

Review

T2-low: what do we know?

Past, present, and future of biologic therapies in noneosinophilic asthma

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Key Messages

- T2-low asthma is an often severe subtype with an unmet need for effective medication.
- Monoclonal antibodies were often trialed in heterogeneous asthma populations.
- T2-low asthma consists of multiple proteomic and transcriptomic subtypes with potentially differential therapeutic responses.
- Biomarker-guided approaches and subgroup analyses are useful to identify responders.
- Existing monoclonal antibodies could be beneficial when administered to the correct target population.

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ABSTRACT

T2-low asthma is an often severe asthma subtype with limited treatment options and biologic therapeutics are lacking. Several monoclonal antibodies (mAbs) targeting non-T2 cytokines were previously reported to be ineffective in asthma. These trials often investigated heterogeneous asthma populations and negative outcomes could be related to unsuitable study cohorts. More tailored approaches in selecting participants based on specific biomarkers have been beneficial in treating severe T2-high asthma. Similarly, mAbs previously deemed ineffective bear the potential to be useful when administered to the correct target population.

Here, we review individual clinical trials conducted between 2005 and 2021 and assess the suitability of the selected cohorts, whether study end points were met, and whether outcome measures were appropriate to investigate the effectiveness of the respective drug. We discuss potential target groups within the T2-low asthma population and suggest biomarkers that may predict a treatment response. Furthermore, we assess whether biomarker-guided approaches or subgroup analyses were associated with more positive study outcomes.

The mAbs directed against alarmins intervene early in the inflammatory cascade and are the first mAbs found to have efficacy in T2-low asthma. Several randomized controlled trials performed predefined subgroup analyses that included T2-low asthma. Subgroup analyses were associated with positive outcomes and were able to reveal a stronger response in at least 1 subgroup. A better understanding of T2-low subgroups and specific biomarkers is necessary to identify the most responsive target population for a given mAb.

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Introduction

Asthma is a heterogeneous inflammatory respiratory disease, affecting approximately 340 million people worldwide.¹ Patients with severe asthma respond poorly to existing treatment and continue to experience an immense disease burden, contributing to disproportionately high health care costs.² There is an unmet need for novel medication to improve asthma outcomes in these patients and to reduce asthma-related impacts on the health care system.

Recent breakthroughs in asthma therapy were achieved through more targeted approaches. In T2-high asthma, the biomarker-guided administration of monoclonal antibody (mAb) therapies improved asthma outcomes and allowed a reduction of systemic corticosteroids in target populations.^{3–7} A number of approved biologic therapies are available as add-on medications to treat T2-high asthma, a subtype of asthma orchestrated by type 2 cytokines and characterized by eosinophilic airway inflammation.⁸ These therapies improve lung function and quality of life and reduce eosinophilic inflammation, asthma-related exacerbations, and hospitalization.⁴ Nevertheless, approximately 50% of people with asthma do not exhibit type 2 inflammation (T2-low asthma),⁹ and consequently, these patients do not benefit from such treatment. Even though T2-low asthma is often severe, refractory to inhaled corticosteroid (ICS), and associated with increased health care burden, it remains incompletely understood. Several approaches in therapeutic targeting of potential mediators of T2-low asthma have also been unsuccessful. Negative study outcomes may have been a result of an inappropriate selection of participants and an unfavorable definition of study end points. In this review, we provide an overview of previous clinical trials targeting T2-low asthma and explore whether the selected cohorts and study end points were suitable to assess the efficacy of the investigated mAb. We further discuss which asthma population might benefit most from a given treatment and whether a trial could be optimized, for example, through the adjustment of outcome measures.

What Is T2-Low Asthma?

T2-low asthma was first described in the late 1990s after it was recognized that the T2-high signature was only prevalent in a proportion of patients with asthma.^{10,11} Although it is generally characterized by the absence of type 2 inflammation and the lack of eosinophilia, as yet, there is no widely accepted definition of T2-low asthma. T2-high asthma, however, is defined by an increase of type 2 cytokines (interleukin [IL]-4, IL-5, IL-13), immunoglobulin E (IgE), and subsequent eosinophilic airway inflammation, although the ideal cutoff points for biomarkers are unclear and their levels can vary within the same patient.^{12–14} Clinically useful biomarkers, such as elevated numbers of blood and sputum eosinophils or increased fractional exhaled nitric oxide (FeNO), are indicative of T2-high asthma. Furthermore, eosinophilic airway inflammation and FeNO can predict a response to type 2 biologics.^{15,16} The identification of major mediators of T2-low asthma and easily detectable biomarkers may be the key to achieve similar breakthroughs in T2-low asthma therapy. The explicit roles of inflammatory mediators in T2-low asthma are increasingly investigated.^{17–20} Cytokines, chemokines, and other molecules that were found to be elevated in serum in T2-low asthma^{20,21} bear the potential to serve as both biomarkers and therapeutic targets; however, they are not yet implemented in clinical practice. T2-

low asthma is prevalent in about half the asthma population,⁹ although its exact prevalence depends on its definition and the implemented cutoffs of T2-high biomarkers. This high proportion of those with T2-low asthma and the lack of current treatment highlight the need to develop effective medication. Table 1 illustrates the differences and similarities of T2-high and T2-low asthma.

The pathophysiological mechanisms underlying T2-low asthma are not fully understood. It is thought to involve type 1 cytokines (tumor necrosis factor [TNF] and interferon gamma [IFN- γ]) secreted by type 1 T helper cells (T_H1) and type 1 innate lymphoid cells (ILC1), type 17 cytokines produced by T_H17 and ILC3 (IL-17, IL-22), and other key inflammatory mediators, such as IL-1 β , IL-6, and IL-8²² (Fig 1). At least 2 airway inflammatory phenotypes fall under the umbrella term of T2-low asthma, namely neutrophilic and paucigranulocytic asthma, both of which widely lack eosinophilia but differ regarding the presence of airway neutrophils. Neutrophilic asthma is often severe and associated with corticosteroid resistance, more frequent exacerbations requiring hospitalization, airflow limitation, and increased bacterial burden.^{23–30} As yet, no effective medication is available to treat neutrophilic asthma. Several clinical studies trialed therapeutic blockade of key inflammatory mediators of neutrophilic airway inflammation^{29,31,32}; however, none have been proven to consistently improve asthma outcomes. In a recent study of asthma inflammatory phenotypes, sputum transcriptomics were combined with serum proteomics. While T2-high asthma comprised of a homogenous, single proteomic- and transcriptomic-associated cluster (PTAC), 9 PTACs were identified in T2-low asthma, 2 of which featured airway neutrophilia.³³ This heterogeneity in T2-low asthma limits the identification of suitable biomarkers and therapeutic targets and could explain why previous trials in treating T2-low asthma with biologics did not achieve positive results.

It is also debated whether T2-low asthma exists as a distinct asthma endotype. It is recognized that it may be a feature of T2-high asthma in which type 2 inflammation is suppressed through the overuse of the administered anti-inflammatory medication. Thus, the presence of T2-low asthma may have been overestimated in the past. A multicenter, single-blind, parallel group, randomized controlled trial has revealed that only 5% of 209 patients with severe asthma, opposed to 23% at study entry, appeared T2-low asthma once their corticosteroid dose has been downtitrated.³⁴ This study demonstrated that the T2-low asthma endotype persisted despite the reduction of corticosteroids, although its prevalence was lower than previously thought. On a further note, a large retrospective sputum study has revealed that the proportions of those receiving ICSs did not differ remarkably across asthma inflammatory phenotypes, implying that noneosinophilic, T2-low asthma exists in mild asthma not treated with ICSs.³⁵ Whether or not T2-low asthma is a true inflammatory endotype, its classification is a clinically useful concept, and it should be recognized as an important heterogeneous subtype of asthma.

Table 1
Comparison of T2-high vs T2-low asthma.

Feature	T2-high asthma	T2-high and T2-low asthma	T2-low asthma
Common triggers	Allergens, pathogens		Air pollutants, cigarette smoke
Involved immune cells	T _H 2, ILC2, eosinophils, basophils		T _H 1, T _H 17, ILC1, ILC3, neutrophils, monocytes, macrophages
Inflammatory mediators	IL-5, IL-13, IL-4, IL-9, IgE	TSLP, IL-33, GM-CSF	IL-17, TNF, IL-1 β , IL-6
Biomarkers	PBE, FeNO, serum IgE		Absence of elevated T2-high biomarkers Lack of T2-low biomarkers
Clinical implication	Often ICS-sensitive	Airway inflammation Airway hyperresponsiveness Airway remodeling	Often ICS-resistant

Abbreviations: FeNO, fractional exhaled nitric oxide; GM-CSF, granulocyte-macrophage colony-stimulating factor; ICS, inhaled corticosteroid; IgE, immunoglobulin E; IL, interleukin; ILC, innate lymphoid cell; PBE, peripheral blood eosinophils; T_H, T helper cell; TNF, tumor necrosis factor; TSLP, thymic stromal lymphopoietin.

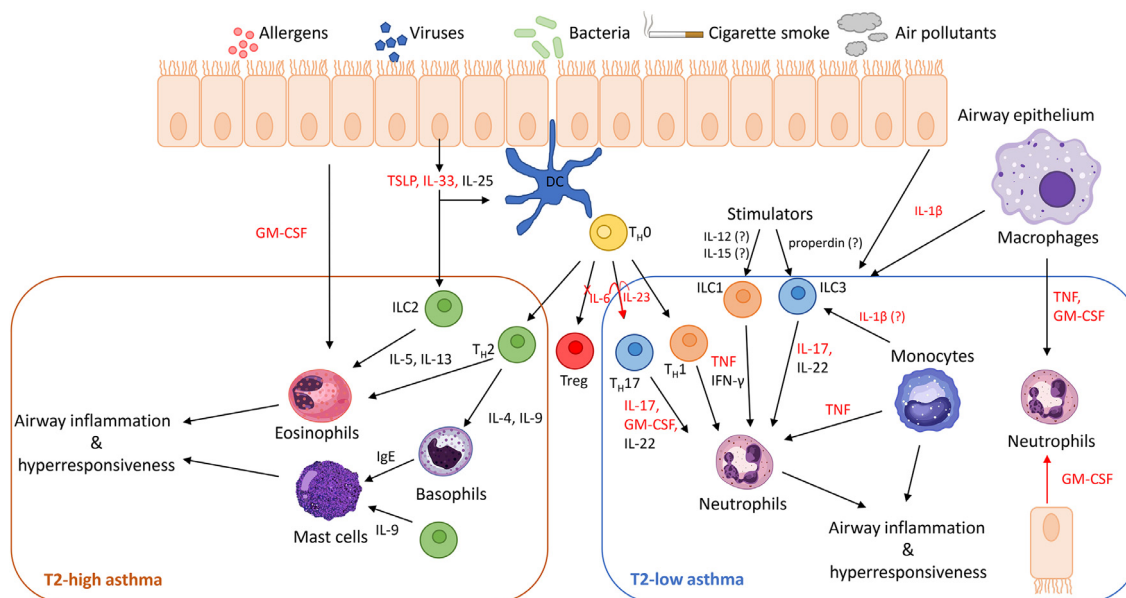


Figure 1. Pathophysiology of T2-high and T2-low asthma. Alarmins secreted by airway epithelial cells activate T cells and innate lymphoid cells. Type 2 cytokines mediate the recruitment of eosinophils/basophils in T2-high asthma. Type 1 and type 17 cytokines are thought to drive T2-low asthma and contribute to neutrophil recruitment. Cytokines discussed in this review are indicated in red. DC, dendritic cell; GM-CSF, granulocyte-macrophage colony-stimulating factor; IgE, immunoglobulin E; IL, interleukin; Th, T helper cell; TNF, tumor necrosis factor; TSLP, thymic stromal lymphopoietin.

Previous Randomized Controlled Trials With Existing Biologics

Alarmins as Novel Targets

Thymic stromal lymphopoietin (TSLP), IL-33, IL-25, and granulocyte-macrophage colony-stimulating factor (GM-CSF) are early mediators released by airway epithelial cells when they encounter environmental triggers, such as pathogens, allergens, or air pollutants (Fig 1). These alarmins mediate the activation and recruitment of innate and adaptive immune cells,³⁶ orchestrating airway inflammation and remodeling. TSLP and IL-33 activate immature dendritic cells, which in turn interact with naive CD4⁺ T cells and promote their differentiation into T_H1, T_H2, T_H17, or regulatory T cells (Treg).^{37,38}

Alarmins further stimulate type 2 innate lymphoid cells to secrete type 2 mediators.³⁹ Thus, TSLP and IL-33 are potentially superior initiators of both T2-low and T2-high asthma. In theory, therapeutic blockade of these alarmins with mAbs may inhibit asthma pathogenesis, prevent trigger related exacerbations, and reduce airway inflammation irrespective of the underlying inflammatory phenotype.

Thymic Stromal Lymphopoietin

Tezepelumab is a mAb directed against TSLP (Fig 2). A phase III clinical trial investigated its effectiveness in severe, uncontrolled asthma (Table 2).⁴⁰ The primary end point was the annualized

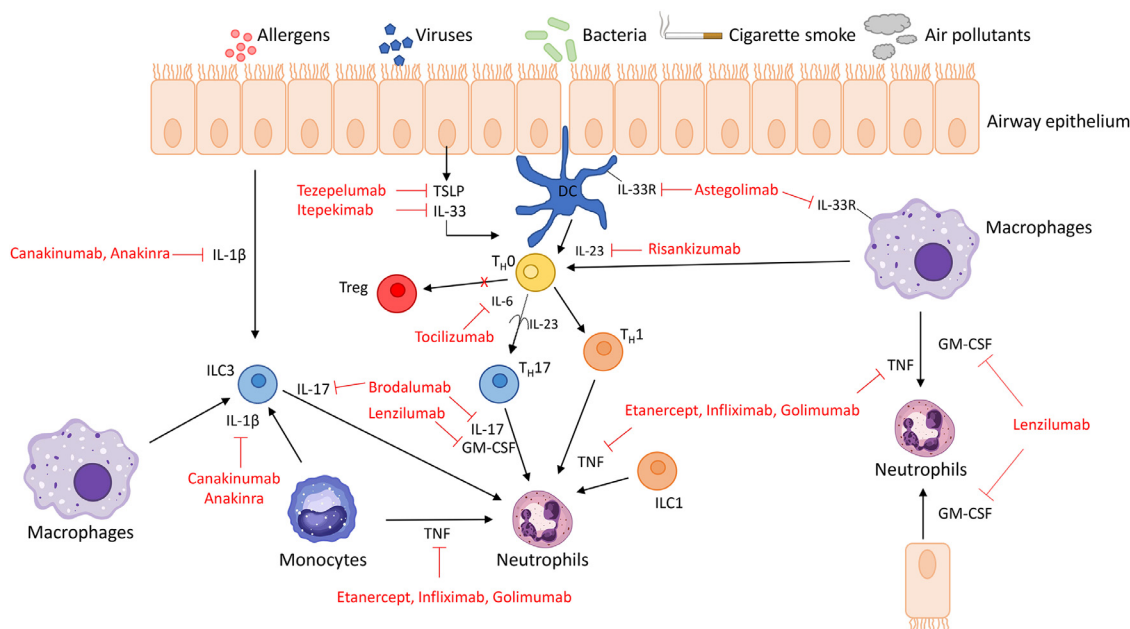


Figure 2. Therapeutic targets of monoclonal antibodies and biological disease-modifying antirheumatic drugs. GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; ILC, innate lymphoid cell; Th, T helper cell; TNF, tumor necrosis factor; TSLP, thymic stromal lymphopoietin.

Table 2
Previous Biologic Approaches in Asthma Management.

Treatment	Target	Selected patients	N and study duration	Primary and secondary outcomes	Outcome	Author, y
Tezepelumab	TSLP Phase III	Adolescents and adults (12-80 y) with severe, uncontrolled asthma Subgroup analyses of EA (T2-high) and non-EA (T2-low): PBE < 150, ≥150, <300, ≥300 cells/μL; FeNO < 25 or ≥25 ppb	N = 1061 Placebo: 532 Tezepelumab: 529 Duration: 52 wk	Primary: AER Secondary: Changes in FEV ₁ , ACQ6 scores, AQLQ, and asthma symptom diary	↓AER, ACQ6 ↑FEV ₁ , AQLQ Greatest improvement in those with ≥300 PBE/μL, but also effective in non-EA No serious treatment-emergent adverse events; 2 severe treatment-emergent adverse events in the healthy group (chest pain for ~10 min) ↑ serum IL-33 ↓ PBE No changes in soluble serum ST2 No antidrug antibody responses ↓ loss of asthma control events ↑ FEV ₁ in single drug vs placebo group	Menzies-Gow et al, ⁴⁰ 2021
Itepekimab	IL-33 Phase I	Healthy adults and adults with moderate asthma	Single dose (healthy, N=40) and multiple doses (asthma, N=23)	Primary: incidence and severity of treatment-emergent adverse events Secondary: pharmacokinetic parameters (serum concentration-time profile of the drug, concentrations of total IL-33 and soluble ST2 in serum, PBE, detection of antidrug antibody responses)	No changes in soluble serum ST2 No antidrug antibody responses ↓ loss of asthma control events ↑ FEV ₁ in single drug vs placebo group	Kosloski et al, ⁴² 2022
Itepekimab	IL-33 Phase II Proof of concept	Adults with moderate-to-severe asthma Subgroup analysis of PBE high (≥300 cells/μL) vs low	N = 296 Placebo: 74 Itepekimab: 73 Itepekimab + dupilumab: 74 Dupilumab: 75 Duration: 12 wk	Primary: loss of asthma control Secondary: change in FEV ₁ Other efficacy end points: change in ACQ5, AQLQ	Greater effects on FEV ₁ in patients with ≥300 PBE/μL ↓AER including eosinophil-low group ↑ time to first exacerbation ↓ risk of asthma exacerbation ↑ AQLQ No remarkable change in FEV ₁	Wechsler et al, ⁴³ 2021
Astegolimab	IL-33 receptor (ST2) Phase IIb	Adults with uncontrolled, severe asthma Subgroup analyses of EA (T2-high) and non-EA (T2-low): PBE < 150, ≥150, <300, ≥300 cells/μL; FeNO < 25 or ≥25 ppb	N = 502 Astegolimab: 375 Placebo: 127 Duration: treatment: 52 wk, whole study including run-in and follow-up: 70 wk	Primary: AER Secondary: Time to first asthma exacerbation during the 52 wk, changes in FEV ₁ , AQLQ, ACQ5, short-acting rescue therapy use, nighttime awakenings, asthma daily symptom severity Other: primary end point assessment by eosinophil subgroups, changes in biomarkers (PBE and FeNO)	Primary: ↓ mTNF on peripheral blood monocytes, improvement in PC ₂₀ , and improved symptom scores (both associated with baseline mTNF on peripheral blood monocytes and the treatment associated change in mTNF from baseline) Secondary: Improvement in post-FEV ₁ , decreased symptom score ↓AHR, ACQ7 ↑AQLQ, FEV ₁	Kelsen et al, ⁴⁴ 2021
Etanercept	TNF	Adults with severe asthma Biomarker: Baseline TNF levels on peripheral blood monocytes	N = 10 Duration: 10 wk etanercept or placebo, 4 wk washout, 10 wk placebo or etanercept	Primary: change in PC ₂₀ between placebo and etanercept treatment phase, change in AQLQ between treatment phases Secondary: change in FEV ₁ , forced expiratory flow at 25%-75% of the vital capacity, and FVC, symptom scores, FeNO, computed alveolar nitric oxide, differential inflammatory cell counts in sputum, mediator concentrations in sputum supernatant	Primary: ↓ mTNF on peripheral blood monocytes, improvement in PC ₂₀ , and improved symptom scores (both associated with baseline mTNF on peripheral blood monocytes and the treatment associated change in mTNF from baseline) Secondary: Improvement in post-FEV ₁ , decreased symptom score ↓AHR, ACQ7 ↑AQLQ, FEV ₁	Berry et al, ⁵¹ 2006
Etanercept	TNF	Adults with severe asthma	N = 17 Duration: 12 wk	Primary: ACQ7 Secondary: change in AHR to inhaled methacholine and FEV ₁	↓AHR, ACQ7 ↑AQLQ, FEV ₁	Howarth et al, ⁵⁰ 2005
Etanercept	TNF	Adults with severe asthma	N = 39 Placebo: 20 ETN: 19 Duration: 12 wk	Primary: Difference in change in mean AQLQ from baseline and end of treatment and mean ACQ from baseline and the last 2 treatment visits Secondary: differences from baseline to end of treatment for bronchial hyperresponsiveness, and the last 2 treatment visits for FEV ₁ % pred, FEV ₁ /FVC, morning, evening, and average daily PEF, diurnal variation in PEF, differences from baseline for laboratory parameters in sputum and serum	↓ACQ ↓ Sputum macrophages and C-reactive protein ↑ Serum TNF and albumin	Morjaria et al, ¹⁰⁷ 2008
Etanercept	TNF Phase 2	Adults (18-70 y) with moderate-to-severe, uncontrolled asthma	N = 132 Placebo: 64 ETN: 68 Duration: 12 wk	Primary: change in FEV ₁ % pred from baseline to wk 12 Secondary: morning PEF, FEV ₁ % pred at wk 2, 4, 8, ACQ5, PC ₂₀ methacholine, AQLQ	No clinical efficacy but well tolerated	Holgate et al, ⁵² 2011
Infliximab (and adalimumab)	TNF	Adults with uncontrolled, severe asthma , no improvement with omalizumab (6 mo)	N = 7 median duration: 17.7 ± 14 mo (3 mo-3 y)	Outcomes: asthma control test, hospitalization rate (conventional and intensive care unit) within the previous year and the year after anti-TNF treatment, weaning from oral corticosteroids	↑Asthma control ↓Exacerbation ↓Oral corticosteroids	Taillé et al, ⁵⁵ 2013
Infliximab	TNF	Adults with moderate asthma	N = 38 Infliximab: 18 Placebo: 20 3 infusions over 6 wk	First: change in morning (PEF) at days 50-56 vs 7 days of the run-in	No remarkable effect on PEF ↓ mean diurnal variation in PEF at wk 8 ↓ patients with exacerbations ↑ probability of freedom of exacerbations ↓ TNF levels	Erin et al, ⁵⁶ 2006

(continued)

Table 2 (Continued)

Treatment	Target	Selected patients	N and study duration	Primary and secondary outcomes	Outcome	Author, y
Golimumab	TNF Phase II	Adults with uncontrolled, severe asthma	N = 309 Golimumab: 231 Placebo: 78 Intended duration of treatment: 52 wk (treatment discontinued after 24 wk) Follow-up at wk 76	First: change from baseline to wk 24 in FEV ₁ and number of severe exacerbations to wk 24	No beneficial effect in study population	Wenzel et al, ⁵⁷ 2009
Canakinumab	IL-1β	Participants with mild asthma No other meds except salbutamol	N = 21 CAM: 16 Placebo: 5 Duration: 2 administrations on days 1 and 15.	Objectives: Establish safety and tolerability Define the pharmacokinetic/pharmacodynamic relationship between ASZ885 and IL-1β in mild asthma Provide estimate of the anti-inflammatory effects of ACZ885 by means of attenuation of LAR after inhalative allergen challenge	↓ free IL-1β Attenuated late asthma response	Pascoe et al, ⁷¹ 2006
Anakinra	IL-1RA binding IL-1R1 Phase I	Healthy volunteers	N = 17	Objectives: Assess safety of the IL-1 receptor antagonist in conjunction with inhaled lipopolysaccharide and test the hypothesis that IL-1 blockade suppresses acute neutrophil response to lipopolysaccharide challenge	↓ airway neutrophilia ↓ IL-1β, IL-6, and IL-8 Well tolerated	Hernandez et al, ⁷³ 2015
Anakinra	IL-1RA binding IL-1R1 Phase 2	Adults (18–45) with mild asthma	N = 25, 12 to receive treatment Single dose	Outcome: Max % FEV ₁ drop from baseline during late phase response (3–10 h)	Withdrawn owing to COVID-19–related safety issues	National Heart, Lung, and Blood Institute, ⁷⁴ 2021
Brodalumab	IL-17R Phase IIa	Adults with inadequately controlled, moderate-to-severe asthma Subgroup analyses based on high bronchodilator reversibility (↑FEV ₁ ≥20%), baseline FEV ₁ % pred (tertiles), ACQ7 (tertiles), ICS dose (≥500 μg or <500 μg), FeNO (≥23.5 or <23.5), PBE (≥6% or <6%), sex, race (White or non-White), weight (≥100 kg or <100 kg)	N = 305 Brodalumab: 228 Placebo: 77 Duration: 10 wk	Primary: Change in ACQ7 total score from baseline to wk 12 (↓ ≥0.5) Secondary: Changes in pre- and post-FEV ₁ from baseline, morning PEF, rescue short-acting β-agonist use, daily asthma symptom score, and symptom-free days	No evident beneficial effect in total cohort ↑ Asthma control in high reversibility subgroup	Busse et al, ⁸¹ 2013
Tocilizumab	IL-6 Phase II	Adults with mild, stable, allergic asthma	N = 11 TCZ: 6 Placebo: 5 Duration: Single dose	Primary: Late asthma response assessed based on FEV ₁ 3 h and 7 h after allergen challenge Secondary: early asthma response (20 min–2 h after post-treatment allergen challenge).	↓ C-reactive protein, IL-6, and soluble IL-6R No effect on allergen-induced bronchoconstriction Single dose had no effect on allergen-induced late asthma response or early asthma response	Revez et al, ³² 2019
Lenzilumab (KB003)	GM-CSF Phase II	Adults with inadequately controlled asthma (ACQ > 1.5) Subgroup analyses: Atopic vs non-atopic, PBE (<300 vs ≥300 cells/μL), medium- vs high-dose ICS, infrequent vs frequent (≥2) exacerbations, high reversibility (<20% vs ≥20%), pre-FEV ₁ pred. (≤50% vs >50%), smoking (≤10 pack-year vs never smoking)	N = 160 Lenzilumab: 78 Placebo: 82 Duration: 20 wk of treatment, 36 wk in total including screening and follow-up visits	Primary: Changes in pre-BD FEV ₁ at wk 24 Secondary: ACQ5, AER, PEF, asthma symptoms, rescue short-acting β-agonist use, safety and tolerability, pharmacokinetics and immunogenicity	No effect on ACQ5 or AER. Lack of effect in neutrophilic asthma possibly dose related ↑ Lung function in EA, in high reversibility group and those with pre-FEV ₁ % pred. ≤ 50	Molfinio et al, ¹⁰³ 2016
Risankizumab	IL-23—Phase IIa	Adults with severe asthma	N = 214 Risankizumab: 105 Placebo: 109 Duration: 24 wk	Primary: Time-to-first asthma worsening: deterioration from baseline for ≥2 consecutive days (≥30% ↓ in morning PEF or ↑ in daily rescue medication ≥50% and at least 4 puffs), an increase in ACQ5 ≥0.75 or a severe asthma exacerbation Secondary: Annualized rate of asthma worsening and severe asthma exacerbations, ACQ5, FEV ₁ . Exploratory biomarkers including sputum cytology were evaluated	Not beneficial in severe asthma with time-to-first asthma worsening and rate of asthma worsening favoring placebo	Brightling et al, ¹⁰⁴ 2020

Abbreviations: ACQ, asthma control questionnaire; AHR, airway hyperresponsiveness; AER, asthma exacerbation rate; AQLQ, asthma quality of life questionnaire; COVID-19, coronavirus disease 2019; EA, eosinophilic asthma; FeNO, fractional exhaled nitric oxide; FEV, forced expiratory volume; FVC, forced vital capacity; ICS, inhaled corticosteroids; LAR, late asthmatic response; PBE, peripheral blood eosinophils; PEF, peak expiratory flow.

Note: Bold represents the asthma population and investigated subgroups.

asthma exacerbation rate in 52 weeks, which was reduced in the tezepelumab treatment group compared with placebo. Secondary end points were the change from baseline in the prebronchodilator (pre-BD) forced expiratory volume in 1 second (FEV₁), 6-item asthma control questionnaire (ACQ6) score, asthma quality of life questionnaire (AQLQ), and asthma symptom diary score. Lung function, asthma control, asthma-related quality of life, and daily asthma symptoms improved more in the tezepelumab vs placebo group. Outcomes were further analyzed relative to the presence of eosinophils. Although tezepelumab was most effective in those patients with at least 300 eosinophils per microliter, corresponding to T2-high asthma, asthma outcomes were also improved in those with less than 300 or 150 eosinophils per microliter relative to the placebo group.⁴⁰ This is consistent with an effect in T2-low asthma. Based on the simultaneous reduction of blood eosinophils, FeNO and IgE, the authors concluded that blockade of TSLP suppresses multiple type 2 inflammatory pathways, resulting in a broader physiological effect than targeting individual cytokines. Although tezepelumab is the first biologic consistently having promising results in T2-low asthma, it would be interesting to further investigate its effect in a broader T2-low asthma population. The authors stated that relevant patient subgroups such as smokers and those with certain coexisting conditions were excluded from the trial. Yet, smokers with asthma often feature neutrophilic, T2-low inflammation,²⁷ and the assessment of the efficacy of tezepelumab would be particularly relevant in this patient group.

Interleukin 33

The mAb itepekimab blocks IL-33, one of the alarmins secreted by airway epithelial cells upon encountering environmental triggers (Fig. 1 and 2). Its administration reduced airway inflammation and tissue damage in a murine model of house dust mite-mediated lung inflammation,⁴¹ suggesting IL-33 might provide a suitable therapeutic target in chronic airway diseases. There were 2 phase I clinical trials which revealed that administration of itepekimab was well tolerated in both healthy adults and those with moderate asthma (Table 2).⁴² Serum levels of IL-33 increased in the itepekimab treatment groups, suggesting that the antibody stabilized the otherwise short-lived cytokine and that detected IL-33 predominantly represented the drug-cytokine complex. In a phase II proof-of-concept study, the administration of itepekimab reduced the proportion of patients with loss of asthma control events and improved pre-BD FEV₁ relative to placebo (Table 2), although the effect on pre-BD FEV₁ was greater in patients with baseline eosinophils greater than or equal to 300 cells/ μ L and numerically not different to the effect of dupilumab.⁴³ The drug was well tolerated over the treatment period of 12 weeks. Although itepekimab does not seem superior to treatment with dupilumab in T2-high asthma, it could be useful in T2-low asthma. Further studies with additional outcome measures would be informative in assessing the suitability of this drug in treating T2-low asthma.

Interleukin 33 Receptor

Astegolimab is a mAb directed against the IL-33 receptor. Suppression of Tumorigenicity (Fig. 2). A double-blind, placebo-controlled, dose-ranging study revealed that astegolimab reduced the asthma exacerbation rate in patients with uncontrolled severe asthma (Table 2).⁴⁴ This effect was comparable in patients with high (>300 cells/ μ L) and low numbers of blood eosinophils. Pre-BD FEV₁ did not change remarkably over the treatment period of 52 weeks, although the group receiving the highest dose was found to have the greatest numerical difference. Interestingly, FEV₁ improvements seemed higher in patients with less than 150 eosinophils per microliter. A

small improvement of the asthma-related quality of life was observed in the highest treatment dose group. Together, these results imply that astegolimab could be useful in treating T2-low asthma.

Targeting Inflammatory Mediators of Neutrophilic Asthma

Tumor Necrosis Factor Alpha

Tumor necrosis factor alpha is a pleiotropic cytokine with pro- and anti-inflammatory functions. It mainly signals through the 2 receptors, TNF receptor 1 (TNFR1) and TNFR2. TNF is primarily produced by activated monocytes and macrophages in response to pathogens and to a lesser extent by T_H1 and other cells of the innate and adaptive immune systems (Fig. 1).^{45–47} Several properties of TNF may be directly or indirectly associated with the pathogenesis or clinical features of asthma. It stimulates chemotaxis of eosinophils and neutrophils, plays a role in T cell activation, can induce airway hyper-responsiveness (AHR), and has been associated with corticosteroid resistance.⁴⁸

Immunotherapies targeting TNF are effective in other chronic inflammatory diseases, such as rheumatoid arthritis and inflammatory bowel disease,⁴⁹ and its implication in asthma suggested it as a promising target. Several clinical trials investigated the effect of therapeutic TNF blockade in asthma, yet their outcomes varied in a population- and drug-dependent manner (Fig. 2, Table 2). At least 3 studies investigated the effect of etanercept, a human recombinant TNF receptor II: IgG Fc fusion protein. In 2 small severe asthma populations, administration of etanercept for 10 and 12 weeks reduced AHR, improved asthma symptom control, quality of life, and lung function, and reduced expression levels of membrane-bound TNF (mTNF) on peripheral blood monocytes.^{50,51} In a larger cohort of patients with moderate-to-severe asthma, no beneficial effects were reported, although the drug was well tolerated.⁵² These results suggest a role for TNF particularly in severe asthma and that only a small population may benefit from such treatment, requiring appropriate screening or phenotyping. Although increased TNF levels on peripheral blood monocytes (PBMcs) predicted a response to etanercept in Berry's trial,⁵¹ a more recent study revealed that mTNF was largely undetectable on circulating monocytes regardless of asthma severity.⁵³ However, mTNF expression levels were elevated on intermediate vs nonclassical monocytes and appeared numerically higher compared with classical monocytes, suggesting that Berry's observation could be subset related. Furthermore, soluble TNF receptor levels were increased in severe vs non-severe asthma, supporting a role of dysregulated TNF signaling in severe asthma.⁵⁴

Other studies investigated the effect of the anti-TNF mAb infliximab in asthma. Administration from 3 to 36 months improved asthma control, reduced exacerbations, and spared oral corticosteroid use in a small cohort of adults with uncontrolled, severe asthma.⁵⁵ A small study of adults with moderate-to-severe asthma demonstrated the safety of infliximab over a 6-week treatment period. Although a decrease in patients with exacerbations was reported, together with a reduction of sputum cytokines including TNF, the primary outcome (change in morning peak expiratory flow [PEF] from baseline at week 8) was not met.⁵⁶ These findings again suggest that TNF blockade is most beneficial in patients with severe asthma and highlights further that a positive study outcome requires a carefully defined clinically useful end point that aligns with the treatment effect.

Administration of the anti-TNF antibody golimumab in a large severe asthma cohort demonstrated an unfavorable risk-benefit profile. While no improvement in asthma outcomes was observed, some patients developed malignancies, requiring the treatment to be terminated early.⁵⁷ An increased risk of malignancies has also been observed in rheumatoid arthritis treated with infliximab and adalimumab, another anti-TNF antibody.⁵⁸ The occurrence of these

adverse effects was dose-dependent, suggesting incomplete blockade and residual TNF signaling may be beneficial and crucial for tumor suppression.

Transcriptomic studies revealed that dysregulation of the TNF signaling pathway is associated with neutrophilic airway inflammation.^{59,60} Yet, no clinical trials investigated the efficacy of anti-TNF mAbs in a neutrophilic asthma cohort, although targeting the correct population is critical for a meaningful study outcome. To circumvent the occurrence of severe adverse effects associated with global TNF blockade, novel therapeutics have been suggested. Agonists or antagonists that selectively inhibit or induce a specific TNF receptor could provide a safer approach in treating autoimmune diseases.^{61–63} A recent study revealed that increased soluble TNFR2 levels in sputum were associated with neutrophilic airway inflammation, severe asthma, a history of frequent exacerbations, reduced lung function, and worse asthma control.⁵⁴ With increased understanding of the role of TNFR2-induced signaling in the context of asthma, this receptor in particular could provide a suitable target for agonists or antagonists.

Interleukin 1 Beta

IL-1 β is thought to be one of the key inflammatory mediators in asthma (Fig 1). In the airways, IL-1 β is released in response to triggers, such as allergens, smoke, and pathogens.⁶⁴ Gene expression and protein levels of IL-1 β are increased in the airways in neutrophilic asthma,^{59,65} and in a mouse model of viral-induced asthma exacerbation, it induced lung neutrophilia.^{66,67} IL-1 signaling has been found to be associated with airway remodeling, smooth muscle activation, airway hyperresponsiveness, and mucus secretion.⁶⁴ Transcriptomic studies revealed an up-regulation of the IL-1 receptor family in severe asthma.⁶⁸ Sputum IL-1 β is associated with frequent exacerbations in chronic obstructive pulmonary disease (COPD) and was suggested as a predictor of future exacerbations.⁶⁹ Considering the overlap of some features in neutrophilic asthma and COPD, IL-1 β could also be a useful biomarker for T2-low, neutrophilic asthma.

The effect of biologics targeting IL-1 β has been investigated in several inflammatory conditions, including asthma and COPD (Fig 2, Table 2). The monoclonal anti-IL-1 β antibody canakinumab is approved for the treatment of periodic fever syndromes and systemic juvenile idiopathic arthritis and is currently trialed in patients with resectable non-small cell lung cancer.⁷⁰ A small study revealed that the administration of canakinumab was safe and well tolerated in patients with mild asthma. It reduced free IL-1 β and attenuated the late asthma response. The authors suggested that 3 to 4 doses per year would be sufficient to reduce IL-1 β for a prolonged period.⁷¹ Despite these promising preliminary results, no further clinical trials were conducted to investigate its effect on asthma outcomes. In COPD, administration of canakinumab for 45 weeks was found to have no clinical efficacy (NCT00581945).⁷²

The IL-1 receptor antagonist anakinra effectively diminished neutrophilic airway inflammation and reduced lipopolysaccharide (LPS)-induced secretion of IL-1 β , IL-6, and IL-8 in healthy volunteers without an occurrence of serious adverse events (Table 2).⁷³ The effected cytokines are all recognized mediators of neutrophilic asthma; thus, administration of anakinra could be particularly beneficial in T2-low, neutrophilic asthma. A clinical trial has been designed to investigate its efficacy in adults with mild asthma. Unfortunately, the study was withdrawn because of coronavirus disease 2019-related risks for the participants (NCT03513458).⁷⁴

Interleukin 17

IL-17 is a key inflammatory mediator produced by T_H17 cells which has been associated with airway hyperresponsiveness and asthma exacerbations (Fig 1).^{75–77} IL-17 contributes to airway

neutrophilia and is further associated with asthma severity and steroid resistance.^{75,77,78} IL-17 has been found to directly induce steroid insensitivity in PBMCs⁷⁹ and neutrophils.⁸⁰

Its association with AHR, airway neutrophilia, asthma exacerbations, and steroid resistance suggests IL-17 as a suitable therapeutic target. A randomized, double-blind, placebo-controlled trial investigated the effect of the human anti-IL-17 mAb brodalumab on asthma control and lung function; however, it did not reveal any beneficial effects in the total study cohort of participants with moderate-to-severe asthma⁸¹ (Fig 2, Table 2). Yet, asthma control improved in those participants with high bronchodilator reversibility (FEV₁ $\geq$ 20%) at baseline, suggesting brodalumab is effective when administered to the correct target population. The authors suggested further investigation of this subpopulation with asthma exacerbations as a more appropriate end point. Prescreening for or subgroup analyses of participants with elevated sputum IL-17 or neutrophils could also be useful to identify responders.

Interleukin 6

IL-6 is a pleiotropic cytokine with pro- and anti-inflammatory properties (Fig 1). It is released as a warning signal in response to pathogenic stimuli and tissue damage.⁸² Furthermore, it is involved in the acute-phase response by inducing mediators, such as C-reactive protein and serum amyloid A. IL-6 also regulates the differentiation of naive T cells into T_H17 cells and thereby contributes to neutrophilic inflammation.^{83,84} It further inhibits transforming growth factor beta (TGF- β)-induced Treg differentiation.⁸⁵ Thus, IL-6 could negatively affect inflammation resolution. Dysregulation of the T_H17-Treg balance is thought to play a role in the pathogenesis of autoimmune diseases and chronic inflammation.^{82,86}

IL-6 is produced by several immune cells, including T cells, monocytes, macrophages, and neutrophils.^{87,88} Classical and intermediate monocytes have been reported to secrete high levels of IL-6 in response to LPS.⁸⁹ Classical monocytes are increased in the airways of asthma patients with neutrophilic airway inflammation,⁹⁰ implying classical monocytes could be a possible source of elevated IL-6 in neutrophilic asthma and targeting IL-6 could potentially be beneficial in this subgroup.

The human anti-IL-6 mAb tocilizumab is used in rheumatoid and juvenile idiopathic arthritis⁸² and has further been found to improve outcomes and survival in critically ill patients with coronavirus disease 2019.^{91,92} A randomized, double-blind, placebo-controlled study investigated the effect of a single dose of tocilizumab on the late asthmatic response (primary end point) and early asthmatic response (secondary end point) after allergen challenge based on lung function outcomes in adults with mild, stable allergic asthma (Fig 2, Table 1).³² The study end point was not met, which, according to the authors, could possibly be explained by an insufficient drug concentration in the lung, inappropriate outcome measures, an unsuitable study population, and lastly, an unfitting drug type and target. Revez's trial was designed to assess the efficacy of the drug to suppress allergic asthma responses. It would be interesting to investigate the effect of tocilizumab on other parameters, such as asthma control or asthma-related quality of life particularly in individuals with T2-low, neutrophilic asthma with increased airway classical monocytes. Considering a half-life of 13 days, fortnightly doses over a longer time period may be necessary to allow meaningful outcome measures.

Clazakizumab, another anti-IL-6 mAb, is part of PrecISE, a clinical trial investigating Precision Interventions for Severe and/or Exacerbation prone Asthma.^{93,94} This study uses a biomarker-guided approach to identify patient groups most likely to respond to 1 of 6 predefined interventions. The target population of clazakizumab is defined as a subgroup featuring obesity and metabolic dysfunction. Plasma levels of IL-6 with a threshold of more than or equal to 3.1 pg/

μL serve as a predictive biomarker, and high-sensitivity C-reactive protein will be used as a monitoring biomarker.

Granulocyte-Macrophage Colony-Stimulating Factor

GM-CSF was originally classified as a hematopoietic growth factor that was later identified as a central mediator of tissue inflammation.⁹⁵ It coordinates immune responses and crosstalk between cells of the myeloid and the lymphoid lineage and is further implicated in several chronic inflammatory diseases, including asthma.⁹⁶ GM-CSF production by lung epithelial cells (Fig 1) is elevated in asthma,⁹⁷ and increased protein levels are found in sputum, bronchoalveolar lavage fluid, and bronchial tissues of both those with asthma and COPD.^{98,99} GM-CSF has anti-apoptotic effects on eosinophils and neutrophils^{100,101} and, thus, could contribute to the higher abundance of these cells in asthma. Murine studies demonstrated a critical role for GM-CSF in orchestrating neutrophil-dendritic cell- $\text{T}_\text{H}17$ -dependent chronic lung diseases,¹⁰² suggesting it as a potential target in T2-low, $\text{T}_\text{H}17$ -high asthma.

A phase 2, randomized, placebo-controlled trial investigated the efficacy and safety of an anti-GM-CSF antibody in patients with inadequately controlled asthma (Fig 2, Table 2).¹⁰³ Participants of the treatment group received 7 doses of lenzilumab over a course of 20 weeks. Although the drug was well tolerated, the primary end point (changes in pre-BD FEV_1) was only met in an eosinophilic subpopulation, but not the entire cohort. The authors suggested that higher or more frequent doses of lenzilumab might be required to neutralize the anti-apoptotic effect of GM-CSF on neutrophils, potentially explaining the lack of efficacy in non-eosinophilic asthma. It is also unclear how many of the non-eosinophilic participants featured paucigranulocytic asthma, in which anti-GM-CSF antibodies might be less beneficial. At some visits, FEV_1 was remarkably improved in those with severe airway obstruction (pre-BD $\text{FEV}_1 \leq 50\%$ predicted) and highly reversible FEV_1 ($\geq 20\%$) at baseline. A critical limitation of the study was that GM-CSF protein levels were not measured, neither in blood nor in sputum, although increased GM-CSF levels could predict a response to its mAb. While the end points of this study were suitable, further improvements could be achieved through adjusted treatment doses or frequencies and a more suitable selection of the study cohort. To further investigate the efficacy of lenzilumab particularly in T2-low asthma, participants could be screened for neutrophilic airway inflammation and increased levels of blood or sputum GM-CSF.

Interleukin 23

IL-23 is a proinflammatory cytokine that plays a crucial role in $\text{T}_\text{H}17$ regulation (Fig 1). It is mainly produced by activated antigen-presenting cells, such as tissue macrophages and dendritic cells. IL-23 is required for the differentiation of naive T cells into $\text{T}_\text{H}17$ cells and is further involved in $\text{T}_\text{H}17$ maintenance, stabilization, and expansion. IL-23 prolongs the secretion of $\text{T}_\text{H}17$ cytokines, including IL-17 and GM-CSF, and, thus, contributes to neutrophilic inflammation in asthma.^{83,84} Targeting IL-23 could be particularly beneficial in T2-low, $\text{T}_\text{H}17$ -high, neutrophilic asthma.

In a phase II, randomized, double-blind, placebo-controlled study, risankizumab, an anti-IL-12-p19 mAb, was administered to adults with severe asthma (Fig 2, Table 2).¹⁰⁴ The primary outcome was the time-to-first asthma worsening ($\geq 30\%$ fall in morning PEF or increase of $\geq 50\%$ and at least 4 puffs of rescue medication for at least 2 consecutive days, an increase in the ACQ5 score by ≥ 0.75 , or a severe asthma exacerbation). The primary end point was not met, with time-to-first and rate of asthma worsening favoring placebo. However, the investigated study population possibly consisted of a mix of participants with T2-high and T2-low asthma with no further

investigation of their IL-23 or IL-17A signature. Median sputum cell proportions suggest that risankizumab recipients predominantly featured eosinophilic airway inflammation (4.5% [0.7–16.4]) rather than neutrophilic airway inflammation (60% [36–76]), and further investigation of a more suitable target population based on sputum neutrophils and $\text{T}_\text{H}17$ inflammatory markers including IL-23 and IL-17A could be considered.

Conclusion

T2-low asthma is a condition with an unmet need for appropriate medication. Although multiple clinical trials investigated the efficacy of novel and existing mAbs, the predominant focus often remains on eosinophilic, T2-high asthma. In this review, we discussed previous trials with respect to the observed study outcomes and the suitability of the selected end points and study populations. Some of the more recent studies demonstrated beneficial effects of mAb administration in T2-low asthma for the first time; however, a better understanding and classification of T2-low subtypes are desirable to better identify the most suitable biologic.

Alarmins provide novel therapeutic targets in asthma. Monoclonal antibodies directed against alarmins bear the potential to interrupt the inflammatory cascade before the involvement of T cells or innate lymphoid cells and, thus, should theoretically be effective irrespective of the type 2 status. Indeed, the 3 introduced mAbs tezepelumab (anti-TSLP),⁴⁰ itepekimab (anti-IL-33),⁴³ and astegolimab (anti-IL-33R)⁴⁴ are the first biologics with beneficial effects in both T2-high and T2-low asthma. Yet, further studies are necessary to understand whether these 3 drugs have differential effects in T2-low asthma and its different subtypes.

A range of previously unsuccessful biologics bear the potential to be useful, when administered to the correct target population. Yet, study participants were often selected based on asthma severity without further classification of inflammatory subtypes, and it is not surprising that “one-size-fits-all” approaches failed to achieve positive outcomes. Of the discussed studies, 5 performed predefined subgroup analyses and 4 of those observed better responses in at least 1 subgroup.^{40,43,44,81,103} Only 1 study was biomarker guided, and the selected cohort responded well to the marker-directed treatment.⁵¹ These observations support the concept of personalized and precision medicine in asthma. Nevertheless, useful biomarkers to identify appropriate T2-low target populations and T2-low specific end points are lacking. Owing to the heterogeneous nature of T2-low asthma, it will be challenging to identify general end points for T2-low asthma, but with increased understanding, more subtype-specific end points are likely to emerge.

There are several promising leads at present. The PrecISE trial will investigate the efficacy of the c-KIT inhibitor imatinib in T2-low asthma with blood eosinophil levels below $300/\mu\text{L}$ as a predictive biomarker and serum tryptase and urinary PGD2 as exploratory monitoring biomarkers.^{93,94} Changes in further secondary biomarkers will be assessed to investigate whether they correlate with improvements in clinical asthma outcomes. This medication has the potential to be beneficial in patients where increased mast cell activity is implicated in the pathogenesis of asthma, and the study design may identify end points that relate to T2-low biology. Other potential T2-low suitable end points could be the reduction of increased immune cells in the airways (although a reduction of neutrophils in neutrophilic asthma did not result in improved asthma outcomes¹⁰⁵) or changes in the levels of inflammatory markers (eg, the targeted cytokine).

There is an additional limitation in T2-low asthma. In T2-high asthma, studies with dupilumab, omalizumab, and mepolizumab have shown that differences between the active treatment and placebo arms are largely driven by increased exacerbations in the placebo group which correlates with elevated blood eosinophils. At

present, there is no equivalent biomarker for T2-low asthma that predicts exacerbations. This may further reduce the likelihood of success in conducting trials with T2-low populations. To address this issue, it will be necessary to identify T2-low biomarkers that are related to asthma exacerbations. The non-T2 markers sTNFR2 and IL-6 are increased in sputum at asthma exacerbation,¹⁰⁶ and increased sputum sTNFR2 is associated with more frequent asthma exacerbations.⁵⁴ With increased understanding of T2-low asthma subtypes, suitable markers might be identified.

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