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### ► To cite this version:

Jérémy Charriot, Engi Ahmed, Arnaud Bourdin. Local targeting of TSLP: feat or defeat. European Respiratory Journal, 2023, 61 (3), pp.2202389. 10.1183/13993003.02389-2022 . hal-04023413

**HAL Id: hal-04023413**

**<https://hal.science/hal-04023413>**

Submitted on 29 Jan 2024

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# **Local targeting of TSLP: feat or defeat**

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The physiological role of airway smooth muscle remains highly elusive given the poor overall understanding of the regulatory elements and their influence in healthy humans [1]. Paradoxically, the pathophysiological contribution of airway smooth muscle contraction to asthma is better understood than the physiological role of this contraction in non-asthmatics: early and late asthmatic responses highlight immediate-immune cell-free and delayed-immune cell-dependent bronchoconstriction, respectively [2].

Excessive early responses typically involve nonspecific mediators such as parasympathic agonists, with methacholine being the agonist most commonly used in routine practice. Mast cell myositis, leading to the release of inflammatory mediators such as histamine, tryptase, reactive oxygen species and lysosomal enzymes, has also been shown to participate in these responses, in addition to the involvement of the nerve growth factor and the synthesis of metabolites from the cyclooxygenase pathway. In contrast, late asthmatic responses could be closely associated with immune events involving T-cells and eosinophils [3]. These two combined events supposedly mirror the two time-scale events that characterise asthma, i.e. excessive and inappropriate bronchoconstriction, as well as chronic inflammation.

Airway hyperresponsiveness (AHR) is suggested to be a “treatable trait” that could be managed by precision medicine in severe asthma patients. Allergen challenge trials have gradually become a requisite benchmark in the development of new asthma drugs [4], while also providing a go/no go signal for new drugs to enter further trial phases. Interestingly, not all anti-asthma drugs have been assessed against this benchmark, even in large-scale phase III trials, but these tests have seldom led to success [5].

The biological revolution in asthma has prompted researchers to be even more imaginative and innovative. Ecleralimab, a fully humanised IgG1 Fab fragment, is the result of an outstanding pharmacology achievement, while successfully demonstrating the feasibility of sufficiently stabilising a monoclonal antibody so as to be suitable for local delivery without losing its specific biological targeting features. Ecleralimab recognises and neutralises thymic stromal lymphopoietin (TSLP), an epithelial cytokine known to be involved in asthma.

In this issue of the European Respiratory Journal, GAUVREAU et al. [6] present the findings of their multicentre double-blind, randomised, placebo-controlled, phase 2 trial investigating the effects of ecleralimab on stable mild atopic asthma. 4 mg of this compound or placebo was administered once daily via inhalation for 12 weeks. The study participants (18–60 years old) were diagnosed with stable mild atopic asthma requiring no asthma medication apart from short-acting  $\beta_2$  agonists, which could be used up to twice weekly. The most striking finding was that by day 84, ecleralimab had reduced the late asthmatic response by 48%.

This TSLP blockade experience suggests that bronchoconstriction should actually be considered as part of the innate warning signal sent by the airway epithelium in response to injury, in the light of the observed decrease in early and late asthmatic responses reported previously with tezepelumab, another monoclonal antibody targeting TSLP, but which is administered subcutaneously. It is indeed quite likely that bronchoconstriction is a physiological response to injury, closely resembling the vasoconstriction response that occurs in the early skin wound phase.

Moreover, ecleralimab tended to decrease airway hyperresponsiveness in comparison to placebo, as highlighted by the increased dose of methacholine needed to induce a 20% decrease in forced expiratory volume in 1 s (PC20). Ecleralimab also significantly decreased allergen-induced sputum eosinophils, fractional exhaled nitric oxide, interleukin (IL)-5 and IL-13.

Mechanistically, the epithelium and smooth muscle are the main TSLP sources in the airways. A functional heterodimeric receptor of TSLP (composed of TSLPR and IL7R $\alpha$ ) is primarily expressed by ILC2, mast cells, eosinophils and airway smooth muscle cells. TSLP may directly affect airway smooth muscles through STAT-3 activation, and indirectly through ILC2 generation of type 2 cytokines [7]. During viral exacerbation, TSLP is essential for ILC2 activation by producing a neurotransmitter, neuromedin U (NMU), that activates the neuromedin U receptor 1 (NMUR1) expressed by ILC2 [8]. TSLP blockade decreases subsequent IL-5 and IL-13 release, airway submucosal eosinophils [9] and airway mast cells [10], thereby jointly providing protection against airway hyperresponsiveness (figure 1).

Multiple questions remain to be addressed in the light of the consistent confirmation of the protective effect of this second TSLP-targeted monoclonal antibody against excessive bronchoconstriction.

First, the inhalation route seems to provide less intense protection against the early asthmatic response as compared to tezepelumab [11], suggesting that the systemic route could potentially be advantageous by facilitating access to the submucosal layer. This scenario should be assessed with caution given the success of many other inhaled drugs in achieving this outcome [12]. This also raises the possibility that: 1) the power of the study was insufficient; 2) the drug could not reach its target; 3) ecleralimab may have been underdosed, i.e. an aspect that would require further investigation; and 4) differential ecleralimab targeting against the short versus long TSLP isoform [13] could also help explain the observed results.

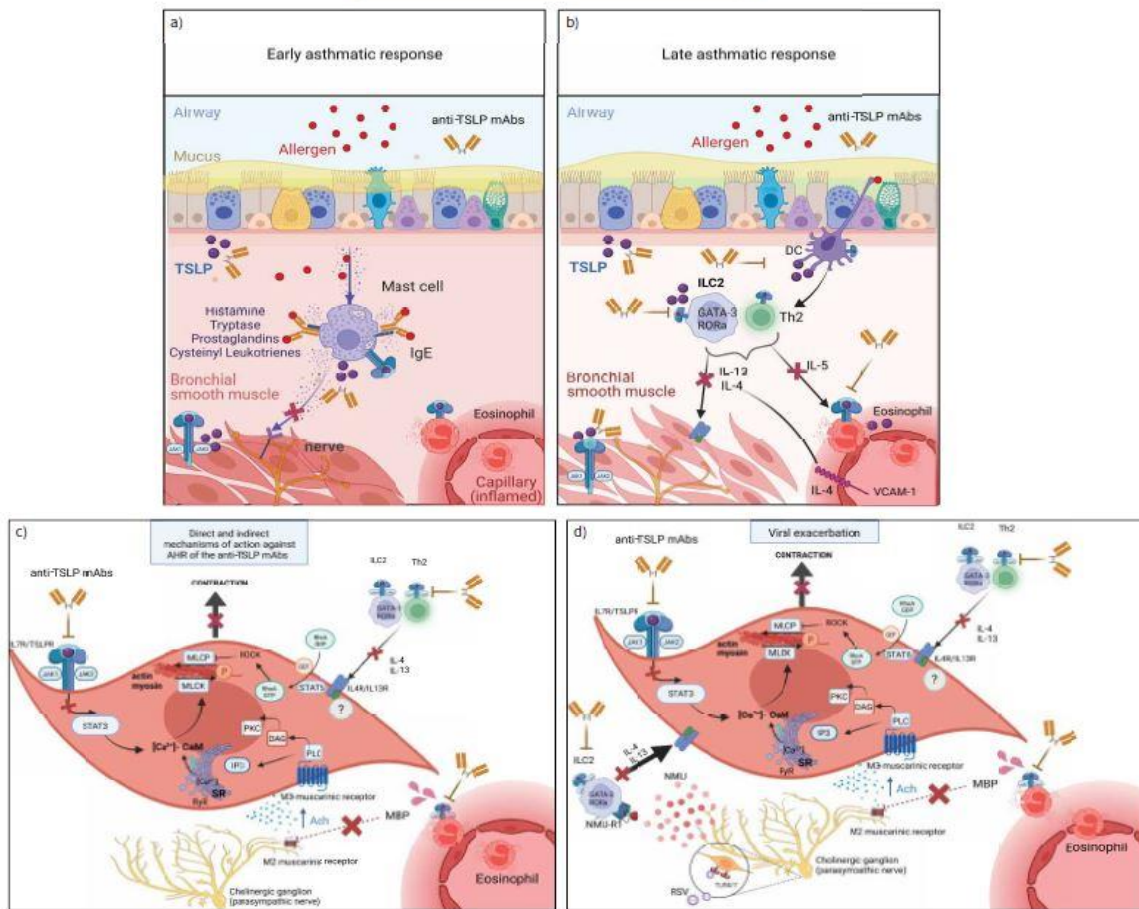
Second, comparisons are hampered by the way outcomes are expressed (i.e. proportion of patients without airway hyperresponsiveness, percentage change in forced expiratory volume in 1 s (FEV1) after challenge, fall in absolute FEV1 expressed in volume or expressed in percent of predicted values, area under the curve, etc.). The European Medicines Agency guidelines for the clinical evaluation of medicinal products for the treatment of asthma states that bronchoprotection (i.e. the ability of a drug to provide protection against bronchial challenge) is an acceptable objective measure of clinical efficacy [14]. Yet the lack of validated minimal clinically important difference and the complexity of assessing the extent of effects would need to be overcome in the future.

Third, it is noteworthy that not more than half of all adults with severe asthma are actually allergic and hence allergen challenge may often inform about non-universal mechanisms, where the contribution of IgE in hyperresponsiveness has been previously suggested [15].

Given the observed benefits of TSLP blockade with tezepelumab in a broad population of asthma subjects with low T2 traits at inclusion, and irrespective of whether or not there is biological evidence of allergy, the allergen challenge test only provides information on one aspect of the asthma mechanism [16]. Interestingly, both allergen and viral challenge can elegantly showcase asthma features, even when administered intranasally [17, 18], which makes sense since the main source of TSLP is airway epithelium, which is embryologically shared by the upper and lower airways.



Mechanisms of action of the anti-TSLP mAbs during the early and late asthmatic responses



**FIGURE 1** a, b) Mechanisms of action of anti-thymic stromal lymphopoietin (anti-TSLP) monoclonal antibodies (mAbs) during early and late asthmatic responses. a) The early asthmatic response (EAR) is characterised by acute airway obstruction beginning within a few minutes and resolving within 1–3 h. EAR is supposedly mainly linked to airway smooth muscle tone. The molecular mechanisms include mast cell degranulation due to IgE crosslinking to their high affinity receptors, leading to the release of histamine, tryptase and lysosomal enzymes, prostaglandins and cysteinyl leukotrienes. Anti-TSLP blocking monoclonal antibodies dampen EAR through the inhibition of epithelial TSLP release and avoid TSLP binding with its TSLPR receptor on mast cells. b) The late asthmatic response (LAR) starts after 3–5 h and peaks at 6–12 h but may persist for up to 24 h. LAR is associated with inflammatory cells, particularly lymphocytes and eosinophils in the bronchial mucosa. TSLP promotes T2 inflammation through activation of type 2 innate lymphoid cells (ILC2), dendritic cells (DC), eosinophils and polarisation of naïve T-helper (Th) cells within T2 cells. Anti-TSLP mAbs directly bind TSLP, thereby avoiding alarmin interaction with target cells such as airway smooth muscle (ASM), eosinophils, ILC2 and Th2 lymphocytes. Blocking TSLP decreased interleukin (IL)-4, -5 and -13 release, thereby decreasing IL-4 facilitation of eosinophil recruitment through VCAM-1. c) Direct and indirect mechanisms of action against airway hyperresponsiveness (AHR) of anti-TSLP mAbs. The molecular mechanism of airway smooth muscle contraction is regulated by myosin light-chain (MLC) phosphorylation, which is controlled by the activity of myosin light-chain kinase (MLCK) and myosin light-chain phosphatase (MLCP). MLCK activation depends on the intracellular  $\text{Ca}^{2+}$  concentration. Cell membrane depolarisation activates calcium channels, resulting in  $\text{Ca}^{2+}$  influx, whereas agonist stimulation mainly activates G protein-coupled receptors (GPCRs) such as the M3 muscarinic receptor, leading to inositol 1,4,5-trisphosphate (IP3) formation and  $\text{Ca}^{2+}$  release

from the sarcoplasmic reticulum (SR). The increase in cytosolic  $\text{Ca}^{2+}$  leads to smooth muscle contraction through MLCK activation by  $\text{Ca}^{2+}$ /calmodulin (CaM) and myosin RLC phosphorylation. Moreover, GPCR activation leads to MLCP inactivation by agonist-induced PKC and RhoA/ROCK activation. Direct effects: TSLP-induced STAT-3 activation was shown to increase ASM migration *via* increased Rac1 activity. *In vitro* evidence suggests that TSLP can stimulate ASM cells to enhance intracellular calcium responses to contractile agonists. Indirect effects: TSLP, which is located upstream in the inflammatory cascade, plays a key role in induction of the T2 response. Moreover, airway eosinophils are especially abundant around airway nerves in asthma, and eosinophils can alter sensory and parasympathetic nerve functions. Eosinophils release the major basic protein (MBP), which is an antagonist of inhibitory M2 muscarinic receptors on parasympathetic nerves, resulting in excessive acetylcholine (ACh) release. ACh therefore binds to muscarinic M3 mAChRs expressed on post-junctional ASM cells, stimulating phospholipase C (PLC) to produce diacylglycerol (DAG), which in turn activates phosphokinase C to promote ASM contraction. TSLP is able to activate ILC2s, which directly induce proliferation of ASM cells through IL-4/-13 release. Both IL-4 and IL-13 can enhance the concentration-dependent mobilisation of intracellular  $\text{Ca}^{2+}$  by histamine in ASM. d) Direct and indirect mechanisms of action against AHR of anti-TSLP mAbs during viral exacerbation: neuroimmune inflammation. TSLP blocking may also attenuate neurogenic inflammation and AHR during viral infections by decreasing the production of neurotransmitter neuromedin U (NMU) in the lungs and the expression of neuromedin U receptor 1 (NMUR1) on ILC2s. The figures were created using BioRender.com.

Finally, other safe and robust challenges warrant development, including viral challenge, which would potentially be able to generate information more accurately on the mechanisms involved during severe exacerbations: the latter are currently understood to be the greatest future risk associated with asthma and hence the exacerbation frequency is the primary endpoint in most clinical trials. Asthma viral challenge studies have only been performed in exceptional circumstances to date, yet they could generate further insight into the mechanism of action of new drugs in broader asthmatic populations.

As multiple biologic drugs for asthma have now been approved, or at least shown to be also efficient, for protecting the upper airways, the challenge for any new biologic agent is to fulfil this dual function. Targeting eculizumab solely towards the lower airways through a so-called pharmacological feat eventually turned out to be a misconception. Initial theoretical safety concerns were raised against systemic TSLP targeting, but recent 2-year assessment findings indicate that the potential advantage of local delivery is unfounded [19]. Although eculizumab has successfully overcome the go/no go hurdle of the allergen challenge study, as elegantly reported in this issue, development of the drug has now been interrupted because, ironically, it seems that the commercial promoter felt that the “go” findings were a warning sign.

### **Acknowledgements:**

We thank David Manley for revising the English in this editorial.

### **Conflict of interest:**

A. Bourdin reports grants from AstraZeneca and Boehringer Ingelheim, consulting fees from AstraZeneca, GSK, Novartis, Sanofi Regeneron, Boehringer Ingelheim, Amgen, AB Science and Med in Cell, lecture honoraria and travel support from AstraZeneca, GSK, Novartis, Sanofi Regeneron, Boehringer Ingelheim and Amgen, and advisory board participation with AstraZeneca, GSK, Novartis, Sanofi Regeneron, Boehringer Ingelheim, Amgen and AB Science, outside the submitted work. J. Charriot reports consulting fees and lecture honoraria from AstraZeneca, GSK, Novartis and Sanofi Regeneron, outside the submitted work. E. Ahmed reports consulting fees from AstraZeneca, GSK, Novartis and Sanofi Regeneron, outside the submitted work.

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