammation can lead to exacerbation of T_H1/T_H17 inflammation. In addition, to off-target effects already described in this review, some more have been described in the context of targeting type 2 inflammation. These include alopecia, eosinophilic conjunctivitis and decreased numbers of goblet cells in the conjunctiva in some patients treated with dupilumab.[346,347] Taking all these effects into account, it is clear that this might not only been seen as off-target, but in many instances are unknown or unforeseen effects. Rare side-effects associated with type 2/eosinophils targeted therapy might still be under the radar. Therefore, it is important to identify as many of these apparent off-target/unforeseen effects as possible as they might help us understand the pathogenetic mechanisms underlying type 2 diseases as well as the molecular mechanisms mediating the different targeted type 2/eosinophils treatments.

6 | CONCLUSIONS AND A LOOK INTO THE FUTURE

It is evident, that the traditional concept of understanding eosinophils has recently changed in the context of newly unveiled cellular functions. Besides well-characterized pro-inflammatory and disease-driving effects of eosinophils, these cells evidently also possess homeostatic, anti-inflammatory and anti-infectious activities. Therefore, these features need to be considered during the process of selecting therapies that affect eosinophils to various degrees: that is from reduction to complete depletion (Table 3). As per the ongoing discussion, it is evident that there has been massive advancement in eosinophil-targeted therapies. All licenced targeted therapies to date have shown a positive treatment effect and improved the disease burden in patients with eosinophil-driven conditions. However, from the perspective of precision medicine, a significant disease burden remains, as evident from the modest reduction in exacerbation rates in most reported studies across different eosinophilic diseases. There are several studies that highlight predictors of good clinical responses to biologics, but few of them focus on those patients who fail to respond adequately despite targeted treatment. This could be due to the involvement of multiple pathways that are activated at the same time in the most severe patients. Phenotyping patients based on blood eosinophils may not be accurate enough for endotypic targeting. For example, in asthma, using blood eosinophils as a (or the only) biomarker often proved inadequate for choosing the right drug for the right patient or for efficiently monitoring the therapeutic response. Moreover, a paradoxical and often transient increase in blood eosinophils can be observed after the initiation of certain mAbs, for example dupilumab. It is therefore pertinent to understand the underlying immunology, and possibly, to perform immune endotyping of patients before prescribing appropriate treatment. For some patients, this may implicate a combination of targeted therapies.

AUTHOR CONTRIBUTIONS

Milos Jesenak, Zuzana Diamant, Edward Knol and Leo Koenderman: Conceptualization; writing—original draft; writing—review and editing; supervision. **Dagmar Simon, Ellen Tufvesson, Ilja Striz, Sven F. Seys and Martina Koziar Vasakova:** Conceptualization; writing—original draft; writing—review and editing. **Manali Mukherjee and Paige Lacy:** Writing—review and editing. **Susanne Vijverberg, Tomas Slisz, Anna Sediva, Hans-Uwe Simon, Jana Plevkova, Jurgen Schwarze, Radovan Kosturiak, Neil E. Alexis and Eva Untersmayr:** Writing—original draft; writing—review and editing.

ACKNOWLEDGMENTS

None.

CONFLICT OF INTEREST STATEMENT

MJ has received consulting fees (ALK-Abello, Stallergenes-Greer, Takeda, Zentiva); honoraria for lectures, presentations (ALK-Abello, Stallergenes-Greer, Takeda, Zentiva, Mundipharma, AstraZeneca, SOBI, Chiesi, CSL Behring, Novartis, Benela, Pfizer, Viatris); support for attending meetings and/or travel (ALK-Abello, Stallergenes-Greer, Takeda, Novartis, Sanofi Pasteur) and honoraria for participation on Advisory Boards (ALK-Abello, Stallergenes-Greer, Chiesi, Novartis, SOBI, Pfizer, Sanofi Genzyme/Pasteur). **ZD** acted as a director respiratory/allergy for QPS-NL (2012–2020); she has received consulting fees from ALK, Antabio, Foresee Pharmaceuticals, GSK, Hippo-Dx, Sanofi-Genzyme, QPS-NL; has participated in the speakers' bureaus of Boehringer Ingelheim, Sanofi-Genzyme; and serves as an Asthma Expert Panel Chair for EUFOREA. **DS** reports a relationship with AbbVie Inc, AstraZeneca, Galderma SA, LEO, Eli Lilly, Novartis, Pfizer, and Sanofi that includes consulting or advisory, and speaking and lecture fees. **ET** has received the independent *Type 2 Innovation Grant* from Sanofi Genzyme. **SFS** is employed by Galenus Health and Hippo Dx; has stocks of Hippo Dx; received honoraria from Teva Pharmaceutical. **MM** supported by an early career award from Canadian Institutes of Health Research and Canadian Asthma Allergy Immunology Foundation; reports grants from CIHR, CAAIF, Methapharm Specialty Pharmaceuticals, Sanofi, and personal fees from AstraZeneca, Novartis, and GlaxoSmithKline. **PL** reports grants from AstraZeneca (ESR-20-20,575, ESR-20-20,718), Natural Science and Engineering Research Council of Canada (NSERC DG RGPIN-2021-02889), and Synergy Respiratory and Cardiac Care, as well as personal fees from GlaxoSmithKline Canada, AstraZeneca Canada, and Synergy Respiratory and Cardiac Care, Canada. **SV** reports no conflict of interest regarding this manuscript. **TS** has received honoraria for lectures and presentations (GlaxoSmithKline). **AS** has received consulting fees (Takeda, Octapharma), honoraria for lectures (Pharming). **HUS** is a consultant for Sanofi and GlaxoSmithKline. **IS** received honoraria for lectures from Sanofi-Genzyme, Thermo Fisher, Astellas, ALK-Abello, Stallergenes-Greer, GSK, Novartis, Ewopharma. **JP** reports no conflict of interest regarding this manuscript. **JS** reports no conflict of interest regarding this manuscript. **RK** has received consulting fees (ALK-Abello, Stallergenes-Greer, Chiesi); honoraria for lectures, presentations (ALK-Abello, Stallergenes-Greer, AstraZeneca, Chiesi, Benela, Pfizer, Viatris); support for attending meetings and/or travel (ALK-Abello, Stallergenes-Greer) and honoraria for participation on Advisory Boards (ALK-Abello, Stallergenes-Greer, Chiesi, Pfizer). **NEA** reports no conflict of interest regarding this manuscript. **EU** has received honoraria for lectures, presentations (Nordmark Pharma GmbH, GEKA mbH, Allergopharma, Bencard GmbH, MacroArray Diagnostics, Nutrica); honoraria for participation on Advisory Boards (Bencard GmbH, Desentum Oy) and is PI of research projects funded by Desentum Oy and Nordmark Pharma GmbH outside the submitted work. **MKV** has received consulting fees (Boehringer Ingelheim, Roche, InterMune, Promedior); honoraria for lectures, presentations (Boehringer Ingelheim, Glaxo Smithkline); support for attending meetings and/or travel (Boehring Ingelheim, Roche) and honoraria for participation on Advisory Boards (Novartis, Boehringer Ingelheim, Roche, Glaxo Smithkline) and independent grant form Roche. **EK** received honoraria for lectures and advisory board membership for Sanofi Genzyme, received honoraria for lectures at Hycor-Abbott, GSK, Thermo Fisher, Novartis, Mark Two Academy and Springer. **LK** received honoraria for lectures and advisory boards of GSK.

DATA AVAILABILITY STATEMENT

Research data are not shared.

ORCID

Milos Jesenak https://orcid.org/0000-0001-7976-2523
Zuzana Diamant https://orcid.org/0000-0003-0133-0100
Dagmar Simon https://orcid.org/0000-0001-8965-9407
Ellen Tufvesson https://orcid.org/0000-0002-4941-332X
Sven F. Seys https://orcid.org/0000-0002-4399-9892
Manali Mukherjee https://orcid.org/0000-0002-7924-7211
Paige Lacy https://orcid.org/0000-0001-8885-6011
Susanne Vijverberg https://orcid.org/0000-0002-4579-4081
Tomas Slisz https://orcid.org/0000-0003-3513-1572
Anna Sediva https://orcid.org/0000-0001-7730-2304
Hans-Uwe Simon https://orcid.org/0000-0002-9404-7736
Ilja Striz https://orcid.org/0000-0001-9269-5652
Jana Plevkova https://orcid.org/0000-0003-2857-0593
Jurgen Schwarze https://orcid.org/0000-0002-6899-748X
Radovan Kosturiak https://orcid.org/0000-0002-6999-4336
Neil E. Alexis https://orcid.org/0000-0002-9417-8269
Eva Untersmayr https://orcid.org/0000-0002-1963-499X
Martina Koziar Vasakova https://orcid.org/0000-0002-0424-9941
Edward Knol https://orcid.org/0000-0001-7368-9820
Leo Koenderman https://orcid.org/0000-0002-5636-6453

REFERENCES

1. Blanchard C, Rothenberg ME. Biology of eosinophils. *Adv Immunol*. 2009;101:81-121.
2. Chusid MJ. Eosinophils: friends of foes? *J Allergy Clin Immunol Pract*. 2018;6:1439-1444.
3. Hillas G, Fouku E, Papaioannou A. Antibodies targeting the interleukin-5 signalling pathway used as add-on therapy for patients with severe eosinophilic asthma: a review of the mechanism of action, efficacy, and safety of the subcutaneously administered agents, mepolizumab and benralizumab. *Expert Rev Respir Med*. 2020;14(4):353-365.
4. Simon D, Simon HU. Therapeutic strategies for eosinophilic dermatoses. *Curr Opin Pharmacol*. 2019;46:29-33.
5. Marichal T, Mesnil C, Bureau F. Homeostatic eosinophils: characteristics and functions. *Front Med*. 2017;4:101.
6. Abdala-Valencia H, Coden ME, Chiarella SE, et al. Shaping eosinophils identity in the tissue contexts of development, homeostasis, and disease. *J Leucoc Biol*. 2018;104(1):95-108.
7. Diamant Z, Vijverberg S, Alving K, et al. Toward clinically applicable biomarkers for asthma: an EAACI position paper. *Allergy*. 2019;74(10):1835-1851.
8. Breiteneder H, Peng YQ, Agache I, et al. Biomarkerdss for diagnosis and prediction of therapy responses in allergic diseases and asthma. *Allergy*. 2020;75(12):3039-3068.
9. Heaney LG, Busby J, Hanratty CE, et al. Composite type-2 biomarker strategy versus a symptom-risk-based algorithm to adjust

corticosteroid dose in patients with severe asthma: a multicentre, single-blind, parallel group, randomised controlled trial. *Lancet Respir Med*. 2021;9(1):P57-P68.
10. Striz I, Golebski K, Strizova Z, et al. New insight into the pathophysiology and therapeutic targets of asthma and comorbid chronic rhinosinusitis with or without nasal polyposis. *Clin Sci (Lond)*. 2023;137(9):727-753.
11. Klion AD, Ackerman SJ, Bochner BS. Contributions of eosinophils to human health and disease. *Annu Rev Pathol*. 2020;15:179-209.
12. Ehrlich P. Beitrage zur Kenntnis der granulirten Bindegewbszellen und der ecosinophilen Leukocythen. *Archiv fur Anatomie und Physiologie, Physiologische Abteilung*. 1879;3:166-169.
13. Gauvreau GM, Denburg JA. Human mast cell and basophil/eosinophil progenitors. *Methods Mol Biol*. 2015;1220:59-68.
14. Hassani M, Tak T, van Aalst C, et al. Differential effects of short- and long-term treatment with mepolizumab on eosinophil kinetics in blood and sputum in eosinophilic asthma. *iScience*. 2021;24(8):102913.
15. Geijsen N, Koenderman L, Coffer PJ. Specificity in cytokine signal transduction: lessons learned from the IL-3/IL-5/GM-CSF receptor family. *Cytokine Growth Factor Rev*. 2001;12:19-25.
16. Johnston LK, Hsu CL, Krier-Burris RA, et al. IL-33 precedes IL-5 in regulating eosinophil commitment and is required for eosinophil homeostasis. *J Immunol*. 2016;197:3445-3453.
17. Mack EA, Pear WS. Transcription factor and cytokine regulation of eosinophil lineage commitment. *Curr Opin Hematol*. 2020;27:27-33.
18. Hartl S, Breyer MK, Burghuber OC, et al. Blood eosinophil count in the general population: typical values and potential confounders. *Eur Respir J*. 2020;55(5):1901874.
19. Dorman SC, Efthimiadis A, Babirad I, et al. Sputum $CD34^{+}IL\text{-}5Ralpha^{+}$ cells increase after allergen: evidence for in situ eosinophilopoiesis. *Am J Respir Crit Care Med*. 2004;169(5):573-577.
20. Steinbach KH, Schick P, Trepel F, et al. Estimation of kinetic parameters of neutrophilic, eosinophilic, and basophilic granulocytes in human blood. *Blut*. 1979;39(1):27-38.
21. Walle AJ, Parwaresch MR. Estimation of effective eosinopoiesis and bone marrow eosinophil reserve capacity in normal man. *Cell Tissue Kinet*. 1979;12(3):249-255.
22. Willebrand R, Voehringer D. Regulation of eosinophil development and survival. *Curr Opin Hematol*. 2017;24(1):9-15.
23. Rodrigo-Munoz JM, Gil-Martinez M, Sastre B, del Pozo V. Emerging evidence for pleiotropism of eosinophils. *Int J Mol Sci*. 2021;22(13):7075.
24. Shah K, Ignacio A, McCoy KD, Harris NL. The emerging roles of eosinophils in mucosal homeostasis. *Mucosal Immunol*. 2020;13(4):574-583.
25. Koenderman L. Priming: a critical step in the control of eosinophil activation. In: Lee JJ, Rosenberg HF, eds. *Eosinophils in Health and Disease*. Academic Press, Elsevier; 2013:170-178. ISBN 978-012-394385-9.
26. Takatsu K, Tominaga A. Interleukin 5 and its receptor. *Prog Growth Factor Res*. 1991;3(2):87-102.
27. Nishinakamura R, Miyajima A, Mee PJ, Tybulewicz VL, Murray R. Hematopoiesis in mice lacking the entire granulocyte-macrophage colony-stimulating factor/interleukin-3/interleukin-5 functions. *Blood*. 1996;88(7):2458-2464.
28. Conus S, Straumann A, Bettler E, Simon HU. Mepolizumab does not alter levels of eosinophils, T cells, and mast cells in the duodenal mucosa in eosinophilic esophagitis. *J Allergy Clin Immunol*. 2010;126(1):175-177.
29. Leckie MJ, ten Brinke A, Khan J, et al. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet*. 2000;356(9248):2144-2148.
30. Flood-Page PT, Menzies-Gow AN, Kay AB, Robinson DS. Eosinophil's role remains uncertain as anti-interleuin-5 only partially depletes numbers in asthmatic airway. *Am J Respir Crit Care Med*. 2003;167(2):199-204.
31. Mesnil C, Raulier S, Paulissen G, et al. Lung-resident eosinophils represent a distinct regulatory eosinophil subset. *J Clin Invest*. 2016;126(9):3279-3295.
32. Liu LY, Sedgwick JB, Bates ME, et al. Decreased expression of membrane IL-5 receptor alpha on human eosinophils: I. loss of membrane IL-5 receptor alpha on airway eosinophils and increased soluble IL-5 receptor alpha in the airway after allergen challenge. *J Immunol*. 2002;169(11):6452-6458.
33. Gigon L, Fettrelet T, Yousefi S, Simon D, Simon HU. Eosinophils from A to Z. *Allergy*. 2023;78(7):1810-1846.
34. O'Connell EM, Nutman TB. Eosinophilia in infectious diseases. *Immunol Allergy Clin North Am*. 2015;35(3):493-522.
35. Klion AD, Nutman TB. The role of eosinophils in host defense against helminth parasites. *J Allergy Clin Immunol*. 2004;113(1):30-37.
36. Bochner BS. The eosinophil: for better or worse, in sickness and in health. *Ann Allergy Asthma Immunol*. 2019;121(2):150-155.
37. Jackson DJ, Akuthota P, Roufosse F. Eosinophils and eosinophilic immune dysfunction in health and disease. *Eur Respir Rev*. 2022;31(163):210150.
38. Rao RR, Long JZ, White JP, et al. Meteorin-like is a hormone that regulates immune-adipose interactions to increase beige fat thermogenesis. *Cell*. 2014;157:1279-1291.
39. Lee MW, Odegaard JI, Mukundan L, et al. Activated type 2 innate lymphoid cells regulate beige fat biogenesis. *Cell*. 2015;160(1–2):74-87.
40. Da Silva FR, Soares Thimoteo D, Ferraz Carbonel A, et al. Histomorphometric analysis of the endometrium in an ectopic model of endometriosis in mice. *Gynecol Endocrinol*. 2022;38:874-878.
41. Lee JJ, Jacobsen EA, McGarry MP, Schleimer RP, Lee NA. Eosinophils in health and disease: the LIAR hypothesis. *Clin Exp Allergy*. 2010;40(4):563-575.
42. Bochner BS, Schleimer RP. The role of adhesion molecules in human eosinophil and basophil recruitment. *J Allergy Clin Immunol*. 1994;94(3 Pt 1):427-438.
43. Knol EF, Tackey F, Tedder TF, et al. Comparison of human eosinophil and neutrophil adhesion to endothelial cells under nonstatic conditions. Role of L-selectin. *J Immunol*. 1994;153(5):2161-2167.
44. Monteiro RC, Hostoffer RW, Cooper MD, Bonner JR, Gartland GL, Kubagawa H. Definition of immunoglobulin A receptors on eosinophils and their enhanced expression in allergic individuals. *J Clin Invest*. 1993;92(4):1681-1685.
45. Koenderman L, Hermans SW, Capel PJ, van de Winkel JG. Granulocyte-macrophage colony-stimulating factor induces sequential activation and deactivation of binding via a low-affinity IgG fc receptor, hFc gamma RII, on human eosinophils. *Blood*. 1993;81(9):2413-2419.
46. Yoon J, Ponikau JU, Lawrence CB, Kita H. Innate antifungal immunity of human eosinophils mediated by a beta 2 integrin, CD11b. *J Immunol*. 2008;181(4):2907-2915.
47. Koenderman L, van der Bruggen T, Schweizer RC, et al. Eosinophil priming by cytokines: from cellular signal to in vivo modulation. *Eur Respir J*. 1996;22:119s-125s.
48. Bracke M, van de Graaf E, Lammers JW, Coffer PJ, Koenderman L. In vivo priming of FcalphaR functioning on eosinophils of allergic asthmatics. *J Leukoc Biol*. 2000;68(5):655-661.
49. Lacy P, Abdel-Latif D, Steward M, Musat-Marcu S, Man SFP, Moqbel R. Divergence of mechanisms regulating respiratory burst in blood and sputum eosinophils and neutrophils from atopic subjects. *J Immunol*. 2003;170(5):2670-2679.
50. Hogan SP, Rosenberg HF, Moqbel R, et al. Eosinophils: biological properties and role in health and disease. *Clin Exp Allergy*. 2008;38(5):709-750.
51. Mukherjee M, Lacy P, Ueki S. Eosinophil extracellular traps and inflammatory pathologies – untangling the web! *Front Immunol*. 2018;9:2763.

52. Bandeira-Melo C, Weller PF. Eosinophils and cysteinyl leukotrienes. *Prostaglandins Leukot Essent Fatty Acids*. 2003;69(2–3):135-143.
53. Davoine F, Lacy P. Eosinophil cytokines, chemokines, and growth factors: emerging roles in immunity. *Front Immunol*. 2014;5:570.
54. Tool AT, Koenderman L, Kok PT, Blom M, Roos D, Verhoeven AJ. Release of platelet-activating factor is important for the respiratory burst induced in human eosinophils by opsonized particles. *Blood*. 1992;79(10):2729-2732.
55. Lacy P, Logan MR, Bablitz B, Moqbel R. Fusion protein vesicle-associated membrane protein 2 is implicated in IFN-gamma-induced piecemeal degranulation in human eosinophils from atopic individuals. *J Allergy Clin Immunol*. 2001;107(4):671-678.
56. Lacy P. The role of rho GTPases and SNAREs in mediator release from granulocytes. *Pharmacol Ther*. 2005;107(3):358-376.
57. Logan MR, Lacy P, Bablitz B, Moqbel R. Expression of eosinophil target SNAREs as potential cognate receptors for vesicle-associated membrane protein-2 in exocytosis. *J Allergy Clin Immunol*. 2002;109(2):299-306.
58. Logan MR, Lacy P, Odemuyiwa SO, et al. A critical role for vesicle-associated membrane protein-7 in exocytosis from human eosinophils and neutrophils. *Allergy*. 2006;61(6):777-784.
59. Willetts L, Felix LC, Jacobsen E, et al. Vesicle-associated membrane protein 7-mediated eosinophil degranulation promotes allergic airway inflammation in mice. *Commun Biol*. 2018;1:83.
60. Radonjic-Hoesli S, Wang X, de Graauw E, et al. Adhesion-induced eosinophil cytolysis requires the receptor-interacting protein kinase 3 (RIPK3)-mixed lineage kinase-like (MLKL) signalling pathway, which is counterregulated by autophagy. *J Allergy Clin Immunol*. 2017;140(6):1632-1642.
61. Simon HU, Yousefi S, Germic N, et al. The cellular functions of eosinophils: collegium Internationale Allergologicum (CIA) update 2020. *Int Arch Allergy Immunol*. 2020;181(1):11-23.
62. Yousefi S, Gold JA, Andina N, et al. Catapult-like release of mitochondrial DNA by eosinophils contributes to antibacterial defence. *Nat Med*. 2008;14(9):949-953.
63. Nogawa H, Suzuki H, Kawabata Y, et al. An unusual case of eosinophilic lung disease with multiple cyst formation. *Respir Med Case Rep*. 2020;31:101300.
64. Fettrelet T, Gigon L, Karaulov A, Yousefi S, Simon HU. The enigma of eosinophil degranulation. *Int J Mol Sci*. 2021;22(13):7091.
65. Ueki S, Konno Y, Takeda M, et al. Eosinophil extracellular trap cell death-derived DNA traps: their presence in secretions and functional attributes. *J Allergy Clin Immunol*. 2016;137(1):258-267.
66. Ueki S, Miyabe Y, Yamamoto Y, et al. Charcot-Leyden crystals in eosinophilic inflammation: active cytolysis leads to crystal formation. *Curr Allergy Asthma Rep*. 2019;19(8):35.
67. Persson EK, Verstraete K, Heyndrickx I, et al. Protein crystallization promotes type 2 immunity and is reversible by antibody treatment. *Science*. 2019;364(6442):eaaw4295.
68. Sabogal Piñeros YS, Bal SM, Dijkhuis A, et al. Eosinophils capture viruses, a capacity that is defective in asthma. *Allergy*. 2019;74(10):1898-1909.
69. Rodrigo-Munoz JM, Sastre B, Canas JA, et al. Eosinophil response against classic and emerging respiratory viruses: COVID-19. *J Investig Allergol Clin Immunol*. 2021;31:94-107.
70. Flores-Torres AS, Salinas-Carmona MC, Salinas E, Rosas-Taraco AG. Eosinophils and respiratory viruses. *Viral Immunol*. 2019;32(5):198-307.
71. LeMessurier KS, Tiwary M, Morin NP, Samarasinghe AE. Respiratory barrier as a safeguard and regulator of defense against influenza A virus and *Streptococcus pneumoniae*. *Front Immunol*. 2020;11:3.
72. Jesenak M, Banovcin P, Diamant Z. COVID-19, chronic inflammatory respiratory diseases and eosinophils – observation from reported clinical case series. *Allergy*. 2020;75(7):1819-1822.
73. Soni M. Evaluation of eosinopenia as a diagnostic and prognostic indicator in COVID-19 infection. *Int J Lab Hematol*. 2021;43(Suppl. 1):137-141.
74. Myari A, Papapetrou E, Tsaousi C. Diagnostic value of white blood cell parameters for COVID-19: is there a role for HFLC and IG? *Int J Lab Hematol*. 2022;44(1):104-111.
75. Cazzaniga M, Fumagalli LAM, D'angelo L, et al. Eosinopenia is a reliable marker of severe disease and unfavourable outcome in patients with COVID-19 pneumonia. *Int J Clin Pract*. 2021;75:e14047.
76. Eijmael M, Janssens N, le Cessie S, van Dooren Y, Koster T, Karim F. Coronavirus disease 2019 and peripheral blood eosinophil counts: a retrospective study. *Infection*. 2021;49:1325-1329.
77. Zein JG, Strauss R, Attaway AH, et al. Eosinophilia is associated with improved COVID-19 outcomes in inhaled corticosteroid-treated patients. *J Allergy Clin Immunol Pract*. 2022;10:742-750.
78. Eger K, Hashimoto S, Braunstahl GJ, et al. Poor outcome of SARS-CoV-2 infection in patients with severe asthma on biologic therapy. *Respir Med*. 2020;177:106287.
79. Francis CHR, Hearn AP, Ratnakumar S, et al. Covid-19 in the absence of eosinophils: the outcome of confirmed SARS-CoV-2 infection whilst on treatment with benralizumab. *Allergy*. 2022;77(8):2558-2560.
80. Huang L, Appleton JA. Eosinophils in helminth infection: defenders and dupes. *Trends Parasitol*. 2016;32:798-807.
81. Cadman E, Thysse KA, Bearder S, et al. Eosinophils are important for protection, immunoregulation and pathology during infection with nematode microfilariae. *PLoS Pathog*. 2014;10:e1003988.
82. Ondari E, Calvino-Sanles E, First NJ, Gestal MC. Eosinophils and bacteria, the beginning of a story. *Int J Mol Sci*. 2021;22(15):8004.
83. Linch SN, Gold JA. The role of eosinophils in non-parasitic infections. *Endocri Metab Immune Disord Drug Targets*. 2011;11:165-172.
84. Morshed M, Yousefi S, Stockle C, Simon HU, Simon D. Thymic stromal lymphopoietin stimulates the formation of eosinophil extracellular traps. *Allergy*. 2012;67:1127-1137.
85. Figueiredo RT, Neves JS. Eosinophils in fungaldiseases: an overview. *J Leukoc Biol*. 2018;104:49-60.
86. Austen KF. Homeostasis of effector systems which can also be recruited for immunologic reactions. *J Immunol*. 1978;121(3):793-805.
87. Lingblom C, Andersson J, Andersson K, Wennerås C. Regulatory eosinophils suppress T cells partly through Galectin-10. *J Immunol*. 2017;198(12):4672-4681.
88. Takemura N, Kurashima Y, Mori Y, et al. Eosinophil depletion suppresses radiation-induced small intestinal fibrosis. *Sci Transl Med*. 2018;10(429):eaan0333.
89. Siddiqui S, Bachert C, Bjermer L, et al. Eosinophils and tissue remodeling: relevance to airway disease. *J Allergy Clin Immunol*. 2023;S0091-6749(23)00800-X. doi:10.1016/j.jaci.2023.06.005
90. Wu D, Molofsky AB, Liang HE, et al. Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis. *Science*. 2011;332(6026):243-247.
91. Brigger D, Riether C, van Brummelen R, et al. Eosinophils regulate adipose tissue inflammation and sustain physical and immunological fitness in old age. *Nat Metab*. 2020;2(8):688-702.
92. Grisaru-Tal S, Itan M, Klion AD, Munitz A. A new dawn for eosinophils in the tumour microenvironment. *Nat Rev Cancer*. 2020;20(10):594-607.
93. Hu G, Wang S, Zhong K, et al. Tumor-associated tissue eosinophilia predicts favourable clinical outcome in solid tumors: a meta-analysis. *BMC Cancer*. 2020;20:454.
94. Simon D, Simon HU. Eosinophilic disorders. *J Allergy Clin Immunol*. 2007;119(6):1291-1300. Erratum in: *J Allergy Clin Immunol*. 2007;120(3):515.
95. Simon SCS, Utikal J, Umansky V. Opposing roles of eosinophils in cancer. *Cancer Immunol Immunother*. 2019;68(5):823-833.

96. Gotlib J. World Health Organization-defined eosinophilic disorders: 2017 update on diagnosis, risk stratification, and management. *Am J Hematol*. 2017;92(11):1243-1259.
97. Valent P, Gleich GJ, Reiter A, et al. Pathogenesis and classification of eosinophil disorders: a review of recent developments in the field. *Expert Rev Hematol*. 2012;5(2):157-176.
98. Valent P, Klion AD, Roufosse F, et al. Proposed refined diagnostic criteria and classification of eosinophil disorders and related syndromes. *Allergy*. 2023;78:47-59.
99. Wang SA. The diagnostic work-up of hypereosinophilia. *Pathobiology*. 2019;86(1):39-52.
100. Radonjic-Hoesli S, Martignoni Z, Cazzaniga S, et al. Characteristics of dermatological patients with blood eosinophilia: A retrospective analysis of 453 patients. *J Allergy Clin Immunol Pract*. 2022;10(5):1229-1237.e8.
101. Leru PM. Eosinophilic disorders: evaluation of current classification and diagnostic criteria, proposal of a practical diagnostic algorithm. *Clin Transl Allergy*. 2019;9:36.
102. Weller PF, Klion AD. Approach to the patient with unexplained eosinophilia. 2022.
103. Hastie AT, Moore WC, Li H, et al. Biomarker surrogates do not accurately predict sputum eosinophil and neutrophil percentages in asthmatic subjects. *J Allergy Clin Immunol*. 2013;132(1):72-80.
104. Jeffery P, Holgate S, Wenzel S, Endobronchial Biopsy Workshop. Methods for the assessment of endobronchial biopsies in clinical research: application to studies of pathogenesis and the effects of treatment. *Am J Respir Crit Care Med*. 2003;168(6 Pt 2):S1-S17.
105. Diamant Z, Boot JD, Mantzouranis E, Flohr R, Sterk PJ, Gerth van Wijk R. Biomarkers in asthma and allergic rhinitis. *Pulm Pharmacol Ther*. 2010;23(6):468-481.
106. Rakowski E, Zhao S, Liu M, et al. Variability of blood eosinophils in patients in a clinic for severe asthma. *Clin Exp Allergy*. 2019;49(2):163-170.
107. Grootendorst DC, Sont JK, Willems LN, et al. Comparison of inflammatory cell counts in asthma: induced sputum vs. bronchoalveolar lavage and bronchial biopsies. *Clin Exp Allergy*. 1997;27(7):769-779.
108. Koenderman L, Hassani M, Mukherjee M, Nair P. Monitoring eosinophils to guide therapy with biologics in asthma: does the compartment matter? *Allergy*. 2021;76(4):1294-1297.
109. Mukherjee M, Nair P. Blood or sputum eosinophils to guide asthma therapy? *Lancet Respir Med*. 2015;3(11):824-825.
110. Felarca AB, Lowell FC. The total eosinophil count in a nonatopic population. *J Allergy*. 1967;40(1):16-20.
111. Schwartz JT, Fulkerson PC. An approach to the evaluation of persistent hypereosinophilia in pediatric patients. *Front Immunol*. 2018;9:1944.
112. Dahl R, Venge P, Olsson I. Blood eosinophil leucocytes and eosinophil cationic protein. Diurnal variation in normal subjects and patients with bronchial asthma. *Scand J Respir Dis*. 1978;59:323-325.
113. Nussbaum JC, Van Dyken SJ, von Moltke J, et al. Type 2 innate lymphoid cells control eosinophil homeostasis. *Nature*. 2013;502:245-248.
114. Gibson PG. Variability of blood eosinophils as a biomarker in asthma and COPD. *Respirology*. 2018;23:12-13.
115. Ullmann N, Bossley CJ, Fleming L, Silvestri M, Bush A, Saglani S. Blood eosinophil counts rarely reflect airway eosinophilia in children with severe asthma. *Allergy*. 2013;68:402-406.
116. Yancey SW, Keene ON, Albers FC, et al. Biomarkers for severe eosinophilic asthma. *J Allergy Clin Immunol*. 2017;140:1509-1518.
117. Wagener AH, de Nijs SB, Lutter R, et al. External validation of blood eosinophils, FE(NO) and serum periostin as surrogates for sputum eosinophils in asthma. *Thorax*. 2015;70(2):115-120.
118. Lim HF, Nair P. Airway inflammation and inflammatory biomarkers. *Semin Respir Crit Care Med*. 2018;39:56-63.
119. Hilvering B, Koenderman L. Quality over quantity: eosinophilactivation status will deepen the insight into eosinophilic diseases. *Respir Med*. 2023;207:107094.
120. Lombardi C, Bert A, Cottini M. The emerging roles of eosinophils: implications for the targeted treatment of eosinophilic-associated inflammatory conditions. *Curr Res Immunol*. 2022;3:42-53.
121. Wenzel SE. Asthma: defining of the persistent adult phenotypes. *Lancet*. 2006;368(9537):804-813.
122. Pizzichini E, Pizzichini M, Efthimiadis A, Hargreave F, Dolovich J. Measurement of inflammatory indices in indices in induced sputum: effects of selection of sputum to minimize salivary contamination. *Eur Respir J*. 1996;9:1174-1180.
123. Dasgupta A, Zhang S, Thabane L, Nair P. Sample size for clinical trials using sputum eosinophils as a primary outcome. *Eur Respir J*. 2013;42:1003-1011.
124. Green RH, Brightling CE, McKenna S, et al. Asthma exacerbations and sputum eosinophils counts: a randomised controlled trial. *Lancet*. 2002;360(9347):1715-1721.
125. Pizzichini MM, Pizzichini E, Clelland L, et al. Sputum in severe exacerbations of asthma: kinetics of inflammatory indices after prednisone treatment. *Am J Respir Crit Care Med*. 1997;155(5):1501-1508.
126. Wang G, Baines KJ, Fu JJ, et al. Sputum mast cell subtypes relate to eosinophilia and corticosteroid response in asthma. *Eur Respir J*. 2016;47(4):1123-1133.
127. Jayaram L, Pizzichini MM, Cook RJ, et al. Determining asthma treatment by monitoring sputum cell counts: effect on exacerbations. *Eur Respir J*. 2006;27(3):483-494.
128. Seys SF. Role of sputum biomarkers in the management of asthma. *Curr Opin Pulm Med*. 2017;23(1):34-40.
129. Gomez JL, Chen A, Diaz MP, et al. A network of sputum microRNAs is associated with neutrophilic airway inflammation in asthma. *Am J Respir Crit Care Med*. 2020;202(1):51-64.
130. Schoenfield JPR, Burg D, Nicholas B, et al. Stratification of asthma phenotypes by airway proteomic signatures. *J Allergy Clin Immunol*. 2019;144(1):70-82.
131. Seys SF, Scheers H, van den Brande P, et al. Cluster analysis of sputum cytokine-high profiles reveals diversity in T(h)2-high asthma patients. *Resp Res*. 2017;18(1):39.
132. Van Rensen ELJ, Hiemstra PS, Rabe KF, Sterk PJ. Assessment of microvascular leakage via sputum induction: the role of substance P and neurokinin A in patients with asthma. *Am J Respir Crit Care Med*. 2002;165(9):1275-1279.
133. Zuiker RHJA, Tribouley C, Diamant Z, et al. Sputum RNA signature in allergic asthmatics following allergen bronchoprovocation test. *Eur Clin Respir J*. 2016;3:31324.
134. Zuiker RGJA, Ruddy MK, Morelli N, et al. Kinetics of TH2 biomarkers in sputum of asthmatics following inhaled allergen. *Eur Clin Respir J*. 2015;2:28319.
135. Lay JC, Peden DB, Alexis NE. Flow cytometry of sputum: assessing inflammation and immune response elements in the bronchial airways. *Inhal Toxicol*. 2011;23(7):392-406.
136. Vidal S, Bellido-Casado J, Granel C, Crespo A, Plaza V, Juarez C. Flow cytometry analysis of leukocytes in induced sputum from asthmatic patients. *Immunobiology*. 2012;217(7):692-697.
137. Abdel-Aziz MI, Vijverberg SJH, Neerincx AH, et al. A multi-omics approach to delineate sputum microbiome-associated asthma inflammatory phenotypes. *Eur Respir J*. 2022;59(1):210603.
138. Huang YJ, Nariya S, Harris JM, et al. The airway microbiome in patients with severe asthma: associations with disease feature and severity. *J Allergy Clin Immunol*. 2015;136(4):874-884.
139. Chami HA, Diaz-Mendoza J, Chua A, et al. Transbronchial biopsy and cryobiopsy in the diagnosis of hypersensitivity pneumonitis among patients with interstitial lung disease. *Ann Am Thorac Soc*. 2021;18(1):148-161.
140. Cottin V. Eosinophilic lung diseases. *Clin Chest Med*. 2016;37(3):535-556.

141. De Giacomi F, Vassallo R, Yi ES, Ryu JH. Acute eosinophilic pneumonia. Causes, diagnosis, and management. *Am J Respir Crit Care Med*. 2018;197(6):728-736.
142. Hetzel J, Eberhardt R, Herth FJF, et al. Cryobiopsy increases the diagnostic yield of endobronchial biopsy: a multicentre trial. *Eur Respir J*. 2012;39(3):685-690.
143. Rosenberg CE, Khoury P. Approach to eosinophilia presenting with pulmonary symptoms. *Chest*. 2021;159(2):507-516.
144. Baines KJ, Simpson JL, Wood LG, et al. Sputum gene expression signature of 6 biomarkers discriminates asthma inflammatory phenotype. *J Allergy Clin Immunol*. 2014;133(4):997-1007.
145. Peters MC, Mekonnen ZK, Yuan S, Bhakta NR, Woodruff PG, Fahy JV. Measures of gene expression in sputum cells can identify TH2-high and TH2-low subtypes of asthma. *J Allergy Clin Immunol*. 2014;133(2):388-394.
146. Seys SF, Grabowski M, Adriaensen W, et al. Sputum cytokine mapping reveals an 'IL-5, IL-17A, IL-25-high' pattern associated with poorly controlled asthma. *Clin Exp Allergy*. 2013;43(9):1009-1017.
147. Henderson AG, Andreson WH, Ceppe A, et al. Mucus hydration in subjects with stable chronic bronchitis: a comparison of spontaneous and induced sputum. *COPD*. 2018;15(6):572-580.
148. Holz O, Seiler T, Karmeier A, et al. Assessing airway inflammation in clinical practice – experience with spontaneous sputum analysis. *BMC Pulm Med*. 2008;8:5.
149. Pizzichini MM, Popov TA, Efthimiadis A, et al. Spontaneous and induced sputum to measure indices of airway inflammation in asthma. *Am J Respir Crit Care Med*. 1996;154(4 Pt 1):866-869.
150. Bakakos P, Schleich F, Alchanatis M, Louis R. Induced sputum in asthma: from bench to bedside. *Curr Med Chem*. 2011;18(10):1415-1422.
151. Pizzichini E, Pizzichini MM, Efthimiadis A, et al. Indices of airway inflammation in induced sputum: reproducibility and validity of cell and fluid-phase measurements. *Am J Respir Crit Care Med*. 1996;154(2 Pt 1):308-317.
152. Green R, Brightling CE, Woltmann G, Parker D, Wardlaw AJ, Pavord ID. Analysis of induced sputum in adults with asthma: identification of subgroup with isolated sputum neutrophilia and poor response to inhaled corticosteroids. *Thorax*. 2002;57(10):875-879.
153. Pavord ID, Pizzichini MM, Pizzichini E, Hargreave FE. The use of induced sputum to investigate airway inflammation. *Thorax*. 1997;52(6):498-501.
154. De Corso E, Lucidi D, Battista M, et al. Prognostic value of nasal cytology and clinical factors in nasal polyps development in patients at risk: can the beginning predict the end? *Int Forum Allergy Rhinol*. 2017;7(9):861-867.
155. Miman MC, Uzun O, Gurses I, Kuku I, Ozturan O, Akarcay M. The sensitivity of nasal eosinophilia in allergic rhinitis. *Eur Arch Otorhinolaryngol*. 2007;264(9):1013-1018.
156. Zhu Z, Wang W, Zhang X, et al. Nasal fluid cytology and cytokine profiles of eosinophilic and non-eosinophilic chronic rhinosinusitis with nasal polyps. *Rhinology*. 2020;58(4):314-322.
157. Howarth PH, Persson CG, Meltzer EO, Jacobson MR, Durham SR, Silkoff PE. Objective monitoring of nasal airway inflammation in rhinitis. *J Allergy Clin Immunol*. 2005;115(3 Suppl 1):S414-S441.
158. Siddiqui S, Wenzel SE, Bozik ME, et al. Safety and efficacy of dexpramipexole in eosinophilic asthma (EXHALE): a randomized controlled trial. *J Allergy Clin Immunol*. 2023;S0091-6749(23)00709-1. doi:10.1016/j.jaci.2023.05.014
159. Amorim MM, Araruna A, Caetano LB, Cruz AC, Santoro LL, Fernandes AL. Nasal eosinophilia: an indicator of eosinophilic inflammation in asthma. *Clin Exp Allergy*. 2010;40(6):867-874.
160. Amorim MM, Fernandes PBL, Caetano LB, Dracoulakis S, Santoro IL, Fernandes ALG. Nasal lavage is better than blood count in predicting sputum eosinophilia. *Clin Exp Allergy*. 2015;45(5):1006-1008.
161. Radonjic-Hoesli S, Brüggen MC, Feldmeyer L, Simon HU, Simon D. Eosinophils in skin diseases. *Semin Immunopathol*. 2021;43(3):393-409.
162. Wolff K, Goldsmith L, Katz S, Gilchrest B, Paller AS, Leffell D. *Fitzpatrick's Dermatology in General Medicine*. 8th ed. McGraw-Hill; 2011.
163. Martin LB, Kita H, Leiferma KM, Gleich GJ. Eosinophils in allergy: role in disease, degranulation and cytokines. *Int Arch Allergy Immunol*. 1996;109(3):207-215.
164. Leiferman KM, Peters MS. Eosinophil-related disease and the skin. *J Allergy Clin Immunol Pract*. 2018;6(5):1462-1482.e6.
165. Long H, Zhang G, Wang L, Lu Q. Eosinophilic skin diseases: A comprehensive review. *Clin Rev Allergy Immunol*. 2016;50(2):189-213.
166. Zhou Y, Barnett MJ, Rivers JK. Clinical significance of skin biopsies in the diagnosis and management of graft-vs-host disease in early postallogeneic bone marrow transplantation. *Arch Dermatol*. 2000;136(6):717-721.
167. Graeff-Teixeira C, da Silva AC, Yoshimura K. Update on eosinophilic meningoencephalitis and its clinical relevance. *Clin Microbiol Rev*. 2009;22(2):322-348.
168. Fulkerson DH, Boaz JC. Cerebrospinal fluid eosinophilia in children with ventricular shunts. *J Neurosurg Pediatr*. 2008;1(4):288-295.
169. Heidemann SM, Fiore M, Sood S, Ham S. Eosinophil activation in the cerebrospinal fluid of children with shunt obstruction. *Pediatr Neurosurg*. 2010;46(4):255-258.
170. Salafia CM, Popek EJ. Inflammatory and vascular placental pathology. *Glob Libr Women's Med*. 2009. doi:10.3843/glowm.10152
171. Koh GC, Shek LP, Goh DY, Van Bever H, Koh DS. Eosinophil cationic protein: is it useful in asthma? A systematic review. *Respir Med*. 2007;101:696-705.
172. Schmekel B, Ahlner J, Malmstrom M, Venge P. Eosinophil cationic protein (ECP) in saliva: a new marker of disease activity in bronchial asthma. *Respir Med*. 2001;95(8):670-675.
173. Prehn A, Seger RA, Torresani T, Molinari L, Sennhauser FH. Evaluation of a clinical algorithm involving serum eosinophil cationic protein for guiding the anti-inflammatory treatment of bronchial asthma in childhood. *Pediatr Allergy Immunol*. 2000;11:87-94.
174. Munthe-Kaas MC, Gerritsen J, Carlsen KH, et al. Eosinophil cationic protein (ECP) polymorphisms and association with asthma, s-ECP levels and related phenotypes. *Allergy*. 2007;62(4):429-436.
175. Jonsson UB, Bystrom J, Stalenheim G, Venge P. Polymorphism of the eosinophil cationic protein-gene is related to the expression of allergic symptoms. *Clin Exp Allergy*. 2002;32(7):1092-1095.
176. Noguchi E, Iwama A, Takeda K, et al. The promoter polymorphism in the eosinophil cationic protein gene and its influence on the serum cationic protein level. *Am J Respir Crit Care Med*. 2003;167(2):180-184.
177. Nair P, Ochkur SI, Protheroe C, et al. Eosinophil peroxidase in sputum represents a unique biomarker of airway eosinophilia. *Allergy*. 2013;68(9):1177-1184.
178. Venge P. Monitoring the allergic inflammation. *Allergy*. 2004;59(1):26-32.
179. Kristjánsson S, Strannegård IL, Strannegård Q, Peterson C, Enander I, Wennergren G. Urinary eosinophil protein X in children with atopic asthma: a useful marker of anti-inflammatory treatment. *J Allergy Clin Immunol*. 1996;97:1179-1187.
180. Kim CK, Callaway Z, Fletcher R, Koh YY. Eosinophil-derived neurotoxin in childhood asthma: correlation with disease severity. *J Asthma*. 2010;47(5):568-573.
181. Oymar K. High levels of urinary eosinophil protein X in young asthmatic children predict persistent atopic asthma. *Pediatr Allergy Immunol*. 2001;12:312-317.
182. Ochkur SI, Kim JD, Protheroe CA, et al. A sensitive high throughput ELISA for human eosinophil peroxidase: a specific assay to

quantify eosinophil degranulation from patient-derived sources. *J Immunol Methods*. 2012;384(1–2):10-20.
183. Rank MA, Ochkur SI, Lewis JC, et al. Nasal and pharyngeal eosinophil peroxidase levels in adults with poorly controlled asthma correlate with sputum eosinophilia. *Allergy*. 2016;71(4):567-570.
184. Acharya KR, Ackerman SJ. Eosinophil granule proteins: form and function. *J Biol Chem*. 2014;289(25):17406-17415.
185. Johansson MW. Eosinophil activation status in separate compartments and association with asthma. *Front Med (Lausanne)*. 2017;4:75.
186. Carr TF, Zeki AA, Kraft M. Eosinophilic and noneosinophilic asthma. *Am J Respir Crit Care Med*. 2018;197(1):22-37.
187. Khatri SB, Iaccarino JM, Barochia A, et al. American Thoracic Society assembly on allergy, immunology and inflammation. *Am J Respir Crit Care Med*. 2021;204(10):e97-e109.
188. Banovcin P, Jesenak M, Michnova Z, et al. Factors attributable to the level of exhaled nitric oxide in asthmatic children. *Eur J Med Res*. 2009;14(Suppl 4):9-13.
189. Alving K, Diamant Z, Lucas S, et al. Point-of-care biomarkers in asthma management: tome to move forward. *Allergy*. 2020;75(4):995-997.
190. Bjermer L, Alving K, Diamant Z, et al. Current evidence and future research needs for FeNO measurement in respiratory diseases. *Respir Med*. 2014;108(6):830-841.
191. Dweik RD, Boggs PB, Erzurum SC, et al. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. *Am J Respir Crit Care Med*. 2011;184(5):602-615.
192. Izumo T, Tone M, Kuse N, et al. Effectiveness and safety of benralizumab for severe asthma in clinical practice (J-BEST): a prospective study. *Ann Transl Med*. 2020;8(7):438.
193. Maglio A, Vitale C, Pellegrino S, et al. Real-life effectiveness of mepolizumab on forced expiratory flow between 25% and 75% of forced vital capacity in patients with severe eosinophilic asthma. *Biomedicine*. 2021;9(11):1550.
194. Crespo A, Giner J, Torrejon M, et al. Clinical and inflammatory features of asthma with dissociation between fractional exhaled nitric oxide and eosinophils in induced sputum. *J Asthma*. 2016;53:459-464.
195. Gao J, Wu F. Association between fractional exhaled nitric oxide, sputum induction and peripheral blood eosinophil in uncontrolled asthma. *Allergy Asthma Clin Immunol*. 2018;14:21.
196. Korevaar DA, Westerhof GA, Wang J, et al. Diagnostic accuracy of minimally invasive markers for detection of airway eosinophilia in asthma: a systematic review and meta-analysis. *Lancer Respir Med*. 2015;3(4):290-300.
197. Couillard S, Laugerud A, Jabeen M, et al. Derivation of a prototype asthma attack risk scale centred on blood eosinophils and exhaled nitric oxide. *Thorax*. 2022;77(2):199-202.
198. Menzies-Gow A, Mansur AH, Brughtling CE. Clinical utility of fractional exhaled nitric oxide in severe asthma management. *Eur Respir J*. 2020;55(3):1901633.
199. Sakkal S, Miller S, Apostolopoulos V, Nurgali K. Eosinophils in cancer: favourable or unfavourable? *Curr Med Chem*. 2016;23(7):650-666.
200. Della Valle L, Gatta A, Farinelli A, et al. Allergooncology: an expanding research area. *J Biol Regul Homeost Agents*. 2020;34:319-326.
201. Carretero R, Sektioglu IM, Garbi N, Salgado OC, Beckhove P, Hämmerling GJ. Eosinophils orchestrate cancer rejection by normalizing tumor vessels and enhancing infiltration of CD8(+) T cells. *Nat Immunol*. 2015;16:609-617.
202. Zaynagetdinov R, Sherrill TP, Gleaves LA, et al. Interleukin-5 facilitates lung metastasis by modulating the immune microenvironment. *Cancer Res*. 2015;75:1624-1634.
203. Jacquelot N, Seillet C, Wang M, et al. Blockade of the co-inhibitory molecule PD-1 unleashes ILC2-dependent antitumor immunity in melanoma. *Nat Immunol*. 2021;22(7):851-864.
204. Radonjic-Hoesli S, Valent P, Klion AD, Wechsler ME, Simon HU. Novel targeted therapies for eosinophil-associated diseases and allergy. *Annu Rev Pharmacol Toxicol*. 2015;55:633-656.
205. Cross NC, Reiter A. Fibroblast growth factor receptor and platelet-derived growth factor receptor abnormalities in eosinophilic myeloproliferative disorders. *Acta Haematol*. 2008;119:199-206.
206. Cools J, DeAngelo DJ, Gotlib J, et al. A tyrosine kinase created by fusion of the PDGFRA and FIP1L1 genes as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome. *N Engl J Med*. 2003;348(13):1201-1214.
207. Simon HU, Klion A. Therapeutic approaches to patients with hypereosinophilic syndromes. *Semin Hematol*. 2012;49:160-170.
208. Klion AD, Robyn J, Maric I, et al. Relapse following discontinuation of imatinib mesylate therapy for FIP1L1/PGGFRA-positive chronic eosinophilic leukemia: implications for optimal dosing. *Blood*. 2007;110(10):3552-3556.
209. Gleixner KV, Peter B, Blatt K, et al. Synergistic growth-inhibitory effects of ponatinib and midostaurin (PKC412) on neoplastic mast cells carrying KIT D816V. *Haematologica*. 2013;98(9):1450-1457.
210. Bleecker ER, Menzies-Gow AN, Price DB, et al. Systematic literature review of systemic corticosteroid use for asthma management. *Am J Respir Crit Care Med*. 2020;201:276-293.
211. St. Germain O, Lachapelle P, Pavord ID, Couillard S. Tackling "people remodelling" in corticosteroid-dependent asthma with Type-2 targeting biologics and a formal corticosteroid weaning protocol. *touchREVIEWS Respir Pulm Dis*. 2022;7:44-47.
212. Belvisi MG. Regulation of inflammatory cell function by corticosteroids. *Proc Am Thorac Soc*. 2004;1:207-214.
213. Ortega H, Llanos JP, Lafeuille MH, et al. Effects of systemic corticosteroids on blood eosinophil counts in asthma: real-world data. *J Asthma*. 2019;56(8):808-815.
214. Schleimer RP, Bochner BS. The effects of glucocorticoids on human eosinophils. *J Allergy Clin Immunol*. 1994;94(6):P1202-P1213.
215. Mukherjee M, Sehmi R, Nair P. Anti-IL5 therapy for asthma and beyond. *World Allergy Organ J*. 2014;7(1):32.
216. Fitzgerald JM, Bleecker ER, Nair P, et al. Benralizumab, an anti-interleukin-5 receptor α monoclonal antibody, as add-on treatment for patients with severe, uncontrolled, eosinophilic asthma (CALIMA): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet*. 2016;388(10056):2128-2141.
217. Haldar P, Brightling CE, Hargadon B, et al. Mepolizumab and exacerbations of refractory eosinophilic asthma. *N Engl J Med*. 2009;360(10):973-984.
218. Nair P, Pizzichini MM, Kjarsgaard M, et al. Mepolizumab for prednisone-dependent asthma with sputum eosinophilia. *N Engl J Med*. 2009;360(10):985-993.
219. Gevaert P, Van Bruaene N, Cattaert T, et al. Mepolizumab, a humanized anti-IL-5 mAb, as a treatment option for severe nasal polyposis. *J Allergy Clin Immunol*. 2011;128(5):989-995.
220. Buttgereit T, Bonnekoh H, Church MK, Bergmann KC, Siebenhaar F, Metz M. Effective treatment of a lymphocytic variant of hypereosinophilic syndrome with reslizumab. *J Dtsch Dermatol Ges*. 2019;17(11):1171-1172.
221. Kuang FL, Legrand F, Makiya M, et al. Benralizumab for PDGFRA-negative hypereosinophilic syndrome. *N Engl J Med*. 2019;380(14):1336-1346.
222. Plötz SG, Simon HU, Darsow U, et al. Use of an anti-interleukin-5 antibody in the hypereosinophilic syndrome with eosinophilic dermatitis. *N Engl J Med*. 2003;349(24):2334-2339.
223. Rothenberg ME, Klion AD, Roufosse FE, et al. Treatment of patients with the hypereosinophilic syndrome with mepolizumab. *N Engl J Med*. 2008;358(12):1215-1228.

224. Oldhoff JM, Darsow U, Werfel T, et al. Anti-IL-5 recombinant humanized monoclonal antibody (mepolizumab) for the treatment of atopic dermatitis. *Allergy*. 2005;60(5):693-696.
225. Straumann A, Conus S, Grzonka P, et al. Anti-interleukin-5 antibody treatment (mepolizumab) in active eosinophilic oesophagitis: a randomised, placebo-controlled, double-blind trial. *Gut*. 2010;59(1):21-30.
226. Simon D, Yousefi S, Cazzaniga S, et al. Mepolizumab failed to affect bullous pemphigoid: a randomized, placebo-controlled, double-blind phase 2 pilot study. *Allergy*. 2020;75(3):669-672.
227. Castro M, Mathur S, Hargreave F, et al. Reslizumab for poorly controlled, eosinophilic asthma: a randomized, placebo-controlled study. *Am J Respir Crit Care Med*. 2011;184(10):1125-1132.
228. Ortega HG, Liu MC, Pavord ID, et al. Mepolizumab treatment in patients with severe eosinophilic asthma. *N Engl J Med*. 2014;371(13):1198-1207.
229. Farne HA, Wilson A, Powell C, Bax L, Milan SJ. Anti-IL5 therapies for asthma. *Cochrane Database Syst Rev*. 2017;9(9):CD010834.
230. Agache I, Akdis CA, Akdis M, et al. EAACI biologicals guidelines – recommendations for severe asthma. *Allergy*. 2021;76(1):14-44.
231. Golebski K, Dankelman LHM, Bjorkander S, et al. Expert meeting report: towards a joint European roadmap to address the unmet needs and priorities of paediatric asthma patients on biologic therapy. *ERH Open Res*. 2021;7(4):00381–2021.
232. Gevaert P, Lang-Loidolt D, Lackner A, et al. Nasal IL-5 levels determine the response to anti-IL-5 treatment in patients with nasal polyps. *J Allergy Clin Immunol*. 2006;118(5):1133-1141.
233. Gibson PG, Prazma CM, Chupp GL, et al. Mepolizumab improves clinical outcomes in patients with severe asthma and comorbid conditions. *Respir Med*. 2021;22(1):171.
234. Han JK, Bachert C, Fokkens W, et al. Mepolizumab for chronic rhinosinusitis with nasal polyps (SYNAPSE): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Respir Med*. 2021;9(10):1141-1153.
235. Bachert C, Han JK, Wagenmann M, et al. EUFOREA expert board meeting on uncontrolled severe chronic rhinosinusitis with nasal polyps (CRSwNP) and biologics: definition and management. *J Allergy Clin Immunol*. 2021;147(1):29-36.
236. Fokkens WJ, Lund VJ, Hopkins C, et al. Executive summary of EPOS 2020 including integrated care pathways. *Rhinology*. 2020;58(2):82-111.
237. Hellings PW, Fokkens WJ, Orlandi R, et al. The EUFOREA pocket guide for chronic rhinosinusitis. *Rhinology*. 2023;61:85-89.
238. Reddel HK, Bacharier LB, Bateman ED, et al. Global initiative for asthma strategy 2021: executive summary and rationale for key changes. *Eur Respir J*. 2021;59(1):2102730.
239. Gevaert P, Calus L, Van Zele T, et al. Omalizumab is effective in allergic and nonallergic patients with nasal polyps and asthma. *J Allergy Clin Immunol*. 2013;131(1):110-116.e1.
240. Bachert C, Han JK, Desrosiers M, et al. 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*. 2019;394(10209):1638-1650.
241. Boyle JV, Lam K, Han JK. Dupilumab in the treatment of chronic rhinosinusitis with nasal polyposis. *Immunotherapy*. 2020;12(2):111-121.
242. Menzies-Gow A, Flood-Page P, Sehmi R, et al. Anti-IL-5 (mepolizumab) therapy induces bone marrow eosinophil maturational arrest and decreases eosinophil progenitors in the bronchial mucosa of atopic asthmatics. *J Allergy Clin Immunol*. 2003;111(4):714-719.
243. Kelly EA, Esnault S, Liu LY, et al. Mepolizumab attenuates airway eosinophil numbers, but not their functional phenotype, in asthma. *Am J Respir Crit Care Med*. 2017;196(11):1385-1395.
244. Smith SG, Chen R, Kjarsgaard M, et al. Increased numbers of activated group 2 innate lymphoid cells in the airways of patients with severe asthma and persistent airway eosinophilia. *J Allergy Clin Immunol*. 2016;137(1):75-86.e8.
245. Mukherjee M, Forero DF, Tran S, et al. Suboptimal treatment response to anti-IL-5 monoclonal antibodies in severe eosinophilic asthmatics with airway autoimmune phenomena. *Eur Respir J*. 2020;56(4):2000117.
246. McDowell PJ, Diver S, Yang F, et al. The inflammatory profile of exacerbations in patients with severe refractory eosinophilic asthma receiving mepolizumab (the MEX study): a prospective observational study. *Lancet Respir Med*. 2021;9(10):1174-1184.
247. Pavord ID, Buhl R, Kraft M, et al. Evaluation of sputum eosinophil count as a predictor of treatment response to mepolizumab. *ERJ Open Res*. 2022;8(2):00560–2021.
248. Barretto KT, Brockman-Schneider RA, Kuipers I, et al. Human airway epithelial cells express functional IL-5 receptor. *Allergy*. 2020;75(8):2127-2130.
249. Buchheit KM, Laidlaw TM, Levy JM. Immunology-based recommendations for available and upcoming biologics in aspirin-exacerbated respiratory disease. *J Allergy Clin Immunol*. 2021;148(2):348-350.
250. Levy BD, Noel PJ, Freemer MM, et al. Future research directions in asthma. An NHLBI working group report. *Am J Respir Crit Care Med*. 2015;192(11):1366-1372.
251. Akdis CA. Does the epithelial barrier hypothesis explain the increase in allergy, autoimmunity and other? *Nat Rev Immunol*. 2021;21(11):739-751.
252. Gleich GJ, Klion AD, Lee JJ, Weller PF. The consequences of not having eosinophils. *Allergy*. 2013;68(7):829-835.
253. Lugogo N, Domingo C, Chanez P, et al. Long-term efficacy and safety of Mepolizumab in patients with severe eosinophilic asthma: A multi-center, open-label, phase IIIb study. *Clin Ther*. 2016;38(9):2058-2070.
254. Murphy K, Jacobs J, Bjermer L, et al. Long-term safety and efficacy of Reslizumab in patients with eosinophilic asthma. *J Allergy Clin Immunol Pract*. 2017;5(6):1572-1581.
255. Roufosse FE, Kahn JE, Gleich GJ, et al. Long-term safety of mepolizumab for the treatment of hypereosinophilic syndromes. *J Allergy Clin Immunol*. 2013;131(2):461-467.
256. Jacobsen EA, Jackson DJ, Heffler E, et al. Eosinophil knockout humans: uncovering the role of eosinophils through eosinophil-directed biological therapies. *Annu Rev Immunol*. 2021;39:719-757.
257. Jackson DJ, Pavord ID. Living without eosinophils: evidence from mouse and man. *Eur Respir J*. 2023;61(1):2201217.
258. Wechsler ME, Ackerman SJ, Weller PF. In reply – are eosinophils needed for normal health? *Mayo Clin Proc*. 2022;97(4):805-807.
259. Gupta A, Ikeda M, Geng B, et al. Long-term safety and pharmacodynamics of mepolizumab in children with severe asthma with an eosinophilic phenotype. *J Allergy Clin Immunol*. 2019;144(5):1336-1342.
260. Khatri S, Moore W, Gibson PG, et al. Assessment of the long-term safety of mepolizumab and durability of clinical response in patients with severe eosinophilic asthma. *J Allergy Clin Immunol*. 2019;143(5):1742-1751.e7.
261. Kolbeck R, Kozhich A, Koike M, et al. MEDI-563, a humanized anti-IL-5 receptor alpha mAb with enhanced antibody-dependent cell-mediated cytotoxicity function. *J Allergy Clin Immunol*. 2010;125:1344-1353.
262. Mathur SK, Modena BD, Coumou H, Barker P, Kreindler JL, Zangrilli JG. Postbronchodilator lung function improvements with Benralizumab for patients with severe asthma. *Allergy*. 2020;75(3):669-672.
263. Nair P, Wenzel S, Rabe KF, et al. Oral glucocorticoid-sparing effect of Benralizumab in severe asthma. *N Engl J Med*. 2017;376(25):2448-2458.

264. Ferguson GT, FitzGerald JM, Bleecker ER, et al. Benralizumab for patients with mild to moderate, persistent asthma (BISE): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Respir Med*. 2017;5(7):568-576.
265. Laviolette M, Gossage DL, Gauvreau G, et al. Effects of benralizumab on airway eosinophils in asthmatic patients with sputum eosinophilia. *J Allergy Clin Immunol*. 2013;132(5):1086-1096.
266. Lommatzsch M, Marchewski H, Schwefel G, Stoll P, Virchow JC, Bratke K. Benralizumab strongly reduces blood basophils in severe eosinophilic asthma. *Clin Exp Allergy*. 2020;50(11):1267-1269.
267. Sehmi R, Lim HF, Mukherjee M, et al. Benralizumab attenuates airway eosinophilia in prednisone-dependent asthma. *J Allergy Clin Immunol*. 2018;141(4):1529-1532.
268. Pham TH, Damera G, Newbold P, Ranade K. Reductions in eosinophil biomarkers by benralizumab in patients with asthma. *Respir Med*. 2016;111:21-29.
269. Poznanski S, Mukherjee M, Zhao N, et al. Asthma exacerbations on benralizumab are largely non-eosinophilic. *Allergy*. 2021;76(1):375-379.
270. Criner GJ, Celli BR, Brightling CE, et al. Benralizumab for the prevention of COPD exacerbations. *N Engl J Med*. 2019;381(11):1023-1034.
271. Criner GJ, Celli BR, Singh D, et al. Predicting response to benralizumab in chronic obstructive pulmonary disease: analyses of GALATHEA and TERRANOVA studies. *Lancet Respir Med*. 2020;8(2):P158-P170.
272. Singh D, Bafadhel M, Brightling CE, et al. Blood eosinophil counts in clinical trials for chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2020;202(5):660-671.
273. Busse WW, Bleecker ER, FitzGerald JM, et al. Long-term safety and efficacy of benralizumab in patients with severe, uncontrolled asthma: 1-year results from the BORA phase 3 extension trial. *Lancet Respir Med*. 2019;7(1):46-59.
274. Geng B, Bachert C, Busse WW, et al. Respiratory infections and anti-infective medication use from phase 3 dupilumab respiratory studies. *J Allergy Clin Immunol Pract*. 2022;10(3):732-741.
275. Kuo CHS, Pavlidis S, Loza M, et al. T-helper cell type 2 (Th2) and non-Th2 molecular phenotypes of asthma using sputum transcriptomics in U-BIOPRED. *Eur Respir J*. 2017;49(2):1602135.
276. Busse W, Corren J, Lanier BQ, et al. Omalizumab, anti-IgE recombinant humanized monoclonal antibody, for the treatment of severe allergic asthma. *J Allergy Clin Immunol*. 2001;108(2):184-190.
277. Milgrom H, Berger W, Nayak A, et al. Treatment of childhood asthma with anti-immunoglobulin E antibody (omalizumab). *Pediatrics*. 2001;108(2):E36.
278. Solèr M, Matz J, Townley R, et al. The anti-IgE antibody omalizumab reduces exacerbations and steroid requirement in allergic asthmatics. *Eur Respir J*. 2001;18(2):254-261.
279. Bousquet J, Rabe K, Humbert M, et al. Predicting and evaluating response to omalizumab in patients with severe allergic asthma. *Respir Med*. 2007;101(7):1483-1492.
280. Djukanović R, Wilson SJ, Kraft M, et al. Effects of treatment with anti-immunoglobulin E antibody omalizumab on airway inflammation in allergic asthma. *Am J Respir Crit Care Med*. 2004;170(6):583-593.
281. Van Neerven RJJ, Knol EF, Ejrnaes A, Wurtzen PA. IgE-mediated allergen presentation and blocking antibodies: regulation of T-cell activation in allergy. *Int Arch Allergy Immunol*. 2006;141(2):119-129.
282. Hanania NA, Wenzel S, Rosén K, et al. Exploring the effects of omalizumab in allergic asthma. *Am J Respir Crit Care Med*. 2013;187(8):804-811.
283. Mukherjee M, Kjasgaard M, Radford K, et al. Omalizumab in patients with severe asthma and persistent sputum eosinophilia. *Allergy Asthma Clin Immunol*. 2019;15:21.
284. Chapman KR, Albers FC, Chipps B, et al. The clinical benefit of mepolizumab replacing omalizumab in uncontrolled severe eosinophilic asthma. *Allergy*. 2019;74(9):1716-1726.
285. Clayton F, Fang JC, Gleich GJ, et al. Eosinophilic esophagitis in adults is associated with IgG4 and not mediated by IgE. *Gastroenterology*. 2014;147(3):602-609.
286. Heil PM, Maurer D, Klein B, Hultsch T, Stingl G. Omalizumab therapy in atopic dermatitis: depletion of IgE does not improve the clinical course – a randomized, placebo-controlled and double-blind pilot study. *J Dtsch Dermatol Ges*. 2010;8(12):990-998.
287. Rocha R, Vitor AB, Trindade E, et al. Omalizumab in the treatment of eosinophilic esophagitis and food allergy. *Eur J Pediatr*. 2011;170(11):1471-1474.
288. Foroughi S, Foster B, Kim N, et al. Anti-IgE treatment of eosinophil-associated gastrointestinal disorders. *J Allergy Clin Immunol*. 2007;120(3):594-601.
289. Cruz AA, Lima F, Sarinho E, et al. Safety of anti-immunoglobulin E therapy with omalizumab in allergic patients at risk of geohelminth infection. *Clin Exp Allergy*. 2007;37(2):197-207.
290. Abraham I, Alhossan A, Lee CS, Kutbi H, MacDonald K. Real-life' effectiveness studies of omalizumab in adult patients with severe allergic asthma: systematic review. *Allergy*. 2016;71(5):593-610.
291. Gasser P, Tarchevskaya SS, Guntern P, et al. The mechanistic and functional profile of the therapeutic anti-IgE antibody ligelizumab differs from omalizumab. *Nat Commun*. 2020;11(1):165.
292. Trischler J, Bottoli I, Janocha R, et al. Ligelizumab treatment for severe asthma: learnings from the clinical development programme. *Clin Transl Immunol*. 2021;10(3):e1255.
293. Maurer M, Gimenez-Arnau AM, Sussman G, et al. Ligelizumab for chronic spontaneous urticaria. *N Engl J Med*. 2019;381:1321-1332.
294. Domingo C, Maspero JF, Castro M, et al. Dupilumab efficacy in steroid-dependent severe asthma by baseline oral corticosteroid dose. *J Allergy Clin Immunol Pract*. 2022;10(7):1835-1843.
295. Wechsler ME, Ford LB, Maspero JF, et al. Long-term safety and efficacy of dupilumab in patients with moderate-to-severe asthma (TRAVERSE): an open-label extension study. *Lancet Respir Med*. 2022;10(1):11-25.
296. Beck LA, Thaçi D, Hamilton JD, et al. Dupilumab treatment in adults with moderate-to-severe atopic dermatitis. *N Engl J Med*. 2014;371(2):130-139.
297. Silverberg JI, Rubini NPM, Pires MC, et al. Dupilumab treatment reduces hospitalizations in adults with moderate-to-severe atopic dermatitis. *J Allergy Clin Immunol Pract*. 2022;10(5):1279-1285.e1.
298. Fujieda S, Matsune S, Takeno S, et al. Dupilumab efficacy in chronic rhinosinusitis with nasal polyps from SINUS-52 is unaffected by eosinophilic status. *Allergy*. 2022;77(1):186-196.
299. Wechsler M, Klion A, Paggiaro P, et al. Effect of dupilumab treatment on blood eosinophil levels in patients with asthma, chronic rhinosinusitis with nasal polyps (CRSwNP), eosinophilic esophagitis (EoE), or atopic dermatitis (AD). *J Allergy Clin Immunol*. 2021;147(2):AB140.
300. Weinstein SF, Katial R, Jayawardena S, et al. Efficacy and safety of dupilumab in perennial allergic rhinitis and comorbid asthma. *J Allergy Clin Immunol*. 2018;142(1):171-177.
301. Hirano I, Dellon ES, Hamilton JD, et al. Efficacy of Dupilumab in a phase 2 randomized Trial of adults with active eosinophilic esophagitis. *Gastroenterology*. 2020;158(1):111-122.e10.
302. Bochner BS, Klunk DA, Sterbinsky SA, Coffman RL, Schleimer RP. IL-13 selectively induces vascular cell adhesion molecule-1 expression in human endothelial cells. *J Immunol*. 1995;154(2):799-803.
303. Dubois GR, Schweizer RC, Versluis C, Bruijnzeel-Koomen CA, Bruijnzeel PL. Human eosinophils constitutively express a functional interleukin-4-induced priming of chemotactic responses and induction of PI-3 kinase activity. *Am J Respir Cell Mol Biol*. 1998;19(4):691-699.

304. Castro M, Corren J, Pavord ID, et al. Dupilumab efficacy and safety in moderate-to-severe uncontrolled asthma. *N Engl J Med*. 2018;378(26):2486-2496.
305. Olaguibel JM, Sastre J, Rodriguez JM, Del Pozo V. Eosinophilia induced by blocking the IL-4/IL-13 pathway: potential mechanisms and clinical outcomes. *J Investig Allergol Clin Immunol*. 2022;32(3):165-180.
306. Lommatzsch M, Stoll P, Winkler J, et al. Eosinophilic pleural effusion and stroke with cutaneous vasculitis: two cases of dupilumab-induced hypereosinophilia. *Allergy*. 2021;76(9):2920-2923.
307. Marcant P, Balaye P, Mehri R, et al. Dupilumab-associated hypereosinophilia in patients treated for moderate-to-severe atopic dermatitis. *J Eur Acad Dermatol Venereol*. 2021;35(6):e394-e396.
308. Wollenberg A, Beck LA, Blauvelt A, et al. Laboratory safety of dupilumab in moderate-to-severe atopic dermatitis: results from three phase III trials (LIBERTY AD SOLO 1, LIBERTY AD SOLO 2, LIBERTY AD CHRONOS). *Br J Dermatol*. 2020;182(5):1120-1135.
309. Bakker DS, Ariens LFM, van Luijk C, et al. Goblet cell scarcity and conjunctival inflammation during treatment with dupilumab in patients with atopic dermatitis. *Br J Dermatol*. 2019;180(5):1248-1249.
310. Wechsler ME, Klion AD, Paggiaro P, et al. Effect of dupilumab on bloode osinophil counts in patients with nasal polyps, atopic dermatitis, or eosinophilic esophagitis. *J Allergy Clin Immunol Pract*. 2022;10:2695-2709.
311. Caminati M, Olivieri B, Dama A, et al. Dupilumab-induced hypereosinophilia: review of the literature and algorithm proposal for clinical management. *Expert Rev Respir Med*. 2022;16:713-721.
312. https://ginasthma.org
313. Verstovsek S, Tefferi A, Kantarjian H, et al. Alemtuzumab therapy for hypereosinophilic syndrome and chronic eosinophilic leukemia. *Clin Cancer Res*. 2009;15(1):368-373.
314. Strati P, Cortes J, Faderl S, Kantarjian H, Verstovsek S. Long-term follow-up of patients with hypereosinophilic syndrome treated with Alemtuzumab, an anti-CD52 antibody. *Clin Lymphoma Myeloma Leuk*. 2013;13(3):287-291.
315. Simpson EL, Parnes JR, She D, et al. Tezepelumab, an anti-thymic stromal lymphopoietin monoclonal antibody, in the treatment of moderate to severe atopic dermatitis: a randomized phase 2a clinical trial. *J Am Acad Dermatol*. 2019;80(4):1013-1021.
316. Corren J, Parnes JR, Wang L, et al. Tezepelumab in adults with uncontrolled asthma. *N Engl J Med*. 2017;377(10):936-946.
317. Emson C, Diver S, Chachi L, et al. CASCADE: a phase 2, randomized, double-blind, placebo-controlled, parallel-group trial to evaluate the effect of Tezepelumab on airway inflammation in patients with uncontrolled asthma. *Respir Med*. 2020;21(1):265.
318. Menzies-Gow A, Corren J, Bourdin A, et al. Tezepelumab in adults and adolescents with severe, uncontrolled asthma. *N Engl J Med*. 2021;384(19):1800-1809.
319. Diamant Z, Sidharta PN, Singh D, et al. Setipiprant, a selective CRTH2 antagonist, reduces allergen-induced airway responses in allergic asthmatics. *Clin Exp Allergy*. 2014;44(8):1044-1052.
320. Singh D, Cadden P, Hunter M, et al. Inhibition of the asthmatic allergen challenge response by the CRTH2 antagonist OC000459. *Eur Respir J*. 2013;41(1):46-52.
321. Gonem S, Berair R, Singapuri A, et al. Fevipiprant, a prostaglandin D2 receptor 2 antagonist, in patients with persistent eosinophilic asthma: a single-Centre, randomised, double-blind, parallel-group, placebo-controlled trial. *Lancet Respir Med*. 2016;4(9):699-707.
322. Pettipher R, Hunter MG, Perkins CM, et al. Heightened response of eosinophilic asthmatic patients to the CRTH2 antagonist OC000459. *Allergy*. 2014;69(9):1223-1232.
323. Horak F, Zieglmayer P, Zieglmayer R, et al. The CRTH2 antagonist OC000459 reduces nasal and ocular symptoms in allergic subjects exposed to grass pollen, a randomised, placebo-controlled, double-blind trial. *Allergy*. 2012;67(12):1572-1579.
324. Straumann A, Hoesli S, Ch B, et al. Anti-eosinophil activity and clinical efficacy of the CRTH2 antagonist OC000459 in eosinophilic esophagitis. *Allergy*. 2013;68(3):375-385.
325. Brightling CE, Gaga M, Inoue H, et al. Effectiveness of fevipiprant in reducing exacerbations in patients with severe asthma (LUSTER-1 and LUSTER-2): two phase 3 randomised controlled trials. *Lancet Repir Med*. 2021;9(1):43-56.
326. Legrand F, Cao Y, Wechsler JB, et al. Sialic acid-binding immunoglobulin-like lectin (Siglec) 8 in patients with eosinophilic disorders: receptor expression and targeting using chimeric antibodies. *J Allergy Clin Immunol*. 2019;143(6):2227-2237.
327. Accessed August 13, 2023. https://investor.allakos.com/news-releases/news-release-details/allakos-announces-topline-phase-3-data-enigma-2-study-and-phase/
328. Rasmussen HS, Chang AT, Tomasevic N, Bebbington C. Phase 1 double-blind, placebo-controlled, ascending dose study of Siglec-8 selective mAb AK002 in healthy subjects. *J Allergy Clin Immunol*. 2018;141(2):AB403.
329. Panch SR, Bozik ME, Brown T, et al. Dexpramipexole as an oral steroid-sparing agent in hypereosinophilic syndromes. *Blood*. 2018;132(5):501-509.
330. Laidlaw TM, Prussin C, Panettieri RA, et al. Dexpramipexole depletes blood and tissue eosinophils in nasal polyps with not change in polyp size. *Laryngoscope*. 2019;129(2):E61-E66.
331. Chen YL, Gutowska-Owsiak D, Hardman CS, et al. Proof-of-concept clinical trial of etokimab shows a key role for IL-33 in atopic dermatitis pathogenesis. *Sci Transl Med*. 2019;11(515):eaax2945.
332. Schmid-Grendelmeier P, Altznauer F, Fischer B, et al. Eosinophils express functional IL-13 in eosinophilic inflammatory diseases. *J Immunol*. 2002;169(2):1021-1027.
333. Hanania NA, Noonan M, Corren J, et al. Lebrikizumab in moderate-to-severe asthma: pooled data from two randomised placebo-controlled studies. *Thorax*. 2015;70(8):748-756.
334. Panettieri RA Jr, Sjöbring U, Péterffy A, et al. Tralokinumab for severe, uncontrolled asthma (STRATOS 1 and STRATOS 2): two randomised, double-blind, placebo-controlled, phase 3 clinical trials. *Lancet Respir Med*. 2018;6(7):511-525.
335. Simpson EL, Flohr C, Eichenfield LF, et al. Efficacy and safety of lebrikizumab (an anti-IL-13 monoclonal antibody) in adults with moderate-to-severe atopic dermatitis inadequately controlled by topical corticosteroids: a randomized, placebo-controlled phase II trial (TREBLE). *J Am Acad Dermatol*. 2018;78(5):863-871.
336. Russell RJ, Chachi L, FitzGerald JM, et al. Effect of tralokinumab, an interleukin-13 neutralising monoclonal antibody, on eosinophilic airway inflammation in uncontrolled moderate-to-severe asthma (MESOS): a multicentre, double-blind, randomised, placebo-controlled phase 2 trial. *Lancet Respir Med*. 2018;6(7):499-510.
337. Gutermuth J, Pink AE, Soldbro L, Bjerregard Oland C, Weidinger S. Tralokinumab plus topical corticosteroids in adults with severe atopic dermatitis and inadequate response to or intolerance of ciclosporin A: a placebo-controlled, randomized, phase III clinical trial (ECZTRA 7). *Br J Dermatol*. 2022;186(3):440-452.
338. Jesenak M, Diamant Z. Blood eosinophils: in quest of a holy grail for personalized asthma treatment with biologicals. *Allergy*. 2020;75(6):1294-1297.
339. Wood LJ, Sehmi R, Dorman S, et al. Allergen-induced increases in bone marrow T lymphocytes and interleukin-5 expression in subjects with asthma. *Am J Respir Crit Care Med*. 2002;166(6):883-889.
340. Johansson K, Malmhäll C, Ramos-Ramírez P, Rådinger M. Bone marrow type 2 innate lymphoid cells: a local source of interleukin-5 in interleukin-33-driven eosinophilia. *Immunology*. 2018;153(2):268-278.
341. Hogan MB, Piktel D, Landreth KS. IL-5 production by bone marrow stromal cells: implications for eosinophilia associated with asthma. *J Allergy Clin Immunol*. 2000;106(2):329-336.

342. Bressler RB, Lesko J, Jones ML, et al. Production of IL-5 and granulocyte-macrophage colony-stimulating factor by naive human mast cells activated by high-affinity IgE receptor ligation. *J Allergy Clin Immunol*. 1997;99(4):508-514.
343. Dubucquoi S, Desreumaux P, Janin A, et al. Interleukin 5 synthesis by eosinophils: association with granules and immunoglobulin-dependent secretion. *J Exp Med*. 1994;179(2):703-708.
344. Bhalla A, Zhao N, Rivas DD, et al. Exacerbations of severe asthma while on anti-IL-5 biologics. *J Investig Allergol Clin Immunol*. 2020;30(5):307-316.
345. Andreev D, Liu M, Kachler K, et al. Regulatory eosinophils induce the resolution of experimental arthritis and appear in remission state of human rheumatoid arthritis. *Ann Rheum Dis*. 2021;80(4):451-468.
346. Narla S, Silverberg JI, Simpson EL. Management of inadequate response and adverse effects to dupilumab in atopic dermatitis. *J Am Acad Dermatol*. 2022;86(3):628-636.
347. Murphy MJ, Cohen JM, Vesely MD, Damsky W. Paradoxical eruptions to targeted therapies in dermatology: a systematic review and analysis. *J Am Acad Dermatol*. 2022;86(5):1080-1091.
348. Corren J, Pham TH, Garcia Gil E, et al. Baseline type 2 biomarker levels and response to tezepelumab in severe asthma. *Allergy*. 2022;77:1786-1796.
349. Diver S, Khalfaoui L, Emson C, et al. Effect of tezepelumab on airway inflammatory cells, remodelling, and hyperresponsiveness in patients with moderate-to-severe uncontrolled asthma (CASCADE): a double-blind, randomised, placebo-controlled, phase 2 trial. *Lancet Respir Med*. 2021;9:1299-1312.
350. Dorey-Stein ZL, Shenoy KV. Tezepelumab as an emerging therapeutic option for the treatment of severe asthma: evidence to date. *Drug Des Devel Ther*. 2021;15:331-338.
351. Sverrild A, Hansen S, Hvidtfeldt M, et al. The effect of tezepelumab on airway hyperresponsiveness to mannitol in asthma (UPSTREAM). *Eur Respir J*. 2021;59:2101296.

How to cite this article: Jesenak M, Diamant Z, Simon D, et al. Eosinophils—from cradle to grave. *Allergy*. 2023;78:3077-3102. doi:10.1111/all.15884