

Trials and tribulations in identifying new biologic treatments for asthma

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Drugs used to treat asthma have a long history, beginning with the bronchodilators and evolving into compounds that suppress airway inflammation. Guidelines for treatment of asthma are largely based on disease severity and control, rather than underlying mechanisms. However, identification of biomarkers in the causal pathways of asthma is enabling responders to be differentiated from nonresponders. Initial efforts have focused on biomarkers of the T helper (Th)2 pathway because this is a target of novel therapeutics. A concerted effort is now needed to substratify asthma beyond Th2 pathways, and using appropriate biomarkers, to target only those patients likely to respond to a specific biologic. To achieve this goal, a different type of relationship is needed between academia and industry, and also within industry, to promote collaboration in the precompetitive space.

Evolution in understanding asthma

At one time, asthma was considered a simple disorder in which the conducting airways contract too much and too easily, either spontaneously or upon stimulation. However, the discovery that inflammation underpins airway hyper-responsiveness forms the basis of modern asthma management, which is achieved with anti-inflammatory drugs, such as inhaled corticosteroids referred to as ‘controllers’, and when required, bronchodilator β_2 -adrenoceptor agonists or so-called ‘relievers’. This treatment has done much to reduce asthma mortality and morbidity, however, in many countries, the disease continues to increase both in prevalence and severity [1]. Current treatment has little or no effect on the natural history of asthma, and certainly does not cure the disease. There has been limited success in therapeutically targeting allergic pathways both with small chemical entities and biologics. Many promising targets identified through *in vitro* cell and *in vivo* animal studies have not translated into clinical practice, suggesting that such models are not representative of the chronic disease as it occurs in humans. This review identifies the importance of subphenotyping asthma into different endotypes in which causal pathways differ. Based on early observations identifying new biomarkers for Th2-mediated pathways in asthma, the application of ‘omics’ platforms, to define these endotypes in human asthma will reveal other novel causal pathways against which more stratified therapeutics can be directed. In addition, moving away from

simple animal models of allergic inflammation will enable new models to be developed that better reflect the multiple environmental factors that are associated with the induction and exacerbation of asthma. This new approach to a seemingly simple concept of episodic bronchospasm, which in reality is a complex disease, will uncover novel causative pathways that are much closer to the origins and progression of asthma, thus making prevention and cure a reality rather than simply suppressing the inflammatory and smooth muscle manifestations.

A brief history of asthma therapeutics

Bronchodilators

Asthma has been recognised as a distinct disease entity since the mid 19th century, when the concept of bronchospasm was first introduced [2]. This led to the development of bronchodilators beginning with *Datura stramonium* and ephedrine (also available as the Chinese herbal supplement, Ma Huang) along with theophylline and its more soluble salt, aminophylline. However, it was the discovery in 1904 of adrenaline and its powerful bronchodilator activity that stimulated 100 years of classical pharmacology to improve smooth muscle relaxant selectivity and duration of action. The widespread, unrestricted use of the short-acting bronchodilators in high doses in the 1960s and 1970s was associated with unexpected peaks in asthma mortality, probably resulting from a combination of overuse during exacerbations, β_2 -receptor refractoriness, and cardiotoxicity of the chlorinated fluorocarbon carriers in pressurised metered dose aerosols in conjunction with asthma-related hypoxia [3].

Anti-inflammatories

The efficacy of systemic corticosteroids in controlling inflammation in asthma was first demonstrated by Phillip Hench using cortisone in rheumatoid arthritis (RA) at the Mayo Clinic in 1947 [4]. The application of a locally acting corticosteroid, beclomethasone dipropionate, for administration by inhalation (inhaled corticosteroid; ICS) has transformed asthma management [5]. The many attempts to improve the therapeutic ratio of ICSs have had limited success, despite a range of new chemical formulations and inhalation devices [6]. The main challenge has been trying to separate the anti-inflammatory from the glucocorticoid and mineralocorticoid metabolic effects of corticosteroids, while at the same time maintaining desirable potency, bioavailability and pharmacokinetics [7].

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Antiallergics

The identification that specific immune cells and inflammatory pathways are involved in asthma began with associating eosinophilia with corticosteroid responsiveness. After this, mast cells were identified as the key driver of the acute early allergic response in asthma. In the 1950s, it was discovered that sodium cromoglycate (SCG) inhibited mast cell activation for mediator secretion [8], and inhalation of SCG was found to be active against controlled allergen and exercise challenge, and in asthma clinical trials [9]. Nedocromil sodium (NS) is a second-generation drug that has similar properties to SCG, but is somewhat more active [10]. Although it has been shown to be an effective antiasthmatic drug, especially in children [8], subsequent meta-analysis and Cochrane Review of SCG trials have concluded that, across a wide age range of young people, 'there was insufficient evidence to be sure about the efficacy of SCG over placebo' [11]. SCG has subsequently been withdrawn from the WHO List of Drugs, despite evidence that the conclusions drawn from the Cochrane Review may be challenged [12]. One problem with this class of drugs is that no mechanism has been found to explain inhibition of mast cell activation, apart from inhibiting chloride flux [13]. The recent discovery that both SCG and NS are potent and selective inhibitors of the G protein-coupled receptor (GPR) 35, the receptor for 2-acyl-lysophosphatidic acid, which is linked to mast cell development and activation [14,15], provides a molecular target against which further mast cell inhibitory drugs could be developed [16].

Leukotriene modifiers

In 1989, the discovery that slow reacting substance of anaphylaxis (SRS-A) is a mixture of cysteinyl leukotriene C₄, D₄ and E₄ [17] led to characterisation of the role of cysteinyl leukotriene receptor 1 (Cyst LT₁R) in mediating smooth muscle contractile, vasoactive and mucus secretory activities [18]. The preferential agonist for the Cyst LT₁R is LTD₄, the extracellular cleavage product of LTC₄. However, selective antagonists [leukotriene receptor antagonists (LTRAs), e.g. montelukast, zafirlukast and pranlukast] were developed even before the receptor was structurally characterised, to provide one of the first nonsteroid oral antiasthma controllers. Clinical trials have generally shown that ICSs are more efficacious than the leukotriene modifiers [19], with the exception of in smokers who lose responsiveness to corticosteroids [20]. However, a more careful analysis of comparator trials, such as the Beclomethasone Versus Montelukast trial [21], reveals that the mean response to both drugs includes a spectrum of responses and there are clear improvements in some patients treated with montelukast, whereas other patients show no response or even deteriorate. Thus, there are distinct montelukast responder and nonresponder patient populations [22]. The preferential overexpression of LTC₄ synthase in mast cells and eosinophils in aspirin-intolerant asthma [23] explains why leukotriene modifying drugs are so effective in this patient subgroup [24]. In seeking markers for therapeutic efficacy for LTRAs and ICS, exhaled nitric oxide and the ratio of urinary LTE₄ (the major Cyst LT metabolite)/exhaled NO ratio predicts a better response to montelukast compared with inhaled fluticasone propionate therapy in

children with mild-to-moderate asthma [25]. Also, because LTRAs are administered orally, patients tend to be more adherent to treatment, explaining why real life effectiveness (as opposed to efficacy) studies have shown little difference in asthma outcome measures when oral montelukast has been compared with an ICS as initial therapy or a long acting beta agonist (LABA) as add-on therapy to ICS [26]. Thus, although LTRAs may not appear as efficacious as ICSs in controlled trials, they are effective in selected patients in whom this mediator pathway dominates and in 'real world' practice where adherence to oral therapy is greater than to inhaled therapy [27].

Biologics targeting the immune system in asthma treatment

Anti-IgE monoclonal antibody

The only other new therapy for asthma to emerge over the past 50 years is the IgG1 monoclonal antibody (mAb), omalizumab. This antibody targets a component of IgE (Fc ϵ 3 region) that usually binds to the high affinity receptor (Fc ϵ R1) on mast cells, basophils and dendritic cells (DCs) through formation of tri- and hexameric IgE-containing immune complexes [28]. Upon drug treatment, free circulating IgE falls rapidly, whereas complexed IgE initially increases before being effectively removed by the reticulo-endothelial system [29]. Loss of IgE from Fc ϵ R1 leads to receptor internalisation, thereby enhancing therapeutic efficacy [30]. Loss of IgE deprives mast cells and basophils of their ability to secrete preformed and newly generated mediators and to facilitate allergen uptake by DCs [31]. Omalizumab, administered by subcutaneous injection 2–4 times weekly, almost abolishes the early (mast-cell-dependent) and the late phase (inflammatory-cell-dependent) allergen-provoked bronchoconstriction [32]. Clinical trials in adults and children have confirmed the efficacy of omalizumab in severe allergic asthma [28], but the response was not uniform across all patients: one third of patients responded dramatically, with life-transforming experiences that included being able to withdraw from corticosteroids, one third with less clinically beneficial responses, and one third experienced no response at all [33]. The situation is made more complex by patients varying in the outcome measures that best responded to therapy. Due to variation in clinical responsiveness to omalizumab, it is advised that patients are assessed at 16 weeks after starting therapy, at which stage, a range of outcome measures is used to decide whether to continue or cease therapy [33,34].

The reasons behind the responder/nonresponder outcomes with omalizumab are not known, despite a genetic analysis being carried out by Novartis. For those who respond to anti-IgE therapy, the relation between free serum IgE and asthma outcome measures after starting and stopping therapy are closely paralleled, whereas for the non-responders, no relation exists during therapy induction or withdrawal [35]. There is clearly an urgent need to discover how the immunopathology in the airways differs between these two phenotypes. Omalizumab is also finding a therapeutic place in preventing anaphylactic side effects and increasing efficacy of allergen-specific immunotherapy, chronic urticaria, mast cell disorders (e.g. some forms of mastocytosis) and bronchopulmonary allergic aspergillosis

[36]. The existence of omalizumab responders and nonresponders must have a biological basis, because treatment administered by healthcare professionals has utilized a common qualifying definition based on allergy, IgE and weight, yet so far, there is no clear explanation because both groups appear to have identical disease phenotypes, as defined by these criteria. One potential explanation for the differences in responsiveness to omalizumab is a lack of efficacy at the target site in the airways; a possibility that will be answered by a more potent form of anti-IgE (QGE031) that is in clinical development (<http://clinicaltrials.gov/ct2/show/NCT01451450>).

Cytokines as therapeutic targets

In the 30 years since T cells, mast cells and eosinophils were identified to play a major role in the allergic cascade, many possible therapeutic targets have emerged, including cell surface receptors, cytokines and chemokines; in particular, those associated with the Th2 pathway (reviewed in [37]). From these studies, interleukin (IL)-5, a Th2 cytokine intimately involved in the maturation, activation and survival of eosinophils, presented itself as an obvious candidate to target [38]. Studies in mice have shown that ablation of the IL-5 gene or blockade of IL-5 or its receptor has dramatic effects in abolishing eosinophilic responses in the lungs with acute and repeated allergen challenge [39]. The anti-IL-5 blocking mAb, mepolizumab, gave similarly encouraging results in appropriately challenged non-human primates [40]. However, when mepolizumab was tested in a similar, allergen-induced early and late phase challenge model in human asthma, no significant effect was observed, despite near ablation of both circulating and sputum eosinophils [41]. A subsequent clinical trial of mepolizumab in moderate-to-severe asthma also failed to reveal efficacy for any asthma outcome measure [42], despite showing marked depletion of circulating and sputum eosinophil numbers. Subsequent studies showed that mepolizumab decreased airway tissue [43] and bone marrow [44] eosinophils by only approximately 50%; possibly because only this proportion of the cells expressed the IL-5 receptor [45].

Based on the prior assumptions that had been made from the various animal models, these findings rocked the strong views held about the causative role of the eosinophil in asthma. However, against other measures of disease, such as the deposition of new matrix (e.g. tenascin, lumican, and procollagen III) in the *lamina reticularis* [46] or in the skin after allergen challenge [47], mepolizumab has proved to be remarkably active. Also, in other eosinophil-driven diseases, such as eosinophilic oesophagitis [48], Churg–Strauss syndrome (allergic granulomatosis and angiitis) [49] and some other systemic hypereosinophilic syndromes [50], mepolizumab is highly efficacious.

Of great interest were two publications reporting that mepolizumab has a marked effect in reducing exacerbations in severe asthma, specifically in patients with oral-corticosteroid-dependent asthma with sputum eosinophilia [51,52]. One has to assume that in these patients, which comprise <1% of the asthmatic population, IL-5-responsive eosinophils are more closely associated with disease outcomes [53]. However, identification of this subgroup of

severe asthma and persisting airway eosinophilia despite high dose corticosteroid treatment provides a way of determining those who will benefit from IL-5 blockade.

Use of a second anti-IL-5 mAb, reslizumab (CTx55700), in severe persistent asthma in patients receiving oral or high doses of inhaled steroids did reveal a trend toward improvement in baseline lung function at the highest dose given, but not in other clinical indices of disease activity [54]. In a larger randomised control trial (RCT) of eosinophilic asthma that was poorly controlled by high-dose ICS [55], those on reslizumab showed significantly greater reductions in sputum eosinophils, improvement in lung function, and a trend towards greater asthma control than those on placebo, which was especially apparent in patients with concomitant polyposis. In a separate trial in severe nasal polyposis, the clinical response to reslizumab was proportional to the levels of IL-5 detected in nasal lavage [56]. Both diseases are characterised by mucosal eosinophilia, and a recent small trial with mepolizumab also revealed efficacy against severe nasal polyposis [57].

Taken together, blockade of IL-5, as a therapeutic approach in severe asthma and associated disorders will only be effective if this cytokine is the predominant driver for the eosinophilia beyond corticosteroid suppression, whereas for those who do not respond, other pro-eosinophilic cytokines and chemokines will be important, such as IL-3, granulocyte–macrophage colony-stimulating factor (GM-CSF), chemokine CC ligand (CCL)1, CCL5 and CCL11. With the development of more active biologics, such as MEDI 563, a defucosylated human mAb directed against the IL-5 receptor α , which exhibits greatly enhanced antibody-dependent cell cytotoxicity (ADCC), the role of eosinophils in this disease will be determined [58]. A recently completed phase 1 study of MEDI 563 in mild asthma showed dose-related reductions in circulating eosinophils reaching total ablation at the higher doses and lasting up to 8–12 weeks following a single injection [59].

IL-4 and IL-13 are of interest for design of novel interventions for asthma and allergic disease, not only because they are the principle drivers of Th2-mediated inflammation, but also because they are highly active in promoting mucous metaplasia, and airway wall remodelling in rodent models (reviewed in [60]). There is also strong genetic evidence incriminating this pathway in asthma [61,62]. Blockade of these cytokines in mouse models not only ablates Th2-mediated acute and chronic inflammation, but also prevents mucous metaplasia, smooth muscle proliferation and deposition of new matrix [63,64]. Of the literature published in 2010, when these targets were receiving a great deal of attention, 2968 peer-reviewed publications were listed in Pubmed for IL-4 and/or IL-13. Within these studies, 1000 were related to allergy, 742 to animal models of asthma, and 417 to mouse models of asthma. There were 66 articles on humans; 52 of which were reviews, eight describing genetics and six covering human sputum or biopsy (with two review articles). This left four peer-reviewed studies that examined IL-13 in relation to asthma. Of these four studies, only two were rigorous and both were from the same laboratory in the UK [65,66]. These latter studies indicated that IL-13 could be detected in the sputum of asthmatics irrespective of severity in only ~50% of

patients. Thus, validation of the IL-13 pathway as a therapeutic target in human asthma seems grossly deficient.

Nevertheless, the data sufficiently convinced at least 12 pharmaceutical and biotechnology companies to launch major programmes using biologics to target IL-13 or the IL-4 receptor α , which is the subunit in common for both the IL-4 and IL-13 receptors (Figure 1, Table 1). Aerovance were the first to report, and described pitrakinra, an IL-4 mutein that acts as an IL-4 and IL-3 antagonist, which can be administered by inhalation or systemically, and which shows significant attenuation of the allergen-provoked late asthmatic response [67]. The anti-IL-13 mAb, IMA 638 (Pfizer), also shows an inhibitory effect against the late response but another anti-IL-13 mAb, IMA 026, does not [68]. Some subtleties exist in the way these two mAbs interact with IL-13. IMA-638 permits interaction of IL-13 with IL-13 receptor α_1 or IL-13 receptor α_2 , but inhibits recruitment of IL-4 receptor α to the IL-13/IL-13 receptor α_1 complex, whereas IMA-026 competes with IL-13 interaction with IL-13 receptor α_1 and IL-13 receptor α_2 (Figure 1) [69]. The potency and efficiency of IMA-638 in effectively depleting IL-13 indicates that cell surface IL-13 receptor α_2 acts as an important scavenger for removal of this cytokine, with implications for future design of IL-13 inhibitors.

The first 12-week clinical trial of AMG 317 (Amgen), a biologic targeting IL-4 receptor α in moderate-to-severe asthma [70], was negative for all asthma outcome measures,

Table 1. Biologics designed to target the IL-4/13 signalling pathway currently being investigated for treatment of asthma

Candidate	Specificity	Format
Nuvance (Immunex)	IL-4	siIL-4R
Pitrakinra (Aerovance)	IL-4R α	IL-4 mutein
AMG-317 (AMGEN)	IL-4R α	mAb
QAX-576 (Novartis)	IL-13	mAb
CAT-354 (MedImmune)	IL-13	mAb
IMA-638 (Wyeth)	IL-13	mAb
TNX650 (Genentech)	IL-13	mAb
IL-13R (Merck & Roche)	IL-13R α_1	mAb
DOM1000P	IL-13	mAb fragment
DOM0910	IL-4/IL-13	mAb fragment
UCB	IL-4/IL-13	mAb fragment

although some benefit was suggested in the most severe disease tertile, as was a dose-response effect against total serum levels of IgE [70,71]. Overall, this was an unexpected and disappointing outcome considering the preclinical data including manipulation of genes encoding IL-13 and its receptors [72,73] or intracellular signalling transcription factors signal transducer and activator of transcription 6 (STAT6) [74] and trans-acting T cell-specific transcription factor GATA-3 [75], the administration of either high affinity soluble receptor (IL-13 α_2) [76] or IL-4 receptor α blocking

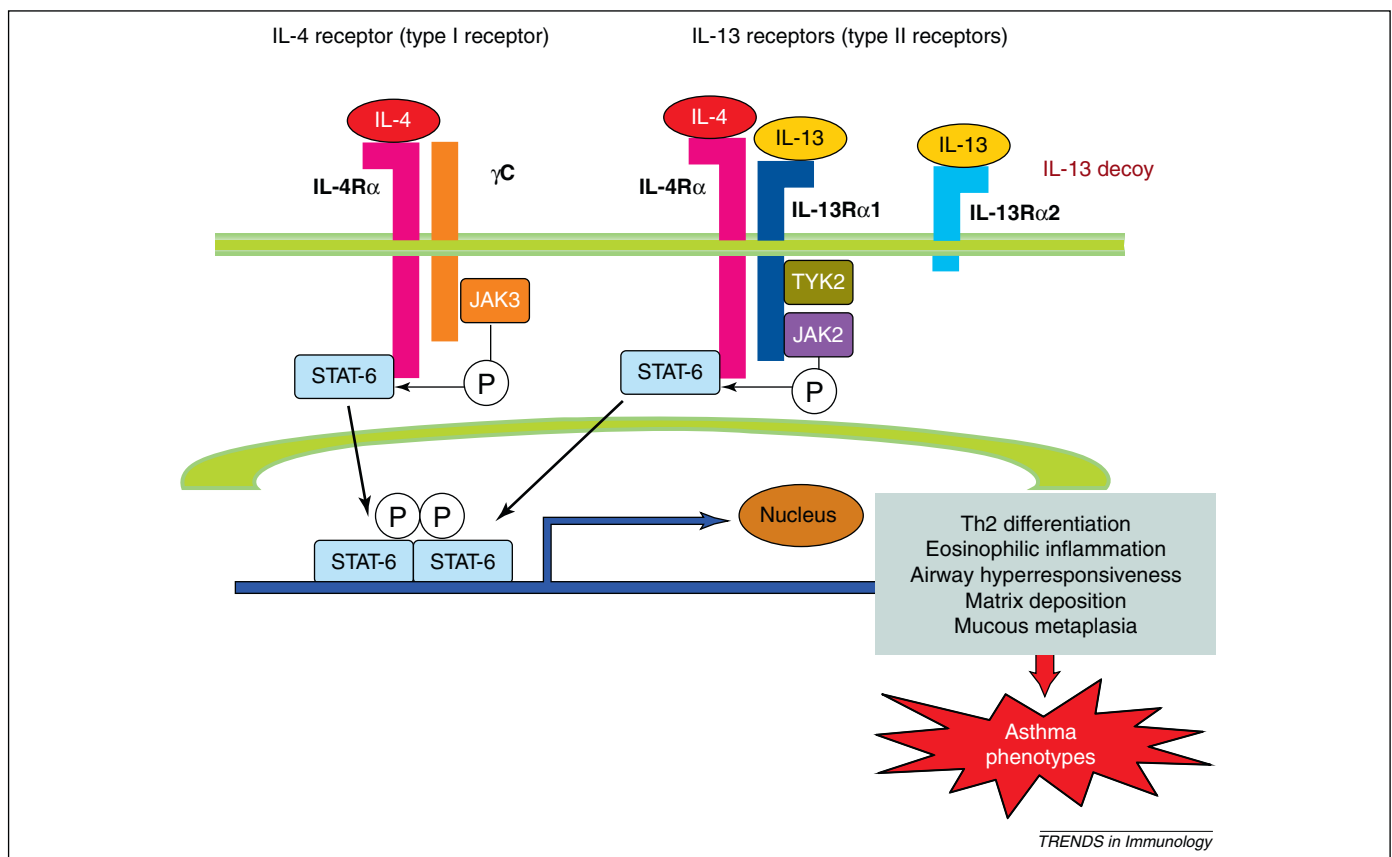


Figure 1. Schematic diagram of the IL-4 and IL-13 signal transduction pathways linked to Th2-type inflammation. IL-4 binds to the IL-4 receptor α subunit that is a component of both the type I (IL-4 receptor α and γ C) and type II receptors (IL-4 receptor α and IL-13 receptor α_1), whereas IL-13 is recognised by the IL-13 receptor α_1 of the type II receptor. IL-13 also binds to the IL-13 receptor α_2 chain with greater affinity than to IL-13 α_1 . IL-13 receptor α_2 lacks a transmembrane-signalling domain and as a consequence functions as a decoy receptor to downregulate IL-13 signalling. γ C activates Janus kinase (JAK) 3 and IL-13 receptor α_1 activates tyrosine kinase 2 (TYK2) and JAK2. Activated JAKs phosphorylate STAT6 which, upon dimerisation, translocates to the nucleus where it binds to the promoters of the IL-4 and IL-13 responsive genes associated with Th2 cell differentiation, airway inflammation, airway hyper-responsiveness, fibrosis and epithelial mucous metaplasia [61].

mAbs [77–79] in mice as well as blocking antibody studies in non-human primates [80,81] used to support the innovation.

Additional proof-of-concept clinical trials with biologics targeting IL-13 and IL-4 are delivering similar disappointing results in related moderate-to-severe asthma populations, including the IgG4 mAb, CAT 354 (MedImmune; Astra Zeneca) [82,83]. However, *post hoc* analysis of the CAT 354 study did show that subjects with elevated levels of IL-13 had the greatest clinical response assessed either by the Juniper Asthma Clinical Questionnaire or improved lung function [84]. This might suggest that sputum IL-13 could be a biomarker for anti-IL-13 responsiveness.

Biomarkers of IL-13 responsiveness

A breakthrough in the understanding of why inhibitors of components of the Th2 pathway were ineffective in atopic asthma, despite showing efficacy in animal models, was provided by a study showing that only 50% of examined patients with active asthma had enhanced activation or suppression of IL-13-responsive genes in airway epithelial brushings [85]. The genes: *PSTN* (periostin), encoding an epithelial secreted matrix component; *CLCA1*, encoding a chloride channel involved in mucous metaplasia; and *SERPINB2*, encoding a plasminogen activator inhibitor type II, were studied more closely and showed consistent expression patterns in different epithelial cell brushings from the same patient, but varied expression between asthma and control patients. Also, there was a broad spectrum of expression of these genes within the asthma population. Thus asthma patients were divided into two subgroups Th2^{high} (IL-13⁺ response) and Th2^{low} depending on their gene expression profiles. Th2^{high} asthmatics had increased numbers of eosinophils in blood and lavage and increased expression of IL-5 and IL-13 in bronchial biopsies. Bronchial hyper-responsiveness (BHR) was also increased in these patients as were the biomarkers, serum IgE, blood and airway eosinophilia, subepithelial fibrosis, airway mucin gene expression [85], intraepithelial mast cell numbers [86] and expression of a variety of chemokines, cytokines and growth factors linked to innate immunity, epithelial repair and remodelling [87]. Asthmatics with high expression of epithelial IL-13-induced genes (Th2^{high}), but not those with low expression (Th2^{low}), were sensitive to ICSs in an RCT [85]. Thus, even for ICSs, responders and nonresponders can be identified through IL-13-linked epithelial biomarkers. Separate studies have shown that epithelial expression of the gene encoding the FK506-binding protein 51 (*FKBP51*) is a particularly strong predictor of corticosteroid responsiveness [88,89].

In bronchial biopsies, *in situ* hybridisation demonstrates an asthma-specific upregulation of *POSTN* that correlates closely with thickening of the *lamina reticularis*, a characteristic morphological feature of asthma. In monolayer airway epithelial cell cultures, periostin protein is basally secreted in response to IL-13 [90], where it activates transforming growth factor (TGF)β-dependent secretion of type 1 collagen by airway fibroblasts/myofibroblasts, and alters collagen fibrillogenesis or crosslinking, leading to stiffening of the matrix. The association of Th2 responsiveness and thickening of the lamina reticularis, and airway remodelling, provides an important causal pathway linking

Th2-mediated inflammation with structural airway changes characteristic of asthma [91]. A proof-of-concept trial of the anti-IL-13 mAb, lebrikizumab (Roche), showed a significant clinical response in moderate-to-severe asthma in terms of lung function, but this clinical response occurred mostly in patients with elevated serum periostin [92].

New biomarkers of severe asthma

Increasingly, it is apparent that asthma is a heterogeneous disorder with many different overlapping causal pathways, phenotypes, treatment responses and natural history [93]. More recently, sputum cells have been used in transcriptomics to identify allergic and nonallergic inflammatory profiles similar to the approach taken using epithelial brushings as already described. Using gene expression profiling of induced sputum and unsupervised hierarchical clustering in moderately severe asthma, three distinct subgroups of asthmatic patients have been identified: Th2^{high} (eosinophil dominant), and two with Th2^{low} characteristics, comprising neutrophilic and macrophage dominant forms [94]. Genes in the IL-1, tumour necrosis factor (TNF)-α and nuclear factor (NF)-κB pathways were overexpressed and correlated with neutrophilic airway inflammation. Neutrophilic-dominant asthma, in common with patients with chronic obstructive pulmonary disease (COPD), also displayed a deficiency in airway and systemic concentrations of soluble RAGE, which is a pattern recognition receptor that interacts with the ligands high-mobility group box-1 and serum amyloid A [95].

In severe corticosteroid asthma, several small trials have revealed efficacy for TNF blockade using etanercept, especially against BHR [96,97]. In patients with RA and concomitant asthma, remission of asthma has also been reported [98]. However, a subsequent, much larger trial of the TNF blocker, etanercept, in moderate-to-severe asthma was negative [99]. A large phase II study over 24 weeks also assessed the efficacy of the anti-TNF mAb, golimumab (Centocor), in a large population of patients with uncontrolled, severe persistent asthma [100]. No significant differences in any asthma outcomes were observed for the group as a whole, but when substratified into those with rhinosinusitis and >12% bronchodilator reversibility, dose-related efficacy was observed. Unfortunately, serious infections occurred more frequently in golimumab-treated patients, halting further use of this drug.

Although initial clinical trials in moderate-to-severe asthma using biologics targeting the IL-4, IL-13 and TNF pathways have so far proven disappointing, disease substratification using biomarkers expressed on the causal pathways of the disease to identify those most likely to respond looks encouraging. It seems that the Th2-dominant disease is the subtype most amenable to this approach, but as other biologics become available directed to non-Th2 mechanisms (e.g. IL-6 and IL-17) greater consideration should be given to identifying appropriate pathway-specific biomarkers to select patients.

Concluding remarks: adopting a stratified approach in the selection of responders to biologics

It is now clear that more effort must be made to identify those asthmatic patients who respond to specific biologic

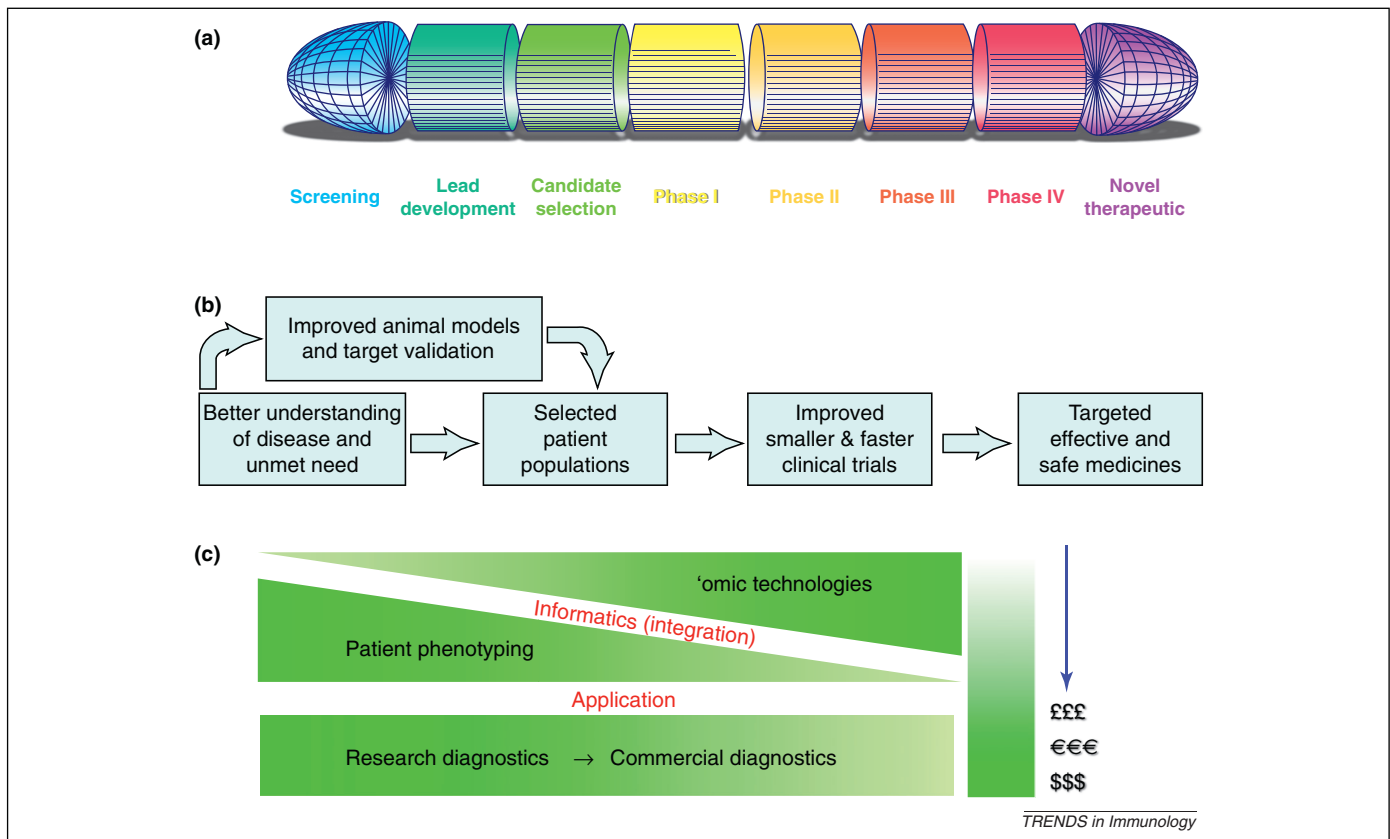


Figure 2. Schematic representation of stratified medicine in the application of biologics to treatment of common chronic diseases such as asthma. **(a)** Traditional model of drug development showing the different steps from concept through to final acceptance of a new therapeutic that is registered for clinical use. **(b)** A stratified or personalised approach to drug or biologic development will require several interacting and iterative processes that lead to faster and more effective clinical trials. **(c)** The new developmental pathway for biologics for treatment of asthma requires the identification of novel causal pathways using different omic technologies in well-phenotyped patients. With the discovery of novel disease-related pathways, biomarkers will need to be found that can then be developed into pathway-specific diagnostic tests. The utility of identifying causal pathways is illustrated with the identification of periostin as a biomarker for IL-13 production, which when released by asthmatic epithelial cells, is causally linked to subepithelial remodelling. High circulating levels of periostin identifies those moderate-to-severe corticosteroid-dependent asthmatic patients most likely to receive therapeutic benefit from blockade of the IL-13-associated signalling pathway. Integration of platforms that can deliver patient phenotyping, omics and informatics, leading to diagnostic tests that will provide the basis for diagnostics to identify those patients most likely to respond to a pathway-specific treatment.

therapy within a larger population. This requires a different approach, in which more attention is given to subphenotyping the disease and defining measures of response to identify pathway-specific diagnostics (Figure 2). The application of nonhierarchical and cluster statistical approaches to subphenotyping asthma has identified at least six different forms in both adults [101] and children [102]. Moreover, these subtypes distinguish themselves from the classical subdivision of asthma by disease severity [101]. Genome-wide association studies are also identifying individual genes that are more strongly linked to some types of asthma than others (e.g. childhood versus adult

onset asthma) [103]. What is now required is a detailed mechanistic appraisal of asthma subtypes, such as the identification of biomarkers in the causal pathways, such as periostin, CCLA1 and serpin B2, that identify Th2-dominant disease. New animal models need to be developed that recapitulate the different disease-related pathways, rather than relying on simple allergen challenge models that are currently used for screening asthma drugs [104]. Epidemiological studies have identified a range of environmental risk factors for asthma beyond allergy, including viral infection, other microbial exposures, air pollutant and environmental tobacco smoke exposure,

Table 2. Possible targets identified from animal models of allergic-type airway inflammation for developing new biologics for treatment of asthma and allied disorders, for which validation in human asthma subtypes is required

Cytokine ^a	Source	Effects	Therapeutic
IL-15 (IL-2 like)	Leukocytes	Th-2, B, NK, macrophage, monocyte ↑	sIL-15rα, mAb
IL-17A	CD4 ⁺ Tcells	Neutrophil ↑	mAb
IL-17E (IL-25)	CD4 ⁺ Tcells	BHR, Th-2 ↑	mAb
IL-33 (IL-1F11)	Epithelium	Th-2, mast cell ↑	mAb
IL-31	Th-2 T cells	Th-2 ↑, pruritis, dermatitis	sIL-31r, mAb
IL-21 (IL-2-like)	CD4 ⁺ Tcells	CD4 ⁺ , CD8 ⁺ T, NK, B cells ↑	mAb
TSLP (IL-7-like)	Epithelium	DC → Th2 cell ↑	mAb

^aAbbreviations: B, B lymphocyte; NK, natural killer; TSLP, thymic stromal lymphopoietin.

and use of certain drugs [105,106]. Once therapeutic candidates have been identified in *in vitro* and animal models, it is essential that before they are taken forward as an attempt to develop new treatments, they are adequately validated in the relevant subtype of asthma in humans (Table 2). To deliver these aims, a new relationship needs to develop in which industrial, academic and clinical teams work together to provide the necessary multidisciplinary environment in the precompetitive space to enable such systems biology approaches to be made [107].

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