CLINICAL INVESTIGATION

MESOS: considerations in designing a mechanistic study for a biologic used to treat asthma

Eosinophils are key effector cells in asthma-associated airway inflammation and remodeling; IL-13 is involved in regulating eosinophil activity. Tralokinumab, currently in Phase III clinical development for patients with severe uncontrolled asthma, is an investigational fully human monoclonal antibody designed to inhibit IL-13. In Phase II studies, tralokinumab improved lung function and had other clinical benefits in those patients with asthma who had an upregulated IL-13 axis. In a subgroup of patients that underwent quantitative computed tomography, there were improvements in airway morphometry, suggestive of a possible effect upon remodeling. The Phase II MESOS study (NCT02449473) aims to better understand the mechanism of action of tralokinumab in improving asthma control, by investigating tralokinumab effects on eosinophil-driven inflammation and airway remodeling.

Keywords: airway remodeling • asthma • biologic • clinical trial design • eosinophil • IL-13 • inflammation • tralokinumab

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Asthma is one of the most common chronic respiratory diseases, affecting approximately 300 million people worldwide [1]. The pathophysiology of asthma is complex and variable among patients. Primarily, asthma is an airway inflammatory disease. Airway inflammation is typically characterized by infiltration of the submucosa of airway walls by eosinophils, neutrophils, lymphocytes (especially T-helper 2 type [Th2]), mast cells, macrophages and dendritic cells [2–4] and these cells, as well as airway epithelial cells, release a variety of inflammatory mediators [5,6]. Other characteristic features of asthma pathophysiology are histopathologic changes in the airways of patients with asthma [7], which contribute to the clinical features of the disease; airway hyper-responsiveness [8], cough with mucus production [9] and progressive airway obstruction [10]. A remarkable feature of asthma is that the clinical manifestations can vary widely among patients, with 5–10% of patients with asthma displaying severe, persistent disease [11]. Emerging evidence strongly supports heterogeneity in asthma with the presence of distinctive inflammatory phenotypes [12]; there is currently interest in whether observed variability in clinical response to currently available treatments for asthma may also be related, in part, to these distinctive inflammatory phenotypes.

Eosinophils are considered key effector cells of both airway inflammation and the airway remodeling process in asthma [13]. In particular, eosinophils release granule proteins [6], cysteinyl leukotrienes [14] and reactive oxygen species [15], which can cause tissue damage. Eosinophil activity is highly regulated by Th2 inflammatory cytokines including IL-5 and IL-13 [16,17]. After exposure to inhaled allergens in sensitized asthma patients there is an increase in bone marrow eosinophil progenitor cells and the percent of bone marrow cells that are IL-5 mRNA$^+$ within 24 h [18]. These changes are associated with an increase in blood eosinophils, indicating that IL-5 plays an important role in mediating bone marrow differentiation and maturation of eosinophils in atopic disease [18]. IL-5 is

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also known to be involved in mediating eosinophil migration to tissue sites such as the lung and in the prevention of eosinophil apoptosis [17,19]. IL-13 is also known to prolong the survival of eosinophils [20] and, furthermore, induces production of CCL11, the eosinophil chemotaxin, by airway epithelial cells [21,22]. In addition, IL-13 stimulates endothelial cell-production of VCAM-1 and P-selectin [23,24], which promote eosinophil adherence to endothelial cells. These effects of IL-13 favor the directed migration of eosinophils to the airways in asthma.

Despite the clinical and pathophysiologic heterogeneity of asthma, international guidelines are consistent in recommending the use of inhaled corticosteroids (ICS) as the standard approach to controlling airway inflammation in all patients with persistent asthma, due to the demonstrated efficacy of ICS [25]. However, it is known that a substantial minority of patients will continue to experience symptoms or exacerbations, despite use of medium-to-high dose ICS and other controller therapies, or long-term oral corticosteroids (OCS) [26]. Consequently, more effective therapies are needed for adequate control of airway inflammation in asthma. The identification of cytokines involved in the pathophysiology of asthma has enabled the development of biologic agents, as novel precise therapies, directed against the components of the underlying inflammatory pathway [27–29].

Of the biologic agents currently under development, those closest to regulatory approval (mepolizumab, reslizumab and benralizumab) are directed against IL-5-driven airway inflammation [28–30]. The anti-IL-5 monoclonal antibodies fall into two separate categories. Benralizumab binds directly to the IL-5 receptor on eosinophils resulting in eosinophil depletion via antibody-dependent cell-mediated cytotoxicity [31], whereas mepolizumab and reslizumab bind to IL-5 thus preventing it from interacting with its receptors and reducing eosinophils in blood, tissue and sputum [32,33]. Studies that have used bone marrow and bronchoscopy biopsies to investigate eosinophil dynamics after treatment with anti-IL-5 agents have consistently shown that these biologics induce partial maturational arrest of the eosinophil lineage in the bone marrow, reduce blood eosinophils and decrease submucosal airway eosinophil infiltration [28,34–37]. These findings support the hypothesis that anti-IL-5 biologics reduce airway inflammation by depleting eosinophil production.

Another group of biologics being developed for use in severe asthma are lebrikizumab, a humanized monoclonal antibody, and tralokinumab, a fully human monoclonal antibody, targeting IL-13 [38,39]. Tralokinumab differs from lebrikizumab in that it blocks IL-13 interaction with both the IL-13Rα1 and IL-13Rα2 receptors [38]; lebrikizumab blocks only the interaction of IL-13 with IL-4Rα [39]. A signaling role for IL-13Rα2 remains controversial. It may act as a decoy receptor, because its 17 amino acid cytoplasmic tail contains no known signaling motifs and it does not, as yet, appear to form complexes with other cell surface proteins capable of signaling [40–43]. Studies with small interfering RNAs [67] or oligonucelotides directed against IL-13Ra2 [68] have suggested a signaling role for IL-13Ra2. More recently, preliminary studies may suggest that under some circumstances, signaling activity may be detected in cellular systems relevant to the lung [69]. Unlike anti-IL-5 monoclonal antibodies, therapeutic use of both lebrikizumab and tralokinumab in patients with severe asthma has been associated with an increase in blood eosinophils [27,44,45]. This observation is postulated to be due to a reduction in eosinophil–endothelial adhesion and airway-derived chemokines resulting from IL-13 blockade. Preclinical evidence studies of tralokinumab have shown a reduction in IL-13 mediated CCL11 production and bronchoalveolar lavage eosinophilia [38,46], supporting this hypothesis.

Tralokinumab is currently in Phase III trials (STRATOS 1 [NCT02161757] [70]; STRATOS 2 [NCT02194699] [71]; TROPOS [NCT02281357] [72]) in patients with severe uncontrolled asthma. To support future development of tralokinumab, a fuller understanding of its effects on airway inflammation is needed. Consequently, the MESOS study has been designed to enable biopsy of airway walls in patients treated with tralokinumab. The hypothesis of this study is that tralokinumab treatment will decrease eosinophil submucosal airway infiltration by interfering with eosinophil trafficking to the lung. It is expected that blood eosinophils will increase and that there will be no effect of tralokinumab on bone marrow production of eosinophils.

Study design

This is a Phase II, randomized, double-blind, parallel group, multicenter, placebo-controlled study in patients with uncontrolled asthma requiring continuous treatment with ICS (≥250 mcg fluticasone dry powder formulation equivalents total daily dose), with or without other asthma controllers (NCT02449473) [73]. The aim of the MESOS study is to further understand the mechanism of effect of tralokinumab in improving asthma outcomes. The study will primarily investigate the effects of tralokinumab both on airway submucosal eosinophil infiltration and on the numbers of activated and nonactivated eosinophils in the blood, sputum and airway submucosa of adults with

asthma inadequately controlled on ICS. As eosinophil movement is driven by organ-specific production of molecules such as CCL11 [21,22], which is induced by IL-13, VCAM and P-selectin [23,24], it is expected that eosinophil migration across endothelial surfaces will be directed to the airways in asthma patients. Therefore, this study will not investigate eosinophil transport across endothelial surfaces in other organs. Exploratory objectives include an evaluation of the effect of tralokinumab on large and small airway structure and function in the study population.

Potentially eligible patients will complete a 4-week run-in period to assess suitability for randomization. Following confirmation of eligibility, patients will be supplied with an electronic handheld peak expiratory flow meter for monitoring lung function at home and an eDiary for recording asthma symptoms and completing relevant questionnaires. Patients who meet the eligibility criteria at week 0 will be randomized to treatment. During the 12-week treatment period, tralokinumab 300 mg (150 mg/ml) or placebo, will be administered subcutaneously using two accessorized 1 ml prefilled syringes, every 2 weeks (Q2W), with an end-of-treatment (EOT) visit at week 12 (Figure 1). There will be a post-treatment follow-up period of 14 weeks following the EOT visit, with a follow-up visit over the telephone at week 16. For female patients of child-bearing potential, there will be a second follow-up visit onsite at week 26. During the run-in and treatment period, the patients will continue receiving their currently prescribed ICS and any other additional asthma controller medication.

This study is sponsored by AstraZeneca. Written informed consent will be obtained from all patients before initiation into the study. The study will comply with the Declaration of Helsinki, the International Conference on Harmonisation/Good Clinical Practice guidelines, applicable regulatory requirements and the AstraZeneca policy on Bioethics and Human Biological Samples.

Outcome measures

Efficacy end points

The primary end point is the change, expressed as a ratio, from baseline to week 12 in airway submucosal eosinophils per mm^2 from bronchoscopic biopsies. Bronchoscopy biopsies will be handled in standard histologic fashion by fixing them immediately in 10% neutral buffered formalin and then imbedding them in paraffin. Paraffin sections will be obtained for light microscopic analysis, and eosinophil counts will be performed on hematoxylin and eosin stained slides. The secondary end points are the changes, expressed as a ratio, from baseline to week 12 in absolute blood and differential sputum eosinophil counts, and blood and sputum eosinophil cationic protein concentrations.

Exploratory end points

The MESOS study will also assess a number of exploratory outcomes (Box 1). These exploratory objectives are grouped around four main themes: large airway structure and function; small airway structure and function; the relationship between inflammation control, and asthma symptoms; and biomarkers. Large airway structure and function will be evaluated using computed tomography (CT) and spirometric measures of large airway obstruction (e.g., FEV_1, FVC and FEV_1/FVC), respectively. Novel CT techniques to be used in this study will allow the airway wall area and airway lumen area to be measured in segmental and subsegmental airways, including the same bronchial segments from which bronchoscopy biopsies have been obtained. The impact of lumen area changes on the estimated airway resistance will also be calculated. Small airway structure and function will be assessed using indirect CT and physiologic measures. As small airways cannot be directly visualized by CT with current technology, the ratio of lung density measures obtained from inspiratory and expiratory CT scans can be used to reflect gas trapping and thus small airway obstruction. Plethysmography will measure lung volumes, and a number of innovative physiologic measures, such as impulse oscillometry and nitrogen wash-out, will be used as complimentary indicators of ventilation heterogeneity and small airway physiology. The study will incorporate standard measures of asthma symptoms to allow a correlation to be made between changes in airway inflammation and asthma control in individual patients. In addition, methacholine challenge will be used to measure bronchial hyper-responsiveness.

A variety of measures will be incorporated into these studies to explore the value of newer biomarkers. These newer biomarkers are clustered into those exploring the effects on inflammatory cells (major basic protein, CD3, CD4, CD8, neutrophil elastase, mast cell tryptase, mast cell chymase and macrophage CD68), mesenchymal cells and vessels (smooth muscle actin, CD34, collagen 1 and endothelial EN4), epithelium (mucin 5AC, involucrin, cytokeratin, ecadherin, clara cell protein 16 and protein 63), matrix (tenascin, collagen 4, lumican and fibronectin) and remodeling activation (periostin, TGF-β and caspase 3).

Safety/tolerability

Safety end points will be assessed by adverse events (AEs), serious adverse events (SAEs) and laboratory tests, including vital signs, electrocardiogram, clini-

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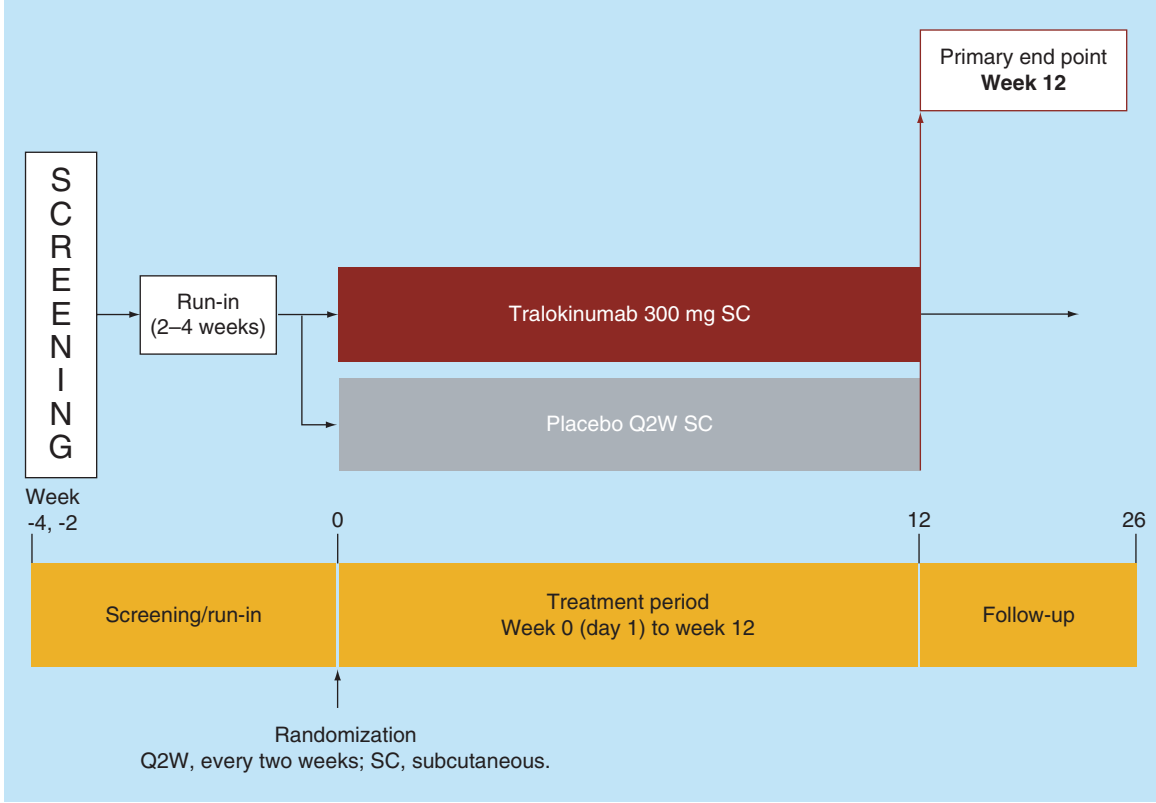

Figure 1. MESOS design.

cal chemistry/hematology/urinalysis parameters and physical examination. AEs will be collected from the time the patient signs the informed consent form, throughout the treatment period, until the first follow-up visit at week 16. Any unresolved AEs at the first follow-up visit will be followed up by the Investigator for as long as they are medically indicated without any further recording in the electronic case report form (eCRF). All SAEs will be reported, whether or not they are considered causally related to the investigational drug or the study procedure, and all SAEs will be recorded in the eCRF. Potential immunogenicity will also be assessed by the incidence rate of positive antidrug antibodies and characterization of their neutralizing potential at week 0 and week 12.

Inclusion & exclusion criteria

Patients will be assessed for their eligibility at visit 1, according to their asthma disease state, the requisite level of severity based on maintenance medication and exacerbation history. The key inclusion and exclusion criteria are shown in Box 2.

Sample size estimate

The sample size is based on the primary end point; change from baseline to week 12 in airway submucosal eosinophils. The study is powered to show a reduction in airway submucosal eosinophils, from baseline to week 12, for tralokinumab compared with placebo in the overall study population. As the assumed deviation of the log values in the two treatment groups are 1.62 and 1.82, it is estimated that 31 patients in each treatment arm will be sufficient to achieve at least 80% power to detect a 3.5-fold difference compared with placebo using a two-sided test at 5% significance level. The assumptions are based on the change in number of eosinophils per mm^2 of subepithelial tissue in bronchial biopsies in previous studies [28,47]. To account for the fact that a proportion of the patients will not have an evaluable primary end point value due to failed biopsies, 40 patients per treatment arm will be randomized.

Conclusion & discussion

Airway inflammation and airway remodeling are key features of asthma. Control of asthma symptoms and prevention of asthma exacerbations may be linked to controlling airway inflammation [48]. A key component of the inflammatory response in asthma is eosinophil submucosal airway infiltration [4,13]. The standard of care for persistent asthma has been regular use of ICS [25]. Although some studies have shown that ICS treatment can reduce airway eosinophil infiltration [49–52], the evidence is not consistent [47]. Newer biologic

agents being developed include monoclonal antibodies directed against IL-5; these agents have been shown to deplete the bone marrow of eosinophil progenitors with secondary decreases in blood and airway eosinophils [28,34,36]. Biologic agents are also being developed which are directed against IL-13. Interestingly, early studies with lebrikizumab and tralokinumab have shown that these biologics are associated with an increase in blood eosinophils [27,44,45]. The hypothesis of the MESOS study is that tralokinumab acts as an anti-inflammatory agent in asthma, but in a fundamentally different way to the anti-IL-5 biologics. It increases blood eosinophils because it interferes with eosinophil trafficking from the blood to the affected tissues, such as the airways in asthma. It is expected that bronchoscopy biopsies of airways in patients with asthma treated with tralokinumab will show reduced submucosal airway infiltration, along with increased blood eosinophils.

IL-13 is a central mediator of asthma pathophysiology with effects including promotion of IgE production [53], increased airway hyper-responsiveness [54], mucus production and smooth-muscle prolifera-

Box 1. Exploratory outcomes.

Outcomes & assessments

- Other biomarkers of airway inflammation
  - **Change from baseline to week 12 in:**
    - The numbers of inflammatory cell counts ($CD3^+$, $CD4^+$, $CD8^+$ lymphocytes, neutrophils, macrophages and mast cells) per mm^2 of epithelium, bronchial submucosa (lamina propria) and airway smooth-muscle bundle, from bronchoscopic biopsies (expressed as a ratio)
    - Differential and total sputum cell counts
    - Soluble biomarkers including, but not limited to: histamine, leukotrienes, IL-13 and IL-5 in the sputum, and DPP-4, periostin, CCL2, CCL11, CCL13, CCL17, IL-33, STAT6, IL-13Rα2, CLCA1 and SERPINB2 in the serum, as well as other standard biomarkers of tissue destruction
    - Nasosorption (biomarkers to be determined)
    - Blood total IgE
    - Fractional exhaled nitric oxide, measured by an electrochemical sensor (NIOX®, Aerocrine)
- Large airway remodeling
  - **Change from baseline to week 12 in:**
    - Airway epithelial cell integrity, lamina reticularis and reticular basement membrane thickening, mucus glands, MUC5A and deposition of periostin in the basement membrane
    - Biomarkers of tissue remodeling and/or destruction, which may include, but are not limited to, α-SMA and collagen type IV, fibronectin, lumican, tenascin and TGF-β, as well as epithelial differentiation markers
    - Large airway dimensions and estimated airway resistance (computed tomography)
    - Airway epithelial gene expression
- Small airway remodeling
  - **Change from baseline to week 12 in:**
    - Resistance at 5–20 Hz and AXH (impulse oscillometry) [59]
    - Sacin (multiple breath washout) [60]
    - Computed tomography measures of small airway disease; air trapping (expressed as a percentage of the lung with expiratory density less than -856 HU and as expiratory-to-inspiratory ratio of mean lung density on computed tomography) [61], and parametric response mapping [62]
- Asthma symptoms and other asthma control metrics
  - **Change from baseline to week 12 in:**
    - Daily asthma symptom scores (combined daytime and nighttime score)
    - Rescue medication use (as recorded in the Asthma Daily Diary)
    - Home peak expiratory flow (morning and evening)
    - Number of nighttime awakenings due to asthma
    - Asthma Control Questionnaire-6 scores [63,64]
- Lung function and bronchial hyper-responsiveness
  - **Change from baseline to week 12 in:**
    - Forced expiratory volume in 1 s (FEV_1), forced vital capacity and forced expiratory flow between 25–75% of the forced vital capacity [65]
    - PC_{20} (methacholine concentration causing 20% drop in FEV_1)
- Rhinosinusitis metrics
  - **Change from baseline to week 12 in:**
    - Rhinosinusitis symptoms (sino-nasal outcome test-20 [66] total score)

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Box 2. Key inclusion and exclusion criteria.

Key inclusion criteria

- Female or male, aged 18–75 years inclusive, with weight ≥40 kg and <150 kg
- Physician-diagnosed asthma for ≥12 months prior to enrollment with requirement for inhaled corticosteroid (minimum dose of ≥250 μg fluticasone proportionate or equivalent delivered dose) alone or in combination, for ≥6 months, with stable dose for ≥1 month prior to enrollment
- Additional maintenance asthma controller medications allowed, at a stable dose for ≥1 month prior to enrollment. Must remain unchanged during the study
- Morning prebronchodilator forced expiratory volume in 1 s (FEV_1) >50% of the predicted normal value and >1 l and postbronchodilator reversibility in FEV_1 of ≥12% and ≥200 ml at enrollment†

Prior to randomization at week 0

- No requirement for a change in inhaled corticosteroid, other asthma controller medications and/or the requirement to add asthma controller medications during the run-in period
- Minimum 70% compliance‡ with usual asthma controller medications and eDiary completion
- Asthma control questionnaire 6 ≥1.5 at weeks -4 or -2
- Successful bronchial biopsy

Key exclusion criteria

- History of interstitial lung disease, chronic obstructive pulmonary disease, or other clinically significant lung disease other than asthma
- Any disorder that is either not stable or could, in the opinion of the Investigator, affect the safety of the patient or influence the study findings
- Any clinically significant abnormal findings during the run-in period
- Current tobacco smoking or a history of tobacco smoking for >10 pack-years
- Chronic oral corticosteroid use
- Hospitalization or oral corticosteroid requirement ≤6 weeks prior to enrollment and a history of ≥3 exacerbations requiring corticosteroid treatment in the previous year
- History of cancer, HIV, hepatitis B or C
- Use of immunosuppressive medication <3 months prior to informed consent
- History of clinically significant infection requiring antibiotics or antiviral medication <30 days prior to informed consent or during the run-in period
- Helminth parasitic infection, diagnosed <6 months prior to informed consent, that is untreated or unresponsive to standard of care
- Women of child-bearing potential must use a highly effective form of birth control from enrollment (visit 1), throughout the study duration and within 16 weeks after last dose of investigational drug, and have a negative serum pregnancy test result at visit 1
- †If not met at week -4, these criteria must be met at week -2. The patient should withhold bronchodilator prior to the lung function measurement for the effect duration specific to the bronchodilator.
- ‡Compliance is defined as completing 10 out of the last 14 days between week -4 and week -2 as reported by the patient in the eDiary.

tion [55], production of inducible nitric oxide synthase by airway epithelial cells [56] and basement membrane thickening [57]. In addition, IL-13 may promote airway inflammation via effects on eosinophils [20–24].

Tralokinumab inhibits IL-13 and has shown clinical benefits in patients with severe uncontrolled asthma [44,45]. In a Phase IIa study (NCT00873860) [74], the primary end point (ACQ-6) was not met, but there was a clear dose-response in lung function measures, with a significant improvement in FEV_1 at the highest dose (600 mg) [44]. In a Phase IIb study (NCT01402986) [75], addition of 300 mg tralokinumab Q2W to high-dose ICS–LABA resulted in significant improvements in lung function in the overall population [45]. In a *post hoc* analysis, additional benefits were seen in lung function and asthma control in a subpopulation of patients who were 'reversible' at baseline (≥12% increase in postbronchodilator FEV_1), not receiving long-term maintenance OCS, and who also had high baseline serum concentrations of surrogate biomarkers for enhanced IL-13 pathway activation [45]. Although there was slightly greater benefit in the Phase IIa study with tralokinumab dosed as 600 mg Q2W compared with the Phase IIb study, where tralokinumab was dosed as 300 mg Q2W, the 600 mg dose was not taken forward because formulation issues required that it would be administered as four separate injections. A subsequent pharmacokinetic/pharmacodynamic analysis of the Phase IIa and IIb results confirmed that the tralokinumab 300 mg dose Q2W provided near maximal dose-plateau effects on FEV_1 [58]. An important question is whether tralokinumab improves asthma out-

comes by reducing airway inflammation. Unfortunately, neither of these Phase II studies incorporated markers of airway inflammation. The MESOS study aims to investigate the mode of action of tralokinumab and test whether it exerts its effect in patients with asthma by reducing inflammation and airway remodeling. This study will be an important compliment to the already initiated Phase III program (STRATOS 1 and 2).

Future perspective

Chronic airway inflammation in asthma may result in airway remodeling, which is associated with poor outcomes in patients. There is an unmet need for therapies that can reduce airway inflammation and, in turn, airway remodeling. New biologics that target specific inflammatory pathways may directly reduce airway inflammation. If the improvements in lung function that have been observed with tralokinumab are driven by reducing inflammation and airway remodeling, it may suggest that anti-IL13 therapies might alter the underlying pathology of the disease.

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Executive summary

Background

- Inflammation and airway remodeling are central processes in the pathophysiology of asthma.
- Eosinophils are key effectors in both processes and their activities are regulated by Th2 cytokines, including IL-5 and IL-13.
- Tralokinumab is an investigational fully human monoclonal antibody that has been designed to specifically target and inhibit IL-13 signaling.

Study rationale

- Preclinical studies suggest tralokinumab may have effects in airway inflammation.
- Phase II studies (NCT00873860, NCT01402986) have shown that tralokinumab treatment is associated with elevated levels of eosinophils in the blood, which could be due to inhibition of eosinophil migration from the blood to the lungs.
- The MESOS study aims to better understand the mode of action of tralokinumab in improving asthma outcomes and to address whether tralokinumab exerts its effect by reducing airway inflammation and airway remodeling.

Study design

- This is a Phase II, randomized, double-blind, parallel-group, multicenter, placebo-controlled study in patients with uncontrolled asthma requiring continuous treatment with inhaled corticosteroids (ICS), with or without other asthma controllers.
- The study will primarily investigate the effects of tralokinumab both on airway submucosal eosinophil infiltration and on the numbers of activated and nonactivated eosinophils in the blood, sputum and bronchial mucosa of adults with asthma inadequately controlled on ICS.
- The effect of tralokinumab on large and small airway remodeling in the study population will also be evaluated.

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