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Revisiting the NIH Taskforce on the Research needs of Eosinophil-Associated Diseases (RE-TREAD)

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DISCLOSURE

P.A. has received honoraria for advisory boards for G.S.K. and AstraZeneca, receives royalties from UpToDate™, and has consulted for Ambrx and Advance Medical. J.R.A. is an employee of Genentech, Inc., holds stock/stock options in Roche, and is a named inventor on patent applications related to biomarkers of eosinophilic disorders. S.J.A. is a co-inventor of the Esophageal String Test (EST) for EoE and co-founder of EnteroTrack, LLC that is developing the EST for clinical use, and is the CSO and a paid consultant for EnteroTrack. He has had recent consulting agreements with and received honoraria from Receptos (Celgene) and Knopp Biosciences, and is a member of the APFED Medical Advisory Panel. B.S.B. receives research funding from the National Institutes of Health and Acerta Pharma. He has current or recent consulting or scientific advisory board arrangements with, or has received honoraria from Sanofi-Aventis, TEVA, GSK, AstraZeneca, and Allakos, Inc., and owns stock in Allakos. He receives publication-related royalty payments from Elsevier and Wolters Kluwer, and is a co-inventor on existing Siglec-8-related patents and thus may be entitled to a share of royalties received by Johns Hopkins University on the potential sales of such products. B.S.B. is also a co-founder of Allakos, Inc. which makes him subject to certain restrictions under University policy. The terms of this arrangement are being managed by the Johns Hopkins University and Northwestern University in accordance with their conflict of interest policies. MHC is a consultant for Shire, Regeneron, Receptos; has received research funding from Shire, Regeneron, Receptos; is Chair for a Medical Advisory Panel, American Partnership for Eosinophilic Disorders (APFED); and is Member of The International Gastrointestinal Eosinophil Researchers (TIGERS). P.C.F. has received grants from the National Institutes of Health, has served as a consultant for Genentech, Inc., and has received research funding from Knopp Biosciences, LLC. J.E.K. has received honoraria for advisory boards from GSK. G.J.G. and K.M.L. (spouses) receive royalties or have royalty sharing agreements with Ception, Cephalon, Teva, Mayo Foundation, and UpToDate™, have consulted for GSK and Knopp, have received grants from AstraZeneca, GSK, and NIH, and serve on the board of APFED and the Beiersdorf Dermatology Advisory Panel. S.K.M. has served on advisory boards for Astra-Zeneca. C.P. is an employee of Knopp Biosciences and has equity interest in the company.

M.E.R. is a consultant for Pulm One, Spoon Guru, Celgene, Shire, Astra Zeneca, GSK, Allakos, Adare, Regeneron, and Novartis, and has an equity interest in the first 3 listed and immune pharmaceuticals, and royalties from Reslizumab (Teva Pharmaceuticals). M.E.R. is an inventor of patents, owned by Cincinnati Children's. F.R. has received honoraria for serving on advisory boards from GSK and Knopp. H.U.S. has received honorarium for serving on a scientific advisory board for Knopp. J.S. is a full-time employee and shareholder of GSK. A.J.W. has received honorariums for advisory boards from GSK, Astra Zeneca, TEVA, and Knopp. M.E.W. has received honoraria from Astra-Zeneca, Teva, GSK, Boehringer Ingelheim, Novartis, Boston Scientific, Sanofi, and Regeneron. P.F.W. has received honorariums for advisory boards from GSK and Knopp.

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AUTHORSHIP

P.K., P.A., and A.K. wrote and edited the manuscript. All listed authors were either participants, speakers, or key experts in the development of this manuscript and have critically evaluated, edited (B.S.B., S.J.A.), and/or written parts of this review.

SUPPORTING INFORMATION

Additional information may be found online in the supporting information tab for this article.

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Abstract

Eosinophil-associated diseases (EADs) are rare, heterogeneous disorders characterized by the presence of eosinophils in tissues and/or peripheral blood resulting in immunopathology. The heterogeneity of tissue involvement, lack of sufficient animal models, technical challenges in working with eosinophils, and lack of standardized histopathologic approaches have hampered progress in basic research. Additionally, clinical trials and drug development for rare EADs are limited by the lack of primary and surrogate endpoints, biomarkers, and validated patient-reported outcomes. Researchers with expertise in eosinophil biology and eosinophil-related diseases reviewed the state of current eosinophil research, resources, progress, and unmet needs in the field since the 2012 meeting of the NIH Taskforce on the Research of Eosinophil-Associated Diseases (TREAD). RE-TREAD focused on gaps in basic science, translational, and clinical research on eosinophils and eosinophil-related pathogenesis. Improved recapitulation of human eosinophil biology and pathogenesis in murine models was felt to be of importance. Characterization of eosinophil phenotypes, the role of eosinophil subsets in tissues, identification of biomarkers of eosinophil activation and tissue load, and a better understanding of the role of eosinophils in human disease were prioritized. Finally, an unmet need for tools for use in clinical trials was emphasized. Histopathologic scoring, patient- and clinician-reported outcomes, and appropriate coding were deemed of paramount importance for research collaborations, drug development, and

approval by regulatory agencies. Further exploration of the eosinophil genome, epigenome, and proteome was also encouraged. Although progress has been made since 2012, unmet needs in eosinophil research remain a priority.

Keywords

biomarkers; eosinophil-related disorders; eosinophilia; hypereosinophilic syndromes; murine models; translational research

1 | INTRODUCTION

In June 2012, a meeting of the National Institute of Health (NIH) Taskforce on the Research of Eosinophil-Associated Diseases (TREAD) was convened with the aim of defining, clarifying, and prioritizing the unmet research and supportive needs of EADs, defined as rare conditions presenting with eosinophils in the peripheral blood and/or tissues with associated pathology. Although the number of citations with “eosinophil” in the subject or as a keyword has increased in the past 5 years (Fig. 1) and the clinical development and approval of therapeutic agents targeting eosinophils in more common eosinophil-related diseases, such as asthma, has brought new focus to the eosinophil, unmet needs remain in research of basic eosinophil biology and rare EADs. The Taskforce consisted of nationally and internationally recognized, basic, and clinical eosinophil researchers and patient advocacy groups (PAGs). The following 7 common unmet needs across the spectrum of EADs were identified: (1) creation of diagnostic codes to identify and define patient subsets; (2) in depth analysis of biological samples (to help standardize markers of eosinophilic tissue involvement and better understand disease pathogenesis); (3) development and validation of reliable testing for diagnosis and assessment of exacerbation of eosinophilic disease; (4) mechanistic understanding of the role of eosinophils in various EADs; (5) establishment of patient registries; (6) improved guidance and success of relevant clinical trials in rare disease patient populations; and (7) expansion of the number of useful therapeutics for treating EADs.[1] In response to this publication, the National Institute of Allergy and Infectious Diseases (NIAID) issued two funding announcements, which were co-sponsored by the National Heart Lung and Blood Institute, the National Institute of Arthritis and Musculoskeletal and Skin Diseases, and the National Institute of Diabetes and Digestive and Kidney Diseases. These announcements covered both exploratory (R21) and more established (R01) investigator-initiated basic and clinical/translational projects.

In 2017, an updated taskforce, “RE-TREAD”, was organized during a meeting of the International Eosinophil Society to bring together a multidisciplinary group of clinicians and scientists with expertise in rare eosinophil-related disorders to discuss issues related to patient care and clinical trial design. A second meeting goal was to continue to foster collaborative relationships between multidisciplinary clinical and basic science researchers with the aim of improving diagnosis and treatment of EADs. This publication summarizes the conclusions of RE-TREAD and the state of eosinophil research in 2017. It also creates a road map for clinicians, PAGs, and other entities concerned with the health and treatment of patients with rare EADs.

2 | METHODS

RE-TREAD was convened during the biennial meeting of the International Eosinophil Society in Göthenburg, Sweden in July 2017. Presentations covered the current status of eosinophil clinical research and disease categorization including diagnostic codes, use of registries, tissue markers of eosinophil pathogenesis including granule proteins, “omics”, histopathology, basic mechanisms in humans and animal models, biomarkers of eosinophilia and eosinophil-associated clinical outcome metrics, clinical trials, and novel therapeutics. Focus groups discussed prioritization of unmet needs and identification of action items for clinicians, researchers, and stakeholders involved in eosinophil-related research.

3 | RESULTS

In contrast to TREAD, which took an organ and disease-specific approach to define unmet needs in individual EADs, RE-TREAD focused on the following 3 main areas important to EADs as a whole: (1) The role of eosinophils and eosinophil activation in the pathogenesis of EADs, (2) clinical and translational studies in EADs, and (3) development and standardization of novel techniques for the study of eosinophils and eosinophil-mediated pathogenesis.

3.1 | The role of eosinophils in the pathogenesis of EADs

Despite the presence of eosinophils and evidence of eosinophil activation in the blood and tissues of patients with EADs, the precise role of eosinophils in disease pathogenesis remains unclear. The reasons for this are multifactorial and include the heterogeneity and complexity of these disorders, the lack of standardized methods to evaluate and target eosinophil-associated pathology, limitations in the recapitulation of human EADs in animal models, and gaps in our understanding of the basic biology of human eosinophils. Recent progress in these areas in the context of novel therapeutics targeting eosinophils, as well as recommendations for future research, are summarized in the next section.

3.1.1 | Eosinophil histopathology—Standardization of methods for evaluation of eosinophil involvement in tissue pathology was identified by TREAD as an unmet need common to all EADs. Specific suggestions for eosinophilic esophagitis (EoE) included counting of eosinophils in biopsies as cells per high power field (/(HPF)) or per unit area, use of mean versus peak counts, consideration of pathologic features other than eosinophil numbers, and detection and validation of eosinophil involvement using immunohistochemical staining for eosinophil granule proteins. Despite significant progress, a number of issues remain. For example, although interobserver agreement among pathologists trained in the counting of eosinophils in esophageal biopsies (per unit area and per HPF) was high,[2] re-review of slides reported to have peak eosinophil counts 1-14/HPF yielded higher counts in >20% of the cases.[3] To diminish over-reliance on a single pathologic feature, an EoE histology scoring system (EoEHSS) was developed. The EoEHSS scores 8 features (eosinophil inflammation, basal zone hyperplasia, eosinophil abscess, eosinophil surface layering, dilated intercellular spaces, surface epithelial alteration, dysker-atotic epithelial cells, and lamina propria fibrosis) for both severity (grade) and extent (stage) of disease and was shown to outperform peak eosinophil count in differentiating

treated from untreated EoE patients.[4] Using a different approach, immunohistochemical staining for eosinophil peroxidase, EoE could be differentiated from gastroesophageal reflux disease (GERD),[5] although the sensitivity and specificity of this assay has still not been directly compared to EoEHSS.

Standardized methods to evaluate eosinophils, or their products, outside of the esophagus, were recognized as an unmet need by TREAD, but still do not exist. Comprehensive evaluations of eosinophilic gastritis (EG) and eosinophilic colitis (EC) biopsies by a panel of pathologists are underway in the Consortium of Eosinophilic Gastrointestinal Researchers (CEGIR (Table 1)). It is anticipated that data generated from this consortium will help standardize methods to evaluate gastrointestinal biopsies across multiple centers and identify the relationship, if any, between eosinophilic gastrointestinal disorders (EGID) and other inflammatory bowel diseases.

TREAD also recommended the standardization and validation of methods to evaluate tissues to improve the differential diagnosis and treatment for organ-restricted EAD. Some progress has been made. For example, using stains to identify Th1 (T-bet and CD4) or Th2 (GATA and CD4), a significantly lower Th1/Th2 cell ratio was identified in morphea compared to eosinophilic fasciitis, facilitating the distinction between these two disorders that share multiple histopathologic features (dermal inflammation including eosinophil infiltration and fibrosis extending into fascia).[6] Similarly, in the esophagus, T-bet (Th1) and GATA-3 (Th2) expression may help distinguish between EoE and gastroesophageal reflux disease (GERD). Finally, additional noninvasive approaches have been developed since TREAD, including the Cytosponge[7,8] and esophageal string test (EST).[9] Assessment of eosinophil granule protein levels using either of these methods has been shown in preliminary studies to correlate with eosinophil counts in matched biopsies.

Future efforts should aim to (i) continue to standardize and validate general methods for evaluating tissue eosinophilia and eosinophil-mediated pathology; (ii) develop additional noninvasive approaches for use as histopathologic surrogates in eosinophil-related diseases; (iii) standardize and validate methods for evaluation of eosinophils in organ-specific conditions (in particular EG, EC), but also in other organs, including skin; and (iv) examine the relationships between EADs and other overlapping inflammatory conditions with associated eosinophilia (e.g., EGID and inflammatory bowel disease, or hypereosinophilic syndromes (HES), EGPA, and IgG4-related disease).

3.1.2 | Animal models of eosinophilic disease—At the time of TREAD, experts felt that existing murine models did not accurately recapitulate EADs, although they were felt to be important in understanding the basic biologic functions of eosinophils. TREAD recommended utilization of “humanized” murine models to better model human disease but recognized that species differences in soluble mediators and tissue proteins needed to be addressed. Since 2012, significant progress has been made in both the development of new murine models and their use in elucidating the complex role of eosinophils in allergic diseases. Further, these models have facilitated the recognition of beneficial functions of eosinophils, including their role in metabolism, tissue regeneration, immune homeostasis, and putative functions for eosinophils in non-eosinophilic diseases (reviewed in Ref. 10).

Because murine models can be manipulated in ways that are not currently possible in humans, they are critical to studies of eosinophil function.[11] Murine models have provided significant insight into allergic inflammation,[12] including knowledge of eosinophil structure and function.[11] Importantly, mice deficient in IL-5,[13] overexpressing IL-5,[14,15] or injected with IL-5[16] have provided information relevant to development of biologics targeting the IL-5 axis. Eosinophils in mice contribute to maintenance of long-lived plasma cells in the bone marrow,[17] IgA production in the intestine,[17,18] modulation of the intestinal microbiome,[19] the metabolic activity of alternatively activated macrophages in adipose tissue,[20] and tissue regeneration in skeletal muscle[21] and liver.[22] Murine models (as well as some data in humans) suggest that recruited, IL-5 dependent, inflammatory eosinophils in allergic inflammation are distinct from IL-5 independent, resident lung eosinophils.[23] Since the last TREAD meeting, novel strains of mice, including iPHIL[12] and eoCre,[24] have been developed to manipulate the kinetics or gene-specific expression within eosinophils (Table 1). Together, these models and earlier strains (for review Ref. 25,26) highlight underappreciated roles for mouse eosinophils, both in disease and in the maintenance of normal tissue homeostasis.

Eosinophils have multifaceted interactions with the immune system, which have been studied extensively in mice. Eosinophils have been shown to exhibit phenotypic changes in response to signals from the extracellular matrix in murine asthma models.[27,28] They can recruit Th2 $CD4^+$ T cells,[29] activate dendritic cells,[30] polarize M2 macrophages[31] cause nerve cell branching,[32] recruit and activate CD8 T cells,[33,34] and secrete nerve growth factor or eosinophil peroxidase which activate mast cells through TrkA[35] or Mas-related gene X2 (MrgX2)[36] receptors, respectively. The interactions between eosinophils and other cells are just starting to be explored in humans.

In a mouse model of severe asthma, EPX, but not eosinophil granule major basic protein-1 (eMBP-1) induced goblet cell metaplasia,[37] suggesting that the pattern of eosinophil granule protein release may be an important driver of a particular disease pathology. As such, granule specific assays, such as the EPX ELISA or EPX immunohistochemistry (Table 1, Supplementary Table S1), will help clarify these relationships and are likely to play a significant role in future assessments of eosinophil function in human eosinophilic and non-eosinophilic disorders.

Despite expanded availability of murine models (Table 1, Supplementary Table S2 and S3) for the study of eosinophil biology, participants recognized the need for better recapitulation of human pathology. For example, although murine models have provided unique insight into roles of individual granule proteins, murine eosinophils are relatively refractory to degranulation in mostmodels. Additional issues that complicate extrapolation of murine findings to humans include differential activation of Th1/Th2/Th17 pathways between the species, altered expression patterns and function of murine and human surface receptor paralogues and orthologues (e.g., Siglec-F and Siglec-8, EGF-like module-containing mucin-like hormone receptor-like 1 (EMR-1) and F4/80), and the absence of murine genes encoding soluble mediators, such as eotaxin-3, EoE gene products, such as calpain-14,[38] and Charcot-Leyden Crystal (CLC) protein/Galectin-10[39] for which intracellular and extracellular roles, for example, in granulogenesis during human eosinophil development,

secretion of granule cationic proteins, and glycan-containing ligands, remain to be determined. Finally, no murine models exist that create “humanized” eosinophil mice as has been done, for example, with mast cells,[40] and current data from newly derived murine models (e.g., non-irradiated NBSGW mice engrafted with hematopoietic cells) have not been specifically evaluated for engraftment of eosinophils using available eosinophil cell surface markers.[41] None of the currently available models adequately replicate the various subtypes of HES or EGPA.

Future efforts should aim to further refine and recapitulate eosinophil biology in murine models including (i) improvement of murine models of eosinophil degranulation in tissues; (ii) further assessment of unique human targets (e.g., eotaxin-3, calpain-14, Charcot-Leyden Crystal Protein (CLC)/Galectin-10, and ECP); (iii) recapitulation of EADs in murine models; (iv) investigations of hematopoietic transfer models in mice to evaluate eosinophil engraftment with the hope that humanized eosinophil mice might allow better investigation of the development, maintenance, and inflammatory responses of human eosinophils; and (v) further assessment of the role of eosinophils, both beneficial and harmful, in homeostasis and non-EADs.

3.1.3 | Eosinophils in human disease—Delineating the basic biology of human eosinophils remains an important goal. Many critical insights into the basic biology of human eosinophils or other cells that interact with eosinophils have been gained since the TREAD meeting in 2012, including the recognition that eosinophils may: (1) interact with and activate innate type 2 lymphoid cells (ILC2s),[42] (2) serve as important sources of cytokines and other mediators, (3) possess the ability to secrete functional, cell-free, tissue-deposited granules, (4) exhibit differential phenotypes (e.g., resident and inflammatory subsets), and (5) exhibit crosstalk with other cells, such as mast cells,[43] and other non-hematopoietic cells (though a discussion of all these interactions is beyond the scope of this review [reviewed in Ref. 44-46], and 6) may be beneficial in some non-EADs (e.g., in some cancers [reviewed in Ref. 47]. Despite this progress, there is still much to learn regarding the interaction of eosinophils and their response to tissue components, such as matrix proteins,[48] fibroblasts,[49] epithelium, and other tissue-resident cells.[50] It is known that the engagement and expression of integrins can engage matrix proteins, such as fibronectin and laminin, and that these interactions alter eosinophil biology including degranulation and survival.[51,52] Whether other changes in the tissue microenvironment (e.g., during morphogenesis, aging, wound development, and repair) influence the biology of resident and recruited eosinophils is less clear. Cell surface lectin receptors, such as Siglec-8, can engage specific glycan ligands on tissue and may selectively influence eosinophils.[53,54] This bidirectional response and understanding of tissue microenvironments will be an important concept for future study in EADs.

Whereas a better understanding of basic eosinophil function is clearly a priority, well-designed translational studies are required for the application of basic science advances to human disease. These, in turn, depend on the development of clear diagnostic criteria, consistent nomenclature for standardization of acquisition, analyses, and histopathologic evaluation of biopsies and establishment of validated biomarkers of disease activity and treatment outcomes in clinical trials and daily practice. Although blood biomarkers tend to

be favored for simplicity and ease of access, biomarkers based on other biological specimens, such as urine or nasal secretions, and organ-specific biomarkers and scoring systems are also needed.

3.1.4. | Eosinophil granule proteins—Eosinophils store a diverse array of cytokines and chemokines preformed within intracellular granules. Tissue eosinophils respond to external stimuli with rapid and differential secretion of eosinophil granule-derived cytokines through a vesicle-mediated process called piecemeal degranulation, ultrastructural evidence of which is readily observed in tissue eosinophils across all EADs. In addition, tissues and secretions from multiple EADs exhibit cell-free, membrane-bound eosinophil granules (reviewed in Ref. 10) that express ligand-reactive receptors on their outer membranes and remain competent to undergo stimulus-dependent, differential secretion.[55] Therefore, there is evolving recognition that eosinophil-derived organelles (i.e., vesicles and free granules) are deposited within tissues as a consequence of cytolytic modes of eosinophil cell death and function as instruments of local tissue damage and/or immunoregulation even in the absence of intact eosinophils. Since the 2012 TREAD report, several studies have begun to identify mechanisms that regulate the expulsion of eosinophil cell-free granules in tissues, both independent from and in association with the extrusion of DNA "traps".[56-60] A more comprehensive understanding of regulated cell death pathways contributing to this process, and the physiological consequences of tissue-deposited eosinophil cell-free granules is needed. Moreover, efficacies of new and existing proposed therapies (further described in the section on *Expansion of new Therapeutic Areas in EADs*) must be measured by criteria not only based on depletion of intact eosinophils, but also with careful attention paid to the presence of residual free granules within tissues.

3.1.5 | Tissue eosinophils and eosinophil subtypes—Eosinophils are produced in the bone marrow from $CD34^+$ eosinophil lineage-committed progenitors (EoP) that can mobilize from the bone marrow and accumulate at sites of allergic inflammation.[61] EoPs may undergo in situ differentiation (i.e., extramedullary hematopoiesis [EMH])[62,63] into mature cells and can contribute to tissue inflammation through production of locally produced cytokines and promotion of EMH with resultant tissue eosinophilia. Better understanding of the role of locally produced cytokines that promote EoP differentiation, EoP accumulation, and blockade of this process is needed to help clarify the processes underlying maintenance and resolution of tissue eosinophilia in EADs.

Although it has long been recognized that eosinophils can have an activated phenotype upon stimulation with IL-5, IL-3, IL-33, or GM-CSF, recent data[23] suggest that there may be differential eosinophil activation by cytokines,[64] and perhaps different subtypes in the human lung. Resident lung eosinophils from non-asthmatic lungs have distinct surface marker expression (Siglec-$8^+$$CD62L^+$IL-$3R^{lo}$) in comparison to inflammatory eosinophils in the sputum of subjects with asthma (Siglec-$8^+$$CD62L^{lo}$IL-$3R^{hi}$), and different eosinophil subtypes may exist in human skin under pathological conditions.[65] The functional consequences of these differences in surface marker expression have not been determined definitively in humans, although corresponding murine data suggest that resident eosinophils have a regulatory phenotype. Recent and prior studies have noted differential gene

expression patterns between tissue-resident eosinophils,[23,66] suggesting that the local environment promotes an eosinophil phenotype with tissue-specific functions. Colonic eosinophils have been shown to be protective in *Clostridium difficile* infections in both mice and humans.[67] Notably, eosinophils promoted intestinal barrier integrity and survival in mice that was independent of eosinophil-derived IL-4 or bactericidal activity. The specific protective mechanism remains unknown, highlighting the need for a deeper understanding of the factors regulating the development of functional eosinophil subtypes, their responses to environmental signals, and the beneficial and harmful consequences of their activities in eosinophil homeostasis, pathogenesis, and trafficking. Although the mechanisms of trafficking have begun to be delineated[68-70] in asthma, additional focus on mechanisms of cell trafficking in specific EADs would be timely. Utilization of single cell analyses, including single cell RNAseq and CyTOF mass spectroscopy, to tease out clusters in heterogeneous populations will likely be helpful in this regard.

Future efforts should aim to (i) delineate mechanisms that control cardinal eosinophil functions (e.g., migration and survival), that both elicit and shape the differential secretion of functionally diverse preformed mediators; (ii) identify subtypes and functions of eosinophils unique to different tissues and mechanisms of eosinophil recruitment to specific organs; (iii) delineate the contribution of local eosinophilopoiesis in EAD pathogenesis via identification of EoP-specific targeting strategies in affected tissues; (iv) better characterize the normal homeostatic, protective, and pathogenic functions of eosinophils in EADs and non-EADs; (v) better characterize cell death pathways of eosinophils, particularly the mechanisms and triggers for, and outcomes of, deposition of cell-free granules in humans; and (vi) continue to explore the relationships between eosinophils and other cells, including lymphocytes, mast cells, ILC2s, fibroblasts, adipocytes, mesenchymal stem cells, and epithelial cells, and their role in EADs.

3.2 | Clinical and translational studies in EADs

Since the TREAD meeting, there has been a dramatic increase in the development and approval of novel therapies for treatment of both common and rare EADs. While this is exciting, some rare EADs remain understudied due to (1) difficulties in recruitment of rare patients and collecting of prospective samples during multicenter studies from which to perform mechanistic research, (2) adequately powered studies from which to draw evidence-based conclusions, and (3) logistical challenges that arise from attempts to create cohesive research plans with uniform collection of biological samples, data-coordination, and analysis. Investigators studying rare EADs would benefit from the creation of research networks and registries for collaboration and pooling of resources. In addition, a comprehensive guide to the resources (Table 1) and organizations that provide funding (Table 3) to researchers in rare diseases, specifically in EADs, would help foster increased engagement of new investigators in this area. Recommendations for overcoming these challenges are summarized in the next section.

3.2.1 | Registries—The Rare Disease Clinical Research Network (RDCRN) program allows multidisciplinary teams to partner across several institutions to facilitate patient recruitment, accelerate young investigator training, and engage patient support through

partnership with PAGs. RDCRN has collaborated with PAGs and the pharmaceutical industry on government-sponsored clinical research studies to be completed in a timely fashion and to bring novel therapeutics to market. An EAD-associated registry was borne out of RDCRN through the CEGIR (U54 AI117804), an initiative of the Office of Rare Disease Research (ORDR) that is jointly funded by NCATS, NIAID, and NIDDK.[71] This registry (part of a longitudinal observational study) is designed to understand the natural history of EGID and will help standardize histopathologic evaluation and assessment of these rare disorders. Although some EADs are included in larger registries (e.g., EGPA is included in the Vasculitis Clinical Research Consortium (VCRC)), the only network to date that attempts to capture all of the varied forms of HES is the French network, Centre de Référence National des Syndromes Hyper-eosinophiliques. This network has recruited more than 800 patients to date and has resulted in the publication of several large clinical series.[72,73] Replication of these efforts in other countries would allow confirmation of the French findings in different epidemiologic settings, identify pools of patients for potential participation in clinical studies, and provide patient data to address important issues in the diagnosis and treatment of EADs.

3.2.2 | Patient and clinician reported outcome measures—The development and validation of disease-specific quality of life (QOL) and disease control indices are needed to measure the patient's perspective of symptoms and disease impacts, and to evaluate the efficacy of treatment and/or other interventions in EADs. These measures, whether patient reported outcomes (PRO) or clinician-reported outcomes, are often required by regulatory authorities for use in clinical licensing trials. Currently validated metrics exist for only a handful of EADs and focus only on symptoms related to the affected organ system. In asthma, previously validated endpoints (such as AQLQ/ACQ)[74,75] and exacerbation rates have been used for trials of eosinophilic asthma; whereas, newly developed PROs and QOLs had to be created for eosinophilic esophagitis (PedsQL (Pediatric Quality of Life Inventory),[76] EoE-QOL-A (EoE Quality of Life-Adults),[77] and PEESS v2.0 (Pediatric Eosinophilic Esophagitis Symptom Severity)).[78] The lack of similar metrics in other EADs, including EGID involving the stomach, small bowel, and colon, are due, in large part, to the heterogeneity of symptoms when multiple organs are affected. The confounding effects of medication-related side effects can also hinder the development of PROs. Because of the limited opportunities for validation of instruments in rare diseases, careful consideration of the design and choice of instruments across centers of excellence in EADs are important so that results from different studies can be compared. In a recent double-blind, placebo controlled trial evaluating mepolizumab in patients with EGPA,[79] a clinician reported outcome measure (EGPA-CAS) specific for EGPA was developed and is being compared to the Birmingham Vasculitis Activity Scale(BVAS) that was developed for use in vasculitis in general. Results from this study, when available, may provide additional tools for study of eosinophil-related symptoms.

3.2.3 | Academia-industry collaborations—The importance of leveraging clinical trials to collect data to enhance understanding of underlying disease mechanisms in EADs was uniformly endorsed by the RE-TREAD participants. One example of this is the EGPA study mentioned above that was co-sponsored by the NIH and GlaxoSmithKline (GSK), and

included extramural NIH-funding to support mechanistic studies at 5 US academic sites.[79] Standard operating procedures were implemented for tracking blood, sputum, and urine samples through a data coordinating center in order to collect blood samples for quantification of cytokines and other mediators for analysis of treatment response and evaluation of exacerbations, and to collect blood and tissue for transcriptome profiling for exploration of mechanisms of disease. During the conduct of the study, special attention was paid to operating procedures for internal governance and transparency requirements, especially in consideration of FDAsubmission of this pivotal phase 3 study. This collaboration serves as a model for future design of clinical studies in EADs with incorporation of a priori defined research goals, and pooling of resources, expertise, and rare-disease patients across multiple institutions.

3.2.4 | Creation of more specific diagnostic codes for EADs—The United States applies a clinical modifier (ICD10-CM) to the International Classification of Diseases (ICD) codes approved by the World Health Organization. These are then approved by major organizations, including the American Hospital Association and Centers for Medicare and Medicaid Services, and govern classification of disease and reasons for visits in most health care settings. ICD-CM codes are important for payors and providers of healthcare services, but, more importantly, availability of specific ICD codes allows clinicians to identify and accurately diagnose rare diseases. Unique ICD codes can improve disease management and treatment choices, and may result in improved resource allocation and understanding of unmet needs in rare EADs. From a research perspective, utilization of more specific codes is important for better understanding of prevalence, treatment, and healthcare utilization associated with these diseases as well as the identification of subjects for potential recruitment into clinical trials. Once codes are available, dissemination of this key change will be important for incorporation by clinicians into workflows.

In the 2012 TREAD, the lack of unique codes for EADs was identified as an unmet need since most EADs, with the exception of Löffler's syndrome and Pulmonary Eosinophilia, would have been classified under the umbrella ICD9 code of "Eosinophilia". Fortunately, since 2012, more specific codes for EGIDs (eosinophilic esophagitis, eosinophilic gastritis or gastroenteritis, and eosinophilic colitis) have been incorporated into ICD-10-CM. Unfortunately, little progress had been made for other EADs as of the 2017 RE-TREAD meeting. A clinical workgroup was convened to prioritize additional specific codes. Within a few months of the meeting, 11 EADs (Table 2) were suggested to APFED (American Partnership for Eosinophilic Disorders) in light of their prior success of this organization in advancing codes for specific EGIDs.

3.2.5 | Biomarkers—Although peripheral absolute eosinophil counts (AEC) have traditionally been used for diagnosis and monitoring of treatment in EADs, their use as biomarkers in the setting of clinical trials has not been validated. Nevertheless, placebo-controlled studies of mepolizumab for HES[80] and EGPA[79] that used a central laboratory to standardize counts across centers demonstrated a reduction in AEC associated with disease improvement and control, suggesting that reduction of the AEC is a useful biomarker of disease activity in certain EADs. Although intuitively it would seem that tissue eosinophilia

should be a superior biomarker of eosinophil-mediated pathology, technical issues, including the lack of standardization across affected tissues and variability between readers (see Histopathology section above), as well the requirement for biopsy samples, have precluded the widespread use of tissue eosinophilia as a biomarker in clinical trials. Where this has been attempted, the results have been mixed. For example, clinical trials of mepolizumab and reslizumab in EoE did not meet their primary endpoints despite reduction in tissue eosinophilia in esophageal biopsies.[81-83] Whether this was due to lack of sufficient tissue depletion, irreversible structural changes in the esophagus, a lack of direct eosinophil contribution to disease pathogenesis, or other factors is unclear.

Markers of eosinophil activation, including eosinophil granule proteins levels in blood, tissue, and body fluids, may be more easily quantifiable markers of disease activity and have been used as biomarkers in some studies, including a multicenter, double-blind, placebo-controlled trial of mepolizumab in HES,[80] asthma,[84,85] and EoE.[9] However, differences in the specific granule proteins measured, and methodologies used between studies, currently make it difficult to draw generalizable conclusions.

A major advancement since the TREAD meeting is the development of noninvasive techniques to assess eosinophil granule deposition. These include the use of body fluids, such as saliva, stool, and urine, as surrogates for tissue biopsies and the esophageal string test.[9] To capture a standardized metric of blood eosinophilia and activation states, a gene expression metric was derived for blood samples collected in PAXgene RNA tubes as part of a clinical trial of anti-IL-13 antibody in asthma.[37] This eosinophil-related gene signature metric is neither age dependent nor lab dependent and may capture activation states as well as proportions of eosinophils in a peripheral blood sample. The applicability of this metric to other EADs remains to be seen.

Several biomarkers have been developed to characterize type 2/eosinophilic inflammation in asthma, including fractional exhaled nitric oxide (FeNO), and serum or plasma periostin protein levels.[86] These biomarkers correlate with eosinophilic airway inflammation in asthma, but have not been explored more broadly in EADs. Additional examples of biomarkers with applicability restricted to a single or selected group of EADs include TARC/CCL17,[87] or quantification of the clonal population[88] in lymphoid HES, functional or structural markers of disease in EoE (e.g., EndoFLIP to measure esophageal distensibility), and tyrosine kinase mutations in myeloid HES. Further demonstration of the validity of these biomarkers is needed.

3.2.6 | Expansion into new therapeutic areas in EADs—Since the TREAD meeting in 2012, 3 biologics that specifically target eosinophils by binding IL-5 (mepolizumab, reslizumab) or its receptor (benralizumab) have been approved for the treatment of eosinophilic asthma.[89-91] Despite this exciting advance in therapeutic options for asthma and the recent FDA approval of mepolizumab for adult patients with EGPA (at a dose of 300 mg sc monthly), progress in the clinical development of targeted therapies for other rare EADs has been slow. The safety and efficacy of mepolizumab (Nucala®, GlaxoSmithKline) as a steroid-sparing agent in HES was demonstrated in a multicenter, double-blind, placebo-controlled phase 2 trial nearly 10 years ago;[80] yet, mepolizumab at

the dose used in this study is currently available only off-label or in the setting of a compassionate use protocol for HES. Both mepolizumab and reslizumab (Cinqair®; Teva Pharmaceuticals) have been studied in eosinophilic esophagitis, although results have been equivocal (see above). Benralizumab (Fasenra™, AstraZeneca), a mAb against the IL-5 receptor that causes antibody-dependent cell-mediated cytotoxicity, has been shown to deplete blood and tissue eosinophils in patients with asthma.[89,92] A phase 2 trial was recently completed in patients with treatment-refractory HES (NCT02130882), and pilot studies are currently underway in EGPA and EGID (Clinicaltrials.gov NCT03010436).

Unrelated to the IL-5 axis, a new small molecule agent, dex-pramipexole (Knopp Biosciences), was noted to have coincidental eosinophil-lowering activity during its initial clinical development in amyotrophic lateral sclerosis.[93] In an open-label phase 2 study in patients with chronic rhinosinusitis with nasal polyps (CRSwNP), dexpramipexole lowered blood eosinophils by 94% from baseline ($P < 0.001$) and polyp eosinophils by 97% ($P = 0.001$).[94] Promising results of a recently completed phase 2 trial in HES should be available soon.[95] Clinical trials of other agents that target eosinophils, including antibodies to Siglec-8, are planned or ongoing for non-EADs (NCT02734849; NCT02808793; NCT03379311) and are likely to provide additional insights into EADs in future planned trials.

Finally, a number of agents that target pathways important in eosinophilic inflammation, rather than eosinophils themselves, are being studied or have been approved for a variety of common disorders where eosinophils may play a role. These include anti-IL-13 (lebrikizumab, tralokinumab, RCP4046), anti-IL-4Rα (dupilumab), anti-TSLP (tezepelumab),[96] and anti-IL-31 (nemolizumab), various of which have demonstrated efficacy in eosinophilic asthma,[97] atopic dermatitis (NCT02525094[98],[99]), nasal polyposis,[100] and EoE (NCT02379052[101]).

An important secondary benefit of clinical trials of targeted agents in these disorders is the mechanistic insights provided regarding the role of eosinophils in disease pathogenesis. For example, the efficacy of mepolizumab, reslizumab, and benralizumab in eosinophilic asthma confirmed a role for eosinophils in asthma exacerbations, but also helped to promote the concept of asthma endotypes.[102] In contrast, the demonstration that antibodies to IL-13 and IL-4Rα are also effective in reducing exacerbations and FeNO levels in eosinophilic asthma, despite a transient increase in circulating eosinophils,[34] highlights the differences between blood and tissue eosinophils and their roles in disease. As the numbers of targeting therapies continue to expand, it will become increasingly important to determine the EADs, and perhaps even subtypes of EADs, that are most likely to benefit from available agents. Future studies should address the positive and negative consequences of targeting multiple pathways in EADs.

Future efforts in clinical and translational research should aim to (i) discover biomarkers of tissue specificity and scoring systems for assessment of disease activity and treatment outcomes for use in clinical trials and daily practice; (ii) delineate epidemiology, diagnostic criteria, organ-specific biomarkers, and endotypes of EADs so that a personalized medicine approach can be used; (iii) identify novel biomarkers of eosinophil involvement, activation

status, and disease activity in prospectively collected and archived human samples; (iv) design adequately powered clinical studies to identify biomarkers for disease management and for discovery of potential novel therapeutic approaches, for example, by inhibition of additional key players in eosinophil biology such as eosinophil chemoattractants, as well as IL-33 and TSLP; (v) facilitate interactions between academia and industry early in clinical trial development for biomarker discovery. Of particular importance is the comparison of the efficacy of conventional and novel therapies for specific EADs and the creation of optimized treatment algorithms and personalized approaches to patient selection for novel therapeutics; (vi) advance new codes for EADs for incorporation and use in ICD10-CM and ICD11-CM; (vii) promote funding guidelines that include support for eosinophil mechanistic studies not strictly aligned to a particular EAD entity; and (vii) perform clinical trials for comparison of the efficacy of conventional and novel therapies for specific EADs and for creation of optimized treatment algorithms.

3.3 | New biological techniques applied to EADs

Major advances in biomedical technology, bioinformatics, and their application since 2012 have led to novel approaches to study EADs. A significant reduction in the cost of genome sequencing and a corresponding ability to aggregate and analyze large datasets has made the application of these approaches to the “eosinophilome” possible. The potential of these developments to deliver precision and personalized care[103] in EADs cannot be overemphasized.

3.3.1 | “Omics”—Notable advances include application of “omic” approaches, particularly proteomics and transcriptomics to human eosinophil immunobiology. An in-depth assessment of the eosinophil proteome with demonstration of upregulation of phosphoproteins with IL-5 stimulation was described.[104] The proteome and phosphoproteome were also noted to change after stimulation, including responses to IL-5, IL-3, and GM-CSF.[105] Other studies show the value of applying transcriptomics to human eosinophil biology, with evolution from gene array studies to RNAseq methodology. Gene array analysis showed upregulated transcripts in bronchoalveolar lavage eosinophils after segmental allergen challenge,[106] and TGF-β target genes in peripheral blood eosinophils were shown to be expressed by RNAseq.[107] Transcriptome analysis of esophageal tissue specimens was shown to be effective in making molecular diagnoses of EoE and differentiating EoE from reflux disease,[108] and glycomics analyses are being used to identify glycoproteins in mucins and other endogenous tissue structures capable of directly interacting with eosinophil lectin receptors[109] further paving the way for personalized approaches to diagnosis of EADs. Identified future challenges include mapping the interaction between the microbiome and eosinophil functional responses and characterization of eosinophil-specific and eosinophil subset-specific signatures in healthy and diseased tissues using single cell sequencing and other novel techniques.

The use of laser capture dissection and single cell sequencing,[110] multiplex florescence in-situ hybridization analysis,[111] and omics[112] have already proven useful in the study of new targets in murine models. Gene editing and clustered regularly interspaced short palindromic repeat (CRISPR) and CRISPR-associated (Cas) technology provide faster generation of

floxed mice to genes of interest in eosinophils, and in cell lines CRISPR/Cas has been used to study the *FIP1L1-PDGFRA* fusion gene to further understand mechanisms in myeloid HES.[113] Conceivably, CRISPR may be used with humanized mice in the future as a tool to manipulate transferred human eosinophils.

Outstanding questions in the field of EADs include the lack of predictors for the development of EADs, as well as optimal treatments for tissue specific manifestations, such as in EGIDs. Using an omics approach, it is hopeful that phenotypic and genomic data, can be analyzed as a function of environment, epigenome, transcriptome, proteome, metabolome, glycome, and microbiome. Indeed, genomic data has already identified regions that confer susceptibility to eosinophilia.[114] The key transcriptional programs responsible for eosinophil development are emerging.[115] The eosinophil proteome has been preliminarily mapped out,[104] and eosinophils have been shown to regulate the intestinal microbiome.[18] De novo mutations and genome wide methylation patterns responsible for HES have been initiated.[116] The EoE transcriptome has been uncovered, providing new insight into disease pathogenesis and providing the framework for a diagnostic PCR-based diagnostic panel.[108,117] Such an approach has facilitated new drug development such as anti-Type 2 cytokine therapy.[118]

Future efforts in the area of technology, biomedical advances, and "big data" should aim to (i) expand and further analyze the eosinophil epigenome, including eosinophil subtypes; (ii) characterize the metabolome of eosinophils, including eosinophil subtypes; (iii) define the environmental factors that may interact with eosinophilomes; (iv) integrate the eosinophil genome, transcriptome, epigenome, proteome, glycome, and metabolome and share these data as a resource to eosinophil researchers; (v) apply the eosinophilome to EADs to improve fundamental knowledge of mechanisms of disease onset, prognosis, and response to treatment; and (vi) explore the role of paradigm-shifts in research as they relate to the diagnosis of EADs and incorporation of these practices into clinical practice (e.g., EoE Diagnostic Panel[108]).

4 | DISCUSSION

The goals of both the prior TREAD meeting[1] and the most recent RE-TREAD meeting were to convene leading international experts in eosinophil biology and EADs to work together to help move the field toward improved understanding, diagnosis, and treatment of EADs. The topics considered ranged from basic eosinophil biology to clinical trials. While progress has been made during these past 5 years, significant gaps remain in our understanding of EAD initiation, development, evolution, progression, organ specificity, and remission. With the unique exception of the highly effective and perhaps "curative" use of tyrosine kinase inhibitors for *PDGFR*-associated myeloid neoplasms,[119,120] treatment paradigms for HES remain mostly empiric.[121] Fortunately, the last 5 years have witnessed the sequential FDA approval of 3 new-in-class anti-eosinophil biologics that target IL-5 or its receptor.[122] Such drugs are not only a welcome addition to the arsenal of therapies for EADs but are highly targeted and specific pharmacologic tools for dissecting the contribution of eosinophils to disease, adding timeliness to RE-TREAD. A prime example is the successful use of mepolizumab to treat EGPA,[79] which provided some mechanistic insight into the question of

whether eosinophils truly play a role in the pathogenesis of a complex multisystem vasculitic disorder. At a minimum, withdrawal of corticosteroids did not result in worsening of vasculitis as has occurred with various other steroidsparing medications used for asthma.[123] Although not yet approved for use in eosinophilic disorders other than asthma and EGPA, there is strong evidence that mepolizumab (and other biologics targeting the IL-5 axis) have clinically beneficial activity in HES, lymphoid HES, and perhaps EGID.[122,124] Since their cost and the paucity of available data, including uncertainty on optimal dosing, are likely to make off-label use in these disorders difficult, the importance of well-designed clinical trials, especially in rare EAD, remains a priority. Moreover, as the number of approved targeted agents increases, direct comparisons between the different agents and the safety and efficacy of combination therapies will be essential to create optimized outcomes based recommendations to help guide physicians.

It is hoped that this RE-TREAD report will once again help to keep the field of basic eosinophil immunobiology and translational efforts aimed at EADs focused on common goals that will benefit patients. Outlined in this document are specific recommendations addressing a wide range of research issues that can directly or indirectly impact the development of new therapeutic modalities and their implementation for the diagnosis and treatment of patients with EADs. Although great strides have been made since the 2012 TREAD report, significant unmet needs remain.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

AEC	absolute eosinophil count

CEGIR	Consortium of Eosinophilic Gastrointestinal Researchers
CRISPR	clustered regularly interspaced short palindromic repeat
EAD	eosinophil-associated diseases
EC	eosinophilic colitis
EG	eosinophilic gastritis
EGID	eosinophilic gastrointestinal disorders
EGPA	eosinophilic granulomatosis with polyangiitis
EMH	extramedullary hematopoiesis
EMR-1	EGF-like module-containing mucin-like hormone receptor-like 1
EoE	eosinophilic esophagitis
EoP	eosinophil progenitor
HES	hypereosinophilic syndromes
HPF	high power field
NCATS	National Center for Advancing Translational Science
NIAID	National Institute of Allergic and Infectious Diseases
NIH	National Institute of Health
ORDR	Office of Rare Diseases Research
PAG	Patient Advocacy Group
PRO	patient reported outcome
QOL	quality of life
RDCRN	Rare Disease Clinical Research Network
TREAD	Taskforce on the Research of Eosinophil-Associated Diseases
TRND	therapeutics for rare and neglected diseases

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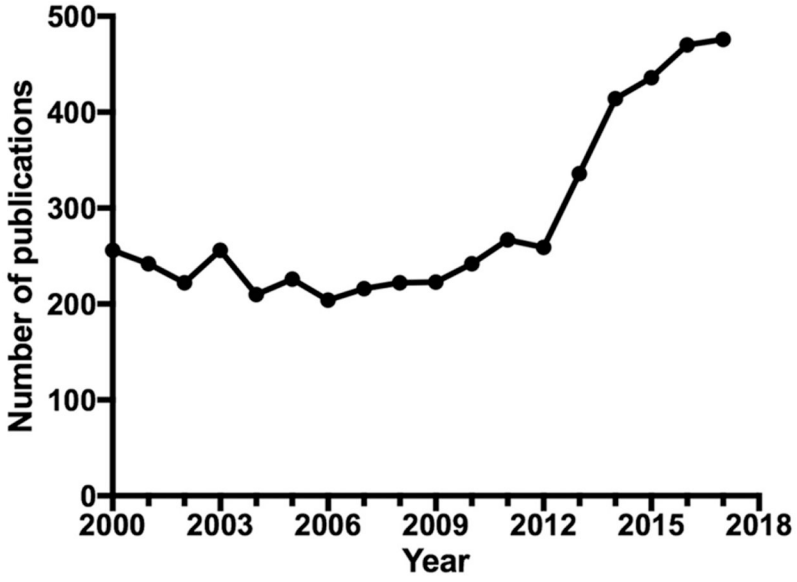

FIGURE 1.
Publications for “eosinophil”, “eosinophilia”, or “eosinophils” as the subject or keyword from 2000 until 2017 (Pubmed)

TABLE 1

Clinical, scientific, and translational resources for eosinophil researchers

Tool	Description	Resource
Rare Diseases Research Tools		
RDCRN program	Resource with support for clinical studies, consortia, collaborations, data sharing and management in Rare Diseases	http://rarediseasesnetwork.epi.usf.edu/ Example Consortia: CEGIR: https://www.rarediseasesnetwork.org/cms/cegir/About-Us/CEGIR VCRC: https://www.rarediseasesnetwork.org/cms/vcrc
Tissue Chip Program	Funded projects for development of 3-D platforms (Chips) to evaluate safety of new drug candidates	https://ncats.nih.gov/tissuechip/about
TRND	Program supports pre-clinical development of therapeutic candidates for diseases with unmet needs	https://ncats.nih.gov/trnd/about
NCATS New Therapeutic Uses program	Resource for repurposing therapeutic agents for new indications	https://ncats.nih.gov/ntu/about
Omics Databases		
NCBI GEO Profiles and Datasets	Searchable database of individual gene expression profiles from curated DataSets in the Gene Expression Omnibus (GEO) repository	https://www.ncbi.nlm.nih.gov/gds
Haemosphere	Searchable gene expression profiles of hematopoietic cell types	haemosphere.org[125]

Strain	Description	Reference
Mouse models[a]		
iPHIL	Eosinophil depletion via inducible diphtheria toxin receptor knockin (EPX promoter driven DTR expression)	12
eoCre	Removal of floxed gene of interest specifically in eosinophils upon crossing (EPX promoter driven Cre)	24
PHIL	Congenital eosinophil deficiency mediated EPX-promoter driven expression of Diphtheria Toxin A chain	126
EPX−/− and MBP-1−/−	Mice with knockout of EPX, MBP-1 or both	127-129
NJ.1638/hE2 or NJ.1726	Mice with chronic severe asthma, overexpression of IL-5 (Cd3δ promoter) and eotaxin-2 (CC10 promoter) or IL-5 from CC10 promoter	15,130,131
Reagents		
IHC/IF[a]	Antibodies to MBP-1 (mouse) and to EPX (Human/mouse) available	5,132
EPX ELISA[a]	EPX specific ELISA (mouse and human) patient samples	84,85,133,134
MBP-1 and CLC/Gal-10 ELISAs[b]	MBP-1 and CLC/Galectin-10 specific ELISAs (human patient samples)	9
Eosinophil Granule protein Multiplex[c]	Simultaneous measurement of MBP, ECP, EDN and EPO in a multiplex suspension array system.	135

CEGIR, Consortium for Eosinophilic Gastrointestinal Disease Researchers; CLC, Charcot-Leyden crystal; ELISA, enzyme-linked immunosorbent assay; EPX, eosinophil protein X; GEO, Gene Expression Omnibus; IF, immunofluorescence; IHC, immunohistochemistry; MBP, major basic protein; RDCRN, Rare Diseases Clinical Research Network; TRND, Treatment for Rare and Neglected Diseases; VCRC, Vasculitis Clinical Research Consortium.

[a] May be performed in collaboration or obtained by contacting Jacobsen.elizabeth@mayo.edu;

[b] May be performed or obtained by contacting sackerma@uic.edu;

[c] May be performed in collaboration with aklion@niaid.nih.gov.

TABLE 2

2017 IES clinical workgroup proposed list of ICD-CM codes

1. Idiopathic Hypereosinophilic Syndrome
2. Lymphocytic Variant Hypereosinophilic Syndrome
3. Myeloid Hypereosinophilic Syndrome with mutations in *PDGFRA, PDGFRB, FGFR1*, or *JAK2*[a]
4. Myeloid Hypereosinophilic Syndrome without a known molecular abnormality
5. Hypereosinophilic Syndrome NOS
6. Eosinophilic Hepatitis
7. Eosinophilic Fasciitis
8. Acute Eosinophilic Pneumonia
9. Chronic Eosinophilic Pneumonia
10. Eosinophilic Granulomatosis with Polyangiitis (EGPA)
11. Drug Rash with Eosinophilia and Systemic Symptoms (DRESS)

Abbreviations: HES, hypereosinophilic syndrome, *PDGFR*, platelet derived growth factor, *FGFR*, fibroblast growth factor receptor, *JAK*, janus associated kinase, NOS, not otherwise specified.

[a] with consideration of modifiers for specific mutations.

TABLE 3

Patient advocacy groups,funding organizations,and other groups relevant to EAD research

Organization	Website
AAAAI[a]	http://www.aaaai.org/professional-education-and-training/grants-awards
APFED[a]	http://apfed.org/research/
AUSEE[a]	http://www.ausee.org/medicalresearchgrants.htm
Burroughs Wellcome Fund	https://www.bwfund.org/feature-research-rare-diseases
CURED[a]	https://curedfoundation.org/
EFC	http://eoscoalition.org/
FARE	https://www.foodallergy.org/
NORD[a]	https://rarediseases.org/for-clinicians-and-researchers/research-opportunities/research-grant-program/
Vasculitis Foundation[a]	http://www.vasculitisfoundation.org/research/

Abbreviations: APFED, American Partnership for Eosinophilic Disorders; CURED, Campaign Urging Research for Eosinophilic Disease; EFC-Eosinophilic Family Coalition; FARE-Food Allergy Research & Education; NORD-National Organization for Rare Disorders.

[a]Organization or Patient Advocacy Group that has provided funding for rare disease or EAD research.