Eosinophil-associated diseases (EADs) are a group of disorders with multisystem manifestations, where eosinophils play a primary role. These disorders can impact the skin, upper and lower airways, cardiovascular system, connective tissues, gastrointestinal tract, the hematopoietic and immune system as well as other organs. Due to paucity of diagnostic and treatment options and limited awareness of EADs among clinical researchers and treating physicians, there is usually poor quality of life outcome. Corticosteroids are the mainstay of therapy for EADs, but toxicity due to long-term use and lack of a durable response necessitate the need for targeted therapies. Allogenic bone marrow/peripheral blood stem cell transplantation may be considered in patients who are refractory to drug therapy. The pace of clinical research and emergence of novel targeted therapies for EADs is accelerating; however, progress remains hindered by knowledge gaps in the eosinophil biology and pathophysiology of EADs.

EADs compromise quality of life and are associated with significant morbidity and occasional fatal outcomes. Eosinophilia patients face diagnostic and therapeutic challenges. Evaluation requires a multidisciplinary approach where a hematologist plays a central role. Optimal diagnostic evaluation and long-term monitoring are essential for managing such patients.

Eosinophilia is established when the absolute eosinophil count (AEC) in the peripheral blood is ≥500 eosinophils/µL. The degree of eosinophilia is categorized into mild (500 to 1500 eosinophils/µL), moderate (1500 to 5000 eosinophils/µL), or severe (>5000 eosinophils/µL).1 Hypereosinophilia (HE) is defined as moderate to severe eosinophilia (≥1500 eosinophils/µL). End-organ manifestations may be present but are not essential for the HE designation. Hypereosinophilic syndrome (HES) is a diagnosis of exclusion that must fulfill two criteria, i.e., HE (AEC ≥1500/µL) on at least two occasions and signs of organ dysfunction attributable to the eosinophilia.1,8,9

Patients with moderate to severe eosinophilia may remain asymptomatic or develop signs of organ dysfunction many years after eosinophilia is first detected. With the availability of newer diagnostic tests and targeted therapies, identification of specific etiologies of HES continues to grow.

The eosinophilic granulocyte or the eosinophil—first described by Paul Ehrlich in the 19th century—remains shrouded in mystery even two centuries later. The eosinophil is a type of white blood cell that has a pro-inflammatory role in the immune system such as movement to inflamed areas, trapping substances, killing cells, antiparasitic and bactericidal activity, participating in immediate allergic reactions, and modulating inflammatory responses. Eosinophils have been considered to be terminally differentiated cytotoxic effector cells that exert their action by cytolytic degranulation (lysis of eosinophils to release their contents).1,2
Recent studies have transformed this simple view of eosinophils and their function. Fresh understanding of the molecular pathways that control the development, trafficking, and degranulation of eosinophils points toward specialized immunomodulatory functions of the eosinophils and their role in promoting homeostasis.

Current studies reveal a controlled, piecemeal degranulation (release of specific mediators packed in vesicles in response to stimuli) that leaves the eosinophils viable. Eosinophils have a highly evolved role in the pathogenesis of diseases such as asthma, HESs and esophagitis.

Eosinophils develop in the bone marrow from pluripotent progenitors and are released into the blood in a mature state. They migrate to the thymus, gastrointestinal tract, mammary glands, and uterus to reside there under homeostatic conditions. Eosinophils are activated and recruited into tissues in response to stimuli such as Interleukin-5 (IL-5) and the eotaxin chemokines. It is understood that chromosomal abnormalities in myeloproliferative hypereosinophilic syndrome (MHES) cause clonal proliferation of eosinophils. For most of the other EADs, the cause of dysregulated eosinophil function and the subsequent disease state remains to be defined.

### EOSINOPHILIA BY ORGAN SYSTEM

**Hematopoietic system**
- Chronic myeloid disorders (molecularly defined)
  - Bcr/Abl+ chronic myeloid leukaemia
  - PDGFRα-rearranged eosinophilic disorder (SM-CEL)
  - PDGFRβ-rearranged eosinophilic disorder
  - KIT-mutated systemic mastocytosis
  - 8p11 syndrome
- Acute leukaemia-myeloid

**Respiratory system**
- IEP (Idiopathic eosinophilic pneumonia)
- Churg–Strauss syndrome
- Hypereosinophilic syndrome
- Tropical pulmonary eosinophilia
- ABPA (Allergic broncho-pulmonary aspergillosis)
- Radiation induced pulmonary eosinophilia
- Bronchial asthma
- Allergic rhinitis
- Episodic angioedema with eosinophilia
- Occupational lung disease
- Chlamydial pneumonia of infancy
- Coccidiomycosis

**Skin**
- Atopic dermatitis
- Exfoliative dermatitis
- Dermatitis herpetiformis
- Pemphigus
- Psoriasis
- Urticaria

**GI tract**
- Eosinophilic gastrointestinal diseases (EGID)-Eosinophilic esophagitis, Eosinophilic gastritis, Eosinophilic gastroenteritis, Eosinophilic colitis
- Hepatobiliary disease-Eosinophilic hepatitis
- Food allergy (milk-protein allergy)
- Helminthic infestation

**Cardiovascular system**
- Endomyocardial fibrosis
- Pericarditis
- Eosinophilic coronary arteritis

**Immune system**
- Eosinophilia-myalgia syndrome, toxic oil syndrome
- Eosinophilic fascitis (Schulman syndrome), Kimura disease, Wells syndrome, Omenn syndrome
- Eosinophilic synovitis
- Connective tissue diseases (scleroderma, polyarteritis nodosa)
- Sarcoidosis, SLE, Sjogren’s syndrome, Rheumatoid arthritis
- Inflammatory bowel disease
- Chronic pancreatitis
- Congenital immunodeficiency syndrome
- Graft-vs-host-disease
Eosinophilia may not be taken into account during routine clinical encounters due to lack of awareness of EADs. There is a general lack of understanding of the role of eosinophil-derived mediator cytokines such as eosinophil cationic protein (ECP), eosinophil major basic proteins (MBP1 and MBP2), eosinophil peroxidase (EPO), and eosinophil-derived neurotoxin (EDN) in the development of HES-related symptoms and organ damage such as fibrosis and tissue remodeling. ICD 10 coding is not available for some eosinophilic disorders, which makes reporting and tracking of such disorders a challenging task. This limits the determination of true incidence and prevalence, thereby causing a negative impact on the development of novel therapies and medical reimbursement for EADs.

**Diagnosis**

The final diagnosis of EADs is based on clinical, molecular, and histopathological (biopsy) findings. Diagnosis of clonal or primary eosinophilia requires investigations such as blood smear and bone marrow morphology, cytogenetic and immunophenotypic analysis. Screening for molecular aberrations such as FIP1L1-PDGFRA/B gene fusion, or FGFR1 gene fusion is undertaken. Based on the underlying etiology, diagnosis of secondary eosinophilia could need stool examination, stool culture, and antibody testing for parasites. Lung manifestations are investigated with pulmonary function tests, bronchoscopy, and sputum testing for eosinophilia, chest x-ray, serology aspergillosis and CT scan of chest. Cardiac complications are confirmed with EKG, echocardiography, chest X-ray and MRI Scan. Endoscopy and colonoscopy are essential while investigating eosinophilic esophagitis/colitis.

**Advances in diagnostics**

The development of antibodies for interleukin-5 receptor subunit-α (IL-5R), chemokine receptor 3 (CCR3), eosinophil peroxidase (EPX), and eosinophil derived neurotoxin (EDN) for detection of eosinophils by flow cytometry, immunohistochemistry, and ELISA will fast track basic and clinical research to understand the role of eosinophils in disease states.

**Eosinophilia: Molecular aberrations, cytokines and the immune system**

Understanding and characterizing associations of eosinophilia with various molecular aberrations, cytokines and immune profile is expected to lead to a more appropriate patient segmentation, and devising of appropriate individualized patient management strategies in the future.

**Molecular aberrations and eosinophilia**

FIP1L1-PDGFRα is the most frequently identified fusion gene and has been identified as a clonal marker associated with the myeloproliferative features of HE. The FIP1L1–PDGFRA fusion has also been found in cases of acute myeloid leukemia (AML) and T-cell lymphoblastic lymphoma, which are characterized by eosinophilia either preceding or around the time of diagnosis, or due to persistence of eosinophilia even after treatment. Patients with FIP1L1-PDGFRα respond well to treatment with tyrosine kinase inhibitors (TKIs) such as imatinib. Classically, acquired eosinophilias may be clonal or reactive/secondary in origin. Most common molecular aberrations noted in clonal eosinophilias are tyrosine kinase (TK) fusion genes leading to constitutive activation of TK activity. Frequently identified fusion genes are PDGFRA (platelet-derived growth factor receptor-α), e.g., FIP1L1-PDGFRα (FIP1-like–1–platelet-derived growth factor receptor-α); PDGFRB (platelet-derived growth factor receptor-β), e.g., ETV6-PDGFRB and; FGFR1, e.g., ZNF198-FGFR1.

Significant eosinophilia is also a common feature for BCR-ABL1-positive chronic myeloid leukemia (CML) at diagnosis. KIT D816V and JAK2 V617F mutations have also been observed in a small percentage of patients with HE. These molecular aberrations have a diagnostic, prognostic, and therapeutic relevance since they can be targeted for development of therapies. Treatment options for patients without underlying imatinib-sensitive PDGFRA or PDGFRB TK fusion genes are limited. However, for patients with KIT D816V or JAK2 V617F point mutations, the TK inhibitors (ruxolitinib or midostaurin) may offer potential targeted treatment.
Cytokines and eosinophilia

In secondary (reactive) eosinophilias, there is a complex interplay of cytokines released from eosinophils and other inflammatory cells. Eosinophilic inflammation is considered to be triggered by the release of type 2 cytokines (IL-5, IL-4, and IL-13). Mast cells are also a potential source of type 2 cytokines. IL-5 is the major cytokine that influences eosinophilopoiesis and in combination with IL-9, it helps to recruit mast cells and eosinophils to an affected tissue site. Inflammatory reactions also result in immunoglobulin class switch and IgE production by B cells. IgE subsequently binds to mast cells and eosinophils residing in the tissue, enabling them to release their toxic granules on antigen binding.

Of the multiple receptors and cytokines that modulate the functioning of eosinophils, the key receptors that define their specialized function are interleukin-5 receptor subunit-α (IL-5Rα), chemokine receptor 3 (CCR3), sialic acid-binding immunoglobulin-like lectin 8 (SIGLEC-8), and pattern recognition receptors (PRRs) such as toll like receptor-7 (TLR7). Humanized IL-5-specific monoclonal antibodies (mepolizumab and reslizumab) have been approved for eosinophilic asthma, while a humanized IL-5Rα-specific monoclonal antibody (benralizumab) is undergoing clinical trials for dysregulated eosinophilia.

IL-5, IL-25, IL-33, and IL-23 are some of the key cytokines that play a central role in the development, activation, and survival of eosinophils. Like IL-5, the receptors for these cytokines could be exploited for therapeutic purposes.

The immune system and eosinophilia

Eosinophils cause antigen-specific stimulation of T cells and B cells leading to cytokine release and humoral immune response. Eosinophil interactions with macrophages, dendritic cells, and mast cells prolong eosinophil survival.

Eosinophil research has made considerable advances and based on the current understanding, the immune response mounted by the eosinophils is far more complex than previously considered. Targeted and piecemeal degranulation by eosinophils in response to the specific stimuli and their complex interaction with other leukocytes and pathogens needs to be studied further.

Attempts at targeting eosinophils for therapeutic purposes have led to better understanding and segmentation of diseases such as bronchial asthma and HESs. The role of exogenous and endogenous PRR ligands such as receptor for advanced glycation end products (RAGE), TLR, and vascular endothelial growth factor (VEGF) in eosinophilic responses and the relationship between piecemeal degranulation and tissue remodeling could be the next frontiers of eosinophil research.

A task force on research needs for EADs (TREAD) was instituted by the National Institutes of Health (NIH) and the Office for Prevention of Diseases for the purpose of defining, clarifying, and prioritizing the unmet research and supportive needs of EADs. The purpose of the workshop report was to promote the translation of research into clinical practice and health policy.

**UNMET NEED ACROSS EOSINOPHILIC DISEASES**

<table>
<thead>
<tr>
<th>Specific ICD 10 codes</th>
<th>Unavailability of specific ICD 10 codes for all EADs; Patient Advocacy groups have succeeded in getting several EADs enlisted in the ICD coding.</th>
</tr>
</thead>
<tbody>
<tr>
<td>FDA-approved therapies</td>
<td>FDA-approved therapies for majority of EADs required as it is challenging for patients to obtain reimbursement for expensive therapies when used off-label.</td>
</tr>
<tr>
<td>Well characterized incidence and prevalence data for EADs</td>
<td>Uncertainty about EAD patient numbers (due to lack of well conducted epidemiology studies) lowers the incentive for pharmaceutical companies to invest in such niche indications.</td>
</tr>
</tbody>
</table>
| Collaborative approach for guidance and resources for diagnosis and management of patients | Limited patient numbers and a general lack of understanding of pathogenesis of EADs requires better access to
  - **Diagnostic and treatment** guidelines; referral to EAD experts will improve standard of care and access to clinical trials
  - **Information on diagnostic testing methodologies** such as immunostaining, molecular diagnostics at commercial testing sites or research-oriented medical institutions will facilitate patient care |

(Contd..)
Robust EAD pipeline includes 21 assets

Five assets (mepolizumab, benralizumab, budesonide, fevipiprant, and masitinib) are in Phase III, and 16 assets are in Phase I or Phase II of clinical development.

Biologics – front runners in management of EADs?

Corticosteroids or chemotherapy for treating EADs may be associated with resistance and/or toxicity issues. Biologics have a targeted approach and a steroid-sparing effect.

Additionally, eosinophil depletion has a minimal impact on host immune functions. With such advantages, biologics, when driven by distinct clinical phenotypes and biomarkers, could emerge as favored therapies in the management of EADs. Nucala (mepolizumab) and Cinqair (reslizumab) are approved for add-on maintenance therapy in severe asthma patients with eosinophilic phenotype.

| Understanding of the pathophysiology of EADs | • Greater understanding of the pathophysiology of EADs would aid in development of non-invasive and reliable biomarkers for better diagnosis, patient management, and development of new therapies
| Collaboration among clinicians, researchers, and pharmaceutical companies | • Agreement on clinically relevant endpoints for clinical trials and with advocacy groups to obtain orphan status for various EADs can help in development of targeted therapies.
| A centralized storage facility for preservation of bio-specimens | • A centralized storage facility and a digital warehouse for collation of data for identification of biomarkers for EADs will help to
| Development of new patient-based registries | • Development of new registries or expansion of an existing registry such as REGID (registry for eosinophilic gastrointestinal disorders) to connect patients, clinical researchers, patient advocacy groups, research funding agencies, and EAD research centers.
| Duration of disease and severity of eosinophilia | • Predicting the duration of disease and severity of eosinophilia that would result in tissue damage

### BIOLOGICS TARGETING IL-5

| Asthma | • IL-5 plays a pivotal role in eosinophil differentiation, activation, and survival. Anti-IL-5 antibodies, mepolizumab and reslizumab, are approved for eosinophilic asthma.
| HES | • Mepolizumab has shown efficacy in clinical trials in patients with FIP1L1/PDGFRA-negative HES but was not approved in EU by CHMP due to lack of evidence that mepolizumab was effective in reducing the need for corticosteroid therapy. In the US, a pivotal Phase III trial is underway. It is currently available only for compassionate use in patients with life-threatening, treatment-refractory disease.
| Eosinophilic granulomatosis with polyangiitis (EGPA) | • Mepolizumab has shown benefit in corticosteroid-dependent EGPA and is undergoing trials.
| Eosinophilic esophagitis (EoE) | • Studies have failed to show efficacy of mepolizumab or reslizumab in adult or pediatric patients with EoE.
| Atopic dermatitis | • Mepolizumab has been ineffective in controlling symptoms of severe atopic dermatitis despite marked reduction of peripheral eosinophilia.
| Nasal polyposis | • Reslizumab and mepolizumab could emerge as novel treatment options in patients with severe nasal polyposis. A European multicenter, placebo-controlled study of mepolizumab for nasal polyposis is underway.
Eosinophil modulating therapies

A number of soluble mediators such as IgE, IL-4, IL-13, thymic stromal lymphopoietin (TSLP), IL-25, and IL-33 are associated with eosinophilic inflammation. Therapies targeting these mediators are under development.

**EOSINOPHIL MODULATING THERAPIES**

**IgE**
- The anti-IgE antibody, omalizumab (Xolair), decreases peripheral blood eosinophilia but has failed to show benefit toward resolution symptoms in eosinophilic gastritis, duodenitis, or esophagitis.

**IL-4 and IL-13**
- IL-4 and IL-13 cytokines are produced by a variety of cell types such as CD4+ Th2 lymphocytes, type 2 innate lymphoid cells (ILC2), mast cells, basophils, and eosinophils. Based on reduction in blood and airway eosinophilia in murine models of allergic inflammation, trials of monoclonal antibodies to IL-4, IL-13, and their receptors have been initiated in asthma, atopic dermatitis, and EoE.
- Clinical trials of monoclonal antibodies targeting IL-4 (pascolizumab) in asthma have not been encouraging. Clinical trials of anti-IL-13 antibody (tralokinumab, lebrikizumab) have shown benefit only in a subset of asthma patients.

**IL-4Rα**
- Antibody to IL-4Rα (dupilumab) has shown benefit and is undergoing trials in asthma in patients with both low and high levels of eosinophils.

**TSLP (thymic stromal lymphopoietin)**
- TSLP is released from epithelial cells in response to eosinophilia through production of IL-5 by activated Th2 lymphocytes. Single nucleotide polymorphisms in TSLP are associated with increased or decreased susceptibility to asthma, atopic disease, and EoE. A proof-of-concept trial of anti-TSLP antibody in patients with asthma has shown significant reduction of peripheral and sputum eosinophilia, allergen-induced bronchoconstriction, and airway inflammation.

**JAK2**
- Some patients of HE carry the JAK2 V617F-activating mutation. The recently approved JAK1/2 inhibitor ruxolitinib as well as other JAK inhibitors in the pipeline may have a role in treatment of eosinophilic disorders such as clonal eosinophilia with JAK2 rearrangement.
- The JAK2 pathway also mediates anti-apoptosis signals in eosinophils in response to GM-CSF and IL-5. Inhibition of this signaling cascade could be exploited to develop therapies for eosinophilic disorders regardless of their subtype.
Significant efforts are needed to bridge the knowledge gaps in the current understanding of the biology of eosinophils and their pathophysiologic role in the EADs to foster research for novel therapies and improve patient outcomes. The importance of clinical phenotype and biomarkers in the evaluation of therapeutic response in EADs has emerged from post-hoc analyses of clinical trials in bronchial asthma. With the increasing number of biologic therapies, the choice of best-suited therapy will depend on an in-depth understanding of advantages and disadvantages of such therapies with respect to the disease pathophysiology in that indication.

References: