



TSLP Inhibitors for Asthma: Current Status and Future Prospects

Maria Gabriella Matera¹ · Paola Rogliani² · Luigino Calzetta² · Mario Cazzola²

© Springer Nature Switzerland AG 2020

Abstract

Thymic stromal lymphopoietin (TSLP) is an allarmin cytokine whose importance in human asthma has been repeatedly documented. Accordingly, targeting of TSLP and TSLP-mediated signalling is considered as an attractive therapeutic strategy to asthma. Tezepelumab, which is the first-in-class anti-TSLP monoclonal antibodies (mAb), is a fully human IgG2 λ mAb that binds human TSLP, prevents interaction with its receptor and, consequently, inhibits multiple downstream inflammatory pathways. Because of the excellent results of Phase II trials, the Food and Drug Administration granted tezepelumab as a ‘breakthrough’ biological drug for the treatment of severe asthma. Several studies with this mAb are ongoing. CSJ117 is an Ab fragment that binds to TSLP and is delivered by inhalation but there is no published information on this biologic agent. Since new information suggests that targeting TSLP may be more likely to improve day-to-day asthma symptoms, in contrast to targeting mediators of the adaptive immune system, approaches that primarily act to ameliorate asthma exacerbations, novel approaches capable of blocking TSLP (for example, fully human single-chain fragment variables against TSLP, bifunctional drugs such as the one that combines an anti-IL-13 mAb with an anti-TSLP mAb, a fusion protein consisting of the ectodomains of TSLPR and IL-7Ra that extend into the extracellular space, also known as a TSLP-trap, fragments capable of disrupting the TSLP:TSLPR complex) are under preclinical investigation. However, some critical aspects remain to be clarified before being able to define this approach as the one that will probably better help patients suffering from severe asthma because of its holistic effects.

1 Role of TSLP in Asthma

Thymic stromal lymphopoietin (TSLP), which was initially identified in 1994 as a cytokine produced by thymic stromal cells, is a short-chain four α -helical bundle type I interleukin-2 (IL-2) family cytokine that shares homology with IL-7. Similar to IL-25 and IL-33, it is an allarmin cytokine. TSLP is mainly secreted by epithelial cells, airway smooth muscle cells, keratinocytes, stromal cells, fibroblasts, mast cells, macrophages/monocytes, granulocytes and dendritic cells (DCs) [1, 2]. There is ample evidence of its ability to differentiate naïve CD4⁺ T lymphocytes in type 2 cells, produce IL-4, IL-5, IL-13, and reduce the expression of interferon γ (IFN- γ) related to type 1 cells [1, 2].

The expression of TSLP increases in the asthmatic airways and correlates with the expression of type 2-attractive chemokines and the severity of the disease. Cultured primary bronchial epithelial cells from severe asthmatics spontaneously release measurable levels of TSLP [3]. Therefore, it has been suggested that TSLP is involved in the pathogenesis of human asthma [1]. However, there is documentation that aberrant TSLP signalling is closely associated with other inflammatory allergic diseases, including atopic dermatitis, eosinophilic esophagitis and also chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis [4–7]. In any case, its expression is also triggered by infection of viral, bacterial, and parasitic pathogens [8]. Interestingly, it has been documented that respiratory epithelial cells from asthmatics respond potently to viral double-stranded RNA by producing higher levels of TSLP compared with epithelial cells from healthy controls [9].

Short and a long isoforms of TSLP, which consist of 60 amino acids and 159 amino acids, respectively, have been identified. They have distinct gene promoters that are differentially controlled by external stimuli [10]. The short isoform is constitutively expressed in several tissues and has been linked to TSLP homeostatic functions, while

✉ Mario Cazzola
mario.cazzola@uniroma2.it

¹ Chair of Pharmacology, Department of Experimental Medicine, University of Campania “Luigi Vanvitelli”, Naples, Italy

² Chair of Respiratory Medicine, Department of Experimental Medicine, University of Rome Tor Vergata, Rome, Italy

Key Points

The importance of TSLP in asthma has been repeatedly documented. Therefore, targeting of TSLP and TSLP-mediated signalling using anti-TSLP mAbs and TSLP cytokine traps may represent an effective anti-asthma strategy.

Tezepelumab, a mAb that neutralises TSLP activity by interfering with the interaction of TSLP with the TSLPR, and subsequent recruitment of IL-7R α in the signalling complex has proven effective in Phase II clinical trials where it significantly decreases asthma exacerbations in patients without systemic eosinophilia.

Several new novel approaches capable of blocking TSLP, such as fully human single-chain fragment variables against TSLP, bifunctional drugs that combine an anti-IL-13 mAb with an anti-TSLP mAb, a fusion protein consisting of the ectodomains of TSLPR and IL-7R α that extend into the extracellular space, also known as a TSLP-trap, fragments capable of disrupting the TSLP/TSLPR complex, are under preclinical investigation.

However, the potency of TSLP as standalone stimulator of ILC2 and whether therapeutic targeting of TSLP covers both isoforms of TSLP or predominantly one rather than the other must still be established.

expression of the long isoform is inducible, exerts inflammatory effects and appears to correlate with pathologies like asthma, atopic dermatitis or psoriasis [11]. Toll-like receptor ligands, proinflammatory cytokines (IFN- γ , tumour necrosis factor [TNF] and IL-1 β), and also an atopic cytokine milieu (TNF- α + IL-4 + IL-13) or TNF- α alone, are able to upregulate the long form but not the short one [12]. It has been documented that the long form contributes to house dust mite-induced airway epithelial barrier dysfunction and that synthetic short isoforms can prevent these effects [13].

TSLP initiates the intracellular type 2 signalling by binding to its high affinity heterodimer receptor complex that consists of its specific receptor, TSLPR, (encoded by *CRLF2* localised on chromosome 5q22.1), which shares 24% identity with the γ chain of common receptor in IL-2, IL-4, IL-9 and IL-15 [11] and does not contain the δ -chain common to the IL-2 family, and an IL-7R α subunit (CD127) in cells co-expressing TSLPR and IL-7R α [14]. TSLP initially binds to TSLPR, followed by recruiting of the IL-7R α chain. By itself, TSLP has no discernible affinity for IL-7R α .

Forming a triple complex of TSLP/TSLPR/IL-7R α , TSLP activates Janus kinase 1 (JAK1) and JAK2, thereby resulting in phosphorylation of signal transducer and activator of primarily transcription 5A (STAT5A) and STAT5B, as

well as STAT1, STAT3, and other STAT proteins to a lesser extent, and mitogen-activated protein kinases (MAPKs) and nuclear factor kappa B (NF- κ B) depending on the cell type [15, 16] (Fig. 1).

TSLP production and release from airway epithelial cells is often activated through various danger signals such as mechanical injury, infection-related agents, and allergens [1], which supports its function as an alarmin signalling the compromise of epithelial integrity.

The type 2-driven immune response is promoted by TSLP directly by action on T cells, mast cells and natural killer cells and indirectly by activation of CD11c⁺ DCs localised within the pulmonary or skin epithelium that then migrate into the lymph nodes where they activate CD4⁺ T cells to differentiate type 2 cells [17]. The type 2-cell differentiation is mediated through TSLP-induced DC expression of the TNF superfamily protein OX40 ligand (OX40L; CD252) [18]. DC-expressed OX40L interacts with OX40 on naïve T cells, resulting in a type 2 lineage commitment by initiating signalling events. These events lead to the production of large amounts of proinflammatory cytokines such as IL-4, IL-5, and IL-13, which enhance immunoglobulin (Ig)E, mast cell and mucous production and increase airway hyperresponsiveness, but not IL-10 and IFN- γ from multiple types of innate immune cells [19, 20].

In particular, the interactions between TSLP and CD34⁺ cells promotes a proinflammatory phenotype characterised by type 2 cytokines (IL-5 and IL-13) [21], and a proliferative phenotype with the involvement of the eosinophil/basophil lineage [22]. Furthermore, it increases the migration of eosinophils, likely in combination with IL-33 through an IL-13-dependent axis, which enhances IL-5 and IL-13 production [23].

In effect, there is evidence that TSLP released from bronchial epithelial cells through action of allergen proteases and biologic components [24] drives in situ eosinophilopoiesis [25]. Therefore, it can be considered a vital factor in orchestrating, perpetuating and amplifying the inflammatory response in asthma. In fact, in the animal model, overexpression of TSLP in the lungs developed into an asthma-like disease [26]. Furthermore, the local application of an anti-TSLPR antibody (Ab) and an anti-TSLP Ab prevented type 2-mediated airway inflammation [27, 28].

The importance of TSLP in asthma has been repeatedly documented. There is evidence that inhaled allergen challenge in patients with mild allergic asthma induces CD127 eosinophil lineage-committed progenitor cells in peripheral blood [23] and TSLPR expression on basophils in blood and airways [29]. Additionally, airway TSLP expression is directly increased by allergen challenge to a degree correlating with increased late-phase airway obstruction [30]. In the asthmatic patients, the numbers of both epithelial and submucosal cells expressing TSLP mRNA correlate inversely

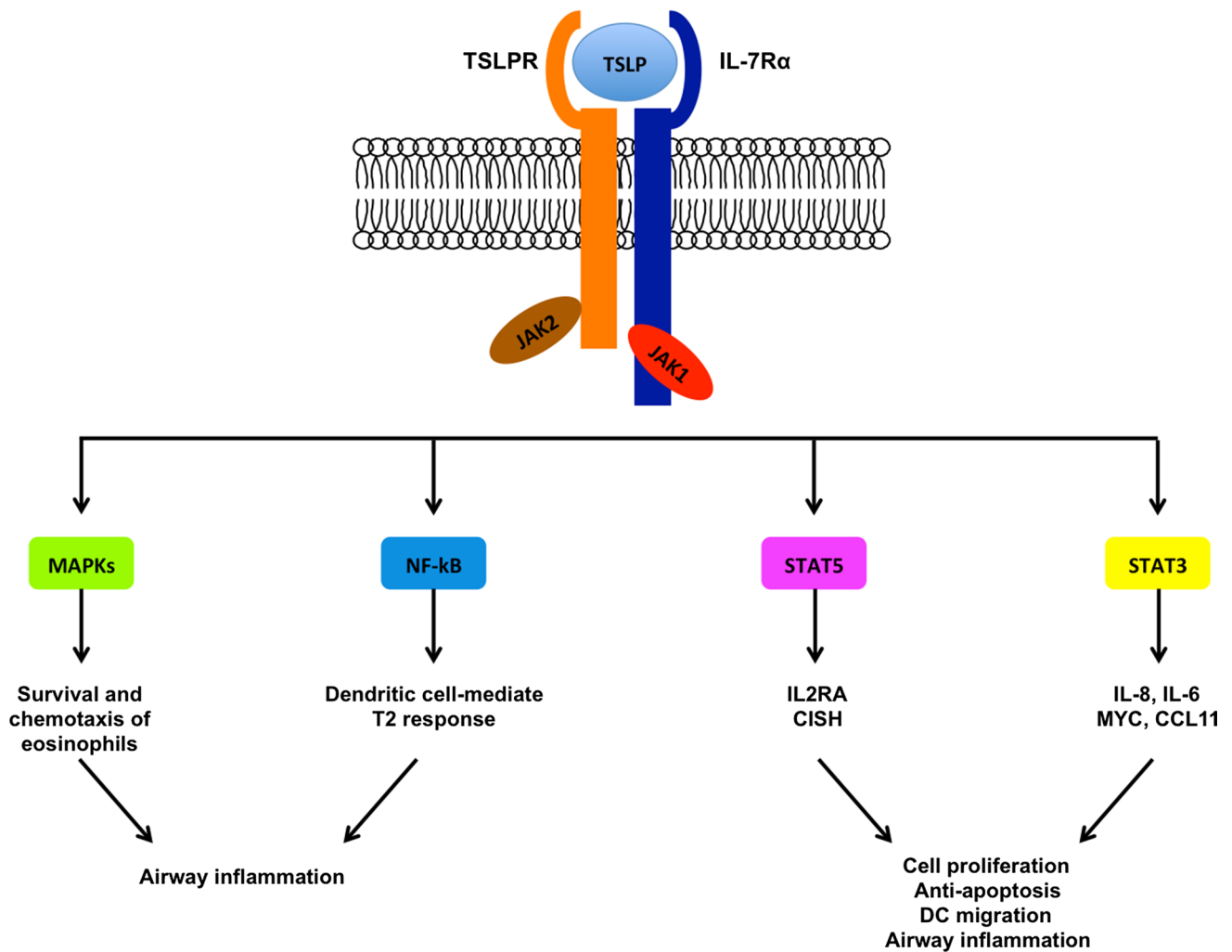


Fig. 1 TSLP function in asthma. TSLP, forming a triple complex of TSLP/TSLPR/IL-7R α , activates JAK1 and JAK2, thereby resulting in phosphorylation of STAT5A and STAT5B, as well as STAT1, STAT3, and other STAT proteins to a lesser extent, and MAPKs and

NF- κ B depending on the cell type. *IL*, interleukin, *JAK* Janus kinase, *MAPKs* mitogen-activated protein kinases, *NF- κ B* nuclear factor kappa B, *STAT* transcription, *TSLP* thymic stromal lymphopoietin

with forced expiratory volume in 1 s (FEV₁) [31]. There is also an association between airway hyperresponsiveness, serum IgE levels, and the number of mast cells expressing TSLP [32]. Even more compelling is the documentation that TSLP expression is increased in a subset of patients with severe asthma in spite of high dose, inhaled or oral corticosteroid therapy [33]. In effect, type 2 innate lymphoid cells (ILC2s) from asthmatic patients with increased TSLP levels are steroid resistant [34].

Consequently, targeting of TSLP and TSLP-mediated signalling is considered as an attractive therapeutic strategy to asthma [35]. It has been suggested that anti-TSLP monoclonal Abs (mAbs) and TSLP cytokine traps may represent effective strategies for therapeutic targeting of TSLP [2].

2 TSLP Inhibitors that have Already Been Under Preclinical or Clinical Development

Modulation of TSLP/TSLPR signalling by a soluble TSLPR, TSLPR-Ig, prepared by fusing the extracellular domain of murine TSLPR with a murine IgG2a Fc tail and administered into mouse airways before sensitisation, resulted in inhibition of the type 2-mediated features of asthma via regulation of pulmonary DC function [36]. As far as we know, this pharmacological approach has not been evaluated in humans.

Several TSLP inhibitors have been or are under clinical development (Table 1).

MK-8226, an intravenous mAb against TSLP, was assessed in a Phase Ib study (ClinicalTrials.gov Identifier:

NCT01732510) that terminated in early testing due to business reasons. The final results were not published.

RG7258, a humanised *mAb* that inhibits binding of TSLP to its receptor, blocked TSLP-mediated responses with high potency and also reduced inflammation and bronchoconstriction in a non-human primate ascaris-sensitive model of allergic lung inflammation [37]. It was in Phase I clinical development (single- and multiple-dose study in asthmatics), but the development was discontinued.

ASP7266, a recombinant human IgG1 *mAb* targeting human TSLPR, was able to inhibit TSLP signalling in human and monkey peripheral white blood cells [38]. It also inhibited human TSLP-stimulated monkey DC-mediated differentiation of naïve CD4⁺ T cells into mature T cells in vitro. When administered intravenously in cynomolgus monkeys, ASP7266 blocked CCL17 mRNA expression in peripheral white blood cells by at least 89% 9 days after administration. It also suppressed ascaris extract-induced skin reactions in sensitised monkeys. Apparently, ASP7266 was in Phase I clinical development, but there is no trace of this study in Clinicaltrials.gov. It is likely that its development was discontinued.

Therefore, tezepelumab (AMG 157, MEDI9929) is the real first-in-class anti-TSLP *mAb*. It is a fully human IgG2 λ *mAb* that binds human TSLP, prevents interaction with its receptor and, consequently, inhibits multiple downstream inflammatory pathways.

Phase I studies aimed to evaluate the initial safety, and pharmacokinetics showed that tezepelumab was well tolerated and exhibited a predictable linear pharmacokinetic profile and acceptable safety and tolerability in healthy and atopic dermatitis adult subjects [39].

In a proof-of-concept study, tezepelumab administered at an intravenous dose of 700 mg every 4 weeks for 12 weeks reduced airway hyperresponsiveness and markers

of systemic (levels of circulating eosinophils decreased compared with placebo, 59% vs 21%, respectively) and airway (the fraction of exhaled nitric oxide [FeNO] and sputum eosinophil levels that dropped from 4.1% at baseline to 0.4% after 6 weeks' inflammation) after allergen challenge in patients with mild allergic asthma [40]. Both the early and late asthmatic responses were significantly attenuated. The early asthmatic response was decreased in the tezepelumab group by 27–31% compared with placebo and also the late response was reduced by 34% and 46% compared with placebo after 6 and 12 weeks, respectively.

In a Phase IIb, randomised, double-blind, placebo-controlled trial, three dose levels of subcutaneous tezepelumab (70 mg every 4 weeks, 210 mg every 4 weeks or 280 mg every 2 weeks) were compared to placebo over 52 weeks in asthma patients treated with medium-to-high doses of inhaled corticosteroids/long-acting β_2 -agonists [41]. They resulted in a reduction in the annualised exacerbation rate by 61%, 71%, and 66% at Week 52, respectively, irrespective of baseline blood eosinophil counts, FeNO, or serum IgE levels, although persistent decreases in blood eosinophil count and FeNO levels were recorded. The pre-bronchodilator FEV₁ at Week 52 was always > 100 mL higher than in the placebo group irrespective of the dose, and symptom control was improved by medium and high dose, but health-related quality of life improved only in the high-dose group. The overall rate of adverse events did not differ in comparison with the rate observed in the placebo group, with discontinuous rates due to adverse events that were 1.1% in patients receiving tezepelumab and 0.7% in the placebo group.

A post hoc analysis of this study showed that tezepelumab decreased not only blood eosinophil count and FeNO levels, but also levels of IL-5, IL-13, periostin, thymus- and activation-regulated chemokine (TARC), and IgE [42]. It was also demonstrated that in patients with severe, uncontrolled

Table 1 TSLP inhibitors that have been or are under clinical development

TSLP inhibitor	Pharmacological activity	Development
MK-8226	Recombinant human IgG1 <i>mAb</i> targeting human TSLPR	A Phase Ib study terminated in early testing for business reasons
RG7258	Recombinant human IgG1 <i>mAb</i> targeting human TSLPR	In a Phase I clinical development (single- and multiple-dose study in asthmatics), but the development was discontinued
ASP7266	Recombinant human IgG1 <i>mAb</i> targeting human TSLPR	In Phase I clinical development, but it is likely that its development was discontinued
Tezepelumab (AMG 157, MEDI9929)	Fully human IgG2 λ <i>mAb</i> that binds human TSLP	The drug is currently in Phase III clinical trials. The FDA granted breakthrough designation for tezepelumab
CSJ117	An Ab fragment that belongs to the immunoglobulin G1/ λ isotype subclass and binds to TSLP. It is delivered by inhalation	A Phase I trial completed, but efficacy and safety of CSJ117 have not yet been established

FDA US Food and Drug Administration, *mAb* monoclonal antibody, TSLP thymic stromal lymphopoietin

asthma, tezepelumab reduced the frequency of hospitalisations and emergency department visits and decreased the number of days in hospital or the intensive care unit [43]. In any case, an exposure–response analysis indicated that 210 mg every 4 weeks is the optimal dose for Phase III trials in severe asthmatics and that the efficacy of tezepelumab on exacerbations and FeNO reduction is not influenced by blood eosinophils or other type 2 biomarkers [44].

It was for the results of this trial that the Food and Drug Administration granted tezepelumab as a ‘breakthrough’ biological drug for the treatment of severe asthma [45]. In effect, a network meta-analysis performed to evaluate effect differences of the mAbs on annualised exacerbation rates in type 2 asthma demonstrated the highest median exacerbation rate among the pooled placebo group and the lowest among the tezepelumab-treated arm [46]. However, the effect estimate of this arm had also the widest surrounding confidence interval.

Several studies are ongoing, mainly to verify the long-term efficacy and safety of tezepelumab in Caucasian or Asian adults and adolescents with severe uncontrolled asthma, and also to evaluate the functionality and performance of accessorised pre-filled syringe or autoinjector for the subcutaneous injections of this mAb, which could allow the patient to take the drug at home (Table 2).

CSJ117 is an Ab fragment that belongs to the immunoglobulin G1/ λ isotype subclass and binds to TSLP. It is delivered by inhalation. CSJ117 has been evaluated in a Phase I bronchoprovocation study that has assessed its safety, tolerability, pharmacokinetics and pharmacodynamics in adult subjects with mild atopic asthma who exhibited early asthmatic response and late asthmatic response after bronchial allergen challenge (ClinicalTrials.gov Identifier: NCT03138811). It has been administered as a once-daily inhaled dose at low, medium or high dose for 12 weeks. Recruitment has been completed but there are no published data regarding this study.

3 Prospects for TSLP Inhibitors

The increasingly solid evidence that TSLP is heavily implicated in asthma pathophysiology and the excellent results obtained with tezepelumab are the likely reason why there is a great interest in identifying new approaches capable of blocking TSLP (Table 3).

There is documentation that the fully human single-chain fragment variables (scFvs) against TSLP are more specific, showing higher affinity but lower repellency, in comparison with mouse and humanised Abs [47]. The scFv29, which has been selected from a fully human Ab library, is specific to TSLP, and does not cross-react with IL-33, IL-4, and IL-13. Initial experiments revealed that scFv29 has a strong

potential to bind to TSLP in competition with the TSLP receptor. It could reduce the maturation rate of DC. Thus, scFv29 has been suggested as a neutralising Ab to block the signalling of TSLP.

Bifunctional drugs, specifically designed to have two mechanisms of action in the same molecule, offer an exciting new approach to the treatment of asthma [48]. There are also attempts to create bifunctional drugs by dual targeting combinations of cytokines with mAbs. A monovalent bispecific Ab format called Zweimab and a bivalent bispecific Ab scaffold termed Doppelmab that are utilised for targeting both TSLP and IL-13 due to great overlap of signalling pathway, have been designed, developed and characterized [49]. The decision to combine an anti-IL-13 mAb with an anti-TSLP mAb in one bispecific format stemmed from the evidence that these two cytokines are co-expressed in the airway epithelium and lamina propria of the lung of severe asthma patients [30]. Zweimabs are heterodimers with knob-in-hole mutations on the heavy chain. Doppelmab scaffold has scFv moiety that is tethered to the CH₃ domain by a short polypeptide linker. Both have stronger affinity to the human targets compared to parental Ab formats.

Fusion proteins, consisting of the ectodomains of TSLPR and IL-7Ra that extend into the extracellular space, also known as TSLP-traps, have been developed by fusing the TSLPR and IL-7R α extracellular regions in both orientations with a flexible (Gly–Gly–Ser)₂₀-linker, termed TSLP-trap1 and TSLP-trap2 [2]. Actually, ‘cytokine traps’ consist of fusions between the constant region of IgG and the extracellular domains of two distinct cytokine receptor components involved in binding the cytokine [50]. Both TSLP-traps bind 250-fold stronger to TSLP than the unlinked receptor ectodomains do and are 20- to 30-fold more potent in inhibiting TSLP-induced STAT5 compared to tezepelumab and its Fab fragment. Furthermore, both TSLP-traps are able to significantly inhibit TSLP-driven DC activation, and are as potent in this regard as tezepelumab [50].

Efforts are also being made to find fragments capable of disrupting the TSLP/TSLPR complex [51]. Four fragments pertaining to diverse chemical classes were identified to reduce TSLP/TSLPR complex formation to less than 75% in millimolar concentrations. All fragments were positively charged, consistent with the cationic nature of the natural ligand TSLP. Fragment bonded at the TSLP binding site that underwent a conformational change.

Recently, baicalein, which is a flavonoid from *Scutellaria baicalensis*, was identified as the first small molecule inhibitor of the human TSLP signalling pathway [52]. In vitro studies showed that this compound blocked the interaction between human TSLP and human TSLPR in a dose-dependent manner. Furthermore, a single treatment with baicalein effectively reduced eosinophil-rich pulmonary inflammation in the ovalbumin-induced animal model. Structure–activity

Table 2 Ongoing trials with tezepelumab

ClinicalTrials.gov Identifier	Title	Phase	Description
NCT03989544	A Study to Evaluate the Pharmacokinetics of Tezepelumab after Being Delivered by an Accessorized Pre-Filled Syringe or Autoinjector Compared with Vial and Syringe in Healthy Adult Subjects (PATH-BRIDGE)	I	A study that designed to compare tezepelumab pharmacokinetic exposure in healthy subjects following single subcutaneous administration of a fixed dose of tezepelumab by using vial and syringe, an accessorised pre-filled syringe or an autoinjector
NCT03688074	Study to Evaluate Tezepelumab on Airway Inflammation in Adults with Uncontrolled Asthma (CASCADE)	II	A 28-week study with a post-treatment follow-up period of 12 weeks is assessing the effect of tezepelumab on airway inflammation in adults with inadequately controlled moderate-to-severe asthma, taking inhaled corticosteroids and at least one additional asthma controller
NCT02698501	Effects of Anti-TSLP in Patients with Asthma	II	A study that aims to investigate whether airway hyperresponsiveness to mannitol is a suitable marker of response to tezepelumab, but also to better understand the anti-inflammatory effects of tezepelumab in the lungs, including whether a reduced airway hyperresponsiveness to mannitol following treatment with tezepelumab is related to a reduction in chymase/CPA-3 positive mast cells
NCT03347279	Study to Evaluate Tezepelumab in Adults & Adolescents with Severe Uncontrolled Asthma (NAVIGATOR)	III	A 52-week study with a post-treatment follow-up period of 12 weeks is evaluating tezepelumab in adults and adolescents with severe uncontrolled asthma, including patients with both type 2-high and type 2-low asthma, on medium to high-dose inhaled corticosteroid and at least one additional asthma controller medication with or without oral corticosteroid
NCT03927157	Study to Evaluate Tezepelumab in Adults with Severe Uncontrolled Asthma (DIRECTION)	III	A 52-week study with a post-treatment follow-up period of 12 weeks is evaluating tezepelumab in Asiatic (China, Korea and Philippines) adults with severe uncontrolled asthma on medium- to high-dose inhaled corticosteroid and at least one additional asthma controller medication with or without oral corticosteroid
NCT04048343	Long-term Safety of Tezepelumab in Japanese Subjects with Inadequately Controlled Severe Asthma (NOZOMI)	III	A 52-week study with a post-treatment follow-up period of 12 weeks is evaluating tezepelumab in Japanese adults and adolescents with severe uncontrolled asthma on medium- to high-dose inhaled corticosteroid and at least one additional asthma controller medication with or without oral corticosteroid
NCT03406078	Study to Evaluate the Efficacy and Safety of Tezepelumab in Reducing Oral Corticosteroid Use in Adults with Oral Corticosteroid-Dependent Asthma (SOURCE)	III	A 48-week study that aims to evaluate the efficacy and safety of tezepelumab in reducing oral corticosteroid use in adults that have received oral corticosteroid for the treatment of asthma for at least 6 months prior to screening and on a stable dose of between ≥ 7.5 to ≤ 30 mg (prednisone or prednisolone equivalent) daily or daily equivalent for at least 1 month
NCT03968978	Tezepelumab Home Use Study (PATH-HOME)	III	A study that is assessing healthcare provider and subject/caregiver reported functionality and performance of subcutaneous injections of tezepelumab administered by accessorised pre-filled syringe or autoinjector in the clinic and in an at-home setting

TSLP thymic stromal lymphopoietin

Table 3 New approaches capable of blocking TSLP

Agent	Pharmacological action
scFvs	Has a strong potential to bind to TSLP in competition with the TSLP receptor
Zweimab	They target both TSLP and IL-13
Doppelmab	
TSLP-traps	They effectively antagonise TSLP-mediated molecular responses relevant for type-2 immunity in human primary cells
Fragments	Bind at the TSLP binding site and the TSLPR site undergoes a conformational change that may be required for fragment binding
Baicalein	Blocks the interaction between human TSLP and TSLPR
2-(4-{2-[(phenylthio)acetyl]carbonohydrazonoyl}phenoxy)acetamide	Inhibits the TSLP mRNA expression and production by the blocking of caspase-1 in mast cells
Linalyl acetate	Inhibits TSLP production and mRNA expression through blockade of the caspase-1/NF- κ B pathway
Catechin	Inhibits the expression of TSLP in epithelial cells by influencing NF- κ B/TSLP pathway
16D10	Inhibits TSLP expression and production selectively via unidentified mechanisms other than direct inhibition of NF- κ B and activation of the Keap1-Nrf2 system

IL, interleukin, *NF- κ B* nuclear factor kappa B, *TSLP* thymic stromal lymphopoietin

relationship studies identified compound 11a, which is a biphenyl flavanone analogue, as a novel human TSLP inhibitor for the discovery and development of new anti-allergic drugs.

In another study, a novel compound, 2-(4-{2-[(phenylthio)acetyl]carbonohydrazonoyl}phenoxy)acetamide (PA), inhibited the TSLP mRNA expression and production by the blocking of caspase-1 in mast cells [53]. Notoriously, caspase-1 is activated by pro-inflammatory stimuli because they increase the level of intracellular calcium and increased intracellular calcium results in the activation of caspase-1. In turn, activated caspase-1 induces the activation of NF- κ B, which induces the expression of TSLP mRNA, followed by the production of TSLP in mast cells. In addition, PA inhibited the production of TSLP in human epidermal cell lines and ear swelling response in mice.

It was also shown that linalyl acetate, one of main constituents of lavender, inhibited TSLP production and mRNA expression through blockade of the caspase-1/NF- κ B pathway [54]. In addition, it decreased the intracellular calcium levels.

Catechin, a main effective component in green tea in doses of 75, 150, or 300 mg/kg, remitted the allergic symptoms in mice with allergic rhinitis, reduced the levels of IL-5, IL-13, and ovalbumin-specific IgE in the serum and restored the T helper type 2/T helper type 1 cell balance by inhibiting the expression of TSLP in epithelial cells by influencing NF- κ B/TSLP pathway [55].

On the contrary, 16D10 (*N*-[2-[2-[(2*E*)-3-(2,5-dimethoxyphenyl)-1-oxo-2-propen-1-yl]-4,5-dimethoxyphenyl]ethyl]acetamide), a chalcone derivative, inhibited TSLP expression in the mouse and human keratinocyte cell lines and its production selectively via unidentified mechanisms

other than direct inhibition of NF- κ B and activation of the Kelch-like ECH-associated protein 1 (Keap1)-nuclear factor erythroid-2-related factor 2 (Nrf2) system [56]. Importantly, 16D10 inhibited TSLP and ovalbumin-specific IgG1 and IgE expression in vivo.

4 Positioning TSLP Inhibitors in Asthma Treatment

Growing evidence suggests that targeting TSLP can provide clinical benefit in asthma by reducing the inflammatory response to a range of environmental insults, including allergens.

Collectively, this new information suggests that targeting TSLP may be more likely to improve day-to-day asthma symptoms, in contrast to targeting mediators of the adaptive immune system, approaches that primarily act to ameliorate asthma exacerbations [57].

It is noteworthy that the effect of the tezepelumab appeared to be the same in the population regardless of the patient's number of eosinophils [41]. This finding suggests that likely this approach can be useful for the treatment of type 2-high and presumably type 2-low asthma because early evidence with compounds that act against upstream targets of type-2 immunity suggests that patients both with and without clinically detectable type 2 disease respond to treatment [58]. Targeting TSLP is thus a promising treatment for various asthmas that are triggered not by only allergens but also viruses, tobacco smoke, and diesel exhaust [59]. Additionally, a possible important advantage of targeting TSLP is the potential to decrease the steroid prescription burden in inflammatory conditions such as asthma.

Obviously, some fundamental aspects remain to be clarified before being able to define this approach as the one that will probably better help our patients suffering from severe asthma because of its holistic effects.

First of all, it is not yet clear the potency of TSLP, IL-25, and IL-33 as standalone stimulators of ILC2 or their potential redundancy with each other. In any case, there is experimental evidence that combined blockade of these three cytokines may better ameliorate airway pathological changes in a murine asthma model, and this could have implications for human asthma [60].

Furthermore, we still do not know if therapeutic targeting of TSLP covers both isoforms of TSLP or predominantly one rather than the other. It is obvious that if the short isoform were also affected, this should be understood as a negative effect and, in any case, it should be quantified. It will, however, be essential to have a precise description of the safety of this therapeutic approach, also considering a possible risk of infections and development of cancer that can be induced by blocking TSLP [60]. In fact, it has been documented that the ablation of the TSLP/TSLPR signalling accelerates tumour growth in different murine models of skin carcinogenesis [61, 62]. Nevertheless, there are data that cast doubt on the pro-tumour role of TSLP [63]. It has been suggested that the role of TSLP as a pro- or anti-tumour factor is dependent on the type of tumour. This effect would depend on the ability of TSLP to directly or indirectly influence the inflammatory milieu of the tumor microenvironment thanks to its ability to regulate the type of response induced by DCs.

In any case, the application of a phenotypic drug discovery strategy to identify biological and chemical starting points for inhibition of TSLP production in lung epithelial cells that could be amenable to further optimisation, providing an opportunity to dissociate TSLP inhibition from other non-desired activities, has been proposed [64].

5 Conclusion

The central role of TSLP in maintaining immune homeostasis and regulating inflammatory responses at mucosal barriers is arguably the reason why tezepelumab proves to be extremely effective in severe uncontrolled asthma. However, it is becoming increasingly evident that the effect of this anti-TSLP Ab involves additional biological functions of the TSLP/TSLPR pathway in other immune cells, such as basophils and non-immune cells such as neurons. Hence, a more in-depth characterisation of the pharmacological actions of tezepelumab and direct comparisons with other mAb that interfere with different pathways are absolutely necessary.

Compliance with Ethical Standards

Conflict of interest Mario Cazzola, Paola Rogliani, Luigino Calzetta and Maria Gabriella Matera have no relevant affiliations or financial involvement with any organization or entity with a financial interest in, or financial conflict with, the subject matter or materials discussed in the manuscript, including employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Funding This manuscript was not funded/sponsored, and no writing assistance was utilized in its production.

References

1. Mitchell PD, O'Byrne PM. Biologics and the lung: TSLP and other epithelial cell-derived cytokines in asthma. *Pharmacol Ther.* 2017;169:104–12.
2. Verstraete K, Peelman F, Braun H, et al. Structure and antagonism of the receptor complex mediated by human TSLP in allergy and asthma. *Nat Commun.* 2017;8:14937.
3. Semlali A, Jacques E, Koussih L, et al. Thymic stromal lymphopoietin-induced human asthmatic airway epithelial cell proliferation through an IL-13-dependent pathway. *J Allergy Clin Immunol.* 2010;125(4):844–50.
4. Ziegler SF. Thymic stromal lymphopoietin and allergic disease. *J Allergy Clin Immunol.* 2012;130(4):845–52.
5. Noti M, Wojno ED, Kim BS, et al. Thymic stromal lymphopoietin-elicited basophil responses promote eosinophilic esophagitis. *Nat Med.* 2013;19(8):1005–13.
6. Redhu NS, Gounni AS. Function and mechanisms of TSLP/TSLPR complex in asthma and COPD. *Clin Exp Allergy.* 2012;42(7):994–1005.
7. Datta A, Alexander R, Sulikowski MG, et al. Evidence for a functional thymic stromal lymphopoietin signalling axis in fibrotic lung disease. *J Immunol.* 2013;191(9):4867–79.
8. Comeau MR, Ziegler SF. The influence of TSLP on the allergic response. *Mucosal Immunol.* 2010;3(2):138–47.
9. Brandelius A, Yudina Y, Calvén J, et al. dsRNA-induced expression of thymic stromal lymphopoietin (TSLP) in asthmatic epithelial cells is inhibited by a small airway relaxant. *Pulm Pharmacol Ther.* 2011;24(1):59–66.
10. Tsilingiri K, Fornasa G, Rescigno M. Thymic stromal lymphopoietin: to cut a long story short. *Cell Mol Gastroenterol Hepatol.* 2017;3(2):174–82.
11. Park JH, Jeong DY, Peyrin-Biroulet L, et al. Insight into the role of TSLP in inflammatory bowel diseases. *Autoimmun Rev.* 2017;16(1):55–63.
12. Xie Y, Takai T, Chen X, et al. Long TSLP transcript expression and release of TSLP induced by TLR ligands and cytokines in human keratinocytes. *J Dermatol Sci.* 2012;66(3):233–7.
13. Dong H, Hu Y, Liu L, et al. Distinct roles of short and long thymic stromal lymphopoietin isoforms in house dust mite-induced asthmatic airway epithelial barrier disruption. *Sci Rep.* 2016;6:39559.
14. Varricchi G, Pecoraro A, Marone G, et al. Thymic stromal lymphopoietin isoforms, inflammatory disorders, and cancer. *Front Immunol.* 2018;9:1595.
15. Rochman Y, Kashyap M, Robinson GW, et al. Thymic stromal lymphopoietin-mediated STAT5 phosphorylation via kinases JAK1 and JAK2 reveals a key difference from IL-7-induced signalling. *Proc Natl Acad Sci USA.* 2010;107(45):19455–60.

16. Yu X, Li H, Ren X. Signalling cascades initiated by TSLP-mediated signals in different cell types. *Cell Immunol.* 2012;279(2):174–9.
17. Borowski A, Vetter T, Kuepper M, et al. Expression analysis and specific blockade of the receptor for human thymic stromal lymphopoietin (TSLP) by novel antibodies to the human TSLPR α receptor chain. *Cytokine.* 2013;61(2):546–55.
18. Ito T, Wang YH, Duramad O, et al. TSLP-activated dendritic cells induce an inflammatory T helper type 2 cell response through OX40 ligand. *J Exp Med.* 2005;202(9):1213–23.
19. So T, Song J, Sugie K, et al. Signals from OX40 regulate nuclear factor of activated T cells c1 and T cell helper 2 lineage commitment. *Proc Natl Acad Sci USA.* 2006;103(10):3740–5.
20. Dorman SC, Efthimiadis A, Babirad I, et al. Sputum CD34⁺IL-5R α ⁺ cells increase after allergen: evidence for in situ eosinophilopoiesis. *Am J Respir Crit Care Med.* 2004;169(5):573–7.
21. Siracusa MC, Saenz SA, Hill DA, et al. TSLP promotes interleukin-3-independent basophil haematopoiesis and type 2 inflammation. *Nature.* 2011;477(7363):229–33.
22. Hui CC, Rusta-Sallehy S, Asher I, et al. The effects of thymic stromal lymphopoietin and IL-3 on human eosinophil-basophil lineage commitment: relevance to atopic sensitization. *Immun Inflamm Dis.* 2014;2(1):44–55.
23. Smith SG, Gugilla A, Mukherjee M, et al. Thymic stromal lymphopoietin and IL-33 modulate migration of hematopoietic progenitor cells in patients with allergic asthma. *J Allergy Clin Immunol.* 2015;135(6):1594–602.
24. Kouzaki H, O'Grady SM, Lawrence CB, et al. Proteases induce production of thymic stromal lymphopoietin by airway epithelial cells through protease-activated receptor-2. *J Immunol.* 2009;183(2):1427–34.
25. Salter BMA, Smith SG, Mukherjee M, et al. Human bronchial epithelial cell-derived factors from severe asthmatic subjects stimulate eosinophil differentiation. *Am J Respir Cell Mol Biol.* 2018;58(1):99–106.
26. Headley MB, Zhou B, Shih WX, et al. TSLP conditions the lung immune environment for the generation of pathogenic innate and antigen-specific adaptive immune responses. *J Immunol.* 2009;182(3):1641–7.
27. Shi L, Leu SW, Xu F, et al. Local blockade of TSLP receptor alleviated allergic disease by regulating airway dendritic cells. *Clin Immunol.* 2008;129(2):202–10.
28. Chen ZG, Zhang TT, Li HT, et al. Neutralization of TSLP inhibits airway remodeling in a murine model of allergic asthma induced by chronic exposure to house dust mite. *PLoS One.* 2013;8(1):e51268.
29. Salter BM, Oliveria JP, Nusca G, et al. Thymic stromal lymphopoietin activation of basophils in patients with allergic asthma is IL-3 dependent. *J Allergy Clin Immunol.* 2015;136(6):1636–44.
30. Wang W, Li Y, Lv Z, et al. Bronchial allergen challenge of patients with atopic asthma triggers an allarmin (IL-33, TSLP, and IL-25) response in the airways epithelium and submucosa. *J Immunol.* 2018;201(8):2221–31.
31. Ying S, O'Connor B, Ratoff J, et al. Expression and cellular provenance of thymic stromal lymphopoietin and chemokines in patients with severe asthma and chronic obstructive pulmonary disease. *J Immunol.* 2008;181(4):2790–8.
32. Ying S, O'Connor B, Ratoff J, et al. Thymic stromal lymphopoietin expression is increased in asthmatic airways and correlates with expression of Th2-attracting chemokines and disease severity. *J Immunol.* 2005;174(12):8183–90.
33. Shikotra A, Choy DF, Ohri CM, et al. Increased expression of immunoreactive thymic stromal lymphopoietin in patients with severe asthma. *J Allergy Clin Immunol.* 2012;129(1):104–11.
34. Liu S, Verma M, Michalec L, et al. Steroid resistance of airway type 2 innate lymphoid cells from patients with severe asthma: the role of thymic stromal lymphopoietin. *J Allergy Clin Immunol.* 2018;141(1):257–68.
35. Park S, Park Y, Son SH, et al. Synthesis and biological evaluation of peptide-derived TSLP inhibitors. *Bioorg Med Chem Lett.* 2017;27(20):4710–3.
36. Zhang F, Huang G, Hu B, et al. A soluble thymic stromal lymphopoietin (TSLP) antagonist, TSLPR-immunoglobulin, reduces the severity of allergic disease by regulating pulmonary dendritic cells. *Clin Exp Immunol.* 2011;164(2):256–64.
37. Fuentes M, Ma X, Zhang J, et al. Anti-TSLPR antibody RG7258 blocks dendritic cell activation, mast cell cytokine release and reduces Th2 inflammation in a non-human primate model of allergic lung inflammation [abstract]. *Am J Respir Crit Care Med.* 2011;183:A2767.
38. Numazaki M, Hanaoka K, Imamura E, et al. ASP7266, a novel antibody against human TSLPR, in the treatment of allergic disease [abstract]. *J Allergy Clin Immunol.* 2018;141(2):AB13.
39. Parnes JR, Sullivan JT, Chen L, et al. Pharmacokinetics, safety, and tolerability of tezepelumab (AMG 157) in healthy and atopic dermatitis adult Subjects. *Clin Pharmacol Ther.* 2019;106(2):441–9.
40. Gauvreau GM, O'Byrne PM, Boulet LP, et al. Effects of an anti-TSLP antibody on allergen-induced asthmatic responses. *N Engl J Med.* 2014;370(22):2102–10.
41. Corren J, Parnes JR, Wang L, et al. Tezepelumab in adults with uncontrolled asthma. *N Engl J Med.* 2017;377(10):936–46.
42. Pham T-H, Ren P, Parnes JR, et al. Tezepelumab reduces multiple key inflammatory biomarkers in patients with severe, uncontrolled asthma in the phase 2b PATHWAY study [abstract]. *Am J Respir Crit Care Med.* 2019;199:A2677.
43. Corren J, Chen S, Callan L, et al. The impact of tezepelumab on hospitalization and emergency department visits in patients with severe uncontrolled asthma: results from the pathway phase 2b trial [abstract]. *Am J Respir Crit Care Med.* 2019;199:A2622.
44. Ly N, Zheng Y, Griffiths JM, et al. Exposure-response analysis of tezepelumab in patients with severe asthma to guide phase 3 dose selection [abstract]. *Eur Respir J.* 2018;52(suppl 62):PA1688.
45. FDAnews. FDA awards AstraZeneca and Amgen's tezepelumab breakthrough designation. <https://www.fda.gov/articles/188355-fda-awards-astrazeneca-and-amgens-tezepelumab-breakthrough-designation>. Accessed 10 Nov 2019.
46. Edris A, De Feyter S, Maes T, et al. Monoclonal antibodies in type 2 asthma: a systematic review and network meta-analysis. *Respir Res.* 2019;20(1):179.
47. Nian S, Zhu J, Yu H, et al. Development and identification of a fully human single-chain variable fragment 29 against TSLP. *Biotechnol Appl Biochem.* 2019;66(4):510–6.
48. Page C, Cazzola M. Bifunctional drugs for the treatment of respiratory diseases. *Handb Exp Pharmacol.* 2017;237:197–212.
49. Venkataramani S, Low S, Weigle B, et al. Design and characterization of Zweimab and Doppelmab, high affinity dual antagonistic anti-TSLP/IL13 bispecific antibodies. *Biochem Biophys Res Commun.* 2018;504(1):19–24.
50. Economides AN, Carpenter LR, Rudge JS, et al. Cytokine traps: multi-component, high-affinity blockers of cytokine action. *Nat Med.* 2003;9(1):47–52.
51. Van Rompaey D, Verstraete K, Peelman F, et al. Virtual screening for inhibitors of the human TSLP:TSLPR interaction. *Sci Rep.* 2017;7(1):17211.
52. Park BB, Choi JW, Park D, et al. Structure-activity relationships of baicalin and its analogs as novel TSLP inhibitors. *Sci Rep.* 2019;9(1):8762.
53. Moon PD, Han NR, Ryu KJ, et al. A novel compound 2-(4-{2-[(phenylthio)acetyl]carbonohydrzonoyl}phenoxy)acetamide

- downregulates TSLP through blocking of caspase-1/NF- κ B pathways. *Int Immunopharmacol.* 2016;38:420–5.
54. Moon PD, Han NR, Lee JS, et al. Effects of linalyl acetate on thymic stromal lymphopoietin production in mast cells. *Molecules.* 2018;23(7):E1711.
 55. Pan Z, Zhou Y, Luo X, et al. Against NF- κ B/thymic stromal lymphopoietin signalling pathway, catechin alleviates the inflammation in allergic rhinitis. *Int Immunopharmacol.* 2018;61:241–8.
 56. Segawa R, Shiraki M, Sudo S, et al. A chalcone derivative suppresses the induction of TSLP in mice and human keratinocytes and attenuates OVA-induced antibody production in mice. *Eur J Pharmacol.* 2019;851:52–62.
 57. Borish L. The immunology of asthma: asthma phenotypes and their implications for personalized treatment. *Ann Allergy Asthma Immunol.* 2016;117(2):108–14.
 58. Marone G, Spadaro G, Braile M, et al. Tezepelumab: a novel biological therapy for the treatment of severe uncontrolled asthma. *Expert Opin Investig Drugs.* 2019;28(11):931–40.
 59. Ohnishi H, Yokoyama A. Future treatment and other new biologics for asthma. In: Yokoyama A, editor. *Advances in asthma, respiratory disease series: diagnostic tools and disease managements.* Singapore: Springer Nature Singapore Pte Ltd; 2019. p. 177–89.
 60. An G, Wang W, Zhang X, et al. Combined blockade of IL-25, IL-33 and TSLP mediates amplified inhibition of airway inflammation and remodelling in a murine model of asthma. *Respirology.* 2019. <https://doi.org/10.1111/resp.13711>.
 61. Demehri S, Turkoz A, Manivasagam S, Yockey LJ, Turkoz M, Kopan R. Elevated epidermal thymic stromal lymphopoietin levels establish an antitumor environment in the skin. *Cancer Cell.* 2012;22(4):494–505.
 62. Di Piazza M, Nowell CS, Koch U, et al. Loss of cutaneous TSLP-dependent immune responses skews the balance of inflammation from tumor protective to tumor promoting. *Cancer Cell.* 2012;22(4):479–93.
 63. Corren J, Ziegler SF. TSLP: from allergy to cancer. *Nat Immunol.* 2019;20(12):1603–9.
 64. Orellana A, García-González V, López R, et al. Application of a phenotypic drug discovery strategy to identify biological and chemical starting points for inhibition of TSLP production in lung epithelial cells. *PLoS One.* 2018;13(1):e0189247.