Treatment options in type-2 low asthma

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Title: Treatment options in type-2 low asthma

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Take home message: 1/3 of severe asthma is type-2 low, presenting a challenge to clinicians. Here we review currently available treatment options including macrolides, bronchodilators, thermoplasty and other treatable traits, and review a range of therapies in development.
Abstract

Monoclonal antibodies targeting IgE or the type-2 cytokines IL-4, IL-5 and IL-13 are proving highly effective in reducing exacerbations and symptoms in people with severe allergic and eosinophilic asthma respectively. However, these therapies are not appropriate for 30-50% of patients in severe asthma clinics who present with non-allergic, non-eosinophilic, ‘type-2 low’ asthma. These patients constitute an important and common clinical asthma phenotype, driven by distinct, though poorly understood pathobiological mechanisms. In this review we describe the heterogeneity and clinical characteristics of type-2 low asthma and summarise current knowledge on the underlying pathobiological mechanisms, which includes neutrophilic airway inflammation often associated with smoking, obesity, occupational exposures and may be driven by persistent bacterial infections and by activation of a recently-described IL-6 pathway. We review the evidence base underlying existing treatment options for specific treatable traits which can be identified and addressed. We particularly focus on severe asthma as opposed to difficult-to-treat asthma, on emerging data on the identification of airway bacterial infection, on the increasing evidence base for the use of long-term low-dose macrolides, a critical appraisal of bronchial thermoplasty, and evidence for the use of biologics in type-2 low disease. Finally we review ongoing research into other pathways including TNF, IL-17, resolvins, apolipoproteins, type I interferons, IL-6 and mast cells. We suggest that type-2 low disease frequently presents opportunities for identification and treatment of tractable clinical problems and is currently a rapidly evolving field with potential for the development of novel targeted therapeutics.
Introduction

Asthma is a complex, chronic disease characterised by heterogeneous airway inflammation. 70-80% of corticosteroid-naïve and 50% of corticosteroid-treated asthma patients have a raised sputum eosinophil count[1], which is generally associated with enhanced expression of the type-2 cytokines interleukin (IL)-4, IL-5 and IL-13[2], increased fractional exhaled nitric oxide (FeNO), peripheral blood eosinophilia and a reproducible type-2 inflammatory epithelial gene signature[3]. This ‘type-2 high’ phenotype is characteristically responsive to treatment with inhaled corticosteroids (ICS), and, in severe disease, to biologic agents targeting these type-2 cytokines[4].

Whilst these treatments are proving highly-effective, there remain a significant proportion of people with ‘type-2 low’ disease characterised by normal sputum and peripheral blood eosinophil counts, and low FeNO, yet with persistent symptoms and airflow limitation and a poor response to corticosteroids (Table 1). Due to a relative paucity of research and limited therapeutic options these patients often present a clinical challenge. However several recent and ongoing studies provide a stimulus to optimism in this rapidly-evolving field. Here we review the heterogeneity and clinical characteristics of type-2 low asthma in adults and adolescents, summarise current knowledge on the underlying pathobiological mechanisms and review the evidence base underlying existing treatment options for specific ‘treatable traits’ within type-2 low asthma. Finally, we review ongoing research into other pathways constituting potential novel therapeutic targets.

How common is type-2 low asthma? A normal sputum eosinophil count is seen in 25% of patients with untreated symptomatic asthma[5] and 40-50% of patients with asthma treated with high doses of ICS[6-8]. Type-2 low asthma may be more common in mild-to-moderate disease, with estimates of 64-73%[9, 10] with a single sputum sample, and even with repeated sampling this may be approximately half of asthmatics[9]. Non-eosinophilic inflammation is also common in irritant-induced occupational asthma[6] and during virus-induced asthma exacerbations[11] and is increasingly seen in biologic-treated type-2 high patients experiencing infective exacerbations.

Type-2 low asthma encompasses both neutrophilic asthma and pauci-granulocytic asthma. Sputum neutrophilia is often defined as ≥61%[9, 12-14] or ≥73%[15-17] neutrophils on a cytospin. The optimal cut-off might differ according to local air pollution levels. For sputum eosinophilia several definitions have been used including cut-offs of 1%[12], 2%[18, 19], 3%[20-22]. Sputum eosinophilia ≥3% identifies individuals with corticosteroid responsive asthma[20, 22], and a definition of sputum eosinophils ≥3%, blood eosinophil ≥0.3×10⁹ or FeNO ≥ 50 ppb identified patients responsive to anti-IL-5 therapy[23]. However for the purposes of identifying non-eosinophilic asthma a lower cut off for sputum eosinophils of <2%[9] may be more specific, and has been adopted in recent clinical trials[10] and GINA guidelines[24]. Whilst some patients may vary around these thresholds over time, in one study 96% of patients without sputum eosinophilia remained non-eosinophilic at 5-year follow-up[19], consistent with a previous report[12]. In a large study with 324 paired sputum samples 47% of participants with mild or moderate asthma had persistently non-eosinophilic samples on multiple occasions over one year, suggesting persistent non-eosinophilic asthma is a common finding[9]. Nonetheless, even in non-eosinophilic asthma, intermittent occurrence of airway eosinophilia is frequently observed[25], with an intraclass correlation coefficient for sputum neutrophils of 0.78[26]. The reasons for this variability are not known, but may be related to changes in treatment,
environmental factors such as allergen exposures, seasonal changes and airway microbiology, and repeated measures may be required to obtain accurate phenotyping[26]. Inhaled and oral corticosteroids being used effectively to treat eosinophilic inflammation may lead to a non-eosinophilic phenotype in some individuals. In a cohort of 26 subjects with stable, persistent asthma and non-eosinophilic sputum at baseline, after 12 weeks withdrawal of maintenance inhaled corticosteroids 80% developed eosinophilic sputum, although it should be noted that the initial phenotype in all but one individual was paucigranulocytic, rather than neutrophilic disease[25]. It is important to recognize that a subset of eosinophilic asthmatics have eosinophil granules, rather than intact eosinophils, present in sputum which may result in misclassifying these patients as pauci-granulocytic asthmatics[27]. Occult eosinophilic inflammation can be identified in these individuals by demonstrating eosinophil granule components, such as eosinophil peroxidase (EPO), in sputum supernatants, or intracellular eosinophil proteins, such as eosinophil cationic protein and EPO, within sputum macrophages that have phagocytosed apoptotic eosinophils [27-29].

What are the clinical characteristics of type-2 low asthma, beyond an absence of type-2 biomarkers? A characteristic feature is a lack of response to systemic corticosteroids. By contrast patients with significant eosinophilic disease typically report symptomatic improvement within 1-2 days of starting oral corticosteroids. Non-eosinophilic asthma is associated with female sex, obesity[30], non-atopic status and adult onset symptoms[5]. It is also associated with smoking, occupational exposures to low-molecular weight compounds[31], and elite athletics[32, 33]. It is useful to enquire about a chronic mucopurulent cough, often a sign of chronic bacterial bronchitis or bronchiectasis, implying the need for additional diagnostic testing such as sputum microbiology (including Mycobacterium tuberculosis and non-tuberculous mycobacteria) and a high-resolution CT thorax. A suggested algorithm for diagnosis of neutrophilic asthma in clinical practice is shown in Figure 1.

Mechanisms
In contrast to type-2 high asthma, less is known about the mechanisms in type-2 low disease. Neutrophilic inflammation is likely due to innate and adaptive cell mediated immune responses (Figure 2). Airway neutrophilia is common during some viral infections and also during stable chronic asthma[2, 6, 8, 2]. A transcriptomic analysis of airway samples in severe neutrophilic asthma found a strong upregulation of mucins, IL-17-inducible chemokines (CXCL1, CXCL2, CXCL3, and CSF-3) and the neutrophil chemoattractants CXCL8 (IL-8), CCL3 and LGALS3 [34]. Signatures of antibacterial responses including CD14, JUN and TLR2, implicate airway bacteria in driving the neutrophilia[34]. Neutrophilic asthma is associated with airway colonisation by bacteria including Haemophilus influenzae and Moraxella catarrhalis[35], which might induce Th17 responses[36]. Other microbiome studies have also linked neutrophilic asthma with the presence of H. influenzae[37] and of a reduced microbial diversity, suggesting dominance of a single airway pathogen[38]. Larger microbiome studies are needed to determine the exact role these bacteria play.

Neutrophilic asthma is also associated with upregulation of oxidative stress responses – potentially driven by smoking or by inflammatory cell-derived reactive oxygen species – and matrix metalloproteases, including MMP-9, a type IV collagenase involved with CXCL8-induced neutrophilia[34]. MMPs are increased in airway samples from asthmatic
smokers[39] and in severe neutrophilic asthma[8], and are implicated in tissue remodelling[8, 40, 41].

Tissue damage driven by stimuli such as viruses, bacteria, smoking or low-molecular-weight agents induces airway epithelium to release thealarmins thymic stromal lymphopoietin (TSLP), IL-25 and IL-33. Whilst these can drive type-2 pathology, they are likely to be released too by drivers of type-2 low pathology, which may explain the observed efficacy of anti-TSLP blockade in non-eosinophilic asthma[42].

Type-2 low asthma is associated with heightened capsaicin cough reflex sensitivity suggesting airway neuronal dysfunction, particularly of transient receptor potential vanilloid-1 (TRPV-1)[43-45]. A range of other immune mechanisms are implicated, including dysregulation of IL-6, IL-17, TNF and type 1 IFN signalling[2], each of which are discussed below.

**Current therapeutic options**

**The role of corticosteroids**

Since the 1950s it has been known that systemic corticosteroids are less effective in patients without sputum eosinophilia[20, 22, 46]. Lack of sputum eosinophilia predicts a lack of response to ICS[30, 47-50]. Indeed corticosteroid treatment can be safely reduced in non-eosinophilic asthma without increasing exacerbation frequency[20].

*Mild asthma*

In mild asthma a recent randomized controlled trial (RCT) found ICS effective in those with sputum eosinophilia, but not in those with <2% sputum eosinophils[10]. In mild-to-moderate asthma ICS didn’t improve lung function in those with type-2 low baseline gene expression profiles[3].

Given the poor steroid efficacy in type-2 low asthma an approach using as-required combination formoterol/corticosteroid inhalers as first line treatment in mild asthma has the dual benefits of increasing ICS delivery in those with type-2 high asthma, whilst reducing unnecessary steroid exposure by 50-80% in those least likely to benefit[51-53].

*Severe asthma*

In difficult-to-treat and severe asthma systemic corticosteroid prescribing is common, with up to 60% of treatment refractory asthmatics in a UK series receiving oral corticosteroids[54]. In symptom-predominant asthma phenotypes[55], or those with fixed airflow limitation[56], overtreatment with systemic corticosteroids can occur with inappropriate escalation of oral corticosteroids[57-59]. Biomarker-directed adjustment of dosing could reduce systemic corticosteroid prescribing. Adjusting ICS doses based on sputum eosinophil or FeNO effectively decreases exacerbations[60, 61]. Whilst induced sputum analysis availability is limited, protocolised reduction of inhaled and systemic steroids based on blood eosinophils, FeNO and serum periostin shows promise and is being evaluated prospectively[62].

**Omalizumab less effective in type-2 low severe allergic asthma**

Omalizumab[63], an injectable monoclonal antibody (mAb) targeting IgE reduces exacerbations in children and adults with severe allergic asthma. Patient selection is typically based on elevated serum IgE, although in severe persistent allergic asthma an
omalizumab RCT found no significant benefit in type-2 low disease, stratified either by FeNO<20 ppb, or blood eosinophils <0.26×10⁹ or periostin <50 ng/mL[64]. In contrast, in a retrospective observational study[65], the response rate was similar in high and low blood eosinophil subgroups. Since this discrepancy might arise from differences in study design, more research is required to delineate the role of type-2 biomarkers in predicting response to omalizumab.

**Macrolides**

Several studies suggested that long-term use of macrolide antibiotics may have steroid-sparing or anti-inflammatory[66] effects in asthma. Macrolides reduce exacerbations in other neutrophilic chronic airways diseases including cystic fibrosis[67], non-CF bronchiectasis[68-70] and COPD[71]. Efficacy in asthma had not been shown in previous small studies[72, 73], however long-term low dose azithromycin has now been shown to be effective in two RCTs. The AZIZAST study randomised 109 adults with exacerbation-prone severe asthma to thrice-weekly azithromycin 250 mg or placebo for 26 weeks. Whilst the primary outcome was not reached in the overall population, in a pre-defined subgroup analysis azithromycin reduced the rate of severe exacerbations in the non-eosinophilic subjects (rate ratio 0.42 versus placebo in those with blood eosinophils <0.2×10⁹/L)[74]. The larger AMAZES study randomised 420 adults with moderate-to-severe asthma to thrice-weekly azithromycin 500 mg or placebo for 48 weeks[75], finding a striking reduction in asthma exacerbations, with the proportion of patients experiencing an exacerbation reducing from 61% to 44%, an incidence rate ratio of 0.59, and with significant improvements in asthma-related quality of life (Figure 3a). This effect was also seen in those without a frequent exacerbation history. Unexpectedly a subgroup analysis found azithromycin effective in both eosinophilic and non-eosinophilic phenotypes. Subsequent meta-analysis of AZISAST and AMAZES confirmed efficacy of maintenance azithromycin as add-on therapy to ICS+LABA[76].

The mechanism of azithromycin efficacy is not understood. Azithromycin has antibacterial, antiviral[77] and anti-inflammatory[66] effects. These include inhibition of cytokines[78], chemokines[79], cytotoxicity[80], biofilms[77], and various immunomodulatory actions on neutrophils and T cells[78], including inhibiting calcineurin[81] and mTOR[82], besides reducing mucus production and stimulating phagocytosis[83]. It is unclear if the clinical efficacy is specific to azithromycin as macrolides differ in these properties[84]. Although short term clarithromycin reduced sputum IL-8[85] studies of other macrolides have been of short duration and small size[72], with a strong evidence base only for azithromycin.

Whilst macrolides are effective and recommended in current American Thoracic Society (ATS) / European Respiratory Society (ERS) and Global Initiative for Asthma (GINA) guidelines for selected persistently symptomatic adults with severe asthma[24, 86], there are several concerns about widespread use. They can cause diarrhoea, usually mild[75]. They have the potential for QT prolongation, although ECG screening excludes this effect[74, 75]. In a COPD trial a slight excess of hearing loss was observed using stringent sequential audiometry data, but this incidence was likely over estimated, was largely reversible[71] and was not observed in the two asthma studies[74, 75] perhaps because the patients were younger, and AMAZES excluded those with hearing loss. The greatest
concern is inducing antimicrobial resistance. Macrolides may predispose to acquisition of mycobacteria by impairing autophagy, and inadvertent monotherapy could induce drug resistance in undiagnosed mycobacterial infection making subsequent treatment difficult, particularly of non-tuberculous mycobacteria[87]. Therefore sputa should be screened for acid fast bacilli prior to therapy. Global macrolide resistance is increasing rapidly amongst other bacteria with 90-100% resistance to *Streptococcus pneumoniae*[88] and *Mycoplasma pneumoniae* in China[89]. Due to its long, 3-day half-life azithromycin poses a particular ecological risk (Figure 3c). Resistance develops rapidly in oropharyngeal flora. Moreover resistance on mobile genetic elements is associated with resistance to other drug classes including chloramphenicol, tetracyclines and penicillins[87].

Azithromycin may act primarily as an antibacterial. Colonisation with *Haemophilus, Streptococci* and *Moraxella* is common in severe asthma [35] and *Haemophilus* may evade penicillins by paracellular and intracellular invasion[90]. In AMAZES the main change in sputum microbiome was a marked reduction in *Haemophilus influenzae* (Figure 3d)[91], perhaps related to high intracellular activity. Furthermore a post hoc analysis of baseline *H. influenzae* abundance by qPCR suggested those with highest *H. influenzae* abundance derived most benefit from the azithromycin therapy in terms of exacerbation reduction[92]. It is unknown whether other intracellular antibiotics like doxycycline are effective. Biomarker-directed antibiotic trials are required to determine which patients respond to antibiotics and the dose and duration of therapy. A greater understanding of their mechanisms could lead to novel macrolides or non-antibiotic macrolide compounds, with less potential for drug resistance[84]. However, we hypothesise their efficacy might be intermediate between placebo and azithromycin.

**Long-acting muscarinic antagonists (LAMAs)**
Several trials investigated addition of long acting anti-muscarinics (LAMAs), predominantly in severe asthma, leading to their inclusion as a step 4 or 5 option in current GINA guidelines[24, 86]. Meta-analyses suggest tiotropium provides significant improvements in lung function including peak expiratory flow and FEV₁, though not in FVC[93, 94]. However, these effects are small, and despite a possible reduction in severe exacerbations LAMAs have not shown a meaningful improvement in quality of life or reduction in hospital admission[93, 94].

**Bronchial Thermoplasty**
Bronchial thermoplasty involves localised radiofrequency ablation of bronchial mucosa applied during three bronchoscopic treatment sessions. The mechanism is poorly understood but may involve reduced smooth muscle mass[95] or inhibition of airway neurons[96] or inflammatory cells[97]. Independence from type-2 pathways makes this theoretically attractive, but efficacy data are limited. Studies were confounded by large placebo responses for subjective patient-reported outcomes, compared with absence of objective effects on lung function[98, 99]. Data are lacking in those with very poor lung function (FEV₁<60%), and treatment is associated with a temporary increase in exacerbations[99]. Long term-follow up data are limited. Current ATS/ERS and GINA guidelines recommend thermoplasty be considered as an add-on therapy for selected adult patients with medically-optimised severe disease, performed within an independent systematic registry or clinical study[24, 100].
Given the lack of many highly-effective options in type-2 low asthma, and especially in paucigranulocytic severe asthma, where airway remodelling may be a key driver of airway hyper-responsiveness (AHR) and symptoms, bronchial thermoplasty research should focus on type-2 low patients and developing predictors of response. Patient selection may be facilitated by emerging bronchoscopic imaging techniques, but must then be validated by long-term prospective registries.

**Other treatable traits**

Symptoms in asthma arise from a complex interplay of inflammation, airway-hyperreactivity and additional factors[101], including co-morbidities and psychological factors, many of which are not associated with airways inflammation[8, 30] (Table 2). Identifying and managing these ‘treatable traits’ may provide significant symptomatic benefit for individuals[56, 102]. Twenty-three treatable traits were identified within the U-BIOPRED cohort, being more common in severe asthma[103]. The most prevalent extra-pulmonary traits were atopy, rhinosinusitis, obesity, reflux and obstructive sleep apnoea. Poor adherence, anxiety and depression were the most common behavioural/ psychosocial treatable traits.

Tobacco smoking is associated with neutrophilic airway inflammation, leads to worse symptoms[104], impaired steroid responsiveness[105], increased bronchial reactivity[106] and rapid lung function decline[104] so every effort should be made to encourage smoking cessation.

Vocal cord dysfunction, also called inducible laryngeal obstruction affects, an estimated 1 in 4 adults with asthma[107] often leading to over treatment. If identified early it responds to speech and language therapy[108]. Dysfunctional breathing is more common in type-2 low asthma[55] and responds to physiotherapy, leading to marked, sustained improvements in symptoms[109].

Type-2 low asthma is associated with obesity[55], perhaps related to increased systemic IL-6 inflammation[110]. Numerous studies have shown that weight loss, particularly when dietary changes are combined with increased exercise, lead to improved asthma control and lung function[111]. The magnitude of improvement is related to extent of weight loss, with at least 5% weight loss required to produce significant improvements[112]. Bariatric surgery is the most effective intervention for achieving sustained weight loss, and is consistently associated with improvements in asthma control, airway reactivity, and lung function[111].

There is less evidence for management of some other treatable traits. Whilst rhinitis, and gastro-oesophageal reflux cause asthma-like symptoms, there is little evidence their treatment improves asthma control[56, 113].

**Anti-TSLP monoclonal antibody tezepelumab**

Upstream targets for therapeutic intervention encompass the epithelial alarmin Thymic Stromal Lymphopoietin (TSLP), which is secreted by airway epithelial cells exposed to noxious stimuli such as cigarette smoke[114], diesel exhaust particles[115], proteases and microbes[116]. Airway TSLP expression is increased in severe asthma, and TSLP has been associated with steroid resistance of airway type 2 innate lymphoid cells in severe
In a phase 2 RCT treatment with subcutaneous tezepelumab, a human anti-TSLP monoclonal antibody reduced asthma exacerbations by 60 to 70%, and improved lung function[42]. Efficacy occurred irrespective of blood eosinophil or FeNO levels, suggesting anti-TSLP might affect disease activity more broadly than inhibition of more downstream pathways. These promising results in type-2 and non-type-2 asthma need replication in larger phase 3 trials.

**Potential Future treatment options**

Development of new therapeutics for type-2 low disease will require a different approach from that taken in type-2 high disease, due to several important differences between these major phenotypes (Table 1). It is unlikely that a simple biologic blocking of a single interleukin pathway will replicate the successes of the anti-type-2 biologics. Neutrophils have a very distinct biology to eosinophils, are far more abundant and critical to many biological processes. Neutrophilia may even be a beneficial response in type-2 low airway inflammation. There is a lack of clinically-available non-invasive biomarkers, a much broader differential diagnosis to be considered and optimal therapeutic targets remain unclear. Nonetheless, several pathways in type 2-low asthma are potential therapeutic targets, which we review next (Table 3). Strategies targeting type-2 low pathways will need to maintain adequate host defence and immune surveillance functions to prevent infectious or neoplastic complications. Furthermore, the successful targeting of specific pathways mediating type-2 low asthma will require the identification of biomarkers to direct treatment in a precision medicine approach to those patients in whom the pathway is active and is mediating disease. Potential new biomarkers include serum and sputum levels of neutrophil products such as neutrophil lipocalin, neutrophil gelatinase-associated lipocalin[119, 120] and myeloperoxidase[8], although these require validation in large cohorts. Another potential approach is the measurement of volatile organic compounds such as in exhaled breath condensate. One study has reported the combination of nonanal, 1-propanol and hexane identifies neutrophilic asthma with 81% sensitivity, although the specificity was low at 43% and these approaches remain at the experimental stage[121].

**Interleukin-1β (IL-1β)**

IL-1β is a pro-inflammatory cytokine that promotes type 2-low neutrophilic asthma. IL-1β generation is mediated by the canonical NLRP3 inflammasome, which activates caspase-1 to process pro-IL-1β into its mature, secreted form[122]. Caspase-1 also cleaves gasdermin-D (GSDMD) into fragments that assemble into a plasma membrane pore releasing mature IL-1β from cells and inducing pyroptotic cell death. A non-canonical inflammasome comprised of caspase-4 and caspase-5 also cleaves GSDMD, with resultant activation of the NLRP3 inflammasome and caspase-1. Caspase-1 activation and GSDMD cleavage also generate neutrophil extracellular traps (NETs) and induce another pro-inflammatory form of lytic cell death, termed NETosis[123, 124].

Sputum levels of IL-1β, IL-1 receptors, NLRP3, caspase-1, caspase-4, and caspase-5 are increased in neutrophilic asthma[125-130]. Sputum IL-1β and NLRP3 correlate with neutrophilic airway inflammation and asthma severity[125, 126, 131]. Ozone exposure increases sputum neutrophils in atopic asthmatics, which correlate with increased sputum IL-1β and IL-8[132, 133]. In severe asthma, sputum neutrophils correlate with sputum
extracellular DNA levels indicative of NET formation, while increased sputum extracellular DNA is associated with increased sputum IL-1β and asthma severity[131].

Murine and human studies support the concept of targeting IL-1β in type 2-low neutrophilic asthma. Administration of either a neutralizing anti-IL-1β antibody or a pharmacological NLRP3 inhibitor (MCC950) suppressed lung IL-1β production and neutrophilic airway inflammation in a murine model of severe, steroid-resistant asthma induced by concurrent allergic airways disease and Chlamydia respiratory infection[125]. Treatment of healthy subjects with the IL-1 receptor antagonist, anakinra, before inhalational endotoxin challenge suppressed sputum neutrophils, IL-1β, IL-6, and IL-8 levels, suggesting anakinra as a candidate treatment[134]. Canakinumab, a neutralizing anti-IL-1β humanized monoclonal antibody has proved safe in a Phase 1/2 clinical trial of mild asthma[135]. Clinical trials utilizing inhalational allergen challenge are underway to assess the effect of anakinra on inflammation and pulmonary function[136, 137]. The development of biomarkers to identify IL-1β-high asthmatics would allow these strategies to be administered in a precision medicine approach.

**Inhibition of the IL-17 pathway**

IL-17A and IL-17F are cytokines produced particularly by innate and adaptive lymphocytes (including Th17 cells, γδ T cells, group 3 ILCs, and mucosal associated invariant T (MAIT) cells) which induce epithelial cells to recruit neutrophils to sites of inflammation[36, 138, 139]. IL-17 cytokines have been implicated in asthma by human genetic studies[140-142], by murine models[143-145] and observations of increased IL-17 levels in human airway samples[146-152], particularly in neutrophilic asthma. However, such weak clinical associations from these small studies have not been replicated consistently. In a bronchoscopy study of 84 volunteers we found no evidence of increased IL-17A protein or Th17 frequencies in asthma in serum, sputum or bronchoalveolar lavage (BAL)[138]. Transcriptomic studies have suggested activation of steroid-resistant IL-17 pathways in severe asthma[34, 153] associated with neutrophilia, smoking, and frequent exacerbations.

It remains unclear whether IL-17 is driving pathology, or may be simply a consequence of epithelial damage or bacterial airway colonisation[36]. Indeed neutrophilic inflammation may promote IL-17 production[2]. Neutrophil cytoplasts (enucleated cell bodies generated when DNA-containing NETs are released) trigger IL-17 production by CD4+ T cells, which suggests neutrophilic inflammation may drive IL-17 expression[2, 154]. IL-17 may be protective in asthma, being important for antibacterial defence, promoting tissue repair, and in maintaining epithelial barrier function[36]. Indeed, the largest clinical trial of an anti-IL-17 receptor A monoclonal antibody (mAb), brodalumab, in >300 patients with moderate-to-severe asthma did not show improvement of symptoms or lung function[155], although patients weren’t selected for airway neutrophilia or IL-17 levels. Similarly, a clinical trial using an anti-IL-23 antibody, risankizumab, which blocks Th17 cell differentiation, worsened asthma control in severe asthmatics who were not selected by cytokine levels or airway neutrophils[156]. A phase 2 clinical trial is ongoing with an anti-IL-17A mAb in moderate to severe asthma[157]. Other strategies to inhibit this pathway include DNAzymes targeting the Th17 transcription factor, RORγt, and small molecule inhibitors[158].
**Resolvins: Lipoxin 4 and Serum Amyloid A**

Lipoxin A4 (LXA₄) is a specialized proresolving mediator (SPM) that is enzymatically derived from arachidonic acid metabolism and promotes resolution of inflammation via interactions with the lipoxin A4/formyl peptide type 2 (ALX/FPR2) receptor[159, 160]. LXA₄ levels are decreased in the blood, sputum, exhaled breath condensate, and BALF from severe adult and paediatric asthmatics, which suggests a causal relationship may exist between reductions in LXA₄ levels and more severe asthma[159, 161-166]. Sputum cells from severe asthmatic children and peripheral blood granulocytes from adults with severe asthma have reduced ALX/FPR2 expression, which may further impair the effects of LXA₄[164, 166]. BALF natural killer (NK) cells from severe asthmatics are skewed towards a cytotoxic CD56dim subset with reduced ALX/FPR2 expression, which might contribute to the impaired resolution of inflammation mediated by NK cell-dependent clearance of T cells and neutrophils[167].

A biochemical endotype of severe asthma has been defined with decreased BALF LXA₄ levels and high BALF levels of the acute-phase protein, serum amyloid A (LXA₄loSAAhi)[168]. Since SAA also signals via the ALX/FPR2 receptor, the increased BALF SAA can compete with and overwhelm the ability of LXA₄ to interact with ALX/FPR2. LXA₄loSAAhi asthmatics have increased BALF neutrophils, more severe asthma, comorbidities, and pruned pulmonary vasculature[168, 169]. BALF from LXA₄loSAAhi asthmatics induced IL-8 production by A549 cells that express ALX/FPR2, which was inhibited by another SPM, 15-epi-LXA₄. This suggests a potential therapeutic role for SPMs in severe, neutrophilic-predominant asthma. In support of this concept, administration of a stable LXA₄ analogue inhibited allergic airway inflammation and AHR in an experimental murine model of OVA-induced airways disease[170], while inhalation of LXA₄ by 5 mild asthmatics antagonized the bronchoconstrictive effects of leukotriene C₄[171]. Inhalation of a stable LXA₄ analogue (5(S),6(R)-LXA₄ methyl ester) was also safe in a small cohort of asthmatic children[172]. Thus, LXA₄, stable LXA₄ analogues, or other SPMs might be utilized in trials assessing efficacy in type 2-low, neutrophilic-predominant asthma[159, 160]. SPM precursors might represent another therapeutic modality based upon a recent study that utilized diet supplementation with n-3 long-chain polyunsaturated fatty acids during the third trimester of pregnancy to reduce the risk of asthma during early childhood[173].

**Apolipoproteins: an inhaled formulation of an APOA1 mimetic peptide**

Studies in murine models of allergen-induced airways diseases identified that APOA1, the major protein component of high-density lipoprotein particles, interacts with ABCA1 transporters on alveolar macrophages and pulmonary vascular endothelial cells, to suppress neutrophilic airway inflammation via a G-CSF-dependent mechanism[174, 175]. In addition, higher serum APOA1 levels are associated with less severe airflow obstruction in allergic asthmatics[176]. These results suggest APOA1 might be beneficial for type 2-low neutrophilic asthma and have supported efforts to develop an inhaled APOA1 mimetic peptide, such as the 5A APOA1 mimetic peptide, that re-capitulates the biological activity of the endogenous APOA1 molecule[158, 177].

**Type I interferons**

People with asthma are predisposed to lower respiratory symptoms during an upper respiratory tract viral infection[178], which has been linked to deficiency of type I/III...
interferons (IFN) IFN-β and IFN-λ [179-183]. This may favour rhinovirus replication, mucin production and impair antimicrobial peptide responses[184]. Whilst these responses may be deficient in an acute exacerbation, in stable state interferon stimulated genes are upregulated in airway epithelial cells in mild asthma, and in peripheral blood in stable severe asthma[185]. Upregulation is not associated with type-2 inflammation. Potentially elevated baseline IFN responses during stable state may lead to desensitisation of type I IFN responses during acute infection[185]. A trial of inhaled IFN-β showed some efficacy in reducing viral-exacerbation induced asthma symptoms in frequently-exacerbating severe asthma, although the trial did not reach its primary endpoint across all asthma[186] and a subsequent trial was negative[187, 188]. It remains unknown if IFN deficiency is a feature of asthma or a consequence of corticosteroid treatment[184], which can suppress IFN-stimulated genes[185].

**Inhibition of the IL-6 pathway**

IL-6 is a biomarker of systemic inflammation, metabolic dysfunction, and obesity. IL-6 is increased in serum and airways in asthma[189-191] and has recently been found to be elevated in plasma in severe asthma associated with obesity, metabolic dysfunction and blood neutrophilia[110]. This may reflect increased IL-6 production by inflammatory macrophages in adipose tissue of obese individuals. This increases asthma severity via an “outside-in” mechanism of lung dysfunction due to systemic inflammation[110]. Furthermore, systemic IL-6 inflammation and obesity are associated with a deficiency of airway cytotoxic CD8+ T cells in type 2-low asthmatics, which may reflect T-cell exhaustion as a mechanism of increased exacerbations due to impaired anti-viral immune responses[192]. The importance of systemic IL-6 in asthma will need confirmation by trials inhibiting IL-6 signalling, such as an ongoing trial of clazakizumab in severe asthma[157]. As anti-IL-6 monoclonals are already in clinical use and high serum IL-6 levels have been identified as a biomarker[110], such clinical translation could follow swiftly.

IL-6 may also play a mechanistic role in severe asthmatics with a SNP in the IL-4 receptor α chain that converts a glutamine to arginine at residue 576[193]. Experiments in mice showed that *Il4r*<sup>R576</sup> promotes conversion of induced regulatory T cells to Th17-like cells by a pathway involving growth-factor-receptor-bound protein 2 (GRB2) adaptor protein, mitogen-activated protein kinase (MAPK) kinase signalling, IL-6, and STAT3, which can be inhibited by a neutralizing anti-IL-6 antibody. The anti-IL-6 antibody also suppressed mixed neutrophilic/eosinophilic airway inflammation and mucous cell metaplasia in a murine model of house dust mite-induced airways disease, while a humanized anti-IL-6 receptor monoclonal antibody, tocilizumab, has been used to successfully treat two paediatric severe asthmatics with the *IL4R*R<sup>576</sup> allele and evidence of mixed Th2/Th17 inflammation[194].

In addition to acting on neutrophils and macrophages, during inflammation IL-6 can also bind to soluble IL-6R shed by inflammatory neutrophils and cause IL-6 trans-signalling on epithelial cells. sIL-6R is increased in serum[195], BAL[195] and sputum[196] in asthma. Activation of IL-6TS reduces epithelial integrity and induces gene signatures associated with airway remodelling. These signatures are expressed in epithelial brushings from frequently-exacerbating, type-2 low asthmatics, associated with submucosal macrophage and T cell infiltration, evidence of impaired epithelial barrier function and induction of the alarmin IL-33[197]. IL-6ST high patients had low expression of the epithelial type-2 gene signature, although did have elevated eosinophils, implying eosinophilia in these individuals is driven
by type-2 independent mechanisms. IL-6TS occurred in the absence of a systemic IL-6 signal, and was associated with TLR signalling and inflammasome activation, suggesting this phenotype was driven by local, likely bacterially-driven inflammation, with pathogens, such as *H. influenzae*. Experiments using human airway smooth muscle cells have also shown that IL-6TS induced proliferative responses, as well as the expression of genes regulating immune responses, airway remodelling, glucose metabolism, and hypoxia[198]. Single nucleotide polymorphisms in the *IL6R* gene are associated with increased serum sIL-6R that may promote IL-6TS[199, 200]. The *IL6R* rs4129267 SNP is associated with both higher serum sIL-6R levels and an increased risk of asthma[199], while the rs2228145 SNP has been associated with worse lung function and severe asthma[201]. Furthermore, elevated serum IL-6R levels were associated with more severe airflow obstruction, which suggests a role for IL-6 trans-signalling in severe asthma.

*Mast cells*

Mast cells contribute to the pathobiology of severe asthma by mediating airflow obstruction and AHR[202]. A role for mast cells in severe asthma was shown with imatinib, which blocks stem cell factor signalling by inhibiting the mast/stem cell growth factor receptor KIT with reductions in airway methacholine reactivity, serum tryptase levels, and airway mast cell counts[203]. Recent studies identified that mast cell-derived tryptase, the dominant secretory granule protein in mast cells, is elevated in BALF and blood from severe asthmatics with either type 2-low or type 2-high disease[138, 202]. Moreover, due to common polymorphisms in the two genes producing β-tryptase, it is possible to have 2, 3 or 4 active β-tryptase alleles, and serum tryptase levels correlate with the number of active β-tryptase alleles[202]. A neutralizing antibody directed against human β-tryptase has been developed that inhibits airway tryptase in non-human primates and can potentially be developed into a new treatment option for type 2-low severe asthmatics. An approach that administers anti-tryptase antibodies to type 2-low asthmatics with increased numbers of active β-tryptase alleles or elevated tryptase levels can potentially target treatment to individuals with a mast cell asthma endotype.

*TH1/ILC1 Cytokines*

Interferon-γ, produced by Th1 CD4+ T cells, type 1 ILCs and NK cells is important in innate immunity[158, 167]. CD4+ T cells from severe asthmatics produce high levels of IFN-γ, which induces increases in CXCL10 (C-X-C motif chemokine ligand 10) that recruits Th1 CD4+ T cells and neutrophils[204, 205]. Murine experiments showed that IFN-γ decreases airway epithelial cell production of secretory leukocyte protease inhibitor (SLPI), which causes increased AHR and steroid resistance[204-206]. Furthermore, SLPI inhibits mast cell tryptase[206]. Therefore, strategies that target the IFN-γ/SLPI pathway might be developed for severe asthmatics with this endotype, however the consequences of impaired IFN-γ activity on airway host defence will need to be considered.

Tumour necrosis factor (TNF), another pro-inflammatory cytokine which recruits pulmonary neutrophils, is increased in BALF from severe asthmatics and causes AHR[158, 167, 207]. Although in a preliminary trial a soluble TNF receptor, etanercept, reduced AHR and improved bronchodilator responsiveness, in a subsequent larger study of severe asthma golimumab, an anti-TNF neutralizing antibody failed to improve asthma control and lung
function, but was associated with serious infections and malignancies[207, 208]. If future anti-TNF trials are considered, strategies to target TNF-high asthma could potentially identify a responsive subset[158].

TL1A (TNFSF15A) is a TNF superfamily member that functions as a ligand for Death Receptor 3 (DR3, TNFRSF25) on T cells and promotes type 2-high allergic lung inflammation in mice[209-213]. TL1A also amplifies Th1 and Th17 responses, which suggests that TL1A inhibition might be considered for the treatment of type 2-low asthma[213-215]. PF-06480605, a monoclonal antibody that neutralizes TL1A, has entered phase 2b clinical trials for inflammatory bowel disease[216, 217] and could be potentially be utilized in studies of type 2-low asthmatics.

**CD6/ALCAM Axis**
CD6 is a T cell co-stimulatory receptor for ALCAM (Activated Leukocyte Cell Adhesion Molecule, CD166) that enhances the activation and differentiation of Th1 and Th17 cells to promote autoimmunity[218]. The CD6/ALCAM pathway also promotes type-2 high, allergic asthma in mice, as well as serum and sputum ALCAM levels are increased in asthmatic children, especially those with severe disease[219]. Furthermore, a genome-wide association study has identified single nucleotide polymorphisms in the region of the ALCAM gene that were associated with an adult-onset, non-allergic asthmatic phenotype[220]. Collectively, these studies suggest that targeting the CD6/ALCAM pathway might be investigated for the treatment of type 2-low asthma[213]. A phase Ib clinical trial is currently in progress to evaluate the safety of an anti-CD6 antibody, itolizumab, in patients with moderate-to-severe uncontrolled asthma, however, additional studies will be required to assess its efficacy in type 2-low asthmatics[221].

**IL-8(CXCL8)/CXCR2 Axis**
Given the key role that IL-8 (CXCL8) plays in neutrophil recruitment and activation, treatments that block binding to its high-affinity C-X-C motif chemokine receptor, CXCR2, have been considered. Although a selective CXCR2 antagonist reduced sputum neutrophils and mild exacerbations in a small trial of severe asthmatics with increased sputum neutrophils, a larger study utilizing a different CXCR2 antagonist did not reduce the frequency of severe exacerbations in severe asthmatics with low peripheral blood eosinophil counts[222, 223]. Although this result called into question the strategy of targeting CXCR2 and neutrophils in uncontrolled asthma, the future development of a biomarker that identifies IL-8-high, neutrophilic asthmatics that can easily be utilized in clinical practice may allow this approach to be re-visited.

**Leukotriene B4 (LTB4)**
5-lipoxygenase-activating protein (FLAP) is a key component of the leukotriene synthetic pathway that generates leukotriene B4 (LTB4), which is both a pro-inflammatory neutrophil product, as well as a potent neutrophil chemoattractant[27, 224, 225]. Administration of the FLAP inhibitor, GSK2190915, to a small cohort of neutrophilic asthmatics for 14 days, suppressed sputum LTB4 levels, but not sputum neutrophils, which suggests that targeting FLAP may not be an effective strategy for treating neutrophilic asthma. Targeting the interaction between LTB4 and its high affinity receptor, BLT1, may represent an alternative strategy to attenuate neutrophilic airway inflammation in asthma[226, 227].
Mitochondrial Reactive Oxygen Species (ROS)

LC28-0126 is a novel mitochondria-targeted scavenger of ROS and reactive nitrogen species that attenuates ischemic-reperfusion injury in cardiomyocytes\cite{27, 228}. LC28-0126 has also been shown to ameliorate neutrophilic airway inflammation and airway hyperresponsiveness in mice. Although LC28-0126 has been administered to healthy volunteers, it has not yet been investigated in clinical trials of type-2 low asthmatics.

Conclusion

Currently the limited treatment options in type-2 low asthma contrast with the dramatic efficacy of novel drugs for type-2 high disease. However, an approach which focuses on identifying specific treatable traits is effective in selected patients with severe type-2 low disease. More trials of biomarker-directed macrolide therapy are required. The need to develop novel biomarkers for specific type-2 low pathways, such as molecular microbiology and exhaled volatile organic compounds\cite{121}, and to understand the underlying pathological mechanisms are recognised research priorities\cite{229}, and the present refocussing of research on type-2 low disease holds genuine promise for novel therapies on the near horizon.
### Tables

**Table 1** Challenges in developing therapeutics for type-2 low neutrophilic asthma, compared with successes in eosinophilic asthma

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<th>Neutrophilic asthma</th>
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<td>Role of neutrophils in the airways of patients with asthma is unknown; neutrophils are beneficial in airway infection.</td>
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<td><strong>Non-invasive biomarkers</strong></td>
<td>Elevated FeNO Blood eosinophils correlate with sputum eosinophils in asthma.</td>
<td>None. Non-invasive biomarkers (e.g. VOC) are not available in clinical practice. Blood neutrophil levels do not correlate with sputum neutrophil levels in asthma.</td>
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<tr>
<td><strong>Heterogeneity of phenotype</strong></td>
<td>Moderate heterogeneity within eosinophilic asthma: allergic versus non-allergic; early-onset versus late-onset.</td>
<td>Huge heterogeneity within neutrophilic asthma; multiple associated factors e.g. smoking, air pollution, obesity, infection.</td>
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<td><strong>Differential diagnosis</strong></td>
<td>Limited: eosinophilic COPD; eosinophilic pneumonia; ABPA; EGPA.</td>
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<td>Clearly delineated: corticosteroids. Type-2 cytokines and their receptors: IL-5, IL-5R and IL-4R. IgE in allergic eosinophilic severe asthma. epithelial alarmins (e.g. TSLP, IL-33).</td>
<td>Less well defined: [see Table 3] pro-inflammatory cytokines such as IL-1β, IL-6, TNF, IL-17, IL-17R, IL-23 CXC chemokines or their receptors β-tryptase, G-CSF, GM-CSF epithelial alarmins (e.g. TSLP; IL-33).</td>
</tr>
</tbody>
</table>

ABPA, allergic bronchopulmonary aspergillosis; COPD, chronic obstructive pulmonary disease; CXC, C-X-C motif chemokine ligand; EGPA, eosinophilic granulomatosis with polyangiitis; FeNO, fractional exhaled nitric oxide; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte/monocyte colony-stimulating factor; IgE, immunoglobulin E; IL, interleukin; NTM, non-tuberculous mycobacteria; TNF, tumour necrosis factor; TSLP, thymic stromal lymphopoietin; VOC, volatile organic compounds.
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<tr>
<th>Treatable trait</th>
<th>Phenotype</th>
<th>Potential Biomarkers</th>
<th>Investigations</th>
<th>Therapeutic option</th>
<th>Comments</th>
</tr>
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<td>Fixed airflow obstruction</td>
<td>Persistent airflow obstruction despite ICS+LABA use.</td>
<td></td>
<td>Spirometry with reduced post-bronchodilator FEV₁/FVC ratio.</td>
<td>Long acting antimuscarinics.</td>
<td>Effect small and may worsen cough so assess response and discontinue if no benefit.</td>
</tr>
<tr>
<td>Chronic bacterial airway colonisation</td>
<td>Persistent mucopurulent cough, frequent infective exacerbations. Bacterial colonisation with potentially pathogenic bacteria (e.g. <em>Haemophilus influenzae</em>).</td>
<td>Typical organisms on sputum culture. Pathogenic specific quantitative PCR.</td>
<td>Sputum culture. Exclude mycobacteria with sputum culture. Consider CT to exclude bronchiectasis.</td>
<td>Long term, low dose azithromycin.</td>
<td>Research needed into optimal patient selection, duration of therapy, potential use of other macrolides.</td>
</tr>
<tr>
<td>Cough reflex hypersensitivity</td>
<td>Female predominant, adult onset.</td>
<td>Capsaicin hypersensitivity.</td>
<td></td>
<td>Discontinue ACEI, treat GORD.</td>
<td>Research needed into cough suppressants including P2X3 inhibitors.</td>
</tr>
<tr>
<td>Airway hyper-reactivity</td>
<td>Marked airway hyperreactivity and inadequate response to other therapies.</td>
<td>Paucigranulocytic.</td>
<td>Reversibility / bronchial hyper-responsiveness testing, CT to exclude bronchiectasis and tracheobronchomalacia.</td>
<td>Consider bronchial thermoplasty in highly-selected patients.</td>
<td>Optimal phenotype, long term outcomes and efficacy of retreatment remain to be defined.</td>
</tr>
<tr>
<td>Steroid over use</td>
<td>Non-eosinophilic, patient reports symptoms are slow to improve after initiation of systemic steroids.</td>
<td>Peripheral blood eosinophil count.</td>
<td></td>
<td>Consider a steroid holiday: cautiously stopping systemic steroids.</td>
<td>Care to avoid iatrogenic adrenal insufficiency.</td>
</tr>
<tr>
<td>Vocal cord dysfunction (ILO)</td>
<td>Episodic, symptoms predominantly inspiratory, inspiratory stridor, minimal response to pharmacotherapy.</td>
<td>Flattened inspiratory flow loop, normal expiratory spirometry.</td>
<td>Laryngoscopy during provocation.</td>
<td>Specialist speech and language therapy.</td>
<td>Often coexists with asthma, triggers include inhalational irritants, exercise, and psychosocial disorders.</td>
</tr>
</tbody>
</table>

**Table 2** Current therapeutic options in type-2 low asthma

ACEi, angiotensin converting enzyme inhibitor; CT, computed tomography; GORD, gastro-oesophageal reflux; ILO, inducible laryngeal obstruction; LABA, long-acting beta-2 agonist; PCR, polymerase chain reaction.
<table>
<thead>
<tr>
<th>Pathway</th>
<th>Pathobiological Mechanism</th>
<th>Potential Biomarkers</th>
<th>Potential Therapeutics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IL-1β</strong></td>
<td>Activation of the NLRP3 inflammasome → NF-κB → cytokines including IL-1β and neutrophil chemokines</td>
<td>IL-1β, IL-1R, NLRP3</td>
<td>Anti-IL-1β (e.g. canakinumab) IL-1β receptor antagonists (e.g. anakinra) NLRP3 small-molecule inhibitors</td>
</tr>
<tr>
<td><strong>IL-17A/F</strong></td>
<td>Th17 / γδ T17 / ILC3 / MAIT cells → IL-17A &amp; IL-17F → epithelial derived neutrophil chemoattractants and antimicrobial defence</td>
<td>IL-17A, IL-17F, IL-23A, RORγt</td>
<td>Anti-IL-17RA (e.g. brodalumab) Anti-IL-23 (e.g. risankizumab) DNAzymes Small-molecule inhibitors</td>
</tr>
<tr>
<td><strong>Alarmins</strong></td>
<td>Epithelial tissue damage → release of alarmins TSLP / IL-33 / IL-25</td>
<td>Low LXA4, High SAA</td>
<td>LXA4 or analogues Specialized proresolving mediator precursors</td>
</tr>
<tr>
<td><strong>Resolvens</strong></td>
<td>Lipoxin A4 promotes resolution of inflammation via ALX/FPR2</td>
<td>Increased serum amyloid A inhibits resolvin signalling via ALX/FPR2</td>
<td></td>
</tr>
<tr>
<td><strong>Colony stimulating factors</strong></td>
<td>Apolipoproteins (e.g. APOA1) → ABCA1 inhibit G-CSF-induced neutrophilia</td>
<td>G-CSF, GM-CSF</td>
<td>Neutralising antibodies APOA1 mimetic peptide</td>
</tr>
<tr>
<td><strong>Type I interferons</strong></td>
<td>Stable state: high ISG → type-2-independent inflammation Acute viral infection: deficient type-I/III IFN → increased viral replication</td>
<td>Blood ISG expression, Low IFN-α / -β / -λ</td>
<td>Inhaled IFN-β</td>
</tr>
<tr>
<td><strong>IL-6</strong></td>
<td>IL-6: obesity / granulocytes → IL-6 → steroid-resistant inflammation IL-6 trans-signalling: bacteria → TLRs → granulocytes shed soluble IL-6R + IL-6 → local epithelial cell inflammation</td>
<td>IL-6, sIL-6R</td>
<td>Anti-IL-6 (e.g. clazakizumab) Anti-IL-6R (e.g. tocolizumab) Antimicrobials</td>
</tr>
<tr>
<td><strong>Mast cells</strong></td>
<td>IgE cross-linking → Mast cell degranulation → mediators including histamine, tryptase, chymase, carboxypeptidase</td>
<td>Tryptase, Chymase</td>
<td>Anti-β-tryptase mAb KIT inhibitors (e.g. imatinib)</td>
</tr>
<tr>
<td><strong>IFN-γ</strong></td>
<td>Th1 / ILC1 / NK cells → IFN-γ → CXCL10 → neutrophilia &amp; ↓ SLPI</td>
<td>TNF, IFN-γ, CXCL10, SLPI, Tbet</td>
<td>Soluble TNFR (e.g. etanercept) Small-molecule inhibitors (JAK1) DNAzyme (Tbet)</td>
</tr>
<tr>
<td><strong>CXCL8 (IL-8)</strong></td>
<td>CXCL8 → CXCR2 → neutrophil recruitment</td>
<td>CXCL8</td>
<td>Small-molecule inhibitors</td>
</tr>
</tbody>
</table>

APOA1, apolipoprotein A1; BET, bromodomain and extraterminal; CXCL, C-X-C motif chemokine ligand; CXCR, C-X-C motif chemokine receptor; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte/monocyte colony-stimulating factor; IFN, interferon; IL, interleukin; ILC, innate lymphoid cell; ISG, interferon-stimulated genes; JAK, Janus kinase; KIT, KIT proto-oncogene receptor tyrosine kinase; LXA, lipoxin A;
mAb, monoclonal antibody; NLRP3, nucleotide-binding oligomerization domain, leucine-rich repeat, and pyrin domain containing; RORγt, retinoic acid-related orphan receptor γ thymus specific; SAA, serum amyloid A; SLPI, secretory leukocyte protease inhibitor; Tbet, T-box transcription factor TBX21; Th1, Th17, helper T-cell type 1 and type 17; TNF, tumour necrosis factor; TSLP, thymic stromal lymphopoietin.
Figure Legends

Figure 1
Suggested algorithm for defining type-2 low asthma in clinical practice.
CF, cystic fibrosis; COPD, chronic obstructive pulmonary disease; Eos, eosinophils; FeNO, fractional exhaled nitric oxide; HRCT, high-resolution computed tomograph; PCD, primary ciliary dyskinesia; PCR, polymerase chain reaction.

Figure 2
Key pathways mediating neutrophilic airway inflammation in type 2-low asthma. APO, apolipoprotein; CXCL, C-X-C motif chemokine ligand; CXCR, C-X-C motif chemokine receptor; FPR, N-formyl peptide receptor; G-CSF, granulocyte colony-stimulating factor; G-CSFR, granulocyte colony-stimulating factor receptor; GM-CSF, granulocyte/monocyte colony-stimulating factor; GM-CSFR, granulocyte/monocyte colony-stimulating factor receptor; gp130, glycoprotein 130; ICS, inhaled corticosteroid; IFN interferon; IL interleukin; ILC, innate lymphoid cell; IL-6TS, interleukin-6 trans-signaling; LXA, lipoxin; NLRP, nucleotide-binding oligomerization domain, leucine-rich repeat, and pyrin domain containing; sIL-6R, soluble interleukin-6 receptor; SAA, serum amyloid A; SLPI, secretory leukocyte protease inhibitor; Th, T helper CD4+ lymphocyte; TNF, tumor necrosis factor; TSLP, thymic stromal lymphopoietin.

Figure 3
Clinical trial data for azithromycin data in severe asthma and for induction of resistance in healthy volunteers. (a) Proportion of subjects with non-eosinophilic severe asthma (FeNO<upper limit of normal and a blood eosinophilia ≤200/ml) free from primary endpoints (severe exacerbations) for 26 weeks, according to study group (azithromycin or placebo) in AZISAST[74]. Azithromycin significantly decreased the number of patients with at least one primary endpoint from 9/27 (33%) azithromycin-treated subjects vs 18/29 (62%) placebo-treated subjects; relative risk 0.54, 95% CI 0.29 to 0.98, p=0.037. Reproduced from Bruselle GG and colleagues[74] with permission from BMJ Publishing Group Ltd. (b) Cumulative severe and moderate asthma exacerbations during 48 weeks of treatment with azithromycin 500 mg, three times per week, or placebo in AMAZES[75]. Reproduced with permission from Elsevier. (c) Temporal changes in the proportion of macrolide-resistant streptococci after azithromycin and clarithromycin use in healthy volunteers. Mean data are shown for 204 volunteers (of 224 recruited) assessed to day 42, and for 99 volunteers assessed to day 180. Error bars are 95% CIs. Reproduced from Malhotra-Kumar and colleagues[230] with permission from Elsevier. (d) Haemophilus influenzae copy number before and after either placebo (left, red) or azithromycin (right, blue) in AMAZES. Reproduced from Taylor SL[91] with permission from American Thoracic Society.
References


44. Satia I, Tsamandouras N, Holt K, Badri H, Woodhead M, Ogungbenro K, Felton TW, O'Byrne PM, Fowler SJ, Smith JA. Capsaicin-evoked cough responses in asthmatic patients:


137. University of North Carolina CH. Late Phase Administration Anakinra as a Rescue Treatment for Inhaled Allergen Challenge-Induced Airway Inflammation (LateAna). 2018 [cited 2020 04/01/2020]; Available from: [http://clinicaltrials.gov/ct2/show/NCT03513458](http://clinicaltrials.gov/ct2/show/NCT03513458)


large high-density lipoprotein particles are positively correlated with FEV1 in atopic asthma. *Am J Respir Crit Care Med* 2015: 191(9): 990-1000.


the prevention of severe asthma exacerbations: results of the INEXAS phase 2a study. *Am J Respir Crit Care Med* 2018: 197.


217. Pfizer. A Study to Evaluate the Efficacy and Safety of PF-06480605 in Adult Participants With Moderate to Severe Ulcerative Colitis. 2020 [cited 2020 19/05/2020]; Available from: https://clinicaltrials.gov/ct2/show/NCT04090411


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<td>Eosinophils:</td>
<td>Neutrophils:</td>
</tr>
<tr>
<td></td>
<td>• long-lived haematopoietic cells</td>
<td>• short-lived haematopoietic cells</td>
</tr>
<tr>
<td></td>
<td>• reside predominantly in mucosal tissues (e.g. airways)</td>
<td>• predominantly circulating in blood</td>
</tr>
<tr>
<td></td>
<td>• absent in sputum and airways in health</td>
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<td></td>
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<td>NLRP3</td>
</tr>
<tr>
<td>IL-17A/F</td>
<td>TH17/γδ T17/ULC3/MAIT cells → IL-17A and IL-17F → epithelial-derived neutrophil chemoattractants and antimicrobial defence</td>
<td>IL17-A, IL-17F</td>
</tr>
<tr>
<td></td>
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<td>IL-23A</td>
</tr>
<tr>
<td>Alarmins</td>
<td>Epithelial tissue damage → release of alarmins TSLP/IL-33/IL-25</td>
<td>RORγt</td>
</tr>
<tr>
<td>Resolvins</td>
<td>Lipoxin A4 promotes resolution of inflammation via ALX/FPR2</td>
<td>Low LXA4</td>
</tr>
<tr>
<td></td>
<td>Increased serum amyloid A inhibits resolvin signalling via ALX/FPR2</td>
<td>High SAA</td>
</tr>
<tr>
<td>Colony stimulating factors</td>
<td>Apolipoproteins (e.g. APOA1) → ABCA1 inhibit G-CSF-induced neutrophilia</td>
<td>G-CSF</td>
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<td></td>
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<td>GM-CSF</td>
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<tr>
<td>Type I interferons</td>
<td>Stable state: high ISG → type 2-independent inflammation</td>
<td>Blood ISG expression</td>
</tr>
<tr>
<td></td>
<td>Acute viral infection: deficient type I/III IFN → increased viral replication</td>
<td>Low IFN-α/β/γ</td>
</tr>
<tr>
<td>IL-6</td>
<td>IL-6: obesity/granulocytes → IL-6 → steroid-resistant inflammation</td>
<td>IL-6</td>
</tr>
<tr>
<td></td>
<td>IL-6 trans-signalling: bacteria → TLRs → granulocytes shed soluble IL-6R + IL-6 → local epithelial cell inflammation</td>
<td>sIL-6R</td>
</tr>
<tr>
<td>Mast cells</td>
<td>IgE cross-linking → mast cell degranulation → mediators including histamine, tryptase, chymase, carboxypeptidase</td>
<td>Tryptase</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chymase</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Th1/ILC1/NK cells → IFN-γ → CXCL10 → neutrophilia and ↓ SLPI</td>
<td>TNF</td>
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<tr>
<td></td>
<td></td>
<td>IFN-γ, CXCL10, SLPI</td>
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<td>Tbet</td>
</tr>
<tr>
<td>CXCL8 (IL-8)</td>
<td>CXCL8 → CXCR2 → neutrophil recruitment</td>
<td>CXCL8</td>
</tr>
</tbody>
</table>