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Toward clinically applicable biomarkers for asthma: An EAACI position paper

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Abstract

Inflammation, structural, and functional abnormalities within the airways are key features of asthma. Although these processes are well documented, their expression varies across the heterogeneous spectrum of asthma. Type 2 inflammatory responses are characterized by increased levels of eosinophils, FeNO, and type 2 cytokines in blood and/or airways. Presently, type 2 asthma is the best-defined endotype, typically found in patients with allergic asthma, but surprisingly also in nonallergic patients with (severe) asthma. The etiology of asthma with non-type 2 inflammation is less clear. During the past decade, targeted therapies, including biologicals and small molecules, have been increasingly integrated into treatment strategies of severe asthma. These treatments block specific inflammatory pathways or single mediators. Single or composite biomarkers help to identify patients who will benefit from these treatments. So far, only a few inflammatory biomarkers have been validated for clinical application. The European Academy of Allergy & Clinical Immunology Task Force on Biomarkers in Asthma was initiated to review different biomarker sampling methods and to investigate clinical applicability of new and existing inflammatory biomarkers (point-of-care) to support diagnosis, targeted treatment, and monitoring of severe asthma. Subsequently, we discuss existing and novel targeted therapies for asthma as well as applicable biomarkers.

KEYWORDS
endotype, eosinophil, FeNO, IgE, phenotype

1 | INTRODUCTION

The hallmarks of asthma include chronic airway inflammation, clinical symptoms and physiological signs including variable airway obstruction and airway hyperresponsiveness (AHR), and structural changes within the lower airways.[1,2] These features differ across the spectrum of asthma, contributing to the variable response to standard anti-inflammatory therapy with inhaled corticosteroids (ICS).[3] Especially severe asthma has been recognized as a highly heterogeneous disorder consisting of multiple overlapping phenotypes, with differences in age of onset, clinical presentation, comorbidities, airway inflammation, responsiveness to ICS, and natural course of disease.[4-6] According to literature, overall approximately 5%-10% of patients either need high doses of ICS and/or oral corticosteroids to control their asthma or have corticosteroid insensitivity, and hence, they are classified as severe asthma patients.[7]

In the past decade, distinct molecular mechanisms have been identified and linked to clinical asthma phenotypes (Box 1).[8-10] The identification of inflammatory subsets and asthma endotypes holds promise to improve asthma management and guidance into selecting the most adequate targeted treatment for each individual patient.[11-13]

Novel approaches to unravel biological asthma networks are emerging, such as the Unbiased BIOmarkers in PREDiction of respiratory disease outcomes (U-BIOPRED) consortium and Severe Asthma Respiratory Program (SARP). With the advent of novel expensive biologicals to treat (severe) asthma (eg, targeting IgE, IL-5, IL-4/IL-13, and others), there is a strong need of clinical and biological markers that can guide the choice of treatment, predict treatment response, and monitor the treatment response. Implementing targeted treatment into daily practice is however challenging and requires biomarker validation and evaluation of the socioeconomic impact.

We reviewed the literature between 1990 and 2018 on non- or semi-invasive sampling methods and biomarkers for the diagnosis, monitoring, and treatment of asthma, with a focus on type 2 inflammation, while non-type 2 inflammation and structural abnormalities are also discussed. In the second part of this paper, we discuss existing and novel targeted therapies for (severe) asthma in context with clinically applicable biomarkers and address unmet needs.

Box 1 Definitions

Phenotype: The observable characteristics in an individual resulting from the expression of genes; the clinical presentation of an individual with a particular genotype (National Institute of Health (NIH) definition).[200]

Endotype: Endotype—a contraction of endophenotype—is a subtype of disease defined functionally and pathologically by a molecular mechanism or by treatment response.[201]

Biomarker: A biomarker is defined as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention (NIH definition).[202]

2 | WHAT IS A CLINICALLY APPLICABLE BIOMARKER?

In order to qualify as a biomarker applicable to evaluate treatment response and monitor disease progression of chronic airway diseases, validation at different levels is required (Figure 1). The so-called "SAVED" model was proposed to describe the characteristics of COPD biomarkers with a high potential to reach clinical translation.[14] This model may also be applicable to validate asthma biomarkers. According to this model, a biomarker should be "Superior" (outperform current practice), "Actionable" (change patient management), "Valuable" (improve patient outcomes), "Economical" (cost-saving or cost-effective), and "Clinically Deployable" (analysis technology available in clinical laboratory).[15]

3 | BIOMARKER SAMPLING METHODS

Inflammatory biomarkers of asthma can be sampled in different body compartments, including the upper and lower respiratory tract, saliva, urine, and peripheral blood.[11,16-18] The first question is whether all these compartments are providing comparable information on the underlying mechanisms of (severe) asthma. This may not be the case as shown for instance by comparative studies from U-BIOPRED on gene expression profiles in sputum, endobronchial biopsies, bronchial brushes, and nasal brushes.[16,17] Therefore, any biomarker should primarily be considered as a representative of a particular sampling site.

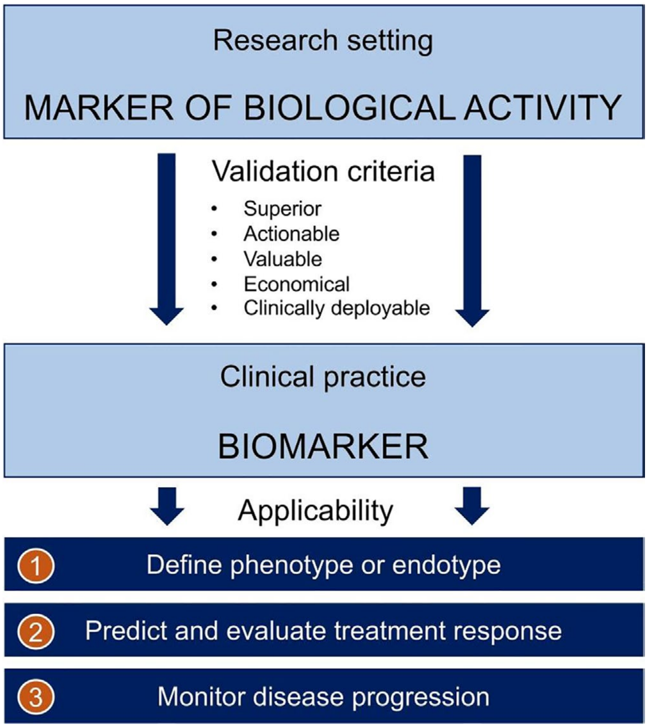

FIGURE 1 Clinical applicability of biomarkers in asthma management. Adapted from Ref.[19] Several studies are screening for markers of biological activity in order to identify markers that discriminate between health and disease, identify disease subtypes, and predict disease progression. However, in order to classify as a clinically applicable biomarker, different validation criteria should be met. The SAVED approach outlines such a validation process in which the following criteria are proposed: "Superior" (outperform current practice), "Actionable" (change patient management), "Valuable" (improve patient outcomes), "Economical" (cost-saving or cost-effective), and "Clinically Deployable" (analysis technology available in clinical laboratory) [14]

In addition, each sampling method has its own advantages and limitations (Table 1). The most tissue-specific and thus presumably most disease-specific method to assess airway inflammation at different sites of the bronchial tree is bronchoscopy combining bronchial biopsies, brushes, and bronchoalveolar lavage (BAL) fluid. However, the invasiveness and potential complications of these procedures preclude bronchoscopy in daily clinical routines.[18] Sputum induction is less invasive allowing repeated and reproducible samplings of (more) central airway inflammation. Nevertheless, it is time-consuming and requires specialized (medical) infrastructure with well-equipped laboratory facilities and personnel.[19,20] Alternatively, sampling biomarkers outside the respiratory tract imply potential drawbacks. Peripheral blood can be easily obtained and blood eosinophils have been shown to correlate with sputum eosinophil counts in some—but not in all—studies.[21-24] The correlation between blood eosinophils and lung tissue eosinophilia is even less clear.[25] In addition, blood eosinophils are subject to significant daily fluctuations,[26] while an unambiguous clinically relevant cutoff value has so far not been established.

During the last decades, several novel, noninvasive methods have been developed, while existing methods have been refined both for online (real-time) assessment of biomarkers (including fractional exhaled nitric oxide [FeNO]) and for offline (delayed analysis) biomarker samplings (such as volatile organic compounds [VOCs]) in exhaled breath and exhaled breath condensate (EBC).[27-29] Despite the simple technology and commercially available analyzers, the interpretation of FeNO is often hampered by several perturbing factors, including age, smoking status, atopy and anti-inflammatory treatment (especially corticosteroids).[30] VOCs are providing a more comprehensive molecular signal and can be analyzed using two different approaches, that is, analytical chemistry techniques, such as gas chromatography with mass spectrometry (GC-MS) to identify individual VOCs or cross-reactive sensor arrays combined with pattern recognition algorithms (electronic noses: eNoses) that can capture complex mixtures of VOCs and are suitable for probabilistic diagnosis or phenotyping. The crucial issues for both VOCs techniques consist of rigorous standardization of sampling, preprocessing, and analysis, including independent external data validation.[31] Particles in exhaled air (PExA) is a novel, noninvasive sampling method of the lining fluid from small airways.[32,33] The potential to identify clinically applicable biomarkers with the PExA method is still evolving. Additionally, biomarkers in exhaled air can also be obtained from EBC, consisting of condensed vapor, as well as nonvolatile molecules. However, this approach is limited due to the lack of standardized methodology for collection as well as variable

TABLE 1 Advantages and limitations of biomarker sampling methods in asthma

Sampling method	Biomarker	Cutoff level	Advantages	Limitations
Bronchoscopy • Biopsy • Bronchoalveolar lavage (BAL) • Bronchial brushings	• Eosinophils • Neutrophils • Total inflammatory cell counts • Cytokines and chemokines • Leakage markers and mediators • Airway remodeling	Clear cutoff values lacking	• Semi-direct readout	• Invasive • Technically complex • Not feasible in very severe disease with compromised lung function and/or with concomitant cardiovascular disorders • Multiple sampling needed to address tissue variation • Potential sampling site bias • Dilution (BAL)
Sputum induction	• Eosinophils • Neutrophils • Total inflammatory cell counts • Cell activation markers • Cytokines, chemokines, leakage markers, and mediators	In general, cutoff of ≥ 3% is used to indicate sputum eosinophilia, and ≥ 61% to indicate sputum neutrophils. However, adapting treatment based on sputum eosinophils has performed with various sputum eosinophil cut-offs, ranging from 2% to 8% [203]	• Semi-direct readout; multiple biomarkers; reproducible readout of cell differentials • Suitable method for disease phenotyping and monitoring in experienced centers	• Semi-invasive • Analyzable samples available in approximately 80%-90% of subjects; • Adapted protocol needed for very severe disease with compromised lung function (contraindicated if FEV_1 <1L [204] and/or with concomitant cardiovascular disorders • Technically complex and time-consuming procedure (soluble markers too variable for daily clinical routine), restricted to specialized centers
Peripheral blood	• Eosinophils • Cell activation markers • IgE (total/specific) • Cytokines, chemokines, and mediators	Various cutoff values, mostly ranging 150-500 cells/µL/1%-4%, are used for blood eosinophils [205]	• Easy to collect	• Semi-invasive • Indirect readout • High intra-subject diurnal variability N.B: blood eosinophils do not adequately reflect airway eosinophilia during systemic corticosteroid treatment [51]
Exhaled breath	• FeNO • Volatile organic compounds (VOCs)	According to clinical guidelines: Low FeNO < 25 ppb (≥12 y), <20 (<12 y), high FeNO > 50 (≥12 y), <35 (<12 y) [57]	• Noninvasive • Simple method allowing repeatable, serial measurements • Suitable method for disease phenotyping and monitoring • Direct readout	• Various perturbing factors affecting FeNO levels • Lack of standardized methods for VOC collection and analysis
Exhaled breath condensate	• pH • Markers of oxidative stress • Leukotrienes • Cytokines or chemokines	No clear cutoff values.Study showed that EBC pH ≤ 7.20 was indicative of not well-uncontrolled asthma [206]	• Noninvasive, allowing serial measurements	• Specialized laboratory needed • Expensive assays • Variable outcomes due to technical issues • Awaits further development and validation
Imaging (eg, qCT, HRCT, (hyperpolarized 3He/129Xe) MRI, PET)	• Airway remodeling	Clear cutoff values lacking	• Noninvasive • Enables to study structural (and functional) aspects	• Standardization is lacking • Radiation exposure (eg, qCT)

biomarker levels with concentrations often under detection limits.[29] Furthermore, new sampling methods and biomarkers obtained from saliva (for genetics and cytokines), nasal swabs (for transcriptomics, epigenetics, and microbiomics), and nasal or bronchial sponges (for transcriptomics and microbiomics) are currently being explored and validated.[34,35] And finally, imaging techniques, including quantitative computed tomography (qCT), magnetic resonance imaging (MRI), and positron emission tomography (PET), are increasingly applied to evaluate "imaging biomarkers" but will not be further discussed in this overview.[36]

4 | BIOMARKERS OF T2 INFLAMMATION

The type 2 (T2) inflammatory pattern is defined by increased T2 cytokine [37,38] or epithelial [39,40] gene expression compared to a reference population. T2 airway inflammation is characterized by increased release of IL-4, IL-5, and/or IL-13 likely from both adaptive (mainly T-helper2) and innate (mainly innate lymphoid cells type 2 [ILC2]) immune cells resulting in eosinophilic airway infiltration (Figure 2). Approximately 50% of asthma patients are identified with T2 airway inflammation equaling the proportion of patients with eosinophilic asthma.[3] T2 asthma is presently the best-characterized endotype within the eosinophilic phenotype, usually associated with allergy, although nonallergic pathways of airway eosinophilia have been proposed (Figure 2).[41] Recently, a subgroup of patients with high FeNO levels (>25 ppb) and low blood eosinophils (<2%) was described. These patients showed a significantly higher number of sensitizations against aeroallergens compared to patients with low FeNO levels.[42] Epithelial-derived cytokines, including thymic stromal lymphopoietin (TSLP), IL-25, IL-33, with subsequent activation of ILC2, may support the underlying pathophysiological event.[43]

Multiple inflammatory components have been evaluated for their potential as a biomarker of T2 (allergic) asthma.[44] Sputum eosinophils are probably the best-characterized and most useful biomarker so far. While in general, eosinophilia suggests corticosteroid responsiveness,[39,45] it may also reflect poor adherence to ICS.[46] Compared to guideline-based management, sputum eosinophil-guided management showed a reduction in exacerbations, especially in patients with more severe asthma.[47] ERS/ATS and recent GINA guidelines now suggest treatment guided by sputum analysis for severe asthma in experienced centers.[7,48] Concomitant systemic eosinophilia and airway eosinophilia have been associated with worse asthma control.[49] However, blood and sputum eosinophils cannot always be used interchangeably, especially in patients on oral corticosteroids.[24,50,51] In children, the presence of blood eosinophilia, especially in combination with allergic sensitization, was found to be a significant predictor of ICS response with respect to both asthma symptoms and exacerbations.[52] Recently, a novel point-of-care method for rapid quantification of eosinophil peroxidase in sputum has been described which can identify patients with airway eosinophilia.[53]

Sputum mRNA analysis is a more sophisticated technique to classify patients into T2 and non-T2 endotypes.[37,38] Inhaled allergen resulted in upregulation of T2 pathway in sputum mRNA.[20] A recent unsupervised sputum analysis of an mRNA panel of 12 cytokines challenged the a priori classification of T2 versus non-T2 asthma.[10] A set of 205 unselected asthma patients could be classified into five clusters with equal proportions of IL-4- and IL-13-high patients, whereas IL-5-high expression was restricted to patients with an IL-25- and IL-17A/F-high pattern. These data confirm earlier reports on a subgroup of patients with concomitant activation of Th2 and Th17 inflammatory pathways.[37,54] Recently, this was reinforced by a complete transcriptomics analysis, showing heterogeneity amongst patients with asthma beyond T2 classification.[9] Profiling serum of T2 cytokine patterns by Meso-Scale multiplex technology may also help to identify eligible patients for biologicals targeting different T2 pathways.[55,56]

FeNO is a reproducible, easily measurable biomarker and a good predictor of ICS response.[57,58] However, FeNO may be affected by several confounders, including demographics, smoking, atopy, and diet.[29,59,60] According to the ATS recommendations, FeNO > 50 ppb (adults) and > 35 ppb (children) is indicative of eosinophilic inflammation, while eosinophilic inflammation is unlikely for FeNO < 25 ppb

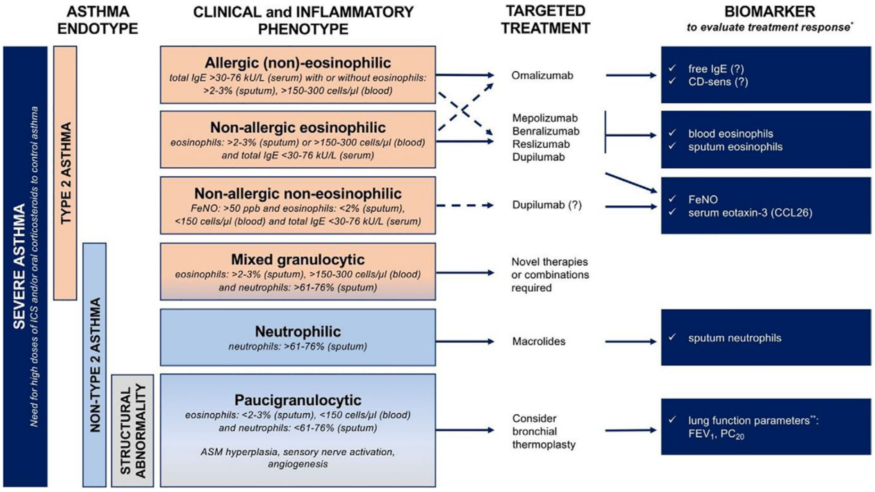

FIGURE 2 Asthma endotypes and targeted treatment approaches

(adults) and < 20 ppb (children).[57] Strategies incorporating FeNO into standard clinical practice allowed reduction in ICS doses in adults (but not in children).[61] In a study in pregnancy, FeNO-guided treatment resulted in a significant reduction in asthma exacerbations and mean ICS dose.[62] Presently, the ERS/ATS severe asthma guidelines do not recommend the use of FeNO to guide therapy in adults or children with severe asthma.[7]

Exhaled VOCs provide a composite biomarker signal, based on pattern recognition. Exhaled VOCs profiles are correlated with blood eosinophil and neutrophil counts [63] and with eosinophils in BAL.[64] Even without information on an individual's molecular pathways, such probabilistic approach can be very powerful in phenotypic classification. Based on the same principle of exhaled VOCs, eNose can predict loss of asthma control [65] and may be more sensitive than FeNO or sputum eosinophilia in predicting clinical efficacy of systemic corticosteroids.[66] However, most studies are small and focused on adults, while scarce data are available in children.[28] Therefore, application of eNose in daily practice requires further validation.

Periostin production by epithelial cells was shown to be induced by IL-13.[39] As such, periostin was proposed as a surrogate marker of T2 inflammation. In the BOBCAT study, serum periostin showed superior prediction of sputum and bronchial tissue eosinophilia than FeNO, blood eosinophils, and serum IgE in 59 patients with uncontrolled severe asthma.[67] However, this was not confirmed in follow-up studies.[21,23,68] Asthma patients with increased serum periostin showed improvements in lung function after treatment with lebrikizumab, an anti–IL-13 monoclonal antibody (mAb), in contrast to patients with low periostin levels.[69] However, lebrikizumab efficacy could not be confirmed in two subsequent phase 3 studies, even not in periostin-high patients.[70] It should be noted that several periostin splice variants exist, complicating its detection by various home-made or commercially available assays with possibly different thresholds for these isoforms. Furthermore, it is unknown whether local sampling is required to obtain a more consistent periostin signal in asthma. Finally, it is unclear whether periostin can be used as potential biomarker in children, since baseline periostin levels are higher in children, probably due to growth.[71]

Dipeptidyl peptidase-4 (DPP-4) has been proposed as a candidate predictive biomarker for the response to anti–IL-13 treatment. Patients with DPP-4 levels above median showed better responses to tralokinumab in lung function and health status.[72] Further studies are needed to confirm the potential role of DPP-4 as a surrogate T2 biomarker.

Urinary leukotriene E4 (LTE4), the end-metabolite of cysteinyl leukotrienes (CysLTs), is a marker of CysLT activity and has been studied in asthma intervention studies with antileukotrienes[73] and in aspirin or NSAID-exacerbated respiratory disease (NERD).[74] Urinary LTE4 could be a potential biomarker in studies involving eicosanoid pathways.[75]

Apart from single biomarkers, composite markers have been applied in some studies. In a systematic review, FeNO, blood eosinophils, and serum IgE showed moderate diagnostic accuracy for identification of sputum eosinophilia.[24] Combining all three markers may be more useful than one. A recent study showed that this approach could accurately identify the presence of ≥ 3% sputum eosinophils in 60% of patients.[76] Using a prediction model in two independent cohorts, FeNO, blood eosinophils, and the activation status of blood eosinophils and neutrophils combined with clinical characteristics could accurately predict sputum eosinophilia (90.5% sensitivity and 91.5% specificity in training cohort; 77% sensitivity and 71% specificity in the validation cohort, respectively).[77] Some clinical trials applying targeted therapies evaluated treatment response in patients based on composite biomarker profiles.[78-80] The role of composite biomarker profiles in asthma phenotyping and management needs to be established.

5 | BIOMARKERS OF NON-T2 INFLAMMATION

The non-T2 endotype consists of patients in whom T2 inflammation is absent or within normal range (eg, T2-low). This endotype covers both patients with a neutrophilic and a paucigranulocytic airway inflammatory pattern.[81] A clear definition of neutrophilic airway inflammation is still lacking since various sputum neutrophil cutoff levels (40%-76%) have been reported.[3,49,82,83] Sputum neutrophilia was found to be associated with (relative) insensitivity to ICS,[3] in smoking [84] and in obese asthma patients.[85,86] Adults with refractory asthma were shown to have higher levels of BAL neutrophils compared to nonrefractory patients with asthma.[87] Apart from reflecting a distinct phenotype, airway neutrophilia often associates with (subclinical) airway infection[88] or oral corticosteroid use.[87] In childhood asthma, neutrophilic airway inflammation seems to play a minor role.[89] In a study in children with severe asthma, therapy resistance was characterized by increased numbers of eosinophils in BAL, endobronchial biopsies, and sputum samples while neutrophil numbers were not increased.[90] Conversely, in a recent study in children with severe treatment-resistant asthma, the presence of intra-epithelial neutrophils and increased IL-17RA expression were associated with better lung function.[91] Recent data however do support the relationship between airway neutrophilia and asthma severity in children. The analysis of the Taiwanese Consortium of Childhood Asthma Study showed that neutrophil-predominant asthma is the most severe asthma phenotype in children with a poor corticosteroid response.[92] In the inner-city study, Th17-related cytokines were associated with difficult-to-control asthma.[55]

Several cytokines associate with sputum neutrophilia (Figure 2). Interleukin-17A, mainly produced by T cells or type 3 ILCs, promotes the production of IL-8, chemoattractant for neutrophils, by structural cells.[43,93,94] Both sputum IL-17A and IL-8 gene expression are positively correlated with sputum neutrophil counts.[45] Gene expression of *CXCR2*, the receptor for IL-8, was found to be increased in neutrophilic compared to eosinophilic asthma.[95] More recently, the inflammasome pathway with increased expression of NLRP3 and IL-1β was found to be associated with neutrophilic asthma.[96,97]

Similar to increased sputum neutrophils, membrane-bound TNF on circulating monocytes was increased in refractory compared to milder asthma,[98] whereas no association was found between free TNF and sputum neutrophils in patients with severe asthma.[99]

Few studies have investigated the potential of serum biomarkers to identify neutrophilic asthma. Serum IL-17 was found to be increased in severe asthma compared to milder forms, and values above 20 pg/mL are an independent risk factor for severe asthma.[100] Increased serum soluble TNF and IL-8 levels accompanied by raised circulating neutrophils have been detected in severe asthma patients compared to healthy controls.[101] A recent analysis showed five biomolecules in serum correlating with BAL neutrophilia.[87] In asthma patients, serum calprotectin (S100A8/A9), a danger molecule released by the airway epithelium, can predict with high sensitivity and specificity in the presence of increased sputum neutrophils (>61%).[102] While blood neutrophils are poor indicators of airway neutrophilia, so far, no serum surrogate biomarkers have been validated for neutrophilic asthma. Interestingly, exhaled hydrogen peroxide (H_2O_2) may be a marker of neutrophilic oxidative burst.[103]

The mechanisms underlying paucigranulocytic asthma are the least defined. Patients with paucigranulocytic phenotype represent approximately 40%-50% of asthma patients and show sputum eosinophil and neutrophil counts within normal ranges.[82] While the majority of these patients are well controlled with a normal lung function, a subgroup (approximately 15%) remains uncontrolled despite normal sputum granulocyte counts.[104] In these patients, a "low-grade" inflammation[76] or structural changes including epithelial cells, airway smooth muscle, nerves and/or vessels may be the underlying pathophysiological substrate.

6 | BIOMARKERS OF STRUCTURAL AIRWAY ABNORMALITIES

Airway remodeling is another key feature of asthma, comprising structural changes (Figure 2) including increased deposition of extracellular matrix proteins in the reticular basement membrane (RBM), increased airway smooth muscle (ASM) mass and/or cell number, goblet cell and glandular hyperplasia and angiogenesis.[105,106] Although bronchial epithelial cell detachment was also claimed to occur in situ, some argued whether this reflects an artifact of bronchoscopy.[107]

Although these features are manifest in adults with chronic asthma, similar changes are already present in childhood asthma,[90,108,109] suggesting that these structural changes may underlie or parallel chronic airway inflammation. Nevertheless, parameters of airway remodeling and pathophysiology are not always concordant and may vary depending on which aspect is assessed. While the ASM mass and collagen deposition[110] have been shown to reflect asthma severity,[111] other associations between markers of airway remodeling and airway obstruction or AHR have been inconsistent.[112,113]

So far, the number of reliable biomarkers reflecting aspects of airway remodeling is scarce. The thickening of the RBM correlates well with eosinophil numbers in bronchial mucosa,[114] and eosinophil-depleting treatments[113,115] showed inhibitory effects on components driving this subepithelial fibrosis. In parallel, reduction in symptoms and asthma exacerbations and improvement in lung function were achieved in adults[114] and in children[116] with protection against methacholine-induced maximal airway narrowing.[117,118] In a biopsy study in severe allergic asthma, apart from anti-eosinophil effects, omalizumab (anti-IgE; [119]) reduced RBM thickening in some patients. In a subsequent analysis, this reduction correlated with galectin-3,[120] which appears to regulate airway remodeling.[121] Chitin and chitinase/chitinase-like proteins have also been found to affect airway remodeling.[122] In a study in children with severe asthma, serum chitinase-like protein YKL-40 correlated with bronchial wall thickening on high-resolution computed tomography (HRCT).[123] Sputum fibroblast growth factor 2 (FGF-2) correlated inversely with the FEV_1/FVC ratio and the severity of asthma which is known to relate to remodeling. This may link to transforming growth factor β (TGF-β), a tissue remodeling factor, which is induced by FGF-2.

Transcriptomics analyses of ASM from asthma patients revealed marked differences compared to healthy controls.[124] In this study, several genes (*RPTOR*, *VANGL1*, *FAM129A*, and *LEPREL1*) differentially expressed in ASM from asthma patients correlated with AHR, linking airway remodeling to pathophysiology.[124] Changes in expression of these genes induced by oral corticosteroids were associated with improvements in airway physiology.[125] These data warrant further investigation.

The precise mechanisms driving ASM hypertrophy and hyperplasia in asthma are less clear. Both the extracellular matrix and the presence of mitogenic compounds may underlie the enhanced ASM mass. Although corticosteroids can attenuate levels of mitogenic compounds, they also directly affect the contractile elements of ASM[126] and the expression of various ASM proteins and airway dynamics.[127] In fact, corticosteroids can affect various cellular programs of ASM and some genetic variants correlated with AHR. Consisting of different components, it is likely that airway remodeling can be evaluated by combining multiple biomarkers generated by unbiased cluster analyses (eg, U-BIOPRED).[9]

Biomarkers of airway remodeling could identify individuals at risk of developing asthma at an early stage.[128] Although controversial, chronic airway inflammation has been considered the major driver of airway remodeling.[113,114] Indeed, anti-inflammatory therapy with corticosteroids has been shown to reduce goblet cell numbers in asthma[129] and airway wall thickening.[130] Hence, some inflammatory markers may be indicative of airway remodeling. In this context, the T2-cytokine IL-13 has been identified as a major driver of airway remodeling in asthma and several proteins induced by IL-13 can be quantified in blood and serve as potential biomarkers. One of these, periostin, has been extensively applied in the context of T2 inflammation and interventions targeting IL-13, while recent studies also underpin its association with bronchial wall thickening in asthma and chronic rhinosinusitis.[131,132]

Biopsies are the gold standard to assess remodeling but depend on invasive technologies and require multiple samples to deal with tissue variation. Still depending on bronchoscopy but covering large areas of the airways in one assessment requiring less extensive processing are imaging techniques that allow for detection of matrix structures such as fibered confocal fluorescence microscopy (FCFM).[133] FCFM visualizes specifically elastic fibers within the airway wall correlating with histological analysis. The link between elastic fiber patterns and lung function is suggestive of structure-function relationship, but requires validation. Besides FCFM, also other light- and laser-based high-resolution imaging techniques like optical coherence tomography (OCT) and confocal laser endomicroscopy (CLE) have recently been explored for assessment of airway remodeling.[134]

7 | BIOMARKERS FOR ASTHMA MANAGEMENT

Novel treatment options have been developed for patients who fail to achieve asthma control despite maximal standard treatment (GINA step 5).[135] The majority of these treatments target T2 inflammation (Figures 2 and 3). In the following sections, we discuss the latest treatment options for severe uncontrolled asthma and applicable or potentially available biomarkers that may guide these treatments. For allergen immunotherapy (AIT), we refer to the recently published EAACI position paper.[136]

7.1 | T2 targeted therapies

7.1.1 | IgE targeted therapies

Omalizumab is the first T2 targeting biological that was approved for severe allergic asthma.[137] This recombinant humanized mAb possesses several activities: binding free serum IgE, decreasing cell-bound IgE, and the expression of high-affinity receptors (FcRI) on inflammatory cells (mast cells, basophils, eosinophils, and dendritic cells).[138] Clinical studies showed that omalizumab as add-on therapy to ICS successfully reduces asthma exacerbations, hospitalizations, and doses of ICS while improving quality of life in adults and children > 12 years of age with moderate-to-severe allergic asthma.[139-141] Whether omalizumab can effectively reduce systemic corticosteroids needs further investigation.[139]

Consistent correlations between treatment response and baseline total serum IgE or antigen specific IgE levels are lacking.[142,143] Serum IgE is used to dose omalizumab, but the cutoff is rather arbitrary.[144] The use of CD-sens (basophil activation threshold) has proven to be useful in monitoring response to omalizumab in allergic asthma.[145] On the other hand, routine measurements of free IgE in serum can identify patients not responding to omalizumab treatment.[143]

Data from the EXTRA study involving 850 patients with uncontrolled severe allergic asthma showed that blood eosinophils, FeNO and serum periostin may potentially predict omalizumab treatment outcomes.[146] In this retrospective analysis, patients were divided

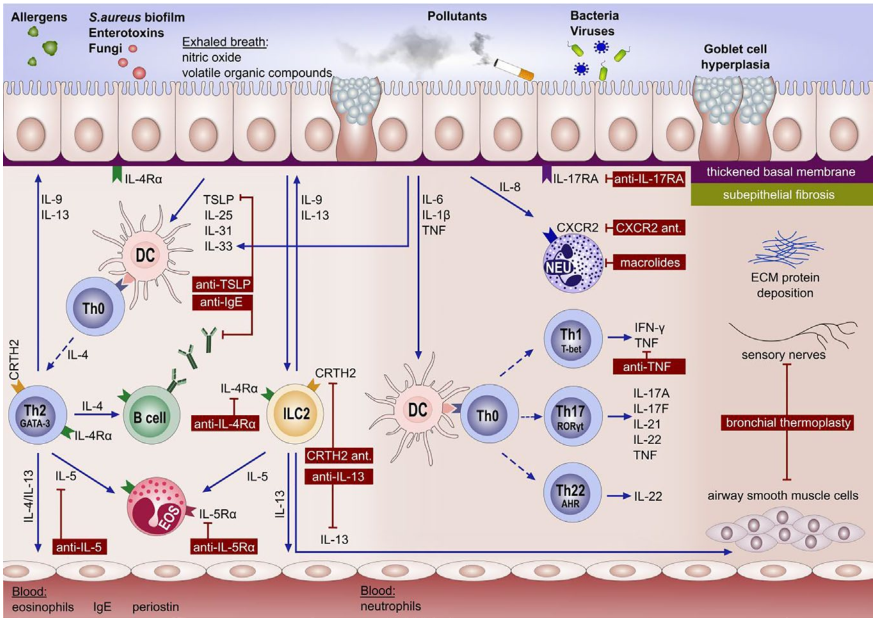

FIGURE 3 Practical flowchart to targeted treatment options for severe asthma according to asthma endotype and applicable biomarkers. *Suggested biomarkers to evaluate treatment response of targeted therapy are complementary to the evaluation of the clinical response evaluation (eg, asthma exacerbation rate, asthma control, and/or asthma quality of life). **For evaluation of therapy-resistant airway obstruction and/or severe airway hyperresponsiveness. Dashed arrow: based on proof-of-concept studies for which additional pragmatic or head-to-head clinical trials are required

into biomarker-high and biomarker-low subgroups based on median biomarker values. Patients treated with omalizumab in the FeNO-high group (≥19.5 ppb) showed more reduction in exacerbations compared to the FeNO-low group (<19.5 ppb): 53% *versus* 16%, respectively. Patients with high baseline blood eosinophils (≥260 cells/µL) showed 32% reduction in exacerbations *versus* 9% in patients with low eosinophils (<260 cells/µL), while patients with periostin high (≥50 ng/mL) had 30% reduction in exacerbations *versus* 3% in the periostin-low group.

Only few studies have investigated the clinical and laboratory predictors of omalizumab efficacy in childhood asthma. The PROSE study showed that children with more severe asthma respond better to omalizumab than those with milder asthma forms.[147] In a smaller study, children with severe asthma who responded to a single dose of 80 mg triamcinolone resulting in a substantial fall in FeNO responded significantly better to omalizumab treatment.[148]

7.1.2 | IL-5 targeted therapies

Interleukin-5 (IL-5) is another promising T2 target. Currently, there are several therapies interfering with the IL-5 pathway available for uncontrolled severe eosinophilic asthma. Current registered treatments comprise mepolizumab and reslizumab, mAb specifically targeting IL-5 and preventing its binding to IL-5 receptors (IL-5R).[149,150] Another anti–IL-5 mAb, benralizumab, directed against the IL-5 receptor α (IL-5Rα), induces a rapid depletion of eosinophils.[151] In several asthma trials, benralizumab showed clinical effectiveness and has been recently registered in several countries.[152]

The first clinical studies of anti–IL-5 in "unphenotyped" mild allergic and moderate asthma were rather disappointing. In these studies, blocking IL-5 had no effect on clinical outcomes, including allergen-induced late asthmatic response, asthma symptoms, lung function and quality of life scores.[153,154] After initial doubts about the importance of eosinophils in asthma, more appropriate target populations and endpoints were selected for subsequent clinical trials. In refractory eosinophilic asthma (sputum eosinophils > 3% or blood eosinophilia 150-400 cells/µL),[149,150,155-158] anti–IL-5 treatment significantly decreased exacerbation rates, improved quality of life, and produced a glucocorticoid-sparing effect. In some studies, even a modest increase in baseline FEV_1 was noted.[156] Similar effects on exacerbations, asthma control, lung function and glucocorticoid-sparing effects have been observed with benralizumab even in the absence of increased baseline eosinophil levels.[157-159] However, the long-term effects of eosinophil depletion remain unclear.

A recent systematic review assessed 13 studies (in total 6000 patients) showing that anti–IL-5 therapy approximately halves the number of exacerbations in uncontrolled eosinophilic asthma.[152] Patients are more likely to respond to anti–IL-5 treatment if they have > 3% of eosinophils in sputum, or ≥ 500 cells/µL blood eosinophils,[21,22,156] although lower eosinophil cutoffs have been used. Nevertheless, more research is needed to identify biomarkers (combinations; cutoffs) that can more accurately predict treatment outcomes.

7.1.3 | IL-4/IL-13 targeted therapies (dual blockade)

Both IL-4 and IL-13 bind to the α chain of type 2 IL-4 receptors (IL-4Rα). Therefore, blocking IL-4Rα affects both IL-4 and IL-13 downstream signaling. Various asthma treatments, such as pitrakinra (mutant form human IL-4) and dupilumab (fully human mAb to IL-4Rα) have been investigated for this purpose.[159,160]

Pitrakinra inhibits IL-4Rα by competing with IL-4. A retrospective analysis of a randomized controlled trial (RCT) in moderate-to-severe asthma showed that pitrakinra dose-dependently decreased exacerbations (from 22%-25% to 11%) in subsets of patients with specific polymorphisms in IL-4Rα genotypes.[163] Pharmacogenetic profiling of these patients might therefore guide pitrakinra treatment.

In the first phase 2 study, dupilumab showed significant reductions in exacerbation rates compared to placebo (6% vs 44%, respectively), and improvement in FEV_1 and ACQ-5 scores after withdrawal of LABA followed by ICS dose tapering and discontinuation in moderate-to-severe asthma with sputum or blood eosinophilia (≥3% and ≥ 300 cells/µL, respectively).[161] In the second phase 2 study in patients with uncontrolled asthma on medium-high ICS doses plus LABA, although improving FEV_1 in those with blood eosinophils ≥ 300 cells/µL, dupilumab reduced severe exacerbations irrespective of blood eosinophil counts at all dose regimen except at a dose of 300 mg every 4 weeks questioning blood eosinophil count as a possible biomarker for responders.[162] Plasma eotaxin-3 is significantly suppressed by dupilumab treatment. As eotaxin-3 is needed for eosinophil chemotaxis, suppression of eotaxin-3 results in a paradoxical increase of blood eosinophils in the early treatment phase.[163] Based on its mode of action, FeNO, serum periostin and/or DPP-4 may serve as potential biomarkers to identify responders to dupilumab[69,72]; this requires further investigation. In two recent phase III studies, in moderate-to-severe uncontrolled asthma and corticosteroid-dependent severe asthma, treatment with dupilumab reduced severe exacerbations and improved lung function and asthma control [160,164] while reducing systemic corticosteroid use.[164] Presently, dupilumab is in registration phase in several countries.

7.1.4 | IL-13 targeted therapies

Human(ized) mAb targeting IL-13 (lebrikizumab and tralokinumab) has been evaluated in phase II and III studies in asthma. In these studies, several biomarkers have been evaluated for their utility to identify potential responders to IL-13–targeting therapy.

Periostin, together with CLCA1 and serpinB2, is co-upregulated in airway epithelial cells from T2-driven asthma patients upon IL-13 stimulation.[39,165] As periostin is secreted at the basolateral side of the epithelium, it may diffuse into the bloodstream and can therefore be quantified in serum.[67]

In phase 2 studies with lebrikizumab, "periostin-high" (and FeNO-high) patients with uncontrolled asthma showed greater improvement in FEV1.[69] This was replicated in uncontrolled severe asthma patients receiving ICS and a second controller, and the periostin-high patients also had a greater reduction in severe exacerbations.[70] However, two subsequent phase 3 trials (LAVOLTA I and LAVOLTA II) failed to demonstrate consistent protection against exacerbations in uncontrolled asthma with high periostin (>50 ng/mL) or blood eosinophilia (≥300 cells/μL).[166]

In a phase 2 study with tralokinumab, periostin-high patients showed nonsignificant improvements in exacerbation rate and FEV_1.[72] In this study, DPP-4-high patients showed improvements in asthma exacerbation rate, FEV_1, ACQ-6, and AQLQ.[72]

Apart from its ability to identify responders to treatment targeting IL-13, increased periostin levels have the potential to predict future asthma exacerbations and also reflected greater FEV_1 decline in asthma patients on prolonged ICS treatment.[167]

7.1.5 | TSLP targeted therapies

Thymic stromal lymphopoietin (TSLP) is an important cytokine centrally involved in first-line immune defense and a recent asthma target. TSLP mediates allergic responses in the skin, gut, and upper and lower airways and is thus considered an upstream "master switch" of T2 inflammation.[168] While constitutive expression is mainly found in epithelial cells, other cells including mast cells, fibroblasts, and ASM can also produce TSLP. This cytokine upregulates OX40L on DCs driving Th2 cell differentiation.[169]

Thymic stromal lymphopoietin expression in bronchial biopsies correlates both with disease severity and with expression of T2 cytokines.[170] Treatment with anti-TSLP (AMG157/tezepelumab) in a cohort of mild atopic asthma patients significantly reduced FeNO and blood eosinophils pre- and postallergen challenge, while the allergen-induced eosinophil response in sputum was completely blocked. These anti-inflammatory effects were associated with reductions in both the early and the late airway responses to inhaled allergen.[171] These data have been replicated in another phase II study in 584 uncontrolled asthma patients on medium- or high-dose ICS plus LABA, where tezepelumab produced dramatic decreases in exacerbation rates across all dose regimen, irrespective of blood eosinophil numbers.[172] Future research should help to identify biomarkers to guide anti-TSLP treatment in subsequent clinical studies.

7.1.6 | CRTH2 antagonists

Chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2) antagonists are small molecules interacting with the prostaglandin D2 receptor (DP2 or CRTH2) on inflammatory cells including Th2 lymphocytes, ILC2s, and eosinophils.[173,174] In proof-of-concept studies, CRTH2 antagonists blocked allergic responses downstream of the Th2 pathway decreasing T(h)2 cytokines, eosinophils, and IgE synthesis.[175,176] However, many CRTH2 antagonists failed in later development phases, possibly due to unselected study populations. In line with emerging evidence of an upregulated PGD2 pathway in severe uncontrolled T2 (eosinophilic) asthma,[177] more recently, several CRTH2 antagonists have been tested in eosinophilic conditions, including allergic and/or refractory eosinophilic asthma, showing improvements in several clinical outcomes.[78,178-182] Using multiple biomarkers in a post hoc analysis of a study in moderate asthma, CRTH2 antagonist OC000459 (Timapiprant) appeared most effective in younger (age ≤ 40 years) patients with uncontrolled, atopic asthma with blood eosinophilia (≥250 cells/μL).[78] Currently, several CRTH2 antagonists are moving into phase 3 studies which should help to consolidate phenotypes and adequate biomarkers responding to these targeted drugs.

7.2 | Non-T2 targeted therapies

7.2.1 | TNF targeted therapies

Tumor necrosis factor (TNF) has been associated with AHR both through its direct effect on ASM cells and indirectly via increased sputum neutrophils.[183] Increased TNF was demonstrated in BAL and bronchial biopsies of patients with severe asthma compared to mild asthma and healthy controls.[184] A placebo-controlled trial with etanercept for 10 weeks in refractory asthma showed beneficial effects on lung function, airway hyperreactivity (AHR), and AQLQ.[98] Post hoc analysis of a phase II study with golimumab in severe persistent asthma showed a longer time to first exacerbation compared to placebo in a subgroup of patients with reversible airway obstruction.[185] However, overall insufficient efficacy and the occurrence of serious infections led to discontinuation of the anti-TNF program.[98,185]

7.2.2 | IL-17RA targeted therapies

IL-17RA is a subunit of the receptor for IL-17A, IL-17F, and IL-25 (also named IL-17E). In addition to its indirect effect on neutrophil recruitment to the airways, IL-17A can increase the contractility and migration of ASM cells, thereby inducing AHR. As such, it is an attractive target for neutrophilic asthma. However, anti–IL-17 treatment with brodalumab showed overall no significant efficacy on clinical parameters including asthma control or lung function.[186]

7.2.3 | CXCR2 antagonists

CXCR2 is the high-affinity receptor of IL-8, which is a known chemoattractant for neutrophils.[187] Two placebo-controlled trials with CXCR2 antagonists have been conducted in patients with uncontrolled asthma.[188,189] Despite dose-dependent reductions in blood neutrophil counts, neither study could demonstrate clinical effectiveness. In line with studies with anti–IL-17RA therapy, these findings challenge a crucial role of neutrophils as potential therapeutic targets in asthma and further research should clarify this.

7.2.4 | Macrolides

Macrolides possess both antimicrobial and nonantimicrobial ("anti-inflammatory") properties and showed clinical effectiveness in distinct asthma populations.[190] Clarithromycin was the first macrolide that was evaluated in a placebo-controlled trial in refractory asthma.[88] Compared to placebo, 8 weeks of treatment with clarithromycin produced significant reductions in sputum neutrophils and IL-8 levels. These effects were paralleled by significant improvements in AQLQ without affecting asthma control or lung function. Azithromycin was assessed in two double-blind placebo-controlled trials. Although in the first study (AZISAST) azithromycin (26 weeks, 250 mg three times a week; n = 109) failed to reduce severe exacerbations and lower respiratory tract infections, there was a significant improvement in clinical endpoints in a subgroup with noneosinophilic asthma.[191] In a recent study (AMAZES) in uncontrolled persistent asthma, azithromycin (48 weeks, 500 mg three times a week; n = 420) on top of ICS plus LABA produced significant improvement in both moderate and severe exacerbations and AQLQ.[192] Remarkably, these beneficial effects were seen in both eosinophilic and noneosinophilic patients with asthma.

7.3 | Targeted therapies for structural abnormalities

7.3.1 | Bronchial thermoplasty

Bronchial thermoplasty (BT) is a relatively novel method that ablates ASM by bronchoscopic intervention involving a localized radiofrequency pulse.[193] Further evidence suggests additional clinical effectiveness from concomitant ablation of sensory nerve fibers within the bronchial epithelium upon BT treatment.[194] Two uncontrolled studies (RISA and AIR) showed improved symptoms, asthma control, quality of life and less mild exacerbations after BT *versus* standard care in symptomatic patients on high-dose ICS and LABA.[195,196] A sham-controlled study (AIR2) showed reduced severe asthma exacerbations and reduced loss of work after BT.[197] A recent 3-year follow-up after BT analysis of two cohorts of symptomatic severe asthma patients (AIR2: n = 190; PAS2: n = 190) showed reduced severe exacerbations, emergency department visits and hospitalizations *versus* the year prior to BT.[198] In these studies, BT did not affect lung function. From a practical perspective including biomarkers, refractory patients with a low PC20 and/or compromised lung function with frequent exacerbations without signs of airway inflammation are likely to be eligible for BT.[199]

8 | CONCLUDING REMARKS AND RECOMMENDATIONS

For efficient and cost-effective adoption of targeted treatment options in daily clinical practice, clinicians need point-of-care, well-defined, and reliable biomarkers to support them in identifying phenotypes and endotypes of asthma most likely to respond.[13,199]

So far, eosinophilic asthma including associated comorbidities (eg, nasal polyposis, NERD) as an inflammatory phenotype responsive to corticosteroids and anti–IL-5 targeted therapy (anti–IL-5, CRTH2 antagonists) has been well defined. Although no absolute/consistent cutoff values have been established, subanalyses show an overall better response in patients with more inflammation, defined by higher blood eosinophil levels. Apart from these observations, so far there is no consensus on a unique lower limit value nor on how exactly blood eosinophil levels relate to other phenotypic features or "treatable traits" nor to concomitant medication within an individual patient.

Eosinophilic asthma comprises different endotypes. Currently, the best point-of-care biomarker to identify the T2 endotype is FeNO, while in more sophisticated settings, serum cytokines or sputum mRNA analysis as part of multidimensional endotyping may help to further characterize the individual profile, while serum periostin and DPP-4 have not been fully validated.

In severe allergic asthma, serum total IgE is useful in identifying patients who could benefit from anti-IgE therapy, but it cannot predict the degree of response after treatment. In patients with concomitant high eosinophil levels who remain uncontrolled, switching to an anti-eosinophilic treatment might be a good option. To guide anti–IL-4/13 targeted (endotypic) therapy, FeNO seems presently the best biomarker as evaluated following the SAVED approach.

Despite recent progress in the identification of other potentially applicable biomarkers in conjunction with targeted treatments, there is still an unmet need to characterize underlying pathways and validate associated biomarkers for distinct asthma pheno/endotypes. So far, T2 asthma has been fairly well characterized including clinically applicable biomarkers, while non-T2 asthma still represents an unmet need lacking adequate biomarkers and targeted treatment options.

Other unmet needs include more differentiating, noninvasive, simply measurable, validated and reliable (composite) biomarkers with well-defined cutoff values and documentation on their stability/behavior over time. In parallel, a consensus on treatment algorithms (which targeted therapy and administration route for which patient, for how long) is urgently needed, as well as longitudinal follow-up of response to novel biologicals in real-life settings, including elderly asthma patients (>60 years) and pediatric populations.

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CONFLICT OF INTEREST

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