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# Precision Medicine for the discovery of treatable mechanisms in severe Asthma

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## SUMMARY

Although the complex disease of asthma has been defined as being heterogeneous, the extent of its endophenotypes remain unclear. The pharmacological approach to initiating treatment has, until recently, been based on disease control and severity. The introduction of antibody therapies targeting the Type2 inflammation pathway for patients with severe asthma has resulted in the recognition of an allergic and an

eosinophilic phenotype, which are not mutually exclusive. Concomitantly, molecular phenotyping based on a transcriptomic analysis of bronchial epithelial and sputum cells has identified a Type-2-high inflammation cluster characterised by eosinophilia and recurrent exacerbations, as well as Type-2-low clusters linked with IL-6 trans-signalling, interferon pathways, inflammasome activation and mitochondrial oxidative phosphorylation pathways. Systems biology approaches are establishing the links between these pathways or mechanisms, and clinical and physiologic features. Validation of these pathways contributes to defining endotypes and treatable mechanisms. Precision medicine approaches are necessary to link treatable mechanisms with treatable traits and biomarkers derived from clinical, physiologic and inflammatory features of clinical phenotypes. The deep molecular phenotyping of airway samples along with non-invasive biomarkers linked to bioinformatic and machine learning techniques will enable the rapid detection of molecular mechanisms that transgresses beyond the concept of treatable traits.

**Key words**: severe asthma, precision medicine, systems biology, eosinophilic asthma,

**Future research perspective**

1. Using systems biology approach to analyse genes, proteins and metabolonomes associated with asthma is the way forward with defining the endotypes of asthma.
2. This approach will lead to the refinement of Type 2-high driving mechanisms and to the definition of Type 2-low endotypes.
3. Both Type 2-high and Type 2-low mechanistic pathways may interact to drive the expression of the endotype
4. This approach will lead to treatable mechanisms for which newer effective treatments will ensue for specific endotypes
5. Precision medicine should define the treatable traits and bedside biomarkers of the endotype. Even for T2-high endotypes, improved bedside biomarkers apart from a blood eosinophil count should become available

**Major milestones discoveries**

1. Blood or sputum eosinophilia in asthma denotes a patient with asthma with more severe disease, recurrent exacerbations and responsive to corticosteroid therapy
2. An endotype of eosinophilic severe asthma as a Type 2-high phenotype driven by IL-5 and characterised by recurrent exacerbations and eosinophilic inflammation has been established
3. Therapies targeting Type 2 pathway with anti-IgE, anti-IL5, anti-IL5Rα and anti-IL-4Rα antibodies reduce severe exacerbations and the maintenance dose of oral corticosteroids with severe asthma with high blood eosinophil counts.

## Introduction

Although asthma is still defined as an ailment recognised by symptoms such as spasms of breathlessness since the time of Hippocrates, the notion of this complex condition being heterogeneous has only been considered in recent years. One important concept emerging from the last 20 years has been the definition of asthma severity and asthma control upon which the pharmacological treatment of asthma has been based. Asthma control has been defined as the extent to which the manifestations of asthma including symptoms and exacerbations have been reduced or removed by treatment, while the severity of asthma is the degree of difficulty in controlling asthma with treatment (1). Both definitions, therefore, relate to the effect of treatments in controlling the manifestations of asthma, which has been the major tenet of pharmacologic therapy. Thus, asthma guidelines emphasise the measure of the level of asthma control, as the sole measure of heterogeneity, to gauge the required level of treatment with controllers such as inhaled corticosteroids (ICS) and long-acting β2-agonists (LABA). A stepwise increase in asthma medication is commensurate with the degree of asthma severity.

This approach has led to the recognition that a substantial proportion of patients fail to achieve guideline-defined control of asthma even at the highest dose of ICS and often in combination with oral corticosteroid (OCS) therapy (2). This, thereby, has led to the recognition of a group of patients with severe asthma who remained relatively resistant to ICS therapy. These patients are defined as suffering

from “asthma which requires treatment with high dose ICS plus a second controller (and/or systemic corticosteroids) to prevent it from becoming ‘uncontrolled’ or which remains ‘uncontrolled’ despite this therapy” (3).

The recognition of asthma being a heterogeneous condition representing several phenotypes has not been considered in the treatment approach to asthma until recently. However, the recognition of the importance of phenotyping has slowly evolved with the initial demonstration that the use of sputum eosinophil counts to indicate corticosteroid responsiveness which in turn was used to dictate the dose of ICS or OCS to be used in the management of patients with symptomatic asthma achieved better asthma outcomes, particularly in terms of exacerbation rates, than using the traditional method of symptomatic and lung function control (4). The application of personalised medicine to asthma management has been initiated with the introduction of monoclonal antibody therapies such as the anti-IgE antibody, omalizumab, followed recently by anti-IL5 antibody therapies (reslizumab and mepolizumab). Serum biomarkers including serum IgE or blood eosinophil count or levels of nitric oxide in exhaled breath (FeNO) have been used to select patients with severe asthma who are most likely to respond to these specific treatments.

While these biologic treatments have defined subsets of patients with allergic asthma and with eosinophilic asthma, the definition of other phenotypes remain unclear. The availability of various *-omics* platforms to define the transcriptomic, proteomic and metabolonomic profiles of patients represent powerful tools to further refine the heterogeneity of asthma, particularly severe asthma where there is a greater need for this refinement and for devising more effective targeted treatments (5). There has already been validation of the Type-2-high inflammation molecular phenotype that has been described in mild asthma and severe asthma based on an analysis of the transcriptome of bronchial epithelial cells (6, 7). However so far, these approaches have hardly influenced clinical practice yet, and delivering precision medicine for asthma has remained very limited. An understanding of the driving mechanisms of disease cannot be gained from just an analysis of symptoms and signs and of physiologic or even inflammatory markers. In addition, the variable nature of the disease is often not considered which means that therapies may need to change according to the disease drivers. With our recent understanding of the

molecular phenotypes of asthma and the increasing numbers of potential treatable mechanisms, our clinical practice will be transformed. Furthermore, it is important to define not only molecular phenotypes but also the 'endo-phenotypes' or endotypes which are the molecular phenotypes defined by a distinct set of pathophysiological mechanisms. Therefore, in order for molecular phenotypes to become endotypes, the associated pathways defined in the phenotype needs to be proven to be the driving mechanism underlying the pathophysiological features of severe asthma.

This review will discuss (i) the latest clinical phenotypes of asthma and their application in daily practice (ii) the approach of precision medicine to defining endotypes which are molecular phenotypes based on mechanisms (8) (iii) how knowledge of endotypes can lead to the discovery of treatable mechanisms and pathways and of new targeted therapies and (iv) how the patient with asthma can benefit from precision medicine.

## Clinical phenotypes

Various clinical phenotypes have been recognised by experienced clinicians but only recently have unbiased methods of clustering been used to dissect severe asthma. Several phenotypes based on clinical and physiologic variables and on inflammatory markers have been reported. In the Unbiased Biomarkers in Prediction of Respiratory Disease Outcomes (U-BIOPRED) cohort of moderate-to severe asthma using a classical partitioning technique of clustering (partition-around-medoids), a well-controlled moderate-to-severe asthma phenotype with three other severe asthma phenotypes were described, which were (i) late-onset asthma with past or current smoking and chronic airflow obstruction with a high blood eosinophil count (ii) non-smoking severe asthma with chronic airflow obstruction and high use of oral corticosteroid therapy and (iii) obese female patients with frequent exacerbations but with normal lung function (9) (**Fig. 1**). There were similarities with the phenotypes described in the American Severe Asthma Research Project (SARP) cohort: (i) early-onset atopic asthma with mild-to-moderate severity, (ii) obese late-onset nonatopic female asthma patients with frequent exacerbations and (iii) those with severe airflow obstruction with daily oral corticosteroid therapy (10). Cluster analysis performed in a Korean adult cohort of asthma reported four asthma subtypes that

were subdivided as 1) smoking asthma; 2) severe obstructive asthma; 3) early-onset atopic asthma; and 4) late-onset mild asthma (11).

Inflammatory markers such as presence of sputum eosinophilia has been used clustering analytics. In the cohorts from the Leicester group, sputum eosinophilia was used as a marker of eosinophilic asthma (12), that led to the description of a cluster of non-eosinophilic inflammation (early-onset, symptom-predominant group in female obese patients) and a cluster of eosinophilic inflammation with late-onset disease, associated with rhinosinusitis, aspirin sensitivity and recurrent exacerbations. The Leicester eosinophilic phenotype was also described in the SARP cohort with late-onset asthma and nasal polyps with exacerbations despite high systemic corticosteroid use which contrasted with the other eosinophilic cohort of early-onset allergic asthma with low lung function (13). It is important to note that, in the U-BIOPRED cohort, in terms of inflammatory characteristics measured in sputum, the eosinophilic group consisted of 50% of patients with severe asthma, while 15% showed a neutrophilic inflammation and 12% a mixed neutrophilic and eosinophilic inflammation. Those with mixed granulocytic disease had the worst lung function and the highest use of regular oral corticosteroids and the highest incidence of nasal polyps, indicating that this group might form a distinct clinical phenotype of severe asthma (14).

In a cohort recruited in China, patients recovering from an exacerbation of asthma were clustered depending on presence of airflow obstruction, sputum neutrophilia or eosinophilia, and smoking status (15). Thus, clusters 1 and 3 were characterized by female patients with sputum neutrophilia, with cluster 1 associated with a small degree of airflow obstruction and early-onset asthma and cluster 3 with a moderate degree of obstruction. Clusters 2 and 4 were associated with high sputum eosinophilia and severe airflow obstruction made of female non-smoking and male smoking subjects, respectively. Several parameters turn out to be important in this clustering exercise including severity of disease, exacerbations, airflow obstruction, age of onset of asthma, cigarette smoking and treatments.

**Precision Medicine needs Systems Biology**

Analysis of clinical and physiologic features on their own (as used in the clinical phenotyping described above) will not be sufficient to derive mechanism-based clusters or to identify the dynamic units and their interactions that underlie the clinical and physiologic features of the disease in a particular patient. Precision medicine has been defined as 'an approach to treat and prevent disease by taking into consideration the individual variability in genes, environment and lifestyle for each individual. Such an approach improves the likelihood of treating 'the right patient with the right drug at the right time', using preventive measures and therapies that are targeted for each particular individual (16). The analysis of all the genes, proteins, lipids and metabolites is more likely to indicate potential pathogenic and causative pathways, that would be necessary for the definition of endotypes by the driving mechanisms (8). In addition, these various platforms may be useful in identifying biomarkers (17). This is a more likely to be a successful strategy to achieve effective treatments that target the mechanisms/pathways driving these phenotypes than an analysis of clinical phenotypes. Precision medicine has been synonymously used as personalised medicine, tailored medicine, stratified medicine or targeted medicine, and will ultimately lead to targeted therapeutics. Future precision medicine will include not only the individual's genome but also his/her exposure to the environment (exposome) and to infectious agents (microbiome) (18) and how these together impact on changes in single cell/tissue immune and inflammatory functions.

In order to achieve this, one needs to approach the lungs and asthma as a complex dynamic system with many interacting units that can even be more complex than each of the constituent units alone. Understanding and analysing/modelling of such systems will need the application of a systems biology (19). Systems biology is an approach for modelling complex biological systems and processes such as those occurring in asthma through the use of multi-level multi-scale mathematical and computing methods in order to integrate the biological networks and pathways involved. This approach is integrative rather than being reductionist. This will allow for the discovery of new properties or mechanisms involved in asthma that have not been evident previously when taking the traditional reductionist approach.Thus, the identification of disease mechanisms should be possible through the statistical

analysis of different levels of -*omics* data such as transcriptomics or proteomics. This can be followed by annotation with up-to-date ontologies to generate biomarker signatures derived from data collected from a single *omics* platform ( called fingerprint) or those biomarker signatures derived from data collected within multiple -*omics* platforms (called handprints) (20). Although multi-level data integration is a major area of research in systems biology, techniques available to do this remain limited and only a handful of papers have been published in relation to respiratory diseases (21) (**Fig 2)**.

**Looking for treatable mechanisms rather than treatable traits**

**Fig 3** illustrates the development of precision medicine to severe asthma through the definition of clinical phenotypes, molecular phenotypes and endotypes. From the patient point of view, clinical phenotyping will lead to the identification of important treatable traits and molecular phenotyping to treatable mechanisms resulting in targeted treatments determined by bedside biomarkers. Treatable traits have been defined within a precision medicine strategy for the management of patients with airways disease based on its description (22). This makes the assumption that a treatable trait is driven by the same causal pathway. However, there is no understanding of the mechanisms underlying treatable traits to be able to direct any targeted therapies against any particular treatable trait. In addition, the definition of a treatable trait is too imprecise to be useful to the individual patient with chronic obstructive lung disease (23). Indeed, twenty-three treatable traits were identified in the U-BIOPRED severe asthma cohort, including seven pulmonary, 11 extra-pulmonary and five behavioural/psychosocial treatable traits (24). Seven out of the ten most prevalent traits in severe asthma were classed as pulmonary treatable traits while the most prevalent extra-pulmonary traits were atopy, rhinosinusitis, obesity, reflux and obstructive sleep apnoea. In addition, there were fewer treatable traits in those with moderately-severe asthma.

Treatable traits are clinical features that should be evident from the clinical phenotyping described above such as chronic airflow obstruction and exacerbations that characterise many of the clinical phenotypes. The qualification of treatable in treatable traits assumes that there already exist effective treatments for that trait, which is usually not always the case. A systems biology approach would open up the way to approach this by identifying the mechanisms for treatable traits (25). For example, gene set variation analysis (GSVA) identified pathways that were differentially expressed in nasal brushings, sputum, bronchial brushings and bronchial biopsy samples obtained from asthmatics with airflow obstruction versus non-airflow obstruction (26). Differentially enriched gene signatures were associated with ICS response, eosinophils, interleukin (IL)-13, interferon-α, specific CD4+ T-cells and airway remodelling. Airflow obstruction in severe asthma is associated with specific underlying gene networks that are associated with treatments, inflammatory pathways and airway remodelling that point towards targets for the therapy of airflow obstruction in severe asthma. These may be considered as being the underlying treatable *mechanisms* of the treatable *trait* of airflow obstruction.

**Molecular phenotypes**

Molecular phenotyping by measuring the expression of genes upregulated by IL-13 in bronchial epithelial cells has defined a group of mild asthmatics (consisting of 50% of patients) characterised by blood and bronchoalveolar lavage eosinophilia, increased levels of serum IgE, increased expression of the mucin MUC5AC, increased expression of IL-5 and IL-13 in biopsies, increased bronchial hyperresponsiveness and response to ICS by an increase in $FEV_1$ when compared to those with low Th2-low expression (6). On the other hand, using GSVA to determine the enrichment of the IL-13 T2-high signature described above, the T2-high phenotype was only found in 37% of patients with severe asthma (7). The T2-high patients were more symptomatic with a higher percentage of patients on oral corticosteroid therapy and with higher levels of nitric oxide in exhaled breath and of blood and sputum eosinophils but not of serum IgE or periostin. Therefore, the majority of patients with severe asthma were T2-low.

However, the prevalence of T2-high or T2-low severe asthma is dependent on the approach taken to measure T2 expression. Using a different approach of defining a composite measure of IL-4, IL-5 and IL-13 gene expression in induced sputum cells, 70% of patients with severe asthma despite high dose ICS were reported to have a T2-high phenotype (27). It is unclear whether this is because of the method used to determine the expression of T2-high or to the separate population of asthma particpants in this report. After treatment with intramuscular triamcinolone, most patients studied in this cohort showed little response in terms of lung function ($FEV_1$), blood eosinophil count or levels of FeNO, accompanied by little change in the expression of IL-4, IL-5 and IL-13 genes in sputum cells, indicating a degree of corticosteroid insensitivity in this group (28).

An unbiased analysis of sputum transcriptomics data to obtaining molecular phenotypes of asthma undertaken in U-BIOPRED using a hierarchical clustering of differentially expressed genes between eosinophilic and non-eosinophilic inflammatory profiles revealed three molecular phenotypes (29)**.** The first transcriptomic-associated cluster (TAC1) was characterized by the immune receptors IL33R, CCR3 and TSLPR with the highest enrichment of gene signatures for IL-13/T2-inflammation-high and for innate lymphoid cell type 2 (ILC-2) associated with the highest sputum eosinophilia: this grouped patients with severe asthma with oral corticosteroid dependency, frequent exacerbations and severe airflow obstruction, which are treatable traits of the severe eosinophilic asthma (**Fig 4**).

The second TAC (TAC2) was characterized by inflammasome-associated genes, interferon-α and tumour necrosis factor-α-associated genes, with sputum neutrophilia, high serum C-reactive protein levels and a higher prevalence of eczema. This phenotype is in agreement with the report of elevated gene expression of NLRP3 inflammasome, caspase-1 and IL-1β in sputum macrophages of patients with neutrophilic asthma (30). The third phenotype (TAC3) was characterized by metabolic pathway genes, ubiquitination and mitochondrial function with pauci-granulocytic inflammation and little airflow obstruction. In addition, these patients showed the highest expression for a signature of exposure to diesel exhaust pollution, indicating that exposure to air pollution may be a causative driver of asthma in this cluster (*unpublished data*). This unbiased approach has provided an

overall idea of the various pathways some of which have not previously been associated with these three molecular phenotypes, with the possibility that each of these phenotypes is underlined by several interacting pathways.

**Late onset severe eosinophilic asthma: an endotype**

Eosinophilic asthma characterized by concomitant high blood and sputum eosinophilia has been associated with very poor asthma control and propensity to asthma exacerbation (13). Unbiased cluster analyses have uncovered patients with late-onset, eosinophilic inflammation-predominant asthma (9, 10, 12), and adult-onset asthma patients with a high blood eosinophil count with frequent exacerbations (31). Many patients also have persistent airflow limitation and distal airway inflammation with air trapping, together with upper airways disease such as chronic rhinosinusitis with nasal polyposis (32). Overall, these patients are defined as having severe asthma, with high load eosinophilic disease, frequent exacerbations and the need for oral corticosteroid therapy to maintain control (33).

Molecular phenotyping using the IL-13-induced genes in bronchial epithelial cells defined a group of mild asthmatics (T2-high) characterised by eosinophilia (6). In patients with moderate-to-severe asthma treated with ICS and LABA, those with T2-high expression had higher levels of symptoms, and showed a greater bronchodilator response to salbutamol (7). This group was composed of a higher number of patients on OCS therapy, and had higher FeNO and serum and sputum eosinophil counts than those with T2-low asthma, characterising a severe eosinophilic phenotype. Thus, the difference between mild versus severe T2-high patient is the presence of corticosteroid insensitivity and oral cortcisoteroid dependence in the severe patient while in the mild patients, there is a good therapeutic response to inhaled corctsioteroid therapy. Finally, targeted treatments such as anti-IL5 and anti-IL5Rα antibodies that caused a reduction in the exacerbation rate and in the blood levels of eosinophils support the concept that this is a severe eosinophilic asthma endotype (34, 35).

**Non-T2 pathways**

GSVA of signatures expressed in bronchial biopsies and airway epithelial brushings identified two distinct asthma subtypes associated with high expression of T-helper cell type 2 cytokines and a lack of corticosteroid response (group 1 and group 3). Group 1 subjects had high levels of submucosal eosinophils, FeNO, exacerbation rates, and oral corticosteroid use, whereas group 3 patients showed the highest levels of sputum eosinophils and had a high body mass index (36). In contrast, group 2 and group 4 patients had an 86% and 64% probability, respectively, of having non-eosinophilic inflammation (36). CD44, a constituent of a corticosteroid insensitivity signature, had the most extensive association and/or interaction with a subset of T2 signature genes including CCL26, IL1R2, and CST2.

MMP-10 and MET genes were overexpressed in patients with high mucosal eosinophilia compared to those with low mucosal eosinophilia (37). The differential gene set characteristic of the high eosinophilia group included extracellular matrix organization, mast cell activation, CC-chemokine receptor binding, circulating immunoglobulin complex, serine protease inhibitors and microtubule bundle formation pathways. In another microarray analysis of endobronchial biopsies of symptomatic asthma, 3 clusters identified by the presence of Th2-high, Th17-high and Th2/Th17-low signatures were described, with an inverse expression of the gene signatures for Th2-high and Th17-high indicating this separation of a T2-high versus T2-low (38).

Coexistence of T2-high and T2-low pathways has also been reported. Thus, RNA-sequencing of airway epithelial brushings from asthmatic and healthy control subjects identified increased expression of T2 markers with IFN-stimulated genes (ISGs), and endoplasmic reticulum (ER) stress-related genes (39). ISGs inversely correlated with T2 markers and defined patients with reduced $FEV_1$. ER stress pathways were enriched in both T2 and ISGs high subjects. In an analysis of the transcriptomic expression of bronchial biopsies and bronchial brushings of patients with moderate-to-severe asthma, the co-existence of T2-pathways with corticosteroid insensitivity gene signatures was reported (36). The existence of a significant correlation between T2 and corticosteroid insensitivity signature expression levels identifies the clinical problem of insensitivity to corticosteroid

treatment in a subgroup of patients with severe asthma. Furthermore, analysis of the genes associated in the corticosteroid insensitivity and T2 signatures in the biopsies and brushings showed an association between CD44 and the T2-associated genes CCL26, IL1R2 and CST2, indicating potential underlying driving mechanisms that should be investigated (36).

**Non-T2 IL-6 associated clusters**

Using an IL-6 trans signalling signature (IL-6TS) that is enriched in genes associated with airway wall remodelling, a subset of patients with IL-6TS-high asthma was identified with an overrepresentation of frequent exacerbators, blood eosinophilia, and submucosal infiltration of T cells and macrophages. Toll-like receptor pathway genes were upregulated in bronchial brushings of these subjects, whereas expression of cell junction genes was reduced. Sputum sIL-6R and IL-6 levels correlated with sputum markers of remodelling and innate immune activation, in particular YKL-40, matrix metalloproteinase 3, macrophage inflammatory protein 1β, IL-8, and IL-1β (40). In addition, a group of patients with high IL6R mRNA level and high IL-6 protein sputum levels associated with higher sputum neutrophil counts regardless of eosinophilic inflammation status has been identified in the same cohort, with poor lung function and higher levels of systemic IL-6 and CRP (41). These subjects resemble the TAC2 group described earlier (29). In a subset of patients with severe asthma with high blood IL-6 levels (42), clinical features of metabolic dysfunction occurred more frequently in a subset of obese and of non-obese patients associated with worse lung function and more frequent asthma exacerbations, together with a higher prevalence of hypertension and diabetes.

Another approach to defining T2-low pathways was to perform hierarchical clustering on the genes in epithelial brushings whose expression correlated with FeNO. This identified nine gene clusters related to type 2 inflammation as well as to non-T2 pathways such as neuronal function, WNT pathways and actin cytoskeleton (43).

**New biologics: towards treatable mechanism**

The biologic treatments that are either currently available or will be available in the near future include anti-IgE antibody (Omalizumab), anti-IL5 antibodies such as mepolizumab and reslizumab, anti-IL5R antibody (benralizumab) and anti-IL4Rα antibody (dupilumab) (**Table 1**). The introduction of these treatments for severe asthma has opened up the era of precision medicine in asthma because, for the first time, treatments are directed towards specific groups using biomarkers. Thus, for omalizumab, patients with severe asthma with circulating IgE levels together with an allergic background are selected for a trial of omalizumab. Importantly, these features are not a biomarker of response but act as a means of stratifying patients as those more likely to respond to therapy. For the anti-IL5 and anti-IL5Rα antibodies, the biomarkers of response to these antibodies in severe asthma includes a history of 2 or more exacerbations and a blood eosinophil count of at least 300 per µl of blood. Studies in U-BIOPRED have shown that using a blood eosinophil count is not a perfect predictor of T2-high asthma (7) and this may not be the best biomarker for selecting the responders to these biologic treatments. Better predictive biomarkers of anti-IL-5/anti-eosinophil responders/non-responders are required.

The severe eosinophilic asthma phenotype can overlap with the severe allergic asthma phenotype as demonstrated in the U-BIOPRED cohort where 37% of patients with severe asthma fell into both phenotypes (*unpublished observations*), indicating that these patients could be suitable for either an anti-IL5 antibody or an anti-IgE antibody treatment. At present, it is not known which antibody treatmnent would be preferable in such patients.

The predictive value of blood eosinophilc ocunt and FeNO has been demonstrated in a Phase 3 study of the anti-IL4Rα antibody, dupilumab, which blocks the effects of the T2-cytokines, IL-4 and IL-13. In those patients with asthma with a baseline blood eosinophil count of ≥300/µl or with a baseline level of nitric oxide in exhaled breath (FeNO) of between 25 and 50 ppb, treatment with dupilumab showed a significant reduction in exacerbation rates compared to those with baseline values of blood eosinophil count <300/µl or < 25 ppb of FeNO. The greatest treatment benefit was observed in those with an elevation of both baseline blood eosinophil count and FeNO (44).

The clinical trials of these T2-biologics have shown that there is a major reduction of severe exacerbations with a small improvement in airflow obstruction in terms of $FEV_1$, together with an improvement in asthma quality of life scores. Therefore, it would be interesting to list the effect of these biologics in terms of their treatable mechanisms and treatable traits as listed in **Table 1.** Thus, we see that inhibition of IL5 and both of IL-4 and IL-13 have effects on exacerbations and airflow obstruction, indicating that there may be overlapping mechanisms or pathways that might regulate different treatable traits. In addition, the contribution of each mechanism or pathway may not completely account for each treatable trait.

It is to be noted that even an average 50-60% reduction in exacerbation rate provided by these T2-targeted biologics still leaves patients with a high disease burden. In addition, some patients do not respond at all. This may be indicative of the concept that there may be also non-T2 pathways interacting with T2-associated pathways.

By contrast, non-T2 directed therapies have not proved to be successful in severe asthma. Brodalumab, a human anti-IL-17RA monoclonal antibody, and the anti-TNFα antibody, golimumab, together with a selective CXCR2 antagonist, AZD5069, which blocks the effect of CXCL8, had no effect on asthma control scores, symptom-free days and $FEV_1$ in uncontrolled moderate-to-severe asthma receiving ICS therapy (45-47). The failure of these therapies may be due to a lack of appropriate patient selection, reflecting a paucity of appropriate biomarkers for non-T2 asthma. Although molecular phenotypes of non-T2 pathways have been proposed, these have not been validated into endotypes, such that the possibility remains that these mechanisms or pathways may not drive the disease or underlie the treatable traits. In addition, T2-pathways may coexist within the non-T2 pathways and interact to result into a complex mechanism such that more than one pathway may be needed to target in order to achieve a significant effect.

**Treatable mechanisms and targeted treatments**

Currently, precision medicine is limited to the use of a blood eosinophil count and FeNO in determining treatment with T2 biologics (**Table 1**). This has resulted in a wide range of treatment effects seen with therapies targeting particular traits. The definition of molecular phenotypes of both T2-high and T2-low asthma, the development of more phenotypic and predictive biomarkers to delineate these endotypes and the validation of specific bedside biomarkers to predict therapeutic outcomes to specific targeted therapies of treatable mechanisms should be the next steps in this process (**Fig 2**).

Selecting discriminating biomarkers measured in serum by looking at the differences in distinctive clinical phenotypes has not been useful. In the U-BIOPRED clinical clusters, the ability of the biomarkers such as serum periostin, blood eosinophil count or FeNO and, serum CCL17, CCL18 and IL-13 in predicting cluster association was very poor (48, 49). In the Wessex severe asthma cohort using 29 clinical, physiologic and cellular parameters, 8 clusters were obtained but there was a lack of association between the pathobiologic biomarkers and clinical features (48). Similarly, using readily-available biomarkers to differentiate clinical clusters (severity, lung function and blood eosinophil counts) was not successful (49).

It is imperative to define our patients in terms of molecular signature(s) that is (are) driving the disease. Thus, T2-high severe asthma could be predicted to some extent from raised levels of FeNO, blood and sputum eosinophil counts, but serum IgE or serum periostin were poor predictors (7). Similarly, blood eosinophil cell counts predicted steroid-resistant T2-high asthma when body mass index was less than 40kg/m$^2$ whereas serum IgE levels strongly predicted this type of asthma when patients were less than 34 years old (28). Similar phenotyping needs to be performed for patients enriched for IL-6, IL-17, inflammasome activation and other molecular mechanisms.

Machine learning techniques use algorithms and statistical methods to perform specific tasks relying on models and inference but not on explicit instructions. Thus, these tools for predictive analytics may be applied to predict asthma phenotypes from clinical biomarkers. Molecular phenotypes originally defined by gene signatures in bronchial biopsies and bronchial epithelial cells in the U-BIOPRED cohort, and by histopathologic results, could be recognised by a clinically-inferable scheme using currently-available inflammatory biomarkers such as sputum eosinophilia and FeNO levels and the presence of oral corticosteroid dependency (36). This approach could define patients who may benefit the most from specific molecular agents that target T2-mediated inflammation and/or relative corticosteroid insensitivity.

Assays involving exhaled breath appear promising in terms of finding out useful predictive bed-side biomarkers. Real-time analysis of metabolites in exhaled breath using a set of 'electronic noses' could discriminate with a high degree of accuracy between the 3 TACs determined by hierarchical clustering of differentially-expressed genes in sputum (36, 50). However, the basis for this good predictability remains unclear.

**Conclusion**

Progress in our treatment of severe asthma of the past 10 years with the introduction of biologic therapies has occurred independently of any systems biology approach to severe asthma. However, the introduction of such specific therapies has necessitated the introduction of some aspects of personalised medicine in the use of biomarkers to define clinical phenotypes that will respond to such precisely-targeted treatments (51). With the introduction of these biologic therapies has emerged the definition of one of the first endotypes of severe asthma, that of the T2-driven severe eosinophilic asthma. Parallel developments in systems biology, machine learning and precision medicine applied to severe asthma are evolving that should help to improve the definition of molecular phenotypes. Increasing number of molecular phenotypes particularly in the T2-low category are being described, although most need to be validated. The impact of these new molecular phenotypes has yet to be felt in clinical practice. This is likely to be due to the fact that no treatments targeted at these phenotypes have emerged. The description of a range of severe asthma

endotypes with distinct treatable driving mechanism(s) should become the future goal of asthma care. This has become imperative due to the lack of precision in our current treatment approaches to the bulk of severe asthma patients who have T2-low pathway-driven disease, particularly with reference to the use of blood biomarkers. Discovery of bedside biomarkers needs to start from a definition of the treatable mechanisms underlying the endotype.

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**Legend to Figures:**

**Fig 1.** Clinical phenotypes defined following clustering of moderate-to-severe asthma using 8 clinico-physiological features. Clustering was performed using partition-around-medoids algorithm with consensus clustering done by random removal of 10% of the data with repeat clustering to achieve cluster stability. From (9).

**Fig 2**. Proposed systems biology approach to asthma. Working from clusters of clinical and molecular phenotypes through to endotypes in order to dissect the biological pathways to understand the drivers underlying a person's asthma. Various approaches are proposed from analysis of multiple omics platforms to functional genomics and validation of endotypes.

**Fig 3**. From systems biology to delivery of precision medicine to asthma. While treatable mechanisms or pathways will follow from a determination of endotypes to form the basis of targeted treatments, treatable traits and biomarkers will be derived from clinical, physiologic and inflammatory features of clinical phenotypes. These will form the basis of clinical trials and ultimately, clinical practice.

**Fig 4**. Description of 3 transcriptomic-associated clusters (TACs) from a hierarchical clustering of sputum transcriptomics analysis that yielded 518 differentially expressed genes comparing sputum eosinophil-high to eosinophil-low severe asthmatic patients. The clinical and inflammatory features of each TAC is shown with TAC1 being Type-2-high and TAC2 and TAC3 being Type-2-low. Adapted from Kuo et al (29)

**Table 1. Biologic therapies targeting treatable mechanisms for severe asthma: biomarker and treatable trait effects**

| Antibody biologic | Biologic Target | Treatable mechanisms/ pathways | Biomarker used | Effects on treatable traits & quality of life measures |
|---|---|---|---|---|
| **Omalizumab** | Anti-T2:<br>Targets IgE | Prevents binding to high affinity receptor | Serum IgE<br>Allergies | Reduces exacerbations<br>Improves AQLQ |
| **Mepolizumab** | Anti-T2: Targets IL-5 | Prevents the terminal differentiation of eosinophil progenitors and reduces the output of eosinophils from bone marrow | Exacerbations ≥ 2;<br>Blood eos ≥150/μL | Reduces exacerbations<br>Reduces airflow obstruction<br>Improves AQLQ.<br>Oral corticosteroid-sparing |
| **Reslizumab** | Anti-T2: Targets IL-5 | As above | Exacerbations ≥ 1;<br>Blood eos ≥400/μL | Reduces exacerbations<br>Reduces airflow obstruction<br>Improves AQLQ |
| **Benralizumab** | Anti-T2: Targets IL-5Rα, consequently, IL-5 | Binds to IL-5Rα and induces eosinophil apoptosis | Exacerbations ≥ 2;<br>Blood eos ≥300/μL | Reduces exacerbations<br>Reduces airflow obstruction<br>Improves AQLQ<br>Oral corticosteroid-sparing |
| **Dupilumab** | Anti-T2: Targets lL4Rα, consequently IL-4 and IL-13 | Prevents the differentiation of naive Th0 cells to Th2 cells. Suppresses IgE synthesis, goblet cell hyperplasia, mucus hypersecretion, airway hyperresponsiveness and fibrosis | Exacerbations ≥ 1<br>Blood eos ≥150/μL | Reduces exacerbations<br>Reduces airflow obstruction<br>Improves AQLQ<br>Oral corticosteroid-sparing |

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