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Towards 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 non-allergic 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 (EAACI) 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.

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

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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, classify as severe asthma patients (7).

In the past decade, distinct molecular mechanisms have been identified and linked to clinical asthma phenotypes (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 (UBIOPRED) consortium and Severe Asthma Respiratory Program (SARP). With the advent of novel expensive biologicals to treat (severe) asthma (e.g., targeting IgE, IL5, IL4/IL13 and others), there is a strong need of clinical and biological markers that can guide the choice of treatment, predict treatment response, as well as monitor the treatment response. Implementing targeted treatment into daily practice is however challenging and requires biomarker validation and evaluation of the socio-economic 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.

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).

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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.

In addition, each sampling method has its own advantages and limitations (Table 1). The most tissue- 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 implies 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 cut-off value has so far not been established.

During the last decades, several novel, non-invasive 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 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 analysed using two different approaches: *i.e.* 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

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for both VOCs techniques consist of rigorous standardization of sampling, pre-processing and analysis, including independent external data validation (31). Particles in exhaled air (PExA), is a novel, non-invasive 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 non-volatile molecules. However, this approach is limited due to the lack of standardized methodology for collection as well as variable 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).

Biomarkers of type 2 (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 IL4, IL5 and/or IL13 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 (39,41). T2 asthma is presently the best-characterized endotype within the eosinophilic phenotype, usually associated with allergy, although non-allergic pathways of airway eosinophilia have been proposed (Figure 3) (42). Recently, a subgroup of patients with high FeNO levels (>25ppb) 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 (43). Epithelial-derived cytokines, including thymic stromal lymphopoietin (TSLP), IL25, IL33, with subsequent activation of ILC2 may support the underlying pathophysiological event (44).

Multiple inflammatory components have been evaluated for their potential as a biomarker of T2 (allergic) asthma (45). Sputum eosinophils are probably the best-characterized and most useful biomarker so far. While in general, eosinophilia suggests corticosteroid responsiveness (39,46), it may also reflect poor adherence to ICS (47). Compared to guideline-based management,

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sputum eosinophil-guided management showed a reduction in exacerbations, especially in patients with more severe asthma (48). ERS/ATS and recent GINA guidelines now suggest treatment guided by sputum analysis for severe asthma in experienced centers (7,49). Concomitant systemic and airway eosinophilia has been associated with worse asthma control (50). However, blood and sputum eosinophils cannot always be used interchangeably, especially in patients on oral corticosteroids (24,51,52). 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 (53). 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 (54).

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 5 clusters with equal proportions of IL4-, IL13-high patients, whereas IL5-high expression was restricted to patients with an IL25-, IL17A/F-high pattern. These data confirm earlier reports on a subgroup of patients with concomitant activation of Th2 and Th17 inflammatory pathways (37,55). Recently, this was reinforced by a complete transcriptomics analysis, showing heterogeneity amongst patients with asthma beyond T2 classification (9). Profiling of T2 cytokine patterns by Meso Scale multiplex technology may help to identify eligible patients for biologicals targeting different T2 pathways (54).

FeNO is a reproducible, easily measurable biomarker and a good predictor of ICS response (58,59). However, FeNO may be affected by several confounders, including demographics, smoking, atopy and diet (29,60,61). 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 (adults) and <20ppb (children) (58). Strategies incorporating FeNO into standard clinical practice allowed reduction in ICS doses in adults (but not in children) (62). In a study in pregnancy, FeNO-guided treatment resulted in a significant reduction in asthma exacerbations and mean ICS dose (63). 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 (64) and with eosinophils in BAL (65). Even without information on an individual's molecular pathways, such

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probabilistic approach can be very powerful in phenotypic classification. Based on the same principle of exhaled VOCs, eNOSE can predict loss of asthma control (66) and may be more sensitive than FeNO or sputum eosinophilia in predicting clinical efficacy of systemic corticosteroids (67). However, most studies are small and focused on adults, while scarce data is 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 IL13 (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 (68). However, this was not confirmed in follow-up studies (21,23,69). Asthma patients with increased serum periostin showed improvements in lung function after treatment with lebrikizumab, an anti-IL13 monoclonal antibody (mAb), in contrast to patients with low periostin levels (70). However, lebrikizumab efficacy could not be confirmed in two subsequent phase 3 studies, even not in periostin-high patients (71). 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 (72).

Dipeptidyl peptidase-4 (DPP-4) has been proposed as a candidate predictive biomarker for the response to anti-IL13 treatment. Patients with DPP-4 levels above median showed better responses to tralokinumab in lung function and health status (73). 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 anti-leukotrienes (74) and in aspirin or NSAIDs-exacerbated respiratory disease (NERD) (75). Urinary LTE4 could be a potential biomarker in studies involving eicosanoid pathways (76).

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 (77). Using a prediction model in two independent cohorts, FeNO, blood eosinophils and the activation status of blood eosinophils and

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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) (78). Some clinical trials applying targeted therapies evaluated treatment response in patients based on composite-biomarker profiles (79–81). The role of composite biomarker profiles in asthma phenotyping and management needs to be established.

Biomarkers of non-type 2 inflammation

The non-T2 endotype consists of patients in whom T2 inflammation is absent or within normal range (e.g. T2-low). This endotype covers both patients with a neutrophilic and a paucigranulocytic airway inflammatory pattern (82). A clear definition of neutrophilic airway inflammation is still lacking since various sputum neutrophil cut off levels (40-76%) have been reported (41,50,83,84). Sputum neutrophilia was found to be associated with (relative) insensitivity to ICS (41), in smoking (85) and in obese asthma patients (86,87). Adults with refractory asthma were shown to have higher levels of BAL neutrophils compared to non-refractory patients with asthma (88). Apart from reflecting a distinct phenotype, airway neutrophilia often associates with (subclinical) airway infection (89) or oral corticosteroid use (88). In childhood asthma, neutrophilic airway inflammation seems to play a minor role (90). 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 (91). Conversely, in a recent study in children with severe treatment resistant asthma, the presence of intra-epithelial neutrophils and increased IL17RA expression were associated with better lung function (92). Recent data however do support the relationship between airway neutrophilia and asthma severity in children. The analysis of the Tawainese Consortium of Childhood Asthma Study showed that neutrophil-predominant asthma is the most severe asthma phenotype in children with a poor corticosteroid response (93). In the inner-city study, Th17-related cytokines were associated with difficult-to-control asthma (56).

Several cytokines associate with sputum neutrophilia (Figure 2). Interleukin 17A, mainly produced by T cells or type 3 ILCs, promotes the production of IL8, chemo-attractant for neutrophils, by structural cells (44,94,95). Both sputum IL17A and IL8 gene expression are positively correlated with sputum neutrophil counts (46). Gene expression of *CXCR2*, the receptor for IL8, was found to be increased in neutrophilic compared to eosinophilic asthma

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(96). More recently, the inflammasome pathway with increased expression of NLRP3 and IL1β was found to be associated with neutrophilic asthma (97,98).

Similarly to increased sputum neutrophils, membrane-bound TNF on circulating monocytes was increased in refractory compared to milder asthma (99), whereas no association was found between free TNF and sputum neutrophils in patients with severe asthma (100).

Few studies have investigated the potential of serum biomarkers to identify neutrophilic asthma. Serum IL17 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 (101). Increased serum soluble TNF and IL8 levels accompanied by raised circulating neutrophils have been detected in severe asthma patients compared to healthy controls(102). A recent analysis showed 5 biomolecules in serum correlating with BAL neutrophilia (88). In asthma patients, serum calprotectin (S100A8/A9), a danger molecule released by the airway epithelium, can predict with high sensitivity and specificity the presence of increased sputum neutrophils (>61%) (103). 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 (104).

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 (83). While the majority of these patients is well-controlled with a normal lung function, a subgroup (approximately 15%) remains uncontrolled despite normal sputum granulocyte counts (105). In these patients a ''low-grade'' inflammation (77), or structural changes including epithelial cells, airway smooth muscle, nerves and/or vessels may be the underlying pathophysiological substrate.

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 (106,107). Although bronchial epithelial cell detachment was also claimed to occur *in situ*, some argued if this reflects an artefact of bronchoscopy (108).

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Although these features are manifest in adults with chronic asthma, similar changes are already present in childhood asthma (91,109,110), 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 (111) have been shown to reflect asthma severity (112), other associations between markers of airway remodeling and airway obstruction or AHR have been inconsistent (113,114).

So far, the number of reliable biomarkers reflecting aspects of airway remodeling are scarce. The thickening of the RBM correlates well with eosinophil numbers in bronchial mucosa (115) and eosinophil-depleting treatments (114,116) showed inhibitory effects on components driving this subepithelial fibrosis. In parallel, reduction in symptoms and asthma exacerbations and improvement in lung function was achieved in adults (115) and in children (117) with protection against methacholine-induced maximal airway narrowing (118,119). In a biopsy study in severe allergic asthma, apart from anti-eosinophil effects, omalizumab (anti-IgE; (120)) reduced RBM thickening in some patients. In a subsequent analysis this reduction correlated with galectin-3 (121), which appears to regulate airway remodeling (122). Chitin and chitinase/chitinase-like proteins have also been found to affect airway remodeling (123). 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) (124). 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.

Transcriptome analyses of ASM from asthma patients revealed marked differences compared to healthy controls (125). 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 (125). Changes in expression of these genes induced by oral corticosteroids were associated with improvements in airway physiology (126). 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 (127) and the expression of various ASM proteins and airway dynamics (128). In fact, corticosteroids can affect various cellular programs of ASM and some genetic variants correlated with AHR. Consisting of different

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components, it is likely that airway remodeling can be evaluated by combining multiple biomarkers generated by unbiased cluster analyses (e.g. U-BIOPRED) (9).

Biomarkers of airway remodeling could identify individuals at risk of developing asthma at an early stage (129). Although controversial, chronic airway inflammation has been considered the major driver of airway remodeling (114,115). Indeed, anti-inflammatory therapy with corticosteroids has been shown to reduce goblet cell numbers in asthma (130), and airway wall thickening (131). Hence, some inflammatory markers may be indicative of airway remodeling. In this context, the T2-cytokine IL13 has been identified as a major driver of airway remodeling in asthma and several proteins induced by IL13 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 IL13, while recent studies also underpin its association with bronchial wall thickening in asthma and chronic rhinosinusitis (132,133).

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) (134). FCFM visualizes specifically elastic fibres within the airway wall correlating with histological analysis. The link between elastic fibre 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 (135).

Biomarkers for management of severe asthma

Novel treatment options have been developed for patients who fail to achieve asthma control despite maximal standard treatment (GINA step 5) (136). The majority of these treatments target T2 inflammation (Figure 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 (137).

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TYPE 2 TARGETED TREATMENT

IgE targeted therapies

Omalizumab is the first T2 targeting biological that was approved for severe allergic asthma (138). 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) (139). Clinical studies showed that omalizumab as add-on therapy to ICS successfully reduces asthma exacerbations, hospitalisations and doses of ICS while improving quality of life in adults and children >12 years of age with moderate to severe allergic asthma (140–142). Whether omalizumab can effectively reduce systemic corticosteroids needs further investigation (143).

Consistent correlations between treatment response and baseline total serum IgE or antigen specific IgE levels are lacking (144,145). Serum IgE is used to dose omalizumab but the cut-off is rather arbitrary (146). The use of CD-sens (basophil activation threshold) has proven to be useful in monitoring response to omalizumab in allergic asthma (144, 146). On the other hand, routine measurements of free IgE in serum can identify patients not responding to omalizumab treatment (146).

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 (148). In this retrospective analysis, patients were divided 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 (≥50ng/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 (149). 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 (150).

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IL5 targeted therapies

Interleukin 5 (IL5) is another promising T2 target. Currently, there are several therapies interfering with the IL5 pathway available for uncontrolled severe eosinophilic asthma. Current registered treatments comprise mepolizumab and reslizumab, mAb specifically targeting IL5 and preventing its binding to IL5-receptors (IL5R) (151,152). Another anti-IL5 mAb, benralizumab, directed against the IL5 receptor α (IL5Rα), induces a rapid depletion of eosinophils (153). In several asthma trials, benralizumab showed clinical effectiveness and has been recently registered in several countries (154).

The first clinical studies of anti-IL5 in ''unphenotyped'' mild allergic and moderate asthma were rather disappointing. In these studies, blocking IL5 had no effect on clinical outcomes, including allergen-induced late asthmatic response, asthma-symptoms, lung function and quality of life scores (155,156). 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) (151,152,157–160), anti-IL5 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 (158). 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 (159–161). However, the long-term effects of eosinophil depletion remain unclear.

A recent systematic review assessed 13 studies (in total 6000 patients) showing that anti-IL5 therapy approximately halves the number of exacerbations in uncontrolled eosinophilic asthma (154). Patients are more likely to respond to anti-IL5 treatment if they have >3% of eosinophils in sputum, or ≥500 cells/μL blood eosinophils (21,22,158), although lower eosinophil cut-offs have been used. Nevertheless, more research is needed to identify biomarkers (combinations; cut-offs) that can more accurately predict treatment outcomes.

IL4/IL13 targeted therapies (dual blockade)

Both IL4 and IL13 bind to the α chain of type 2 IL4 receptors (IL4Rα). Therefore, blocking IL4Rα affects both IL4 and IL13 downstream signaling. Various asthma treatments, such as pitrakinra (mutant form human IL4) and dupilumab (fully human mAb to IL4Rα) have been investigated for this purpose (161,162).

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Pitrakinra inhibits IL4Rα by competing with IL4. 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 IL4Rα genotypes (165). 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), 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) (163). 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 (164). 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 (165). Based on its mode of action, FeNO, serum periostin and/or DPP-4 may serve as potential biomarkers to identify responders to dupilumab (70,166); 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 (167,168) while reducing systemic corticosteroid use (168). Presently, dupilumab is in registration phase in several countries.

IL13 targeted therapies

Human(ised) mAb targeting IL13 (lebrikizumab and tralokinumab) have 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 IL13-targeting therapy.

Periostin, together with CLCA1 and serpinB2, is co-upregulated in airway epithelial cells from T2-driven asthma patients upon IL13 stimulation (39,169). As periostin is secreted at the basolateral side of the epithelium, it may diffuse into the bloodstream and can therefore be quantified in serum (68).

In phase 2 studies with lebrikizumab, 'periostin-high' (and FeNO-high) patients with uncontrolled asthma showed greater improvement in FEV1 (70). This was replicated in

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uncontrolled severe asthma patients receiving ICS and a second controller and the periostin-high patients also had a greater reduction in severe exacerbations (71). 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) (170).

In a phase 2 study with tralokinumab, periostin-high patients showed non-significant improvements in exacerbation rate and FEV_1 (171). In this study DPP-4-high patients showed improvements in asthma exacerbation rate, FEV_1, ACQ-6 and AQLQ (171).

Apart from its ability to identify responders to treatment targeting IL13, 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 (172).

TSLP targeted therapies

Thymic Stromal Lymphopoetin (TSLP) is an important cytokine centrally involved in first line immune defence and a recent asthma target. TSLP mediates allergic responses in the skin, gut, upper and lower airways and is thus considered an upstream "master switch" of T2-inflammation (173). 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 (174).

TSLP expression in bronchial biopsies correlates both with disease severity and expression of T2-cytokines (175). Treatment with anti-TSLP (AMG157/tezepelumab) in a cohort of mild atopic asthma patients significantly reduced FeNO and blood eosinophils pre- and post-allergen 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 late airway responses to inhaled allergen (176). 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 (177). Future research should help to identify biomarkers to guide anti-TSLP treatment in subsequent clinical studies.

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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 (178,179). In proof of concept studies, CRTH2 antagonists blocked allergic responses downstream of the Th2-pathway decreasing T(h)2-cytokines, eosinophils and IgE synthesis (180,181). 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 (182), more recently, several CRTH2 antagonists have been tested in eosinophilic conditions, including allergic and/or refractory eosinophilic asthma, showing improvements in several clinical outcomes (79,183–187). 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) (79). Currently, several CRTH2 antagonists are moving into phase 3 studies which should help to consolidate phenotypes and adequate biomarkers responding to these targeted drugs.

NON-TYPE 2 TARGETED TREATMENT

Anti-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 (188). Increased TNF was demonstrated in BAL and bronchial biopsies of patients with severe asthma compared to mild asthma and healthy controls (189). A placebo-controlled trial with etanercept for 10 weeks in refractory asthma showed beneficial effects on lung function, airway hyperreactivity (AHR), and AQLQ (99). 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 (190). However, overall insufficient efficacy and the occurrence of serious infections led to discontinuation of the anti-TNF program (99,190).

IL17RA targeted therapies

IL17RA is a subunit of the receptor for IL17A, IL17F and IL25 (also named IL17E). In addition to its indirect effect on neutrophil recruitment to the airways, IL17A can increase the contractility and migration of ASM cells, thereby inducing AHR. As such it is an attractive target for

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neutrophilic asthma. However, anti-IL17 treatment with brodalumab showed overall no significant efficacy on clinical parameters including asthma control or lung function (191).

CXCR2 antagonists

CXCR2 is the high-affinity receptor of IL8, which is a known chemo-attractant for neutrophils (192). Two placebo-controlled trials with CXCR2 antagonists have been conducted in patients with uncontrolled asthma (193,194). Despite dose-dependent reductions in blood neutrophil counts, neither study could demonstrate clinical effectiveness. In line with studies with anti-IL17RA therapy, these findings challenge a crucial role of neutrophils as potential therapeutic targets in asthma and further research should clarify this.

Macrolides

Macrolides possess both antimicrobial and non-antimicrobial (''anti-inflammatory'') properties and showed clinical effectiveness in distinct asthma populations (195). Clarithromycin was the first macrolide that was evaluated in a placebo-controlled trial in refractory asthma (89). Compared to placebo, 8 weeks of treatment with clarithromycin produced significant reductions in sputum neutrophils and IL8 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, 250mg 3 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 non-eosinophilic asthma (196). In a recent study (AMAZES) in uncontrolled persistent asthma, azithromycin (48 weeks, 500mg 3 times a week; n=420) on top of ICS plus LABA produced significant improvement in both moderate and severe exacerbations and AQLQ (197). Remarkably, these beneficial effects were seen in both eosinophilic and non-eosinophilic patients with asthma.

TREATMENTS TARGETING AIRWAY REMODELING

Bronchial thermoplasty

Bronchial thermoplasty (BT) is a relatively novel method that ablates ASM by bronchoscopic intervention involving a localized radiofrequency pulse (198). Further evidence suggests

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additional clinical effectiveness from concomitant ablation of sensory nerve fibers within the bronchial epithelium upon BT treatment (199). 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 (200,201). A sham-controlled study (AIR2) showed reduced severe asthma exacerbations and reduced loss of work after BT (202). 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 (203). 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 (204).

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 (13,204).

So far, eosinophilic asthma including associated comorbidities (e.g., nasal polyposis, NERD) as an inflammatory phenotype responsive to corticosteroids and anti-IL5 targeted therapy (anti-IL5, CRTH2 antagonists) has been well-defined. Although no absolute/consistent cut off 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-IL4/13 targeted (endotypic) therapy, FeNO seems presently the best biomarker as evaluated following the SAVED approach.

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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, non-invasive, simply measurable, validated and reliable (composite) biomarkers with well-defined cut-off values and documentation on their stability/behaviour 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

KK was an employee and shareholder of Novartis until 31 October 2018.

References

1. Lambrecht BN, Hammad H. The immunology of asthma. Nat Immunol. 2015;16(1):45–56.

2. Papi A, Brightling C, Pedersen SE, Reddel HK. Asthma. Lancet. 2018;391(10122):783–800.

3. Green RH, Brightling CE, Woltmann G, Parker D, Wardlaw AJ, Pavord ID. Analysis of induced sputum in adults with asthma: identification of subgroup with isolated sputum neutrophilia and poor response to inhaled corticosteroids. Thorax. 2002;57(10):875–9.

4. Moore WC, Meyers DA, Wenzel SE, Teague WG, Li H, Li X, et al. Identification of Asthma Phenotypes Using Cluster Analysis in the Severe Asthma Research Program. Am J Respir Crit Care Med. 2009;181(4):315–323.

This article is protected by copyright. All rights reserved.

Accepted Article

5. Haldar P, Pavord ID, Shaw DE, Berry MA, Thomas M, Brightling CE, et al. Cluster Analysis and Clinical Asthma Phenotypes. Am J Respir Crit Care Med. 2008;178(3):218–224.

6. Fahy JV. Type 2 inflammation in asthma--present in most, absent in many. Nat Rev Immunol. 2015;15(1):57–65.

7. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. Eur Respir J. 2014;43(2):343–73.

8. Wu W, Bleecker E, Moore W, Busse WW, Castro M, Chung KF, et al. Unsupervised phenotyping of Severe Asthma Research Program participants using expanded lung data. J Allergy Clin Immunol. 2014;133(5):1280–8.

9. Kuo C-HS, Pavlidis S, Loza M, Baribaud F, Rowe A, Pandis I, et al. T-helper cell type 2 (Th2) and non-Th2 molecular phenotypes of asthma using sputum transcriptomics in U-BIOPRED. Eur Respir J. 2017;49(2).

10. Seys SF, Scheers H, Van den Brande P, Marijsse G, Dilissen E, Van Den Bergh A, et al. Cluster analysis of sputum cytokine-high profiles reveals diversity in T(h)2-high asthma patients. Respir Res. 2017 23;18(1):39.

11. Richards LB, Neerincx AH, van Bragt JJMH, Sterk PJ, Bel EHD, Maitland-van der Zee AH. Biomarkers and asthma management: analysis and potential applications. Curr Opin Allergy Clin Immunol. 2018;18(2):96–108.

12. Agache I, Akdis C, Jutel M, Virchow JC. Untangling asthma phenotypes and endotypes. Allergy. 2012;67(7):835–846.

13. Muraro A, Lemanske RF, Hellings PW, Akdis CA, Bieber T, Casale TB, et al. Precision medicine in patients with allergic diseases: Airway diseases and atopic dermatitis-PRACTALL document of the European Academy of Allergy and Clinical Immunology and the American Academy of Allergy, Asthma & Immunology. J Allergy Clin Immunol. 2016;137(5):1347–58.

14. Hollander Z, DeMarco ML, Sadatsafavi M, McManus BM, Ng RT, Sin DD. Biomarker Development in COPD: Moving From P Values to Products to Impact Patient Care. Chest. 2017;151(2):455–67.

15. FDA CDER Biomarker Qualification Program [Internet]. Available from: https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DrugDevelopmentToolsQualificationProgram/BiomarkerQualificationProgram/default.htm

16. Hekking P-P, Loza MJ, Pavlidis S, De Meulder B, Lefaudeux D, Baribaud F, et al. Transcriptomic gene signatures associated with persistent airflow limitation in patients with severe asthma. Eur Respir J. 2017;50(3).

17. Hekking P-P, Loza MJ, Pavlidis S, de Meulder B, Lefaudeux D, Baribaud F, et al. Pathway discovery using transcriptomic profiles in adult-onset severe asthma. J Allergy Clin Immunol. 2018;141(4):1280–90.

18. Alexis NE. Biomarker sampling of the airways in asthma. Curr Opin Pulm Med.

This article is protected by copyright. All rights reserved.

Accepted Article

2014;20(1):46–52.

19. Seys SF. Role of sputum biomarkers in the management of asthma. Curr Opin Pulm Med. 2017;23(1):34-40.

20. Zuiker RGJA, Tribouley C, Diamant Z, Boot JD, Cohen AF, Van Dyck K, et al. Sputum RNA signature in allergic asthmatics following allergen bronchoprovocation test. Eur Clin Respir J. 2016;3:31324.

21. Wagener AH, de Nijs SB, Lutter R, Sousa AR, Weersink EJM, Bel EH, et al. External validation of blood eosinophils, FE(NO) and serum periostin as surrogates for sputum eosinophils in asthma. Thorax. 2015;70(2):115–20.

22. Fowler SJ, Tavernier G, Niven R. High blood eosinophil counts predict sputum eosinophilia in patients with severe asthma. J Allergy Clin Immunol. 2015;135(3):822–824.e2.

23. Agache I, Strasser DS, Klenk A, Agache C, Farine H, Ciobanu C, et al. Serum IL-5 and IL-13 consistently serve as the best predictors for the blood eosinophilia phenotype in adult asthmatics. Allergy. 2016;71(8):1192–202.

24. Korevaar DA, Westerhof GA, Wang J, Cohen JF, Spijker R, Sterk PJ, et al. Diagnostic accuracy of minimally invasive markers for detection of airway eosinophilia in asthma: a systematic review and meta-analysis. Lancet Respir Med. 2015;3(4):290–300.

25. Ullmann N, Bossley CJ, Fleming L, Silvestri M, Bush A, Saglani S. Blood eosinophil counts rarely reflect airway eosinophilia in children with severe asthma. Allergy. 2013;68(3):402–6.

26. Spector SL, Tan RA. Is a single blood eosinophil count a reliable marker for “eosinophilic asthma?” J Asthma. 2012;49(8):807–10.

27. Ludviksdottir D, Diamant Z, Alving K, Bjermer L, Malinovschi A. Clinical aspects of using exhaled NO in asthma diagnosis and management. Clin Respir J. 2012;6(4):193–207.

28. Neerincx AH, Vijverberg SJH, Bos LDJ, Brinkman P, van der Schee MP, de Vries R, et al. Breathomics from exhaled volatile organic compounds in pediatric asthma. Pediatr Pulmonol. 2017;52(12):1616–27.

29. Boot JD, de Kam ML, Mascelli MA, Miller B, van Wijk RG, de Groot H, et al. Nasal nitric oxide: longitudinal reproducibility and the effects of a nasal allergen challenge in patients with allergic rhinitis. Allergy. 2007;62(4):378–84.

30. Dweik RA, Sorkness RL, Wenzel S, Hammel J, Curran-Everett D, Comhair SAA, et al. Use of Exhaled Nitric Oxide Measurement to Identify a Reactive, at-Risk Phenotype among Patients with Asthma. Am J Respir Crit Care Med. 2010;181(10):1033–1041.

31. Bos LD, Sterk PJ, Fowler SJ. Breathomics in the setting of asthma and chronic obstructive pulmonary disease. J Allergy Clin Immunol. 2016;138(4):970–6.

32. Almstrand A-C, Ljungström E, Lausmaa J, Bake B, Sjövall P, Olin A-C. Airway monitoring by collection and mass spectrometric analysis of exhaled particles. Anal Chem. 2009;81(2):662–8.

This article is protected by copyright. All rights reserved.

Accepted Article

33. Larsson P, Lärstad M, Bake B, Hammar O, Bredberg A, Almstrand A-C, et al. Exhaled particles as markers of small airway inflammation in subjects with asthma. Clin Physiol Funct Imaging. 2017;37(5):489–97.

34. Farzan N, Vijverberg SJ, Kabesch M, Sterk PJ, Maitland-van der Zee AH. The use of pharmacogenomics, epigenomics, and transcriptomics to improve childhood asthma management: Where do we stand? Pediatr Pulmonol. 2018;53(6):836-845

35. Huang YJ, Boushey HA. The microbiome in asthma. J Allergy Clin Immunol. 2015;135(1):25–30.

36. Trivedi A, Hall C, Hoffman EA, Woods JC, Gierada DS, Castro M. Using imaging as a biomarker for asthma. J Allergy Clin Immunol. 2017;139(1):1–10.

37. Seys SF, Grabowski M, Adriaensen W, Decraene A, Dilissen E, Vanoirbeek JA, et al. Sputum cytokine mapping reveals an "IL-5, IL-17A, IL-25-high" pattern associated with poorly controlled asthma. Clin Exp Allergy J Br Soc Allergy Clin Immunol. 2013;43(9):1009–17.

38. Peters MC, Mekonnen ZK, Yuan S, Bhakta NR, Woodruff PG, Fahy JV. Measures of gene expression in sputum cells can identify TH2-high and TH2-low subtypes of asthma. J Allergy Clin Immunol. 2014;133(2):388-94.

39. Woodruff PG, Modrek B, Choy DF, Jia G, Abbas AR, Ellwanger A, et al. T-helper Type 2-driven Inflammation Defines Major Subphenotypes of Asthma. Am J Respir Crit Care Med. 2009;180(5):388–395.

40. Bhakta NR, Solberg OD, Nguyen CP, Nguyen CN, Arron JR, Fahy JV, et al. A qPCR-based metric of Th2 airway inflammation in asthma. Clin Transl Allergy. 2013;3(1):24.

41. Green RH, Brightling CE, Woltmann G, Parker D, Wardlaw a J, Pavord ID. Analysis of induced sputum in adults with asthma: identification of subgroup with isolated sputum neutrophilia and poor response to inhaled corticosteroids. Thorax. 2002;57(10):875–9.

42. Brusselle GG, Maes T, Bracke KR. Eosinophils in the spotlight: Eosinophilic airway inflammation in nonallergic asthma. Nat Med. 2013;19(8):977–9.

43. Soma T, Iemura H, Naito E, Miyauchi S, Uchida Y, Nakagome K, et al. Implication of fraction of exhaled nitric oxide and blood eosinophil count in severe asthma. Allergol Int. 2018;67:S3–11.

44. Kortekaas Krohn I, Shikhagaie MM, Golebski K, Bernink JH, Breynaert C, Creyns B, et al. Emerging roles of innate lymphoid cells in inflammatory diseases: Clinical implications. Allergy. 2018;73(4):837–50.

45. Zissler UM, Esser-von Bieren J, Jakwerth CA, Chaker AM, Schmidt-Weber CB. Current and future biomarkers in allergic asthma. Allergy. 2016;71(4):475–94.

46. Berry M, Morgan A, Shaw DE, Parker D, Green R, Brightling C, et al. Pathological features and inhaled corticosteroid response of eosinophilic and non-eosinophilic asthma. Thorax.

This article is protected by copyright. All rights reserved.

Accepted Article

2007;62(12):1043–9.

47. Gibson PG. Inflammatory phenotypes in adult asthma: clinical applications. Clin Respir J. 2009;3(4):198–206.

48. Petsky HL, Cates CJ, Lasserson TJ, Li AM, Turner C, Kynaston JA, et al. A systematic review and meta-analysis: tailoring asthma treatment on eosinophilic markers (exhaled nitric oxide or sputum eosinophils). Thorax. 2012;67(3):199–208.

49. 2018 GINA Report, Global Strategy for Asthma Management and Prevention [Internet]. 2018. Available from: www.ginasthma.org

50. Schleich FN, Chevremont A, Paulus V, Henket M, Manise M, Seidel L, et al. Importance of concomitant local and systemic eosinophilia in uncontrolled asthma. Eur Respir J. 2014;44(1):97–108.

51. Kostikas K, Zervas E, Gaga M. Airway and systemic eosinophilia in asthma: does site matter? Eur Respir J. 2014;44(1):14–6.

52. Mukherjee M, Nair P. Blood or sputum eosinophils to guide asthma therapy? Lancet Respir Med. 2015;3(11):824–5.

53. Fitzpatrick AM, Jackson DJ, Mauger DT, Boehmer SJ, Phipatanakul W, Sheehan WJ, et al. Individualized therapy for persistent asthma in young children. J Allergy Clin Immunol. 2016;138(6):1608–1618.e12.

54. Wolfe MG, Mukherjee M, Radford K, Brennan JD, Nair P. Rapid quantification of sputum eosinophil peroxidase on a lateral flow test strip. Allergy. 2018. doi: 10.1111/all.13711. [Epub ahead of print]

55. Choy DF, Hart KM, Borthwick LA, Shikotra A, Nagarkar DR, Siddiqui S, et al. TH2 and TH17 inflammatory pathways are reciprocally regulated in asthma. Sci Transl Med. 2015;7(301):301ra129.

56. Brown KR, Krouse RZ, Calatroni A, Visness CM, Sivaprasad U, Kercsmar CM, et al. Endotypes of difficult-to-control asthma in inner-city African American children. PloS One. 2017;12(7):e0180778.

57. Agache I, Strasser DS, Pierlot GM, Farine H, Izuhara K, Akdis CA. Monitoring inflammatory heterogeneity with multiple biomarkers for multidimensional endotyping of asthma. J Allergy Clin Immunol. 2018;141(1):442–5.

58. Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO, et al. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. Am J Respir Crit Care Med. 2011;184(5):602–15.

59. Bjermer L, Alving K, Diamant Z, Magnussen H, Pavord I, Piacentini G, et al. Current evidence and future research needs for FeNO measurement in respiratory diseases. Respir Med. 2014;108(6):830–41.

60. Kostikas K, Minas M, Papaioannou AI, Papiris S, Dweik RA. Exhaled nitric oxide in

This article is protected by copyright. All rights reserved.

Accepted Article

asthma in adults: the end is the beginning? Curr Med Chem. 2011;18(10):1423–31.

61. Zuiker RGJA, Boot JD, Calderon C, Piantone A, Petty K, de Kam M, et al. Sputum induction with hypertonic saline reduces fractional exhaled nitric oxide in chronic smokers and non-smokers. Respir Med. 2010;104(6):917–20.

62. Petsky HL, Cates CJ, Kew KM, Chang AB. Tailoring asthma treatment on eosinophilic markers (exhaled nitric oxide or sputum eosinophils): a systematic review and meta-analysis. Thorax. 2018;73(12):1110-1119.

63. Powell H, Murphy VE, Taylor DR, Hensley MJ, McCaffery K, Giles W, et al. Management of asthma in pregnancy guided by measurement of fraction of exhaled nitric oxide: a double-blind, randomised controlled trial. Lancet. 2011;378(9795):983–90.

64. de Vries R, Dagelet YWF, Spoor P, Snoey E, Jak PMC, Brinkman P, et al. Clinical and inflammatory phenotyping by breathomics in chronic airway diseases irrespective of the diagnostic label. Eur Respir J. 2018;51(1).

65. Fens N, van der Sluijs KF, van de Pol MA, Dijkhuis A, Smids BS, van der Zee JS, et al. Electronic nose identifies bronchoalveolar lavage fluid eosinophils in asthma. Am J Respir Crit Care Med. 2015;191(9):1086–8.

66. Brinkman P, van de Pol MA, Gerritsen MG, Bos LD, Dekker T, Smids BS, et al. Exhaled breath profiles in the monitoring of loss of control and clinical recovery in asthma. Clin Exp Allergy J Br Soc Allergy Clin Immunol. 2017;47(9):1159–69.

67. van der Schee MP, Palmay R, Cowan JO, Taylor DR. Predicting steroid responsiveness in patients with asthma using exhaled breath profiling. Clin Exp Allergy J Br Soc Allergy Clin Immunol. 2013;43(11):1217–25.

68. Jia G, Erickson RW, Choy DF, Mosesova S, Wu LC, Solberg OD, et al. Periostin is a systemic biomarker of eosinophilic airway inflammation in asthmatic patients. J Allergy Clin Immunol. 2012;130(3):647–654.e10.

69. Simpson JL, Yang IA, Upham JW, Reynolds PN, Hodge S, James AL, et al. Periostin levels and eosinophilic inflammation in poorly-controlled asthma. BMC Pulm Med. 2016;16(1):67.

70. Corren J, Lemanske RF, Hanania NA, Korenblat PE, Parsey MV, Arron JR, et al. Lebrikizumab Treatment in Adults with Asthma. N Engl J Med. 2011;365:1088–1098.

71. Hanania NA, Noonan M, Corren J, Korenblat P, Zheng Y, Fischer SK, et al. Lebrikizumab in moderate-to-severe asthma: pooled data from two randomised placebo-controlled studies. Thorax. 2015;70(8):748–56.

72. James A, Hedlin G. Biomarkers for the Phenotyping and Monitoring of Asthma in Children. Curr Treat Options Allergy. 2016;3(4):439–52.

73. Brightling CE, Chanez P, Leigh R, O'Byrne PM, Korn S, She D, et al. Efficacy and safety of tralokinumab in patients with severe uncontrolled asthma: a randomised, double-blind, placebo-controlled, phase 2b trial. Lancet Respir Med. 2015;3(9):692–701.

This article is protected by copyright. All rights reserved.

Accepted Article

Accepted Article

74. Diamant Z, Timmers MC, van der Veen H, Friedman BS, De Smet M, Depré M, et al. The effect of MK-0591, a novel 5-lipoxygenase activating protein inhibitor, on leukotriene biosynthesis and allergen-induced airway responses in asthmatic subjects in vivo. J Allergy Clin Immunol. 1995;95(1 Pt 1):42–51.

75. Kowalski ML, Agache I, Bavbek S, Bakirtas A, Blanca M, Bochenek G, et al. Diagnosis and management of NSAID-Exacerbated Respiratory Disease (N-ERD)-a EAACI position paper. Allergy. 2019;74(1):28-39.

76. Hagan JB, Laidlaw TM, Divekar R, O'Brien EK, Kita H, Volcheck GW, et al. Urinary Leukotriene E4 to Determine Aspirin Intolerance in Asthma: A Systematic Review and Meta-Analysis. J Allergy Clin Immunol Pract. 2017;5(4):990–997.e1.

77. Demarche S, Schleich F, Henket M, Paulus V, Van Hees T, Louis R. Detailed analysis of sputum and systemic inflammation in asthma phenotypes: are paucigranulocytic asthmatics really non-inflammatory? BMC Pulm Med. 2016;16:46.

78. Hilvering B, Vijverberg SJH, Jansen J, Houben L, Schweizer RC, Go S, et al. Diagnosing eosinophilic asthma using a multivariate prediction model based on blood granulocyte responsiveness. Allergy. 2017;72(8):1202–11.

79. Pettipher R, Hunter MG, Perkins CM, Collins LP, Lewis T, Baillet M, et al. Heightened response of eosinophilic asthmatic patients to the CRTH2 antagonist OC000459. Allergy. 2014;69(9):1223–32.

80. Hanratty CE, Matthews JG, Arron JR, Choy DF, Pavord ID, Bradding P, et al. A randomised pragmatic trial of corticosteroid optimization in severe asthma using a composite biomarker algorithm to adjust corticosteroid dose versus standard care: study protocol for a randomised trial. Trials. 2018;19(1):5.

81. Nagasaki T, Matsumoto H, Kanemitsu Y, Izuhara K, Tohda Y, Horiguchi T, et al. Using exhaled nitric oxide and serum periostin as a composite marker to identify severe/steroid-insensitive asthma. Am J Respir Crit Care Med. 2014;190(12):1449–52.

82. Schleich FN, Manise M, Sele J, Henket M, Seidel L, Louis R. Distribution of sputum cellular phenotype in a large asthma cohort: predicting factors for eosinophilic vs neutrophilic inflammation. BMC Pulm Med. 2013;13:11.

83. Simpson JL, Scott R, Boyle MJ, Gibson PG. Inflammatory subtypes in asthma: assessment and identification using induced sputum. Respirol Carlton Vic. 2006;11(1):54–61.

84. Moore WC, Hastie AT, Li X, Li H, Busse WW, Jarjour NN, et al. Sputum neutrophil counts are associated with more severe asthma phenotypes using cluster analysis. J Allergy Clin Immunol. 2014;133(6):1557–1563.e5.

85. Telenga ED, Kerstjens HAM, Ten Hacken NHT, Postma DS, van den Berge M. Inflammation and corticosteroid responsiveness in ex-, current- and never-smoking asthmatics. BMC Pulm Med. 2013;13:58.

86. Telenga ED, Tideman SW, Kerstjens H a. M, Hacken NHTT, Timens W, Postma DS, et al. Obesity in asthma: more neutrophilic inflammation as a possible explanation for a reduced

This article is protected by copyright. All rights reserved.

treatment response. Allergy. 2012;67(8):1060–8.

87. Marijsse GS, Seys SF, Schelpe A-S, Dilissen E, Goeminne P, Dupont LJ, et al. Obese Individuals with Asthma Preferentially Have a High IL-5/IL-17A/IL-25 Sputum Inflammatory Pattern. Am J Respir Crit Care Med. 2014;189(10):1284–5.

88. Alam R, Good J, Rollins D, Verma M, Chu H, Pham T-H, et al. Airway and serum biochemical correlates of refractory neutrophilic asthma. J Allergy Clin Immunol. 2017;140(4):1004–1014.e13.

89. Simpson JL, Powell H, Boyle MJ, Scott RJ, Gibson PG. Clarithromycin targets neutrophilic airway inflammation in refractory asthma. Am J Respir Crit Care Med. 2008;177(2):148–155.

90. Wang F, He XY, Baines KJ, Gunawardhana LP, Simpson JL, Li F, et al. Different inflammatory phenotypes in adults and children with acute asthma. Eur Respir J. 2011;38(3):567–74.

91. Bossley CJ, Fleming L, Gupta A, Regamey N, Frith J, Oates T, et al. Pediatric severe asthma is characterized by eosinophilia and remodeling without T(H)2 cytokines. J Allergy Clin Immunol. 2012;129(4):974–982.e13.

92. Andersson CK, Adams A, Nagakumar P, Bossley C, Gupta A, De Vries D, et al. Intraepithelial neutrophils in pediatric severe asthma are associated with better lung function. J Allergy Clin Immunol. 2017;139(6):1819–1829.e11.

93. Su M-W, Lin W-C, Tsai C-H, Chiang B-L, Yang Y-H, Lin Y-T, et al. Childhood asthma clusters reveal neutrophil-predominant phenotype with distinct gene expression. Allergy. 2018;73(10):2024–32.

94. Jones CE, Chan K. Interleukin-17 stimulates the expression of interleukin-8, growth-related oncogene-alpha, and granulocyte-colony-stimulating factor by human airway epithelial cells. Am J Respir Cell Mol Biol. 2002;26(6):748–53.

95. Bullens DMA, Decraene A, Seys S, Dupont LJ. IL-17A in human respiratory diseases: innate or adaptive immunity? Clinical implications. Clin Dev Immunol. 2013;2013:840315.

96. Baines KJ, Simpson JL, Wood LG, Scott RJ, Fibbens NL, Powell H, et al. Sputum gene expression signature of 6 biomarkers discriminates asthma inflammatory phenotypes. J Allergy Clin Immunol. 2014;133(4):997–1007.

97. Simpson JL, Phipps S, Baines KJ, Oreo KM, Gunawardhana L, Gibson PG. Elevated expression of the NLRP3 inflammasome in neutrophilic asthma. Eur Respir J. 2014;43(4):1067–76.

98. Rossios C, Pavlidis S, Hoda U, Kuo C-H, Wiegman C, Russell K, et al. Sputum transcriptomics reveal upregulation of IL-1 receptor family members in patients with severe asthma. J Allergy Clin Immunol. 2018;141(2):560–70.

99. Berry MA, Hargadon B, Shelley M, Parker D, Shaw DE, Green RH, et al. Evidence of a Role of Tumor Necrosis Factor α in Refractory Asthma. N Engl J Med. 2006;354(7):697–708.

This article is protected by copyright. All rights reserved.

Accepted Article

100. Manni ML, Trudeau JB, Scheller EV, Mandalapu S, Elloso MM, Kolls JK, et al. The complex relationship between inflammation and lung function in severe asthma. Mucosal Immunol. 2014;7(5):1186–98.

101. Agache I, Ciobanu C, Agache C, Anghel M. Increased serum IL-17 is an independent risk factor for severe asthma. Respir Med. 2010;104(8):1131–7.

102. Silvestri M, Bontempelli M, Giacomelli M, Malerba M, Rossi GA, Stefano D, et al. High serum levels of tumour necrosis factor-α and interleukin-8 in severe asthma: markers of systemic inflammation? Clin Exp Allergy. 2006;36(11):1373–81.

103. Decaesteker T, Seys S, Hox V, Dilissen E, Marijsse G, Manhaeghe L, et al. Serum and sputum calprotectin, a reflection of neutrophilic airway inflammation in asthmatics after high-altitude exposure. Clin Exp Allergy. 2017;47(12):1675–7.

104. Horváth I, Hunt J, Barnes PJ, Alving K, Antczak A, Baraldi E, et al. Exhaled breath condensate: methodological recommendations and unresolved questions. Eur Respir J. 2005;26(3):523–48.

105. Ntontsi P, Loukides S, Bakakos P, Kostikas K, Papatheodorou G, Papathanassiou E, et al. Clinical, functional and inflammatory characteristics in patients with paucigranulocytic stable asthma: Comparison with different sputum phenotypes. Allergy. 2017;72(11):1761–7.

106. Fixman ED, Stewart A, Martin JG. Basic mechanisms of development of airway structural changes in asthma. Eur Respir J. 2007;29(2):379–89.

107. James AL, Elliot JG, Jones RL, Carroll ML, Mauad T, Bai TR, et al. Airway smooth muscle hypertrophy and hyperplasia in asthma. Am J Respir Crit Care Med. 2012;185(10):1058–64.

108. Ordoñez C, Ferrando R, Hyde DM, Wong HH, Fahy JV. Epithelial desquamation in asthma: artifact or pathology? Am J Respir Crit Care Med. 2000;162(6):2324–9.

109. Payne DNR, Rogers AV, Adelroth E, Bandi V, Guntupalli KK, Bush A, et al. Early thickening of the reticular basement membrane in children with difficult asthma. Am J Respir Crit Care Med. 2003;167(1):78–82.

110. Barbato A, Turato G, Baraldo S, Bazzan E, Calabrese F, Panizzolo C, et al. Epithelial damage and angiogenesis in the airways of children with asthma. Am J Respir Crit Care Med. 2006;174(9):975–81.

111. Roche WR, Beasley R, Williams JH, Holgate ST. Subepithelial fibrosis in the bronchi of asthmatics. Lancet Lond Engl. 1989;1(8637):520–4.

112. Benayoun L, Druilhe A, Dombret M-C, Aubier M, Pretolani M. Airway structural alterations selectively associated with severe asthma. Am J Respir Crit Care Med. 2003;167(10):1360–8.

113. Sumi Y, Hamid Q. Airway remodeling in asthma. Allergol Int. 2007;56(4):341–8.

114. Ward C, Pais M, Bish R, Reid D, Feltis B, Johns D, et al. Airway inflammation, basement membrane thickening and bronchial hyperresponsiveness in asthma. Thorax. 2002;57(4):309–

This article is protected by copyright. All rights reserved.

Accepted Article

Accepted Article

16.

115. Sont JK, Willems LN, Bel EH, van Krieken JH, Vandenbroucke JP, Sterk PJ. Clinical control and histopathologic outcome of asthma when using airway hyperresponsiveness as an additional guide to long-term treatment. The AMPUL Study Group. Am J Respir Crit Care Med. 1999;159(4 Pt 1):1043–51.

116. Flood-Page P, Menzies-Gow A, Phipps S, Ying S, Wangoo A, Ludwig MS, et al. Anti-IL-5 treatment reduces deposition of ECM proteins in the bronchial subepithelial basement membrane of mild atopic asthmatics. J Clin Invest. 2003;112(7):1029–36.

117. Nuijsink M, Hop WCJ, Sterk PJ, Duiverman EJ, de Jongste JC. Long-term asthma treatment guided by airway hyperresponsiveness in children: a randomised controlled trial. Eur Respir J. 2007;30(3):457–66.

118. Booms P, Cheung D, Timmers MC, Zwinderman AH, Sterk PJ. Protective effect of inhaled budesonide against unlimited airway narrowing to methacholine in atopic patients with asthma. J Allergy Clin Immunol. 1997 Mar;99(3):330–7.

119. Ulrik CS, Diamant Z. Add-on montelukast to inhaled corticosteroids protects against excessive airway narrowing. Clin Exp Allergy J Br Soc Allergy Clin Immunol. 2010;40(4):576–81.

120. Riccio AM, Mauri P, De Ferrari L, Rossi R, Di Silvestre D, Benazzi L, et al. Galectin-3: an early predictive biomarker of modulation of airway remodeling in patients with severe asthma treated with omalizumab for 36 months. Clin Transl Allergy. 2017;7:6.

121. Mauri P, Riccio AM, Rossi R, Di Silvestre D, Benazzi L, De Ferrari L, et al. Proteomics of bronchial biopsies: galectin-3 as a predictive biomarker of airway remodelling modulation in omalizumab-treated severe asthma patients. Immunol Lett. 2014;162(1 Pt A):2–10.

122. Cortegano I, del Pozo V, Cárdaba B, de Andrés B, Gallardo S, del Amo A, et al. Galectin-3 down-regulates IL-5 gene expression on different cell types. J Immunol Baltim Md 1950. 1998;161(1):385–9.

123. Lee CG, Da Silva CA, Dela Cruz CS, Ahangari F, Ma B, Kang M-J, et al. Role of chitin and chitinase/chitinase-like proteins in inflammation, tissue remodeling, and injury. Annu Rev Physiol. 2011;73:479–501.

124. Konradsen JR, James A, Nordlund B, Reinius LE, Söderhäll C, Melén E, et al. The chitinase-like protein YKL-40: a possible biomarker of inflammation and airway remodeling in severe pediatric asthma. J Allergy Clin Immunol. 2013;132(2):328–335.e5.

125. Yick CY, Zwinderman AH, Kunst PW, Grünberg K, Mauad T, Chowdhury S, et al. Gene expression profiling of laser microdissected airway smooth muscle tissue in asthma and atopy. Allergy. 2014;69(9):1233–40.

126. Yick CY, Zwinderman AH, Kunst PW, Grünberg K, Mauad T, Fluiter K, et al. Glucocorticoid-induced changes in gene expression of airway smooth muscle in patients with asthma. Am J Respir Crit Care Med. 2013;187(10):1076–84.

127. Ammit AJ, Burgess JK, Hirst SJ, Hughes JM, Kaur M, Lau JY, et al. The effect of asthma

This article is protected by copyright. All rights reserved.

therapeutics on signalling and transcriptional regulation of airway smooth muscle function. Pulm Pharmacol Ther. 2009;22(5):446–54.

128. Slats AM, Sont JK, van Klink RHCJ, Bel EHD, Sterk PJ. Improvement in bronchodilation following deep inspiration after a course of high-dose oral prednisone in asthma. Chest. 2006;130(1):58–65.

129. Tufvesson E, Aronsson D, Bjermer L. Cysteinyl-leukotriene levels in sputum differentiate asthma from rhinitis patients with or without bronchial hyperresponsiveness. Clin Exp Allergy J Br Soc Allergy Clin Immunol. 2007;37(7):1067–73.

130. de Kluijver J, Schrumpf JA, Evertse CE, Sont JK, Roughley PJ, Rabe KF, et al. Bronchial matrix and inflammation respond to inhaled steroids despite ongoing allergen exposure in asthma. Clin Exp Allergy J Br Soc Allergy Clin Immunol. 2005;35(10):1361–9.

131. Niimi A, Matsumoto H, Amitani R, Nakano Y, Sakai H, Takemura M, et al. Effect of short-term treatment with inhaled corticosteroid on airway wall thickening in asthma. Am J Med. 2004;116(11):725–31.

132. Hoshino M, Ohtawa J, Akitsu K. Association of airway wall thickness with serum periostin in steroid-naive asthma. Allergy Asthma Proc. 2016;37(3):225–30.

133. Ebenezer JA, Christensen JM, Oliver BG, Oliver RA, Tjin G, Ho J, et al. Periostin as a marker of mucosal remodelling in chronic rhinosinusitis. Rhinology. 2017;55(3):234–41.

134. Yick CY, von der Thüsen JH, Bel EH, Sterk PJ, Kunst PW. In vivo imaging of the airway wall in asthma: fibered confocal fluorescence microscopy in relation to histology and lung function. Respir Res. 2011;2(1):85.

135. Wijmans L, d'Hooghe JNS, Bonta PI, Annema JT. Optical coherence tomography and confocal laser endomicroscopy in pulmonary diseases. Curr Opin Pulm Med. 2017;23(3):275–83.

136. Boyman O, Kaegi C, Akdis M, Bavbek S, Bossios A, Chatzipetrou A, et al. EAACI IG Biologicals task force paper on the use of biologic agents in allergic disorders. Allergy. 2015;70(7):727–54.

137. Shamji MH, Kappen JH, Akdis M, Jensen-Jarolim E, Knol EF, Kleine-Tebbe J, et al. Biomarkers for monitoring clinical efficacy of allergen immunotherapy for allergic rhinoconjunctivitis and allergic asthma: an EAACI Position Paper. Allergy. 2017;72(8):1156–73.

138. Busse WW. Anti-immunoglobulin E (omalizumab) therapy in allergic asthma. Am J Respir Crit Care Med. 2001;164(8 Pt 2):S12-17.

139. Thomson NC, Chaudhuri R. Omalizumab: clinical use for the management of asthma. Clin Med Insights Circ Respir Pulm Med. 2012;6:27–40.

140. Normansell R, Walker S, Milan SJ, Walters EH, Nair P. Omalizumab for asthma in adults and children. Cochrane Database Syst Rev. 2014;(1):CD003559.

This article is protected by copyright. All rights reserved.

Accepted Article

141. Casale TB, Luskin AT, Busse W, Zeiger RS, Trzaskoma B, Yang M, et al. Omalizumab Effectiveness by Biomarker Status in Patients with Asthma: Evidence From PROSPERO, A Prospective Real-World Study. J Allergy Clin Immunol Pract. 2019;7(1):156-164.e1.

142. Pike KC, Akhbari M, Kneale D, Harris KM. Interventions for autumn exacerbations of asthma in children. Cochrane Database Syst Rev. 2018 08;3:CD012393.

143. Normansell R, Walker S, Milan SJ, Walters EH, Nair P. Omalizumab for asthma in adults and children. Cochrane Database Syst Rev. 2014;(1):CD003559.

144. Wahn U, Martin C, Freeman P, Blogg M, Jimenez P. Relationship between pretreatment specific IgE and the response to omalizumab therapy. Allergy. 2009;64(12):1780–7.

145. Korn S, Haasler I, Fliedner F, Becher G, Strohner P, Staatz A, et al. Monitoring free serum IgE in severe asthma patients treated with omalizumab. Respir Med. 2012;106(11):1494–500.

146. Humbert M, Busse W, Hanania NA, Lowe PJ, Canvin J, Erpenbeck VJ, et al. Omalizumab in asthma: an update on recent developments. J Allergy Clin Immunol Pract. 2014;2(5):525–536.e1.

147. Johansson SGO, Lilja G, Hallberg J, Nopp A. A clinical follow-up of omalizumab in routine treatment of allergic asthma monitored by CD-sens. Immun Inflamm Dis. 2018;6(3):382–91.

148. Hanania NA, Wenzel S, Rosén K, Hsieh H-J, Mosesova S, Choy DF, et al. Exploring the effects of omalizumab in allergic asthma: an analysis of biomarkers in the EXTRA study. Am J Respir Crit Care Med. 2013;187(8):804–11.

149. Teach SJ, Gill MA, Togias A, Sorkness CA, Arbes SJ, Calatroni A, et al. Preseasonal treatment with either omalizumab or an inhaled corticosteroid boost to prevent fall asthma exacerbations. J Allergy Clin Immunol. 2015;136(6):1476–85.

150. Fleming L, Koo M, Bossley CJ, Nagakumar P, Bush A, Saglani S. The utility of a multidomain assessment of steroid response for predicting clinical response to omalizumab. J Allergy Clin Immunol. 2016;138(1):292–4.

151. Nair P. Mepolizumab for Prednisone-Dependent Asthma with Sputum Eosinophilia. 2009;360(10):985–993.

152. Haldar P, Brightling CE, Hargadon B, Gupta S, Monteiro W, Sousa A, et al. Mepolizumab and Exacerbations of Refractory Eosinophilic Asthma. N Engl J Med. 2009;360(10):973–984.

153. Laviolette M, Gossage DL, Gauvreau G, Leigh R, Olivenstein R, Katial R, et al. Effects of benralizumab on airway eosinophils in asthmatic patients with sputum eosinophilia. J Allergy Clin Immunol. 2013;132(5):1086–1096.e5.

154. Farne HA, Wilson A, Powell C, Bax L, Milan SJ. Anti-IL5 therapies for asthma. Cochrane Database Syst Rev. 2017 21;9:CD010834.

155. Leckie MJ, ten Brinke A, Khan J, Diamant Z, O'Connor BJ, Walls CM, et al. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. Lancet. 2000;356(9248):2144–8.

This article is protected by copyright. All rights reserved.

Accepted Article

156. Flood-Page P, Swenson C, Faiferman I, Matthews J, Williams M, Brannick L, et al. A Study to Evaluate Safety and Efficacy of Mepolizumab in Patients with Moderate Persistent Asthma. Am J Respir Crit Care Med. 2007;176(11):1062–1071.

157. Bel EH, Wenzel SE, Thompson PJ, Prazma CM, Keene ON, Yancey SW, et al. Oral glucocorticoid-sparing effect of mepolizumab in eosinophilic asthma. N Engl J Med. 2014;371(13):1189–97.

158. Ortega HG, Liu MC, Pavord ID, Brusselle GG, FitzGerald JM, Chetta A, et al. Mepolizumab treatment in patients with severe eosinophilic asthma. N Engl J Med. 2014;371(13):1198–207.

159. Castro M, Zangrilli J, Wechsler ME, Bateman ED, Brusselle GG, Bardin P, et al. Reslizumab for inadequately controlled asthma with elevated blood eosinophil counts: results from two multicentre, parallel, double-blind, randomised, placebo-controlled, phase 3 trials. Lancet Respir Med. 2015;3(5):355–66.

160. Pavord ID, Korn S, Howarth P, Bleecker ER, Buhl R, Keene ON, et al. Mepolizumab for severe eosinophilic asthma (DREAM): a multicentre, double-blind, placebo-controlled trial. Lancet Lond Engl. 2012;380(9842):651–9.

161. Slager RE, Otulana BA, Hawkins GA, Yen YP, Peters SP, Wenzel SE, et al. IL-4 receptor polymorphisms predict reduction in asthma exacerbations during response to an anti-IL-4 receptor α antagonist. J Allergy Clin Immunol. 2012;130(2):516–522.e4.

162. Castro M, Corren J, Pavord ID, Maspero J, Wenzel S, Rabe KF, et al. Dupilumab Efficacy and Safety in Moderate-to-Severe Uncontrolled Asthma. N Engl J Med. 2018;378(26):2486–96.

163. Wenzel S, Ford L, Pearlman D, Spector S, Sher L, Skobieranda F, et al. Dupilumab in persistent asthma with elevated eosinophil levels. N Engl J Med. 2013;368(26):2455–66.

164. Wenzel S, Castro M, Corren J, Maspero J, Wang L, Zhang B, et al. Dupilumab efficacy and safety in adults with uncontrolled persistent asthma despite use of medium-to-high-dose inhaled corticosteroids plus a long-acting β2 agonist: a randomised double-blind placebo-controlled pivotal phase 2b dose-ranging trial. Lancet. 2016;388(10039):31–44.

165. Bachert C, Mannent L, Naclerio RM, Mullol J, Ferguson BJ, Gevaert P, et al. Effect of Subcutaneous Dupilumab on Nasal Polyp Burden in Patients With Chronic Sinusitis and Nasal Polyposis: A Randomized Clinical Trial. JAMA. 2016;315(5):469–79.

166. Brightling CE, Chanez P, Leigh R, O'Byrne PM, Korn S, She D, et al. Efficacy and safety of tralokinumab in patients with severe uncontrolled asthma: a randomised, double-blind, placebo-controlled, phase 2b trial. Lancet Respir Med. 2015;3(9):692–701.

167. Castro M, Corren J, Pavord ID, Maspero J, Wenzel S, Rabe KF, et al. Dupilumab Efficacy and Safety in Moderate-to-Severe Uncontrolled Asthma. N Engl J Med. 2018;378(26):2486–96.

168. Rabe KF, Nair P, Brusselle G, Maspero JF, Castro M, Sher L, et al. Efficacy and Safety of Dupilumab in Glucocorticoid-Dependent Severe Asthma. N Engl J Med. 2018;378(26):2475–85.

169. Woodruff PG, Boushey HA, Dolganov GM, Barker CS, Yang YH, Donnelly S, et al. Genome-wide profiling identifies epithelial cell genes associated with asthma and with treatment

This article is protected by copyright. All rights reserved.

Accepted Article

response to corticosteroids. Proc Natl Acad Sci U S A. 2007;104(40):15858–63.

170. Hanania NA, Korenblat P, Chapman KR, Bateman ED, Kopecky P, Paggiaro P, et al. Efficacy and safety of lebrikizumab in patients with uncontrolled asthma (LAVOLTA I and LAVOLTA II): replicate, phase 3, randomised, double-blind, placebo-controlled trials. Lancet Respir Med. 2016;4(10):781–96.

171. Brightling CE, Chanez P, Leigh R, O'Byrne PM, Korn S, She D, et al. Efficacy and safety of tralokinumab in patients with severe uncontrolled asthma: a randomised, double-blind, placebo-controlled, phase 2b trial. Lancet Respir Med. 2015;3(9):692–701.

172. Kanemitsu Y, Matsumoto H, Izuhara K, Tohda Y, Kita H, Horiguchi T, et al. Increased periostin associates with greater airflow limitation in patients receiving inhaled corticosteroids. J Allergy Clin Immunol. 2013;132(2):305–312.e3.

173. Roan F, Bell BD, Stoklasek TA, Kitajima M, Han H, Ziegler SF. The multiple facets of thymic stromal lymphopoietin (TSLP) during allergic inflammation and beyond. J Leukoc Biol. 2012;91(6):877–86.

174. Ito T, Liu Y-J, Arima K. Cellular and molecular mechanisms of TSLP function in human allergic disorders--TSLP programs the "Th2 code" in dendritic cells. Allergol Int. 2012;61(1):35–43.

175. Ying S, O'Connor B, Ratoff J, Meng Q, Mallett K, Cousins D, et al. Thymic stromal lymphopoietin expression is increased in asthmatic airways and correlates with expression of Th2-attracting chemokines and disease severity. J Immunol Baltim Md 1950. 2005;174(12):8183–90.

176. Gauvreau GM, O'Byrne PM, Boulet L-P, Wang Y, Cockcroft D, Bigler J, et al. Effects of an anti-TSLP antibody on allergen-induced asthmatic responses. N Engl J Med. 2014;370(22):2102–10.

177. Corren J, Parnes JR, Wang L, Mo M, Roseti SL, Griffiths JM, et al. Tezepelumab in Adults with Uncontrolled Asthma. N Engl J Med. 2017;377(10):936–46.

178. Hirai H, Tanaka K, Yoshie O, Ogawa K, Kenmotsu K, Takamori Y, et al. Prostaglandin D2 selectively induces chemotaxis in T helper type 2 cells, eosinophils, and basophils via seven-transmembrane receptor CRTH2. J Exp Med. 2001;193(2):255–61.

179. Xue L, Salimi M, Panse I, Mjösberg JM, McKenzie ANJ, Spits H, et al. Prostaglandin D2 activates group 2 innate lymphoid cells through chemoattractant receptor-homologous molecule expressed on TH2 cells. J Allergy Clin Immunol. 2014;133(4):1184–94.

180. Singh D, Cadden P, Hunter M, Pearce Collins L, Perkins M, Pettipher R, et al. Inhibition of the asthmatic allergen challenge response by the CRTH2 antagonist OC000459. Eur Respir J. 2013;41(1):46–52.

181. Diamant Z, Sidharta PN, Singh D, O'Connor BJ, Zuiker R, Leaker BR, et al. Setipiprant, a selective CRTH2 antagonist, reduces allergen-induced airway responses in allergic asthmatics. Clin Exp Allergy. 2014;44(8):1044–52.

This article is protected by copyright. All rights reserved.

Accepted Article

182. Fajt ML, Gelhaus SL, Freeman B, Uvalle CE, Trudeau JB, Holguin F, et al. Prostaglandin D_2 pathway upregulation: relation to asthma severity, control, and TH2 inflammation. J Allergy Clin Immunol. 2013;131(6):1504–12.

183. Kuna P, Bjermer L, Tornling G. Two Phase II randomized trials on the CRTh2 antagonist AZD1981 in adults with asthma. Drug Des Devel Ther. 2016;10:2759–70.

184. Hall IP, Fowler AV, Gupta A, Tetzlaff K, Nivens MC, Sarno M, et al. Efficacy of BI 671800, an oral CRTH2 antagonist, in poorly controlled asthma as sole controller and in the presence of inhaled corticosteroid treatment. Pulm Pharmacol Ther. 2015;32:37–44.

185. Bateman ED, Guerreros AG, Brockhaus F, Holzhauer B, Pethe A, Kay RA, et al. Fevipiprant, an oral prostaglandin DP2 receptor (CRTh2) antagonist, in allergic asthma uncontrolled on low-dose inhaled corticosteroids. Eur Respir J. 2017;50(2).

186. Gonem S, Berair R, Singapuri A, Hartley R, Laurencin MFM, Bacher G, et al. Fevipiprant, a prostaglandin D2 receptor 2 antagonist, in patients with persistent eosinophilic asthma: a single-centre, randomised, double-blind, parallel-group, placebo-controlled trial. Lancet Respir Med. 2016;4(9):699–707.

187. Diamant Z, Aalders W, Parulekar A, Bjermer L, Hanania NA. Targeting lipid mediators in asthma: time for reappraisal. Curr Opin Pulm Med. 2019;25(1):121-127.

188. Anticevich SZ, Hughes JM, Black JL, Armour CL. Induction of human airway hyperresponsiveness by tumour necrosis factor-alpha. Eur J Pharmacol. 1995;284(1–2):221–5.

189. Howarth PH, Babu KS, Arshad HS, Lau L, Buckley M, McConnell W, et al. Tumour necrosis factor (TNFα) as a novel therapeutic target in symptomatic corticosteroid dependent asthma. Thorax. 2005;60:1012 –1018.

190. Wenzel SE, Barnes PJ, Bleecker ER, Bousquet J, Busse W, Dahlen S-E, et al. A Randomized, Double-blind, Placebo-controlled Study of Tumor Necrosis Factor- Blockade in Severe Persistent Asthma. Am J Respir Crit Care Med. 2009;179(7):549–558.

191. Busse WW, Holgate S, Kerwin E, Chon Y, Feng J, Lin J, et al. Randomized, double-blind, placebo-controlled study of brodalumab, a human anti-IL-17 receptor monoclonal antibody, in moderate to severe asthma. Am J Respir Crit Care Med. 2013;188(11):1294–302.

192. Gernez Y, Tirouvanziam R, Chanez P. Neutrophils in chronic inflammatory airway diseases: can we target them and how? Eur Respir J. 2010;35(3):467–9.

193. Nair P, Gaga M, Zervas E, Alagha K, Hargreave FE, O'Byrne PM, et al. Safety and efficacy of a CXCR2 antagonist in patients with severe asthma and sputum neutrophils: a randomized, placebo-controlled clinical trial. Clin Exp Allergy. 2012;42(7):1097–103.

194. O'Byrne PM, Metev H, Puu M, Richter K, Keen C, Uddin M, et al. Efficacy and safety of a CXCR2 antagonist, AZD5069, in patients with uncontrolled persistent asthma: a randomised, double-blind, placebo-controlled trial. Lancet Respir Med. 2016;4(10):797–806.

195. Shinkai M, Henke MO, Rubin BK. Macrolide antibiotics as immunomodulatory medications: proposed mechanisms of action. Pharmacol Ther. 2008;117(3):393–405.

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196. Brusselle GG, Vanderstichele C, Jordens P, Deman R, Slabbynck H, Ringoet V, et al. Azithromycin for prevention of exacerbations in severe asthma (AZISAST): a multicentre randomised double-blind placebo-controlled trial. Thorax. 2013;68(4):322–9.

197. Gibson PG, Yang IA, Upham JW, Reynolds PN, Hodge S, James AL, et al. Effect of azithromycin on asthma exacerbations and quality of life in adults with persistent uncontrolled asthma (AMAZES): a randomised, double-blind, placebo-controlled trial. Lancet Lond Engl. 2017;390(10095):659–68.

198. Trivedi A, Pavord ID, Castro M. Bronchial thermoplasty and biological therapy as targeted treatments for severe uncontrolled asthma. Lancet Respir Med. 2016;4(7):585–92.

199. Facciolongo N, Di Stefano A, Pietrini V, Galeone C, Bellanova F, Menzella F, et al. Nerve ablation after bronchial thermoplasty and sustained improvement in severe asthma. BMC Pulm Med. 2018;18(1):29.

200. Cox G, Thomson NC, Rubin AS, Niven RM, Corris PA, Siersted HC, et al. Asthma control during the year after bronchial thermoplasty. N Engl J Med. 2007;356(13):1327–37.

201. Pavord ID, Cox G, Thomson NC, Rubin AS, Corris PA, Niven RM, et al. Safety and efficacy of bronchial thermoplasty in symptomatic, severe asthma. Am J Respir Crit Care Med. 2007;176(12):1185–91.

202. Castro M, Rubin AS, Laviolette M, Fiterman J, De Andrade Lima M, Shah PL, et al. Effectiveness and safety of bronchial thermoplasty in the treatment of severe asthma: a multicenter, randomized, double-blind, sham-controlled clinical trial. Am J Respir Crit Care Med. 2010;181(2):116–24.

203. Chupp G, Laviolette M, Cohn L, McEvoy C, Bansal S, Shifren A, et al. Long-term outcomes of bronchial thermoplasty in subjects with severe asthma: a comparison of 3-year follow-up results from two prospective multicentre studies. Eur Respir J. 2017;50(2).

204. Svenningsen S, Nair P. Asthma Endotypes and an Overview of Targeted Therapy for Asthma. Front Med. 2017;4:158.

205. NIH-National Cancer Institute [Internet]. Available from: https://www.cancer.gov/publications/dictionaries/genetics-dictionary/def/phenotype

206. Anderson GP. Endotyping asthma: new insights into key pathogenic mechanisms in a complex, heterogeneous disease. Lancet. 2008 Sep;372(9643):1107–19.

207. Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther. 2001;69(3):89–95.

208. Petsky HL, Kew KM, Turner C, Chang AB. Exhaled nitric oxide levels to guide treatment for adults with asthma. Cochrane Database Syst Rev. 2016;9:CD011440.

209. Pizzichini E, Pizzichini MMM, Leigh R, Djukanović R, Sterk PJ. Safety of sputum induction. Eur Respir J Suppl. 2002;37:9s–18s.

210. van Bragt JJMH, Vijverberg SJH, Weersink EJM, Richards LB, Neerincx AH, Sterk PJ, et al.

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Accepted Article

Blood biomarkers in chronic airways diseases and their role in diagnosis and management. Expert Rev Respir Med. 2018;12(5):361–74.

211. Kostikas K, Papaioannou AI, Tanou K, Giouleka P, Koutsokera A, Minas M, et al. Exhaled NO and exhaled breath condensate pH in the evaluation of asthma control. Respir Med. 2011;105(4):526–32.

Figure legends

Figure 1. Clinical applicability of biomarkers in asthma management. Adapted from (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).

Figure 2. Asthma endotypes and targeted treatment approaches.

Figure 3. Practical flow chart 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 (e.g. 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.

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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) (205).

Endotype: Endotype – a contraction of endophenotype – is a subtype of disease defined functionally and pathologically by a molecular mechanism or by treatment response (206).

Biomarker: A biomarker is defined as a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention (NIH definition) (207).

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Table 1. Advantages and limitations of biomarker sampling methods in asthma.

Sampling method	Biomarker	Cut off level	Advantages	Limitations
Bronchoscopy *** Biopsy** *** Broncho-alveolar lavage (BAL)** *** Bronchial brushings**	• Eosinophils • Neutrophils • Total inflammatory cell counts • Cytokines and chemokines • Leakage markers and mediators • Airway remodelling	Clear cut-off values lacking	• Semi-direct read-out	• 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	In general, cut-off of ≥3% are used to indicate sputum eosinophilia, and ≥61% to indicate sputum neutrophils. However, adapting treatment based on sputum eosinophils	• Semi direct read-out; multiple biomarkers; Reproducible readout of cell differentials • Suitable method for disease phenotyping and monitoring in experienced centres	• Semi-invasive • Analysable samples available in approx. 80-90% of subjects; • Adapted protocol needed for very severe disease with compromised lung function (contra-indicated if FEV_1 <1L (209) and/or with concomitant cardiovascular disorders

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	markers and mediators	has performed with various sputum eosinophil cut-offs, ranging from 2-8% (208)		• Technically complex and time-consuming procedure (soluble markers too variable for daily clinical routine), restricted to specialized centres
Peripheral blood	• Eosinophils • Cell Activation markers • IgE (total/specific) • Cytokines, chemokines and mediators	Various cut-off values, mostly ranging 150-500 cells/μL / 1-4% are used for blood eosinophils (210)	• 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 (52)
Exhaled breath	• FeNO • Volatile organic compounds (VOCs)	According to clinical guidelines: Low FeNO < 25 ppb (≥12 yrs), <20 (<12 years), high FeNO >50 (≥12 yrs), <35 (<12 years) (58)	• Non-invasive • 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	No clear cut-off values. Study showed that	• Non-invasive, allowing serial measurements	• Specialized lab needed • Expensive assays • Variable outcomes due to technical issues

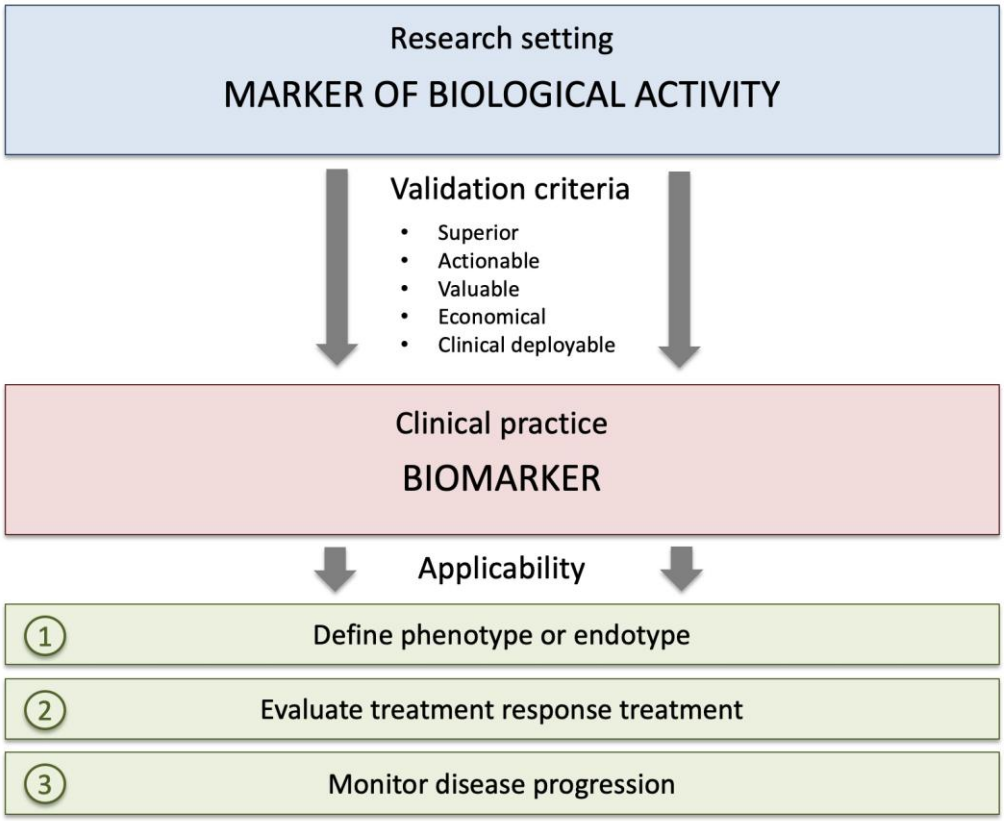
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	• Cytokines or chemokines	EBC pH ≤7.20 was indicative of not well-uncontrolled asthma (211)		• Awaits further development and validation
Imaging (e.g. qCT, HRCT, (hyperpolarized 3He/129Xe) MRI, PET)	• Airway remodeling	Clear cut-off values lacking	• Non-invasive • Enables to study structural (and functional) aspects	• Standardization is lacking • Radiation exposure (e.g. qCT)

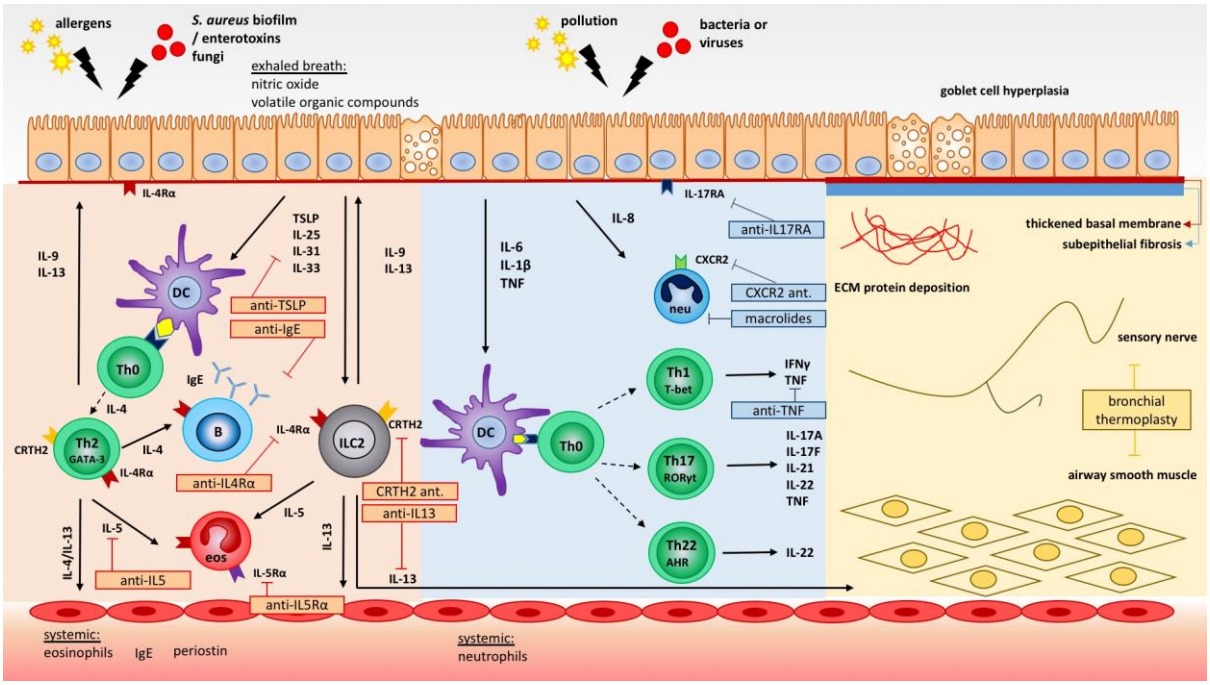
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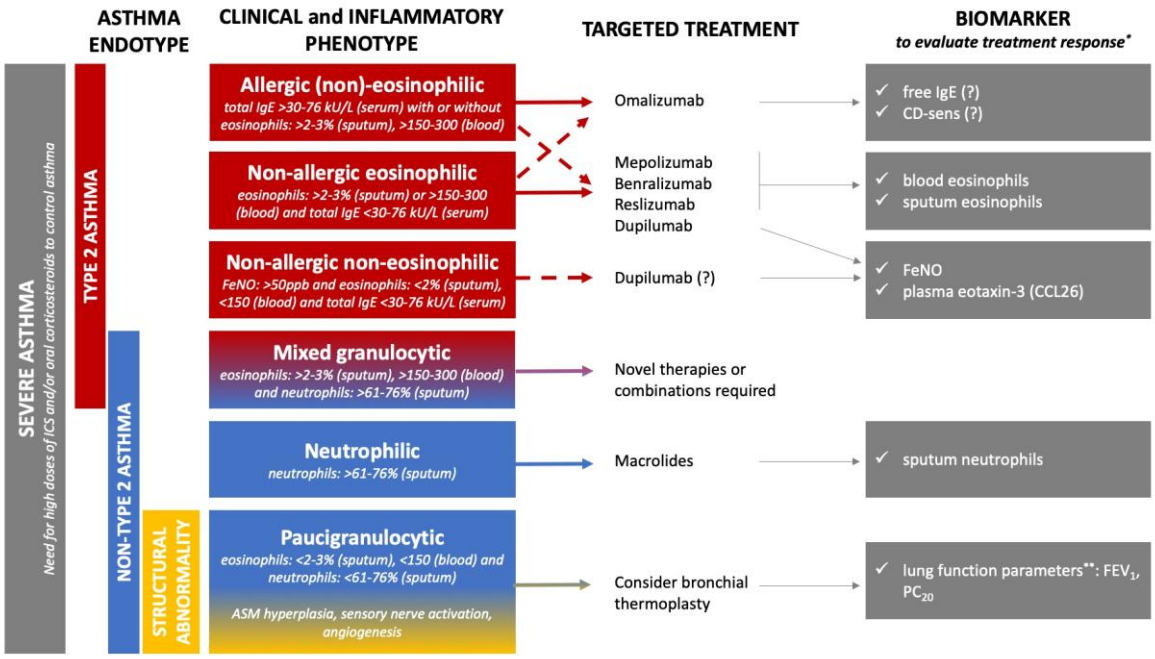
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