**Title**: The bronchodilator and anti-inflammatory effect of long-acting muscarinic antagonists in asthma: an EAACI position paper.

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**Statement of contribution**

IA and IEG designed the work and distributed the tasks among the other authors. The rest of the authors performed the literature review and drafted the corresponding sections. IA and IEG coordinated the work of the rest of authors and wrote the final version of the manuscript which was approved by all the authors.

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**Abbreviations**

ACh: acetylcholine

AEC: airway epithelial cell

BHR: bronchial hyperresponsiveness

BMI: body mass index

ChAT: choline acetyl transferase

COPD: chronic obstructive pulmonary disease

CRP: C-reactive protein

EAACI: European Academy of Allergy and Clinical Immunology

EGF: epithelial growth factor

GINA: Global Initiative for Asthma

GLY: glycopyrronium bromide

GM-CSF: granulocyte and monocyte colony stimulating factor

ICS: inhaled corticosteroids

LABA: long-acting β2 agonist

LAMA: long-acting muscarinic antagonist

LTB4: leukotriene B4

M: muscarinic

MITT: triple therapy with multiple inhaler devices

NAEPP: National Asthma Education and Prevention Program

OCS: oral corticosteroids

PG: prostaglandin

QoL: quality of life

s: allergen-specific

SITT: triple therapy with single inhaler device

SMC: smooth muscle cell

TIO: tiotropium bromide

TT: triple therapy

TXA2: thromboxane A2

**Abstract** (188 words)

As cholinergic innervation is a major contributor to increased vagal tone and mucus secretion, inhaled long-acting muscarinic antagonists (LAMA) area pillar for the treatment of chronic obstructive pulmonary disease and asthma. By blocking the muscarinic receptors expressed in the lung, LAMA improve lung function and reduce exacerbations in asthma patients who remained poorly controlled despite treatment with inhaled corticosteroids and long-acting β2 agonists. Asthma guidelines recommend LAMA as a third controller to be added-on before the initiation of biologicals. In addition to bronchodilation, LAMA also exert anti-inflammatory and anti-fibrotic effects by inhibiting muscarinic receptors present on neutrophils, macrophages, fibroblasts and airway smooth muscle cells. Thus, besides bronchodilation, LAMA might provide additional therapeutic effects, thereby supporting an endotype-driven approach to asthma management. The Position Paper, developed by the Asthma Section of the European Academy of Allergy and Clinical Immunology, discusses the main cholinergic pathways in the lung, reviews the findings of significant clinical trials and real-life studies on LAMA use in asthma, examines the placement of these drugs in asthma clinical guidelines, and considers the potential for personalized medicine with LAMA in both adult and pediatric asthma patients.

**Main text** (4440 words)

1. Introduction:

Asthma is an environmentally-driven chronic inflammatory airway disease displaying a significant heterogeneity in terms of pathophysiology, severity, and evolution [**1**]. Inhaled corticosteroids (ICS) are the cornerstone of asthma treatment in all severity steps, as they block most inflammatory mechanisms elicited by environmental stressors [**2**]. Asthma guidelines recommend increasing the ICS dose from low to medium in case of insufficient control [**3-5**]. Moreover, the combination of ICS with an inhaled bronchodilator, as both controller and reliever medication, is advised from the first treatment step to simultaneously alleviate inflammation and limit bronchoconstriction. In recent years, guidelines favoured the use of formoterol in this respect, as it is a β2 agonist with both a fast onset of action and long duration of effect [**3**]. More recently, several large-scale clinical trials have shown that inhaled long-acting muscarinic antagonists (LAMA) provide additional bronchodilation and protection from exacerbations in asthma patients who remain poorly controlled with ICS/long-acting β2 agonist (LABA) [**6-9**]. Therefore, tiotropium (TIO) was approved for patients with asthma ≥6 years, whereas glycopyrronium (GLY) is indicated in patients≥18 years (**Table 1**). LAMA inhibit acetylcholine (ACh) signalling through the G protein-coupled muscarinic receptors expressed in the lung, thus interfering with the actions of the parasympathetic nervous system [**10**]. LAMA have been a pillar of chronic obstructive pulmonary disease (COPD) treatment for decades [**11**], where ACh-driven bronchoconstriction is the main reversible component of airflow limitation [**12**]. Conversely, both decreased adrenergic stimulation and enhanced muscarinic activation are prominent inducers of bronchoconstriction in asthmatics [**13**]. Thus, in those patients who remain insufficiently controlled with ICS/LABA, asthma guidelines recommend adding-on LAMA, before conducting a phenotypic assessment to decide on a personalized management strategy with biologicals or other targeted therapies [**3-5**]. This Position Paper by the Asthma

Section of the European Academy of Allergy and Clinical Immunology (EAACI) describes the major neuronal and non-neuronal cholinergic pathways in the lung, together with the anti-inflammatory and anti-remodelling effect of muscarinic inhibition in airway diseases. The available evidence support LAMA clinical efficacy and provide further opportunities for an endotype-driven approach to asthma management.

## 2. The heterogeneity of asthma endotypes

In most patients with asthma, airway inflammation arises from mixed exposure (coined as the exposome) to airborne allergens, indoor and outdoor pollutants, microbes, and several other environmental stressors such as extreme weather events [**14**]. These environmental stimuli trigger both stromal cell-dependent and hematopoietic cell-dependent inflammation, which translates into airflow obstruction and bronchial hyperresponsiveness (BHR), via the dysregulation of adrenergic and cholinergic innervation [**1**]. Ach signalling in airway epithelial cells (AEC), fibroblasts and smooth muscle cells (SMC) induces fibrosis and remodelling without concomitant infiltration by hematopoieticcells [**12**]. Stimulation of the muscarinic receptors on innate lymphoid cells and myeloid dendritic cells activates adaptive T1, T3 or T2 immune responses (dominated by IFNγ, IL-8/IL-17, and IL-4/IL-5/IL-13, respectively), including the synthesis of allergen-specific (s)IgG and sIgE by infiltrating B cells [**15**]. These inflammatory events can drive bronchoconstriction indirectly, via the inhibition of adrenergic receptors [**13**]. Regardless of the involvement of adaptive immune responses, eosinophils and neutrophils are recruited to the airways, under the influence of IL-5, and IL-8, respectively [**16**]. Cholinergic stimulation of hematopoietic and stromal cells further augments granulocyte recruitment [**12**]. Airway remodelling arises from the effects of the mediators produced by recruited and bronchial resident cells [**17**]. In this regard, sIgG can trigger the degranulation of neutrophils, whereas sIgE activates resident mast cells and eosinophils, leading to the release of preformed mediators like proteases [**1**].

The features of the immune response driven by stromal and hematopoietic cells, and of the neural dysregulation do not differ substantially between allergic and non-allergic asthmatics [**17**]. The diversity of mechanisms elicited by environmental exposures in the airways has been recently highlighted in the new EAACI Nomenclature of Allergic Diseases [**18**]. In this regard, it is logical to believe that the relative contribution of these mechanisms varies across the asthma population, and that this heterogeneity accounts for the different disease theratypes [**19**]. Inflammatory events elicited by environmental stressors in asthma patients are summarised in **Figure 1.**

3. <u>The relevance of the acetylcholine/muscarinic axis in asthma pathogenesis</u>

The vagus nerve connects the central nervous system with the parasympathetic airway ganglion where it releases ACh. In the synaptic cleft, this neurotransmitter binds to the nicotinic or muscarinic (M) 1 receptor expressed in postjunctional parasympathetic fibres [**20**]. In turn, these nerves stimulatelung cells expressing M1, M2, or M3 receptors. Of note, both stromal and hematopoietic cells can respond to parasympathetic neurotransmitters [**21**]. M1 and M3 activation in submucosal glands induces mucus secretion, whereas M3 stimulation in SMC drives bronchoconstriction [**20**]. Vagal and parasympathetic fibres also express M2 receptors in the areas exposed to the synaptic cleft, so ACh can be recaptured in an autocrine manner, with subsequent blocking of the stimulation of postjunctional nerves or target cells [**22**]. Moreover, unmyellinated C fibres connect the central nervous system with the subepithelial region of the bronchial mucosa, where they are activated by physical stimuli such as heat or cold [**21**]. Stimulated C fibres secrete neurokinins, which promote neurotransmission in the airway ganglion (peripheral reflex arch). As the neuronal cholinergic system is one of the main regulators of airway homeostasis (**Figure 2**), dysfunction can lead to significant alterations resulting in different asthma endotypes [**20**].

*3.1 Regulation of airway smooth muscle tone by muscarinic receptors in asthma patients*

Airway SMC from the trachea and large bronchi express large numbers of M2 and M3 receptors [**20**]. Although M2 is more abundant, M3 receptor signalling is the main driver of ACh-mediated bronchoconstriction in the large airways, as illustrated by the lack of response to methacholine provocation in M3 knocked out mice [**23**].

Patients with asthma are more sensitive to M3 receptor signalling as compared to healthy subjects, partially due to anenhanced ability to open large $Ca^{2+}$ channels in SMC [**24**]. The intracellular signalling molecules include phospholipase Cβ1, 1,4,5-trisphosphate, CD38 and cyclic adenosine diphosphate ribose. Although the activation threshold of $Ca^{2+}$ channels may be intrinsically decreased in asthmatics, several cytokines (IL-1β, IL-13, TNFα, or IFNγ) are known to potentiate this effect [**25**].

ACh signalling in SMC also increases contractilityby $Ca^{2+}$-independent mechanisms. M2- and M3-receptor activation blocks the function of myosin light chain phosphatase, thus promoting actin cytoskeletal dynamics and bronchoconstriction [**26**]. This effect is mediated by the increased activity of RhoA/Rho kinase cascade pathway which is also stimulated by exposure to allergens, bacteria and cigarette smoke [**26**].

Increased ACh availability also accounts for M1 and M3 heightened signalling in asthmatics. ACh is synthetized in vagal or parasympathetic fibres by the enzyme choline acetyl transferase (ChAT), the expression of which is boosted by prostaglandins (PG) or thromboxane A2 (TXA2) [**27**]. Conversely, ACh is metabolized in the synaptic cleft by acetylcholinesterase, an enzyme which is inhibited by allergen exposure [**28**]. Moreover, the ability of presynaptic M2 receptor to recapture ACh is decreased in asthmatics exposed to several environmental triggers. For example, major basic proteins released from eosinophils recruited to the airways upon allergen- or ozone-induced inflammation compete with ACh to bind to M2 receptors in the synaptic clefts [**29**]. Similarly, neuraminidases and IFNγ produced during viral infections cleave M2 receptor and prevent the recapture of ACh [**30**].

In this regard, asthmatics are more responsive to antimuscarinic bronchodilation during viral infections than during steady state [**31**].

The epithelial barrier defect in patients with asthma facilitates the interaction between subepithelial C fibres and the environmental stressors [**32**]. C fibres respond with the production of neurokinins, which boosts the release of histamine, PG, TXA2, or bradykinin to the airway ganglion, and favours M1 receptor activation in the postjunctional parasympathetic fibre [**33**]. **Figure 3** depicts the mechanisms driving cholinergic bronchoconstriction in asthma patients.

*3.2. Regulation of mucus secretion by muscarinic receptors in asthmatics*

Airway mucus is a heterogenous gelatinous mix of secretions and cell debris that facilitates homeostatic clearance of external agents from the lumen. Goblet cells and submucosal glands are the primary source of the secretions. Besides water (98%) and electrolytes (1%), secretions contain glycoproteins like mucins, especially MUC5AC and MUC5B [**34**]. These glycopolymers are held together by disulfide bonds and contribute substantially to the viscosity of airway mucus [**34**]. As submucosal glands from the trachea and large bronchi express high amounts of M1 and M3 receptors, ACh is the main driver of mucus secretion in the central airways [**35**]. Isolated M3 signalling stimulates mucin secretion, whereas the co-activation of M1 and M3 receptors results in the release of water and electrolytes by submucosal glands [**36**]. Conversely, direct goblet cell stimulation in the central airways require relatively high amounts of ACh [**37**], which probably implies a minor role for this pathway in physiological conditions.

Asthma patients have an altered mucus composition with increased MUC5AC and additional mucin types, together with less abundant water and electrolytes [**38**]. Moreover, M1 and M3 stimulation in submucosal glands transactivates the receptor for epithelial growth factor (EGF) in goblet cells, making them more sensitive to EGF-mediated goblet cell hyperplasia and mucus hypersecretion [**39**]. Thus, ACh pathway indirectly contributes to goblet cell alterations in asthma patients.

*3.3 Regulation of airway inflammation and remodelling by muscarinic receptors*

Hematopoietic cells infiltrating the asthmatic airways express ChAT and can synthetize ACh, while their exposure to inflammatory milieu upregulates the expression of muscarinic receptors [**40**]. Indeed, the non-neuronal cholinergic system is believed to regulate airway inflammation in an autocrine/paracrine manner. For example, major basic protein from eosinophils favour ACh release, which in turn activates group 2 innate lymphoid cells, thus furthering eosinophil activation [**41**, **42**]. Airway lymphocytes also express M1-M3 receptors, although high inter-individual variability exists [**43**]. The activation of M3 receptors on CD8+ T cells enhances their cytotoxicity and cytokine production, whereas cholinergic stimulation in T and B cells promotes their proliferation [**44**]. M1 receptors are present on airway mast cells and eosinophils, while neutrophils, macrophages and monocytes display M1-M3 receptor expression [**45**]. ACh promotes the activation of intracellular MAP kinases in macrophages and neutrophils, leading to leukotriene (LT) B4 synthesis and chemotaxis of hematopoietic cells [**46**]. Bronchial stromal cells also express ChAT and muscarinic receptors. M1/M3 receptor activation in AEC triggers the release of LTB4 among other chemotactic factors for eosinophils, monocytes and neutrophils [**47**]. Moreover, the stimulation of nicotinic receptors in AEC increases granulocyte and monocyte colony stimulating factor (GM-CSF) production [**48**]. Thus, the epithelial cholinergic system becomes a potent initiator of inflammatory responses.

Besides promoting contractility, the M3 receptor activation enhances the expression of IL-6, IL-8, and cyclooxygenase 1, and the responsiveness to mitogenic factors such as EGF in SMC [**49**]. Moreover, M2 and M3 signalling in mesenchymal cells triggers the expression of contractile proteinsvia RhoA/Rho kinase and phosphoinositide 3 kinase pathways, a crucial step in their maturation into SMC [**50**]. Of note, this effect is driven by allergen exposure, due to its capacity to release ACh from airway stromal cells [**51**]. Interestingly, ACh stimulation also induces directly the proliferation of airway fibroblasts[**52**]. Of note, sub-epithelial fibrosis, extracellular matrix deposition, and SMC hyperplasia are cardinal features of airway remodelling in asthma [**53**]. Together with the ability to induce goblet cell hyperplasia and mucus secretion, this evidence indicates the relevance of non-neuronal cholinergic system in airway remodelling [**54**]. The effects of cholinergic stimulation on mucus secretions, airway inflammation and remodelling in asthma patients are detailed in **Figure 4**.

4. *In vivo* and *in vitro* data showing the anti-inflammatory effect of LAMA

A multitude of animal studies, especially mouse models of asthma/COPD exacerbations triggered by bacterial or viral infections, demonstrate the anti-inflammatory effects of LAMA [**55-60**]. Collectively, these models show a reduction of inflammatory mediators and cells in the bronchoalveolar lavage fluid following treatment with LAMA [**61**].

Numerous *in vitro* studies described anti-inflammatory effects for LAMA on primary or immortalised human AEC [**62**]. TIO (100nM) and aclidinium were able to suppress tobacco smoke-induced production of IL-8 by AEC [**63**]. Moreover, TIO attenuated IL-8 and NF-κB expressionon human AEC following their incubation with IL-17A or sputum from COPD patients [**64**]. TIO also reduced the production of chemokines (e.g. CCL2) by AEC after their incubation with TGFβ1, ACh, or sputum from COPD individuals [**65**]. Interestingly, TIO concentrations similar to those usually seen in the sera of patients taking 18 μg/day suppressed the release of IL-1β, IL-6 and IL-8 induced by rhinoviruses in AEC sampled from healthy subjects and from patients with asthma or COPD [**66**]. Similarly, the LPS-triggered production of IL-8 and expression of NF-κB was inhibited by TIO in cultured epithelial cells [**67**]. Moreover, TIO reduced the secretion of IL-8 by human lung fibroblasts following their stimulation by IL-1β, TGFβ1 or cholinergic drugs [**65**].

Importantly, TIO (30nM), but not ipratropium bromide, reduced the activation of neutrophils (TNFα secretion, and expression of adhesion molecules) following their incubation with the supernatant of cultures of LPS-stimulated lung macrophages [**68**]. Moreover, TIO increased the apoptosis of CD3+ and CD8+ T cells in peripheral blood of COPD patients, while inhibiting the release of IL-5 and IL-13, but not IL-4, following the bacterial stimulation of peripheral mononuclear cells from asthmatics [**66**]. Conversely, the synthesis of GM-CSF, IL-6, IL-8 and LTB4 by neutrophils following their incubation with sputum from COPD subjects was not suppressed by TIO [**69**].

The evidence of the anti-inflammatory effect of LAMA other than TIO is much scarcer. In one study, GLY (100nM) prevented both mRNA and protein expression of IL-8 following ACh stimulation of primary AEC [**68**]. Bronchial provocation via histamine in patients with asthma leads to the release of IL-4, IL-5, IL-6, IL-9, IL-13, TNFα, and TSLP in the distal airways [**70**]. Pre-treatment with ICS/LAMA or ICS/LABA/LAMA combinations suppressed the synthesis of these mediators and improved histamine-triggered BHR [**71**].

A recent systematic review identified 49 original articles examining the *in vivo* and *in vitro* effects of LAMA in patients with asthma or on human cells [**72**]. The collective analysis of TIO studies provided low-to-medium quality evidence for an anti-inflammatory effect of LAMA in patients with stable airway disease. A 1-month TIO course (18 μg/day) suppressed LTB4 release by blood neutrophils primed with GM-CSF but did not modify IL-6 and TNFα levels in the exhaled breath condensate [**73**]. Studies with longer treatment periods showed inconsistent results. A 3-month TIO course did not change serum C-reactive protein (CRP) or blood granulocyte and monocyte counts [**74**], whereas a 6-month course reduced blood eosinophil, neutrophil and monocyte counts without affecting CRP, TNFα, or fibrinogen levels in serum [**75**]. Conversely, 1-year TIO treatment (18 μg/day) resulted in enhanced sputum IL-8, while no change was observed for sputum IL-6 or serum CRP or IL-8 [**76**].

In summary, only TIO has shown clear anti-inflammatory effects in animal models of airway diseases, and *in vitro* studies with human cells (mostly AEC), but definitive evidence for a clinical effect of LAMA beyond bronchodilation is still lacking in asthma patients [**77**].

5. Clinical data on the efficacy of LAMA in moderate-to-severe asthmatics

The co-administration of ICS, LABA and LAMA is commonly referred as triple therapy (TT). TT regimens can be administered once or twice daily, via a single inhalation device (SITT) or through multiple inhalers (MITT) [**78**]. In asthma patients, SITT is associated with a higher adherence and persistence as compared to MITT [**79**]. Moreover, several works focusing on COPD individuals demonstrate a higher adherence and persistence, a lower rate of severe exacerbations and reductions in healthcare resource utilization for SITT [**80-82**]. On the other hand, MITT has the advantage of greater flexibility when adjusting for the ICS dose [**78**]. In any case, adherence to both SITT and MITT is relatively low and typically decreases over 12 months [**83**]. Further research is warranted to assess whether once-daily SITT dosing translates into additional clinical benefits.

Four clinical trials assessed the efficacy of SITT administered in different combinations and doses versus medium-to-high dose ICS/LABA, or MITT in asthma patients [**6-9**]. Importantly, no significant differences in exacerbation rate were found between SITT (high dose ICS) and high dose ICS/LABA (same molecules) in the CAPTAIN study (fluticasone furoate plus vilanterol plus UME) [**9**], IRIDIUM study (mometasone furoate plus indacaterol acetate plus GLY) [**7**], and TRIGGER study (beclomethasone dipropionate plus formoterol plus GLY) [**6**]. Nevertheless, a lower number of severe exacerbations were observed for SITT, when compared with dose-matched ICS/LABA combinations with the same (TRIMARAN) [**6**] or different (IRIDIUM) [**7**] molecules. Moreover, a pooled analysis of CAPTAIN study showed that TT was associated with a higher and clinically relevant improvement in asthma control questionnaire at week 24 (63% for TT versus 55% for ICS/LABA) [**9**]. A recent meta-analysis pooling these trials concluded that: (i) SITT with high dose ICS is more effective than SITT with medium dose ICS in reducing exacerbations; (ii) SITT with medium dose ICS is equally effective as high dose ICS/LABA in reducing exacerbations; (iii) SITT with medium dose ICS improves $FEV_1$ significantly more than high dose ICS/LABA [**84**].

Clinical trials assessing the efficacy of adding a LAMA to patients who remained poorly controlled on dose-matched ICS/LABA have shown an increase in $FEV_1$ as compared with placebo [**85-87**]. A reduction of exacerbation risk was also reported for TT by the PrimoTinA-asthma study [**85**]. Moreover, MITT improved control and quality of life (QoL), although the minimal clinically important difference was not reached.

Interestingly, a recent study including >12000 adolescents and adults with uncontrolled asthma indicated that TT did not reduce disease-related hospitalizations [**88**]. On the other hand, the addition of TIO reduced short-acting β2 agonist utilization, and improved lung function and sleep quality in school age children receiving ICS/LABA in the context of persistent moderate asthma [**89**]. The safety profile of ICS/LABA/LAMA is considered favorable in adolescent and adult asthmatics [**90-93**]. A pairwise meta-analysis of clinical trials identified an increased risk (RR 0.74) of vascular serious adverse events for TT [**92**] in this population, but other studies have not confirmed this effect [**93, 94**]. Of note, indirect evidence from systematic reviews including both children and adults with persistent uncontrolled asthma showed that TT was associated with increased dry mouth and dysphonia (high-certainty evidence), but serious adverse events were not significantly different between active and placebo groups (moderate-certainty evidence) [**94**].

The results of major clinical trials of TT in asthma patients are summarised in **Table 2**.

6. <u>Comparative performance of ICS/LABA/LAMA in patients with asthma or with COPD</u>

As a whole, inflammation in asthma is regarded more sensitive to ICS than in COPD [**2**, **90**]. On the other hand, cholinergic stimulation is a relevant driver of bronchoconstriction in both asthma and COPD individuals, whereas decreased adrenergic activation is more prominent in asthma than in COPD [**95**, **96**]. Of note, the performance of LABA and LAMA as an add-on therapy to ICS in asthma patients is comparable in terms of improvement of control, exacerbation rate and need for systemic corticosteroids [**95**, **96**]. On the other hand, LAMA perform slightly better for lung function, while LABA improves the quality of life in asthmatics [**91**, **96**]. Thus, unlike COPD, LAMA are not recommended as monotherapy in asthma [**97-99**].

In patients with COPD, inhaled muscarinic antagonists or β2 agonists in monotherapy are advised for mild patients with ≤1 moderate exacerbations not leading to hospitalization in the previous year (long-acting drugs are preferred) [**11**]. Conversely, patients with moderate-to-severe disease, or those with ≥2 moderate exacerbations or ≥1 exacerbation leading to hospitalization should receive LAMA/LABA combination [**100**]. Of note, the addition of ICS is only encouraged in COPD individuals with frequent exacerbations and a pre-ICS blood eosinophil count ≥300 cells/µL. Indeed, TT in this population may reduce the risk of all-cause mortality [**101**]. LAMA improve $FEV_1$, symptoms (dyspnea, cough, and expectoration), and QoL, while reducing exacerbations, hospitalizations, and death rate in COPD individuals [**102**]. Of note, LAMA enhance the benefits of pulmonary rehabilitation by increasing its ability to reduce symptoms and improve QoL, peripheral muscle function, and exercise capacity in patients with COPD [**100**]. Moreover, LAMA are superior to LABA in decreasing exacerbation and hospitalization rates in moderate-to-severe COPD patients [**102**]. Nevertheless, LAMA/LABA combination is still recommended in this population because both drug types modulate the bronchial tone differently and have an additive effect on the inhibition of $Ca^{2+}$channels and SMC tyrosine kinases [**101**]. No preference for bronchodilator type is given for mild COPD patients with infrequent exacerbations, due to a high inter-patient variability in the responsiveness to these drugs [**11**].

The safety profile of LAMA is considered favorable in COPD individuals [**103**]. A slightly higher incidence (adjusted hazard ratio of 1.28 (95% CI: 1.05-1.55) relative to LABA-ICS) of major adverse cardiovascular events has been recently reported for TT [**104**], but this finding requires confirmation. **Table 3** summarises the comparative efficacy/safety of LAMA, LABA and ICS in asthma and COPD patients.

7. LAMA positioning in asthma guidelines.

The 2024 update of the Global Initiative for Asthma (GINA) [**3**] recommends adding inhaled TIO or GLY to asthma patients insufficiently controlled with medium dose ICS/formoterol (step 5). GINA considers that TT should be advised in every patient before high dose ICS or before the initiation of biologicals, including prior phenotypic assessment. Similarly, the addition of inhaled TIO is regarded as an intermediate step before biologicals for subjects 6-11 years insufficiently controlled with medium dose ICS/LABA.

The Focused Updates of the Asthma Management Guidelines generated in 2020 by the National Asthma Education and Prevention Program (NAEPP) established by the US Government reviewed the evidence regarding LAMA use [**5**]. In patients ≥12 years ICS/TIO is listed as an alternative to ICS/formoterol in step 3 (low dose ICS) and step 4 (medium dose ICS), whereas TT with medium-to-high dose ICS is advised for every individual in step 5. Conversely, LAMA is not recommended for patients 5-11 years, or adolescents and adults on OCS therapy (step 6).

The European Respiratory Society/American Thoracic Society Guideline on the Management of Severe Asthma conducted a systematic review on the use of LAMA in pediatric and adult patients in 2020 [**4**]. The document formulates a strong recommendation for the addition of inhaled TIO to children, adolescents and adults with severe asthma uncontrolled despite GINA steps 4-5 or NAEPP step 5 (moderate quality evidence). **Table 4** summarises the positioning of LAMA in major asthma guidelines.

8. Real-life studies of LAMA in severe asthma

Published registries report that 36-39% of severe asthmatics receive a LAMA [**105**-**107**]. In this regard, the capacity of TT to reduce exacerbations as compared with dose-matched ICS/LABA regimens was reported in a large retrospective real-life cohort of 7857 patients [**105**], and in a real-life prospective study of 2042 asthmatics [**106**]. The previous smoking habit, older age, concomitant bronchiectasis, and body mass index (BMI)>30 kg/m$^2$ were associated with increased LAMA prescription in real-life studies.

9. LAMA guiding precision medicine in asthma

Seventy-five% of severe asthmatics on TT remain uncontrolled, thus leading to >80% of severe asthmatics on biologicals receiving or having received a LAMA [**107**]. The relatively low success rate of LAMA in severe asthma might arise from the lack of a phenotype-guided prescription [**105**-**107**]. In this regard, patients with high total IgE in serum at baseline, are less likely to respond to a 3-month course of inhaled TIO [**108**]. In line with this, a sub-analysis of the CAPTAIN study suggests that increasing ICS dose from medium to high may especially benefit patients with elevated T2 biomarkers, whereas TT with medium dose ICS might be a more meaningful approach to prevent exacerbations in subjects without evidence of T2 biomarkers [**109**]. Conversely, a post hoc analysis of PrimoTinA-asthma trial indicated that the efficacy of add-on TIO to ICS/LABA was independent of gender, BMI, disease evolution, smoking habit, and $FEV_1$ reversibility [**110**]. Smoking habit (current versus former) did not influence the improvement of lung function in COPD individuals treated with GLY [**111**]. On the other hand, a post-hoc analysis of the IRIDIUM trial showed that TT benefits equally asthma patients with/without fixed airflow limitation [**112**].

Interestingly, M1-M3 receptors are more abundant in the central airways which might account for a higher benefit from LAMA in patients with predominant proximal airway obstruction, as compared with those with primary involvement of the distal airways [**20**]. The same rationale might apply for patients with increased mucus production [**34-36**].

Moreover, the relative importance of the vagal tone increases with age [**113**]. Therefore, it is tempting to speculate that asthmatics with older age, increased mucus production, exacerbations triggered by infections, decreased $FEV_1$ (as a measure of central airways obstruction) with preserved FEF25-75 or other measure of the peripheral airways, and with absent T2 biomarkers are particularly good candidates for TT [**114**]. **Table 5** shows the visible properties of an asthma patient theratype benefiting from LAMA addition.

10. Knowledge gaps and research needs

The evidence available from mechanistic and real-life studies points at a phenotype-specific performance for LAMA [**72**, **105-107**]. Thus, it is crucial to investigate whether individuals with non-T2 asthma benefit better from these drugs, and how LAMA interact with other non-T2 interventions (e.g. azithromycin or bronchial thermoplasty) [**115**]. The impact on lung function decline and on other surrogates of remodelling deserves further evaluation. There is also a need to analyze the impact of current and former smoking habit on LAMA performance in asthmatic populations. Of note, most clinical trials of TT in asthmatics exclude current smokers and include only a limited number of former non-heavy smokers [**6, 7, 9**]. Similarly, it would be interesting to investigate if certain patients' profiles on low-to-medium ICS/LABA would improve their asthma control by adding LAMA rather than increasing the ICS dose.

The efficacy and safety of LAMA in children also requires further investigation. The comparative performance of LAMA molecules needs to be tested in head-to-head trials. Similarly, the real capacity of LAMA to delay the introduction of biological in severe asthma patients needs to be established. In this regard, there is a need to analyse the long-term performance of TT, and whether the delay of biologicals is harmful for patients. Overall, the unmet needs about LAMA potential in asthmatics can only be addressed by sufficiently powered clinical trials, registries and real-life studies incorporating long-term surveillance and follow-up.

## 11. Conclusion and final remarks

The therapeutic arsenal for patients with asthma has been recently enlarged by the addition of LAMA, the indication of which was previously restricted to COPD. Cytokine-mediated inflammation and decreased adrenergic stimulation are major drivers of bronchoconstriction and remodelling in most asthmatics, especially in patients with T2 asthma. These traits respond relatively well to the combination of ICS and LABA. Nevertheless, patients with T2 asthma on ICS/LABA can obtain additional benefit from the blockage of muscarinic receptors. In this regard, large scale clinical trials indicate the capacity of TT to improve lung function and decrease exacerbations in poorly controlled moderate-to-severe asthmatics. Moreover, the cholinergic system might be the main driver of bronchoconstriction, mucus secretion and remodelling in some asthma patients of varying phenotypes and severity. Indeed, some data suggest that the addition of LAMA can delay the prescription of biological in some individuals with severe asthma. Further studies are needed to identify the LAMA responsive theratypes, and thus to position LAMA in the personalized and cost-efficient strategies for the management of asthma patients.

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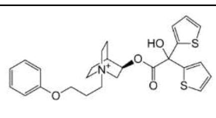
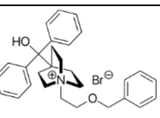
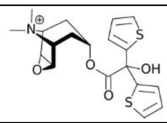
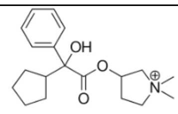
**Table 1. Available LAMA molecules.**

| | | | | |
|---|---|---|---|---|
| |  |  |  |  |
| | **Aclidinium bromide** | **Umeclidinium bromide** | **Tiotropium bromide** | **Glycopyrronium bromide** |
| **Metabolism** | Hydrolysis | CYP2D6 | CYP2D6<br>CYP3A4 | Unknown |

| | | | | |
|---|---|---|---|---|
| **Half-life** (hours) | 5-8 | 11 | 27-45 | 33-57 |
| **Approval** (Europe) | COPD | COPD | COPD<br>Asthma | COPD<br>Asthma |
| **Dose per inhalation** (µg) | 322-340 | 55 | 46-9 | 18-2.5 |
| **LABA combination** | Formoterol | Vilanterol | Olodaterol | Indacaterol |
| **Triple therapy combination** | - | Fluticasone furoate +Vilanterol | - | Mometasone furoate+Indacaterol<br><br>Beclomethasone dipropionate Formoterol fumarate<br><br>Budesonide+ Formoterol+ |
| **Inhalation device** | Genuair® | Ellipta® | Respimat®<br>Zonda®<br>Handihaler®<br>Glenmark® | Breezhaler®<br>Nexthaler®<br>Aerosphere®<br>MDI |
| **Pharmacological features** | Faster onset of action than tiotropium. Rapidly hydrolyzed in plasma to two major inactive metabolites resulting in a low and transient systemic exposure. | Slower dissociation from M3 receptor as compared with M1 and M2 receptors | | Significantly more rapid onset of action than other molecules.<br>It preferentially binds to M3 over M2 receptors.<br>The anticholinergic effects are primarily limited to the airways, thereby reducing systemic adverse events.<br>Shorter absolute dissociation time at both M2 and M3 receptors than aclidinium and tiotropium. |

**Abbreviations:** COPD= chronic obstructive pulmonary disease; CYP = cytochrome; LABA = long-acting beta2 sympathomimetic; LAMA = long-acting muscarinic antagonist;

**Table 3. Comparative efficacy of ICS, LABA and LAMA in patients with asthma or COPD**

| | | **Asthma** | **COPD** |
|---|---|---|---|
| **ICS** | Benefits | Improves lung function, quality of life, and asthma control<br>Decreases exacerbation rate | Decreases exacerbation rate only in patients with high blood eosinophils |

| | | | |
|---|---|---|---|
| | Harm | Depends on the dose<br>Low/medium dose<br>Oral candidiasis<br>Decreased growth in children<br>High dose<br>Osteoporosis/bone fractures<br>Adrenal insufficiency<br>Metabolic syndrome | Severe (increased risk of osteoporosis, bone fractures, and severe infections) |
| **LABA** | Benefits | Improves lung function better than LAMA. Improves quality of life and control. Benefit all patients. | Improves lung function, quality of life and exacerbation rate in all patients, but less than LAMA. |
| | Harm | Mild (tachycardia, muscle-skeletal tremor), but more frequent than LAMA | Mild (tachycardia, muscle-skeletal tremor), but more frequent than LAMA |
| **LAMA** | Benefits | Improves lung function<br>Decreases exacerbation rate in patients on medium-to-high ICS/LABA<br>Decreases mucus production | Improves lung function, quality of life, and symptoms, and decreases exacerbation rate in all patients |
| | Harm | Mild and reversible (dry mouth) | Mild and reversible (dry mouth) |

**Table 4. LAMA positioning in major asthma guidelines. *≥18 years. **≥4 years**

| | **GINA 2024** | **NAEPP 2020** | **ERS/ATS 2020** |
|---|---|---|---|
| *Adolescents and adults* | | | |
| **Add-on preferred** | Medium dose ICS/formoterol | Medium or high dose ICS/LABA | Medium or high dose ICS/formoterol |

| | | | |
|---|---|---|---|
| **Step** | 5 | 5 | GINA 4-5<br>NAEPP 5 |
| **Molecule** | TIO or GLY | TIO or GLY | TIO |
| **Add-on alternative** | Medium/high dose ICS/LABA | Low dose ICS<br>Medium dose ICS | No |
| **Step** | 4<br>5 | 3<br>4 | - |
| **Molecule** | TIO or GLY | TIO or GLY | - |
| *Children 6-11 years* | | | |
| **Add-on preferred** | No | No | Medium dose ICS/formoterol |
| **Step** | - | - | GINA 5 |
| **Molecule** | - | - | TIO |
| **Add-on alternative** | Medium dose ICS/formoterol | No | No |
| **Step** | 4 | - | - |
| **Molecule** | TIO | | |

**Table 5. Visible properties of an asthmatic theratype who would benefit the most from LAMA addition**

| **Domain** | **Visible property** |
|---|---|
| *Age* | Older age |
| *Lung function* | Low $FEV_1$<br>with preserved FEF25-75 or R25-R5 |
| *Mucus production* | Frequent and abundant |
| *Exacerbations* | Triggered by infections |
| *T2 biomarkers* | Low |

**Figure Legends**

Figure 1: Mechanisms elicited by environmental stressors in asthma patients. Allergens can activate tissue-dependent, innate immune system-dependent, and adaptive immune system-dependent mechanisms.

Figure 2: Neuronal cholinergic system in the airways. The vagus nerve connects the central nervous system with the airway ganglion, where cholinergic neurotransmission takes place. Subsequently, parasympathetic fibers stimulate smooth muscle cells and submucosal glands in the airways. Cholinergic neurotransmission in the airway ganglion is enhanced by neurokinins released from unmyellinated C fibers. ACh: acetylcholine; M: muscarinic; NAChR: nicotinic acetylcholine receptor.

Figure 3: Cholinergic control of bronchial smooth muscle tone in asthma patients. Asthmatics display intrinsic abnormalities in the contractility of smooth muscle cells. These alterations arise from calcium-dependent and calcium-independent mechanisms. Increased availability of acetylcholine in the synaptic cleft also accounts for cholinergic bronchoconstriction. In this regard, many environmental stressors can either promote acetylcholine synthesis or decrease acetylcholine metabolism. ACh: acetylcholine; AChE: acetylcholinesterase; cADPR: cyclic adenosine diphosphate ribose; ChAT: choline acetyl transferase; IFN: interferon; IL: interleukin; IP3: 1,4,5-trisphosphate; M: muscarinic; MBP: major basic protein; MLCP: myosin light chain phosphatase; PG: prostaglandin; PLCβ1: phospholipase Cβ1; SMC: smooth muscle cell; TNF: tumor necrosis factor; TXA2: thromboxane A2.

Figure 4: Cholinergic control of mucus secretion, inflammation and remodeling in asthma patients. Cholinergic stimulation increases mucus secretion by submucosal glands together with an altered mucus composition. Moreover, acetylcholine signaling also promotes goblet cell metaplasia in an indirect manner. Airway epithelial cells and most hematopoietic cells present in the airway mucosa express choline acetyl transferase together with muscarinic receptors. The activation of these receptors promotes granulocyte chemotaxis, and

lymphocyte proliferation by direct and indirect mechanisms. Cholinergic stimulation via muscarinic receptors drives the differentiation of mesenchymal cells into smooth muscle cells with a secretory phenotype. Moreover, acetylcholine stimulates directly bronchial fibroblasts to induce sub-epithelial fibrosis. ACh: acetylcholine; ChAT: choline acetyl transferase; COX-1: cyclooxygenase 1; EGF: epithelial growth factor; EGFR: epithelial growth factor receptor; GM-CSF: granulocyte and monocyte colony stimulating factor; IL: interleukin; IP3K: phosphoinositide 3 kinase; LTB4: leukotriene B4; M: muscarinic; MUC: mucin.