Impact of long-acting muscarinic antagonists on small airways in asthma and COPD: A systematic review

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Abstract

Small airway disease is recognized as a cardinal pathological process of chronic obstructive pulmonary disease (COPD), and recently small airways have been recognized as a major site of airflow obstruction also in asthmatic patients. The transversal involvement of small airways in COPD and asthma has warranted research efforts to identify therapeutic strategies able to unlock the small airway compartment. The mainstay of COPD treatment is represented by long acting-acting β_2-adrenoceptor agonists (LABAs) and long-acting muscarinic antagonists (LAMAs). In asthma the efficacy of LAMAs administered add-on to inhaled corticosteroids (ICSs) or ICS/LABA combinations has been investigated only in recent years. The aim of this systematic review was to examine the current literature concerning the impact of LAMAs on small airways and their lung deposition in both COPD and asthma. LAMAs administered either alone or in combination induced an effective bronchorelaxant effect of small airways, however the effectiveness of respiratory medications not only relies on the selected drug, but also on the employed inhalation device and patient's adherence. Tiotropium delivered via Respimat® SMI achieved a superior drug deposition in the peripheral lung compared to HandiHaler® dry powder inhaler and metered-dose inhalers (MDIs). The use of co-suspension™ delivery technology for MDIs and the introduction of the eFlow® nebulizer to deliver glycopyrronium improved aerosol drug delivery to the peripheral lung, by achieving uniform distribution of drug particles. This systematic review provides a synthesis of current literature concerning the impact of LAMAs on small airways and an insight on LAMAs distribution within the lung.

Keywords: COPD; drug deposition; LAMA; Respimat; SAD; small airways; tiotropium.

1. Introduction

Chronic obstructive respiratory disorders, such as chronic obstructive pulmonary disease (COPD) and asthma, represent a serious social and economic healthcare burden worldwide [1]. Although very different in terms of risk factors, symptoms, inflammation, and therapy [2], both diseases share impairment of small airways [3], thus making this region of the lung an important pharmacological target in the long-term [4].

Small airways, defined as having an internal diameter of <2 mm and devoid of cartilage [5], are considered the "silent zone" of the lung, since their obstruction can go unnoticed for years and become detectable through standard lung function tests, only when consistently damaged [3].

Advancement of knowledge concerning small airway disease (SAD) has been hampered by the lack of appropriate tools and markers able to accurately and non-invasively evaluate alterations of small airways [6,7]. The current experimental evidence indicates that in healthy subjects small airways carry a very low resistance, whereas those of patients with asthma and COPD are characterized by substantial increased resistance [3]. Interestingly, such an increase in small airways resistance was related with narrowing and distortion of the airways, coupled with signs of chronic inflammation, fibrosis, and mucus plugging [3]. Small airways obstruction may have little effect on lung mechanics due to the presence of collateral ventilation, however collateral ventilation itself may affect the distribution of inspired gases causing ventilation heterogeneity [3,8].

SAD is a widely recognized feature of COPD that extensively contributes to irreversible airway obstruction [9,10] and findings from ex vivo studies showed that loss of small airways precedes emphysema [10,11]. Moreover, in COPD patients SAD may affect spirometry results, increase lung hyperinflation, and lead to clinical deterioration of health status [12]. Considering that lung hyperinflation represents the main cause of dyspnea and functional limitation, early detection of pathological changes of small airways is particularly important,

making the small airways an important treatment target especially in COPD patients [12]. Asthma has been historically considered a disease predominantly involving large airways and, until few years ago, it was still unclear whether SAD is related with disease severity or with a specific clinical asthma phenotype [13]. Conversely, to date it is extensively recognized that targeting small airways in asthma is pivotal because SAD can be considered a treatable trait, leading to a better asthma management and individualized patient care [14,15].

There is a number of reliable non-invasive methodological tools used to infer the extent of SAD, including body plethysmography, nitrogen washout, impulse oscillometry (IOS), and cross-sectional imaging [3], but currently no non-invasive gold standard tool is available for the assessment or diagnosis of SAD.

The transversal involvement of the peripheral lung in COPD and asthma has warranted research efforts to identify therapeutic strategies able to unlock the silent zone [3,16]. Bronchodilators represent the cornerstone of COPD treatment, with long acting-acting β_2-agonists (LABAs) and long-acting muscarinic antagonists (LAMAs) administered as monotherapies or as together in combination inhalers [17]. On the other hand, in asthma LAMAs were historically believed to be less effective than β_2-agonists, as the cholinergic component of bronchoconstriction was considered negligible compared to the direct constrictor action of inflammatory mediators or leukotrienes [18]. Only quite recently, several studies have evaluated the efficacy of LAMAs as add-on therapy to inhaled corticosteroids (ICSs) or ICS/LABA combinations [19] and the first LAMA recommended by the Global Initiative for Asthma (GINA) as a treatment option for patients at Step 4 or 5 with a history of asthma exacerbations was tiotropium bromide (TIO) [20].

Targeting small airways is challenging and most inhalers fail to emit sufficiently small particles to effectively reach the peripheral lung [21,22]. The therapeutic efficacy of inhaled drugs depends on different factors [3], namely the particle size, measured by the mass

median aerodynamic diameter (MMAD) used to quantify the heterogeneity of particles in an emitted dose [3], the fine-particle fraction (FPF), indicating the proportion of particles within the aerosol that are <5 μm [23], and the geometric standard deviation (GSD), measuring the dispersion of the particle diameter [24].

To date, there are no systematic reviews that exclusively focused on the impact of LAMAs on small airways in pulmonary disorders, therefore the aim of this systematic review was to examine the current literature concerning the impact of LAMAs on small airways and their lung deposition in asthma and COPD.

2. Materials and methods

2.1. Review question

The question of this systematic review was to assess the evidence across literature concerning the impact of current LAMAs on small airways in COPD and asthma and to evaluate the extent of drug deposition within the lungs.

2.2. Search strategy and study eligibility

The protocol of this synthesis of the current literature has been registered to the international prospective register of systematic reviews (PROSPERO, registration ID: CRD42021233191), and performed in agreement with the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) [25], with the relative flow diagram shown in Figure 1. This study satisfied all the recommended items reported by the PRISMA-P checklist [25].

The PICO (Patient problem, Intervention, Comparison, and Outcome) framework was applied to develop the literature search strategy and question, as previously reported [26]. Namely, the "Patient problem" included COPD and asthma; the "Intervention" regarded the administration of current LAMAs administered as monotherapy or in combination; the "Comparison" was performed with respect to active and negative controls; the assessed "Outcome" was the impact on small airways and lung deposition.

A key inclusion criterion for the selection of clinical studies was the use of specific methods for the assessment of ventilation heterogeneity, airway resistance, air trapping, and hyperinflation, considered reflective of small airway patency. In this respect, the review by Usmani et al. [27] was used as a reference to make this selection. The search was performed in MEDLINE in order to provide for relevant studies available with no time limit up to April 19th, 2021.

The research string was as follows: ("tiotropium bromide"[MeSH Terms] OR ("tiotropium"[All Fields] AND "bromide"[All Fields]) OR "tiotropium bromide"[All Fields] OR "tiotropium"[All

Fields] OR ("glycopyrrolate"[MeSH Terms] OR "glycopyrrolate"[All Fields] OR "glycopyrronium"[All Fields]) OR ("glycopyrrolate"[MeSH Terms] OR "glycopyrrolate"[All Fields]) OR "aclidinium"[All Fields] OR ("gsk573719"[Supplementary Concept] OR "gsk573719"[All Fields] OR "umeclidinium"[All Fields])) AND ((("small"[Journal] OR "small"[All Fields]) AND ("airway"[All Fields] OR "airway s"[All Fields] OR "airways"[All Fields])) OR ("deposit"[All Fields] OR "deposit s"[All Fields] OR "deposited"[All Fields] OR "deposites"[All Fields] OR "depositing"[All Fields] OR "deposition"[All Fields] OR "depositional"[All Fields] OR "depositioning"[All Fields] OR "depositions"[All Fields] OR "deposits"[All Fields])).

Citations of previously published relevant reviews were examined to select further pertinent studies, if any [28,29].Two reviewers independently checked the relevant studies identified from the literature search. The studies were selected in agreement with previously mentioned criteria and any difference in opinion about eligibility was resolved by consensus.

2.3. Data extraction

Data from included studies were extracted in agreement with Data Extraction for Complex Meta-anALysis (DECiMAL) recommendations [30], and checked for study references and characteristics, in vitro and in silico models to mimic breathing patterns and measure drug deposition respectively, type of animals, number of patients or animals, gender, treatments and comparators with doses of medications, type of inhaler, route of administration, and investigated outcomes to assess the impact on small airways.

2.4. Endpoints

The primary endpoint of this systematic review was to assess the impact of current LAMAs on small airways in COPD and asthma. The secondary endpoint was to evaluate the extent of lung deposition of LAMAs.

2.5. Strategy for data analysis

Data from original papers were extracted and reported via qualitative synthesis. When superiority was reported between treatments, it was statistically significant ($P<0.05$) in the primary publications.

3. Results

3.1. Study characteristics

Of the 107 potentially relevant records identified in the initial search, 31 studies were deemed eligible for a qualitative analysis. The main characteristics of the included studies are reported in Table 1.

Overall, this systematic review included data obtained from studies evaluating the impact of LAMAs in respect to small airway patency and lung deposition. Ten studies evaluated TIO administered alone [31–40], two investigated TIO in dual combination [33,41], and one in triple combination [42]. A total of thirteen studies investigated the effects of glycopyrrinium (GLY) administered alone [43–48], in dual combination [43,45,46,49–52], and in triple combination [44,53–55]. One study evaluated the ex vivo impact of aclidinium (ACL) administered alone and in dual combination [56] while another study investigated its lung deposition [57]. Two studies performed a comparison across different LAMAs on lung deposition [58,59], and two others compared triple combinations including a LAMA [60,61]. No studies concerning the impact of umeclidinium (UMEC) administered alone on small airways or lung deposition were identified from the literature search.

3.2. Tiotropium

A study performed on both rat and human small airways investigated the cholinergic contraction in response to cumulative concentrations of TIO compared to the M_2 muscarinic acetylcholine (ACh) receptor (mAChR) selective antagonist AF-DX116 and the M_3 mAChR preferential antagonist 4-DAMP [31]. In rat small airways, TIO was more potent at

suppressing the contractile response mediated by carbachol (CCh) (negative logarithm of the half-maximal inhibitory concentration [pIC_{50}] 9.86±0.07) than AF-DX116 (pIC_{50} 6.31±0.19) and it was as potent as 4-DAMP [31]. In human small airways, cumulative concentrations of TIO 30 pM – 1 nM and 4-DAMP 0.3 nM – 30 nM inhibited the CCh induced contraction with pIC_{50} values of 10.35±0.05 and 8.74±0.31, respectively, whereas AF-DX116 showed a competitive antagonist response with a dissociation pK_B value of 6.37±0.13 [31]. Summarizing, these data suggest that M_3, rather than M_2 mAChR antagonists predominantly inhibit the contraction of rat and human small airways [31].

According to a recent ex vivo study performed in human small airways pre-contracted by CCh, TIO administered alone potently relaxed the bronchial tone in a concentration-dependent manner (negative logarithm of the half-maximal effective concentration [pEC_{50}] 7.94±0.25) and completely suppressed the contractility of airway smooth muscle, by producing a supra-maximal bronchorelaxant effect (maximal effect [E_{max}] 121.45±15.64%) [33].

An in vitro study aimed to measure the particle size distribution of TIO 2.5 µg administered via soft mist inhaler (SMI) compared to SAL 100 µg delivered through pressurized metered dose inhaler (pMDI), when inhalers were used either alone or coupled to an inhalation aid [32]. Compared to pMDI, SMI alone generated a lower MMAD (3.40±0.44 µm vs. 1.51±0.70 µm, respectively), a higher extra-FPF (EFPF) (9.5±2.4% vs. 46.8±3.0%, respectively), and an improved GSD (2.72±0.37 vs. 5.04±0.08) [32]. With the use of an inhalation aid, either a spacer or a valved holding chamber (VHC), the MMAD generated from the pMDI was further reduced compared to pMDI alone (pMDI + spacer 2.53±0.10 µm vs. pMDI + VHC 2.30±0.27 µm), whereas adding an inhalation aid on the SMI did not produce any change in the generated MMAD [32]. Compared to the use of the inhalers alone, the EFPF improved with the used of an inhalation aid, for both the SMI (SMI + spacer 53.0±3.2% vs. SMI + VHC 61.2±1.1%) and the pMDI (pMDI + spacer 18.7±1.6% vs. pMDI + VHC 25.3±5.3%) [32]. No

change was detected in the FPF of the delivered dose with both inhalers coupled to an inhalation aid [32]. Summarizing, SMI alone showed a bimodal particle size distribution, with a small MMAD and a high FPF. With the use of either inhalation aid, MMAD decreased for pMDI whereas the SMI showed a marginal improvement on the particle size distribution [32].

In healthy subjects and in mild, moderate, and severe COPD patients, lung deposition of TIO 18 μg administered via dry-powder inhaler (DPI) was in the range of 18.0 – 22.0% of the nominal dose and 41.0 – 43.0% of the delivered dose [38]. The extrathoracic deposition relative to the nominal dose was 23.0 – 28.0% and relative to the emitted dose was 57.0 – 58.0% [38]. Moreover, the central/peripheral (C/P) ratios, evaluating the distribution of drug deposition within lungs, did not differ across the four study groups [38]. Overall, these findings suggest that patients at all stages of COPD may gain full therapeutic benefit from TIO via DPI, without impairing lung deposition or drug systemic exposure in relation with disease severity [38].

In COPD patients characterized by varying degrees of hyperinflation, Verbanck et al. [36] investigated whether the beneficial impact of TIO 18 μg via Handihaler® DPI on FEV_1 and inspiratory capacity (IC) was paralleled by improvements in small airway ventilation heterogeneity [36]. Regardless of the degree of hyperinflation, no improvement from baseline was detected for the multiple-breath washout test (MBW) indices S_{cond} and S_{acin} as well as for the body plethysmography parameters functional residual capacity (FRC) and residual volume (RV), following treatment with TIO [36]. S_{cond} decreased only during the last study visit, 90 minutes after TIO administration, in patients with a high degree of hyperinflation [36]. Summarizing, the consistent increase in FEV_1 and IC induced by TIO and predominantly observed in COPD patients with a high degree of hyperinflation seemed unrelated to small airways ventilation heterogeneity [36].

In patients with stable COPD, a 3-days treatment with either TIO 18 μg quaque die (QD) via DPI or the LAMA oxitropium bromide 800 μg QD did not induce an improvement in small

airway impairment, detected by the body plethysmography parameters RV and FRC [35]. This evidence seemed unexpected to the authors and could be related to the short treatment period that perhaps was not long enough to reach a maximum improvement [35].

Pecchiari et al. [37] conducted a randomized clinical study to investigate for the first time the acute effects of TIO 18 µg HandiHaler® DPI on small airways compared to the LABA indacaterol (IND) 150 µg administered using Breezhaler® DPI in patients with moderate to severe COPD. During the single-breath nitrogen washout (N_2 SBW) test, TIO reduced the slope of Phase III (S_{III}) to a similar extent as IND (-2.9±4.2 N_2%/L vs. -2.1±2.0 N_2%/L) and the closing volume (CV), measured as a percentage of vital capacity (VC) (CV/VC%) (-5.0±4.0% vs. -3.0±5.0%) [37]. TIO was as effective as IND at improving RV (-0.27±0.52 L vs. -0.33±0.42 L) [37]. This is the first-ever evidence that TIO and IND similarly improved the mechanical heterogeneity of peripheral airways and positively affected small airway closure [37].

Iwanaga et al. [34] reported that in asthmatic patients, TIO delivered via Respimat® SMI achieved a higher drug deposition in the whole lung and small airways than when administering the LABA formoterol (FOR) via Flutiform® pMDI, Symbicort® DPI or Relvar® DPI, as detected by functional respiratory imaging (FRI). The deposition fractions for Respimat® SMI in the upper, central, and small airways were in the ranges 41.3 – 44.3%, 13.8 – 22.6%, and 34.6 – 42.4%, respectively, compared to MDIs or DPIs, characterized by a whole lung deposition of 20.0 – 44.3% and peripheral airway deposition of 11.3 – 29.2% [34].

Two recent studies [39,40] confirmed the superiority of TIO Respimat® SMI over HandiHaler® DPI on small airway resistance in mild to moderate COPD patients. TIO Respimat® SMI improved the difference between resistance at 5 Hz (R_5) and 20 Hz (R_{20}) (R_5–R_{20}) by 0.16 kPa/L/s [39] and 0.497±0.148 kPa/L/s [40], although Usmani et al. [40]

failed to show a significant improvement of S_{acin} and S_{cond}, indicative of ventilation heterogeneity in acinar and conducting airways, respectively.

3.3. Glycopyrronium

An ex vivo study [43] performed in human small airways pre-contracted by histamine reported that GLY was more effective at relaxing passively sensitized small airways (E_{max} 70.47±9.36%, pEC_{50} 4.06±0.54) than non-sensitized ones (E_{max} 37.22±3.87%, pEC_{50} 4.37±0.29). Another ex vivo study [44] confirmed such findings on GLY and also showed that the effect on passively sensitized small airways was ≃1.5 logarithm less potent than on medium bronchi, and it was as potent on small COPD airways as on medium ones.

In human small airways pre-contracted by ACh, GLY was as effective as the LABA IND at suppressing the bronchial contractile tone, with an E_{max} of 103.01±1.59% and 94.85±1.70%, respectively, although GLY was more potent than IND at inducing a concentration-dependent relaxant effect (pEC_{50}: GLY 8.45±0.23 vs. IND 6.53±0.18) [45].

Compared to the dual phosphodiesterase 3 and 4 (PDE3/4) inhibitor ensifentrine, GLY was found to be more potent at inducing a concentration-dependent relaxant effect of human small airways pre-contracted by CCh (pEC_{50}: GLY 9.25±0.35 vs. ensifentrine 5.96±0.06) [46].

Pham et al. [47] characterized in vitro the aerosol performance of GLY administered at 25 µg/mL or 50 µg/mL by eFlow® closed system (CS) vibrating membrane nebulizer. The MMAD and FPF were respectively 3.7 µm and 72.0%, both independent of the formulation strength [47]. The mean delivered dose assessed by continuous flow method was 88.0% of the nominal dose for both doses of GLY, and the mean delivered dose assessed by breathing simulation was 56.8% for GLY 25 µg/mL and 62.6% for GLY 50 µg/mL [47]. Overall, the eFlow® CS generated GLY aerosols with high delivered dose and small droplet size with narrow size distribution suitable for central and peripheral airway deposition [47].

According to a Phase IIIB randomized study [48] evaluating the impact on small airway patency in COPD patients, treatment with GLY 18 µg bis in die (BID) MDI did not induced an improvement in FRC, compared to FOR 9.6 µg BID MDI. However, as stated by the authors, the study could have been not sufficiently powered to detect a significant difference with body plethysmography [48].

3.4. Aclidinium

In passively sensitized human small airways pre-contracted by ACh, ACL induced a concentration-dependent relaxant effect as potent as FOR (pEC_{50}: ACL 7.93±0.26 vs. FOR 8.37±0.28), but differently from FOR, ACL did not completely abolish the bronchial contraction elicited by ACh (E_{max}: ACL 68.07±4.47% vs. FOR 98.99±0.28%) [56].

A Phase I study [57] quantified the lung deposition of a single dose of ACL 200 µg delivered through Genuair® DPI, at a targeted peak inspiratory flow rate (PIFR) of 90 L/min, in healthy subjects. Overall, 30.1±7.3% of ACL was deposited in the whole lung, 54.7±7.2% in the oropharynx, 11.5% was retained in the inhaler, and 3.7% was exhaled [57]. The coefficient of variation of drug deposition in the lungs was 24.0% and there was no correlation between whole lung deposition and PIFR over the range 66.0 – 99.9 L/min [57]. ACL reached all lung regions, but the highest drug deposition was achieved in the most central zone and the lowest in the peripheral one (9.9% and 2.6%, respectively) [57]. Summarizing, Genuair® DPI delivered ACL efficiently to the lungs, the drug deposition was independent of PIFR, and the inter-subject variability was low [57].

3.5. Umeclidinium

No available studies concerning the impact of UMEC on small airways were identified from the literature search.

3.6. Combinations including a LAMA

3.6.1. Dual combinations including tiotropium

An ex vivo study [33] characterized the pharmacological interaction between TIO and the LABA OLO in human small airways pre-contracted by CCh. Combining low concentrations of TIO (1.5 – 3.6 nM) and OLO (1.5 – 25 nM) elicited a strong synergistic interaction and induced a maximal enhancement of relaxation by +26.31±12.39%, compared to the expected additive response predicted by the Bliss Independence (BI) theory [33]. These findings indicate that TIO/OLO combination elicits a potent and strong synergistic bronchorelaxation of small airways [33].

Ciciliani et al. [41] conducted a combined in vitro/in silico analysis to compare the lung deposition of TIO/OLO 2.5/2.5 µg fixed-dose combination (FDC) to TIO 2.5 µg and OLO 2.5 µg when administered via Respimat® SMI. Under the very severe COPD breathing pattern, TIO/OLO FDC showed the highest amount of the nominal dose reaching the lung (69.0 – 72.0%), followed by TIO (64.0%) and OLO (58.0%) in the simulation mouth-throat model [41]. Under moderate COPD breathing patterns, the difference between the drug formulations was less prominent, with results ≈50.0% of the nominal dose [41]. The cumulative particle size distribution for the in vitro dose indicated a FPF of 60.0 – 70.0% of the lung fraction and a MMAD of 3.0 – 4.0 µm for all formulations [41]. The regional deposition pattern analysis performed in silico indicated that TIO/OLO FDC showed the lowest amount of particles retained in the oropharyngeal area and the highest amount reaching all regions of the simulation lung model, including small airways [41]. In the periphery, particle deposition for both formulations was highest at lower flow rates and decreased consistently as the flow rate increased [41]. Summarizing, the aerosol delivery via Respimat® SMI achieved high particle deposition deep into the lung periphery with all the evaluated formulations [41].

3.6.2. Dual combinations including glycopyrronium

Taylor et al. [50] performed the first gamma scintigraphy imaging randomized controlled trial (RCT) to assess the lung deposition of GLY/FOR 14.4/10 µg delivered through a pMDI

formulated using co-suspension delivery technology in healthy adults. A total of 38.4% of the emitted GLY/FOR dose was deposited in the lungs and ≤0.25% was retained in the exhalation filter [50]. The mean normalized outer/inner regional airway deposition ratio was 0.57 and the standardized C/P ratio was 1.85 [50]. Summarizing, GLY/FOR pMDI was efficiently and uniformly deposited in the proximal and distal regions of the lungs after a 10-second breath-hold, with a low exhaled fraction [50].

In moderate to severe COPD patients, GLY/FOR 18/9.6 µg BID MDI formulated using co-suspension delivery technology achieved ≃48.0% of the total delivered dose deposited within the lungs, as detected by FRI [49]. Compared to placebo, GLY/FOR reduced FRC and RV by 13.0% and 22.0%, respectively [49].

In human small airways pre-contracted by ACh, combining low concentrations of GLY (0.2 – 1.5 nM) with IND (0.03 – 0.13 µM) induced a synergistic relaxant effect (+28.46±5.35% vs. expected additive effect) [45].

The BRIGHT [52] RCT evaluated the impact of GLY/IND 50/110 µg QD DPI on small airway patency compared to TIO 18 µg QD HandiHaler® DPI and placebo, in moderate to severe COPD patients. Lung function parameters assessed by body plethysmography showed no difference between GLY/IND and TIO in FRC and RV [52]. Compared to placebo, GLY/IND and TIO improved FRC (-0.52 L, 95%CI -0.70 – -0.35 and -0.40 L, 95%CI -0.58 – -0.23, respectively) and RV (-0.52 L, 95%CI -0.70 – -0.35 and -0.41L, 95%CI -0.59 – -0.24, respectively), with maximal effect detected 60 minutes post-dose [52].

By contrast, Molino et al. [51] observed that in moderate to severe COPD patients, GLY/IND 50/110 µg QD administered via Breezhaler® DPI improved peripheral airway resistance, by reducing the IOS indices pre-dosing R_5 (0.16 kPa/L/s, 95%CI -0.283 – -0.037) and R_{20} (-0.066 kPa/L/s, 95%CI -0.1255 − -0.0061), whereas no improvement was observed with TIO 2.5 µg QD Respimat® SMI.

An ex vivo study [43] performed on human small airways pre-contracted by histamine explored whether the combination between GLY and the ICS beclomethasone dipropionate (BDP) induced an additive or even a synergistic interaction. Combining GLY plus BDP administered at low concentrations inducing 30% of E_{max} (EC_{30}) when given as single agents, synergistically relaxed passively sensitized human small airways by 73.30±5.39%, whereas no synergistic interaction was detected in non-sensitized tissues [43]. According to the BI analysis, GLY/BDP elicited a synergistic relaxant effect that was +22.30±5.39% greater than the expected additive response [43]. This is the first study to provide the pharmacological rationale for combining low doses of a LAMA plus an ICS [43].

In human small airways pre-contracted by CCh, GLY plus the dual PDE3/4 ensifentrine administered at low-to-middle concentrations (EC_{30-40}) synergistically relaxed small airways (+21.05±4.02% vs. expected additive effect) and isoeffective concentrations inducing EC_{30} enhanced the intraluminal bronchiole area of 69.08±2.41%, compared with the additive response [46]. When administered in combination at low concentrations (EC_{30}), GLY plus ensifentrine achieved the maximal relaxant response at ≃1 h post-administration (+65.60±9.20% of lumen area enhancement vs. single agents) and the reduced contractile tone lasted up to 6 h post-treatment, when the luminal area was still enhanced by +29.30±2.04% [46]. The maximal synergistic interaction occurred ≃30 min post-administration (+28.04±8.66% vs. expected additive effect) [46]. GLY and ensifentrine demonstrated to interact synergistically by increasing the effectiveness and the duration of bronchorelaxation of human small airways [46].

3.6.3. Dual combinations including aclidinium

An ex vivo study [56] performed in passively sensitized human small airways pre-contracted by ACh evaluated the pharmacological interaction between ACL and the LABA FOR. The BI analysis indicated that combining ACL (3.2 nM – 1.0 μM) with FOR (1.8 nM – 63.0 nM) synergistically relaxed small airways, leading to a maximal bronchorelaxant response of

+19.67±0.85%, compared to the additive effect [56]. The analysis of interaction revealed that low isoeffective concentrations of ACL plus FOR produced a luminal area enhancement of 69.89±2.28%, compared to the effect of the single compounds [56]. This represents the first study to have pharmacologically confirmed under controlled experimental settings the synergistic benefit of combining ACL plus FOR on human small airways relaxation [56].

3.6.4. Triple combinations including tiotropium

A recent RCT [42] investigated the impact of adding OLO 5 µg or TIO/OLO 5/5 µg administered QD via the soft mist Respimat® inhaler to pre-existing treatment with an ICS as extrafine HFA-BDP in current smokers with persistent asthma. Chronic dosing with triple therapy induced a greater improvement in small airway resistance compared to BDP/OLO, by reducing trough IOS index R_5–R_{20} from 0.17±0.02 kPa/L/s to 0.12±0.03 kPa/L/s, whereas no effect was observed with BDP/OLO [42]. No difference in peak R_5–R_{20} was detected after single or chronic dosing with either treatment [42]. Summarizing, these findings demonstrated the superiority of triple therapy over ICS/LABA on trough small airway outcomes [42].

3.6.5. Triple combinations including glycopyrronium

An ex vivo study [44] investigated the triple combination of the ICS BDP with the LABA FOR and GLY, administered at the concentration-ratio 100:6:12.5 and reproducing the TRIMBOW™ formulation [62], in passively sensitized and COPD small airways. In passively sensitized airways, the maximal synergistic interaction was achieved with BDP/FOR/GLY 10/0.6/1.25 ng/mL (+24.95±7.85% vs. expected additive effect). The Unified Theory analysis confirmed the synergism (Log_{10} of CI <0) and the extent of synergistic interaction was very strong at concentrations inducing 25–75% E_{max} (overall CI: 0.066) and strong at concentrations inducing 90% E_{max} (overall CI: 0.145) [44]. In COPD small airways, the maximal synergistic interaction was detected for BDP/FOR/GLY 3/0.18/0.375 ng/ml (+28.85±5.01% vs. expected additive effect) [44]. The Unified Theory analysis confirmed the

synergism and the extent of synergistic interaction was low at concentrations inducing 25% E_{max} (CI: 0.310), strong at concentrations inducing 50% E_{max} (CI: 0.118), and very strong at concentrations eliciting ≥75% E_{max} (overall CI: 0.032) [44]. This study proved that BDP/FOR/GLY administered at the combination ratio 100:6:12.5 induced middle to very strong synergistic bronchorelaxant effect in human small airways [44].

An in silico study performed by Usmani et al. [53] reported that in moderate to severe COPD, the extrafine formulations BDP/FOR/GLY and BDP/FOR pMDIs have similar intrathoracic deposition patterns (31.0±5.7% and 28.1±5.2%, respectively), with a mean deposition ratio of 1.10, as assessed by FRI. The C/P ratio was 0.48±0.13 for BDP/FOR/GLY and 0.62±0.17 for BDP/FOR [53]. Summarizing, both BDP/FOR/GLY and BDP/FOR were effectively delivered to the lung, with a higher drug deposition detected in the small airways than in the large ones [53].

A Phase I gamma scintigraphy RCT [54] investigated the lung deposition patterns for BUD/GLY/FOR 320/14.4/10 µg FDC delivered using a pMDI formulated using co-suspension™ delivery technology in healthy adults. Following a breath-hold period of 3 and 10 seconds, the mean total lung deposition for BUD/GLY/FOR pMDI was 34.5% and 37.7% respectively, and a very low fraction of the dose (≤0.4%) was exhaled for both breath-hold lengths [54]. The normalized outer/inner regional airway deposition ratio was 0.75 and 0.65 for the 3- and 10-second breath-holds respectively, whereas the standardized C/P ratios were 1.40 and 1.79, respectively [54]. Overall, BUD/GLY/FOR pMDI formulated using innovative co-suspension™ delivery technology was efficiently distributed across inner and peripheral regions of the lung, showing similar deposition patterns after a 3- or a 10-second breath-hold [54].

The TRIFLOW RCT [55] compared the impact of extrafine BUD/GLY/FOR 100/10/6 µg via pMDI (Trimbow®) to BDP/FOR 100/6 µg via pMDI (Fostair®) on gas trapping and small airway function in COPD patients with hyperinflation. Body plethysmography showed no

improvement in the area under the curve over 12 h (AUC_{0-12}) of FRC following intervention with both treatments, but compared to BDP/FOR, BUD/GLY/FOR improved RV AUC_{0-12} by -163 mL (95%CI -263 mL – -64 mL) [55]. Triple therapy reduced the IOS index R_5–R_{20} AUC_{0-12} by -0.045 kPa/L/s, while BDP/FOR afforded no improvements [55]. In summary, COPD patients with evidence of hyperinflation showed greater improvements with BUD/GLY/FOR pMDI over BDP/FOR pMDI on gas trapping and small airways resistance, thus indicating that the GLY component had a beneficial effect on small airway physiology [55].

3.7. Comparison across different LAMAs on lung deposition

Ciciliani et al. [59] conducted a combined in vitro/in silico analysis to compare the aerosol deposition of TIO 5 µg QD, GLY 44 µg QD, and ACL 322 µg BID administered respectively via Respimat® SMI, Breezhaler® DPI, and Genuair® DPI in mouth-throat and lung models of COPD. Respimat® SMI delivered a higher modeled dose to the lung reaching all regions of the simulation model (59.0±5.0% and 67.0±5.0% of the nominal dose under moderate and very severe COPD breathing patterns, respectively), than Breezhaler® DPI (43.0±2.0% and 51.0±2.0% of the nominal dose for moderate and severe disease simulations, respectively) and Genuair® DPI (32.0±2.0% and 42.0±1.0% for moderate and very severe disease, respectively) [59]. Respimat® SMI generated the largest particles at the outlet of the throat model (MMAD 3.7 µm) compared to the DPIs Breezhaler® and Genuair® (MMAD ≈2.5 µm) [59]. In summary, Respimat® delivered the lowest amount of particles depositing in the mouth-throat model and the largest drug aerosol reaching all regions of the simulation lung model [59].

Ohar et al. [58] assessed in vitro the aerosol performance and drug delivery of two LAMAs, GLY 25 µg/mL and TIO 18 µg administered respectively via eFlow® CS and HandiHaler® DPI. The MMAD (3.6 – 4.6 µm) and the FPF (48.2 – 63.7%) for GLY with eFlow® CS were generally similar across the different simulated breathing patterns, whereas TIO via HandiHaler® DPI showed variations in both MMAD (3.8 – 5.8 µm) and FPF (16.1 – 32.4%)

[58]. Delivery of GLY via eFlow® CS resulted in a high in vitro deposition of drug particles within the respirable range (<5 μm) whereas the majority of TIO was deposited at the throat/mouthpiece section, irrespective of the breathing patterns [58]. The median residual dose of GLY with eFlow® CS (2.4 – 4.4%) was lower compared to that of TIO with HandiHaler® DPI (40.0 – 67.0%). These results confirmed the different deposition patterns generated by the two different inhaler devices [58].

3.8. Comparison across triple combinations

An in silico study [60] compared the lung deposition of the extrafine BDP/GLY/FOR FDC delivered via pMDI to the non-extrafine fluticasone furoate (FF)/UMEC/vilanterol (VI) FDC delivered via DPI, both approved for the maintenance treatment of COPD [60]. FRI and high-resolution computed tomography lung scans revealed a similar total intrathoracic drug deposition for the LAMA component of both FDCs, but the peripheral deposition was higher with BDP/GLY/FOR than FF/UMEC/VI (GLY: 24.1±5.1 vs. UMEC: 16.8±4.9) [60]. BDP/GLY/FOR was widely delivered throughout all lung regions, with a C/P deposition ratio <1, confirming a greater peripheral than central deposition in the lungs compared to FF/UMEC/VI (GLY: 0.49±0.13 vs. UMEC: 1.20±0.48) [60].

Manoharan et al. [61] observed that in moderate to severe COPD patients already treated with an ICS/LABA, peripheral airway resistance, measured as R_5-R_{20}, improved after chronic dosing with ACL 322 μg BID via Genuair® DPI (-0.06 kPa/L/s, 95%CI -0.11 – -0.01), but not with TIO 18 μg QD via Handihaler® DPI, when both were used as triple therapy.

4. Discussion

Targeting small airways is crucial to manage SAD in COPD patients, as SAD induces airflow limitation and gas trapping which, in turn, may lead to lung hyperinflation. Since lung hyperinflation is correlated with reduced inspiratory capacity and increased functional residual capacity, the overall clinical result of SAD is represented by relevant health status deteriorations associated with dyspnea, physical deconditioning, and reduced quality of life [12]. Interestingly, this systematic review confirmed that LAMAs administered either alone or as dual and triple combinations induce an effective bronchorelaxant effect on small airways, thus leading to an effective management of SAD (Figure 2). Nevertheless, the effectiveness of the LAMAs investigated in this qualitative synthesis not only relies on each specific agent, but also on the employed inhalation device and patient's adherence [63].

In Europe and United States, TIO delivered through Respimat® SMI is approved as maintenance treatment for COPD and as long-term treatment of asthma in patients aged ≥6 years that remain uncontrolled at GINA Steps 4 and 5 with a history of exacerbations [64–67]. The improved drug deposition in the whole lung and small airways characterizing Respimat® SMI [38,41], allowed reduction in the nominal dose of TIO from 18 µg QD delivered through HandiHaler DPI®, to 5 µg QD [68,69]. In both COPD and asthma, Respimat® SMI achieved the greatest drug delivery with respect to DPIs and MDIs [34,59]. Under patients' real-life use conditions, the emission performance of Respimat® SMI was tolerant to environmental temperature and humidity fluctuations [70]. Interestingly, real-life use of Respimat® SMI generated an increase in extra-fine TIO particles compared to controls, therefore it is very important to correctly instruct patients to hold their breath after a slow and deep inhalation, in order to avoid loss of particles due to exhalation shortly after taking a puff [70].

TIO Handihaler® DPI is a single dose, breath-actuated DPI, which provided consistent lung deposition and systemic drug exposure in COPD patients, regardless of the level of disease

severity [38]. Surprisingly, two randomized studies [35,36] indicated that TIO Handihaler® DPI did not improve small airway function and ventilation heterogeneity in COPD patients, although the short observation period of the study by Incorvaia et al. [35] might have been not long enough to detect a significant result. By contrast Pecchiari et al. [37] reported that TIO was as effective as the LABA IND on small airway patency. DPIs have been introduced in the 1980s to avoid some issues associated with pMDIs, including the use of environmentally harmful chlorofluorocarbon propellants and patient hand-lung coordination [71]. Despite the apparent simplicity of DPIs, drug delivery can be affected by different factors, including environmental conditions. In this regard, Ammari et al. [72] evaluated the delivered dose and particle size distribution of TIO Handihaler® DPI after exposure to patients' real-life use environments. The DPI emission performance was resistant to the daily environmental fluctuations in temperature and humidity, thus retaining therapeutic benefits [72].

The introduction of a new co-suspension™ delivery technology pMDI for GLY administered in addition to a LABA or ICS/LABA was found to be particularly effective at achieving a uniform distribution of drug particles within all lung regions [49,54]. As a matter of fact, the PINNACLE RCTs [73,74] reported that GLY/FOR delivered by co-suspension™ delivery technology MDI induced sustained improvements in respiratory outcomes in patients with moderate to severe COPD.

GLY administered using the novel eFlow® CS nebulizer was the first nebulized LAMA approved by FDA for COPD [75]. eFlow® CS uses a vibrating membrane technology to generate a soft aerosol mist of GLY solution, it is portable, light-weight, and virtually silent [76]. eFlow® CS showed to achieve a high delivered dose, with a small droplet size within the respirable range, suitable for central and peripheral airway deposition [47,58].

An effective inhaled delivery of respiratory medications to small airways is crucial to the management of chronic obstructive respiratory disorders, indeed small airways are

recognized as the major site of airflow obstruction in both COPD and asthma [77]. Although SAD is recognized as a pathophysiological feature of COPD and has been already studied over many decades [78], SAD in asthma has been investigated relatively recently [79]. The Assessment of Small Airways Involvement In Asthma (ATLANTIS) prospective cohort study [80] has proffered a different perspective concerning the role of SAD in asthma pathogenesis, by reporting that small airway function is impaired in over 90.0% of patients. Alterations of peripheral lung pathophysiology and biology are representative of an early sign of obstructive disease, to be investigated and assessed in everyday clinical practice [3]. An obstruction of the small airways progressively leads to air trapping during expiration, resulting in hyperinflation [81]. COPD patients are susceptible to develop exercise-induced air trapping [82], a well-known phenomenon also referred to as dynamic hyperinflation, leading to a reduction in IC, with consequent increased dyspnea and exercise limitation [83,84]. Manco et al. [85] recently provided the first evidence in COPD patients that the marker of SAD S_{acin} is an independent predictor of excessive ventilation and dynamic hyperinflation.

Overall, the use of targeted drug delivery to the peripheral lung may result in important clinical implications, such as an improvement in dynamic hyperinflation and small airway air trapping [86]. Singh et al. [87] advanced the hypothesis that the use of Respimat® SMI leads to an early increase in the systemic exposure to, and higher plasma peak concentrations of TIO after dosing, owing to its delivery as a fine mist deep into the lung and thus exposing patients to a higher risk of anticholinergic cardiovascular effects. In this regard, a network meta-analysis [88] confirmed that there was no statistical difference between TIO HandiHaler® DPI and Respimat® SMI with respect to the safety profile, and a low absolute risk of cardiovascular adverse effects was found with both devices.

The main limitation of this systematic review is related with the intrinsic nature of the included studies, mostly RCTs and basic research studies. Therefore, real-life evidence is particularly

needed to further assess the correlation between the pharmacological impact of LAMAs on small airways and patients reported outcomes, although such a kind of study may be challenging by a strict managing point of view.

Concluding, to the best of our knowledge this is the first review to have systematically provided a qualitative synthesis of current literature concerning the impact of LAMAs on small airways, as well as an insight on LAMAs distribution within the lung, by reporting evidence that LAMAs are effective in unlocking the silent zone when administered via inhaler devices effective at delivering the drugs into the small airway compartment, a condition leading to significant clinical improvement especially in COPD patients.

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Conflicts of interest

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B.L.R. has no conflict of interest to declare.

E.P. has no conflict of interest to declare.

M.C. has participated as a lecturer, speaker, and advisor in scientific meetings and courses under the sponsorship of Almirall, AstraZeneca, Biofutura, Boehringer Ingelheim, Chiesi Farmaceutici, GlaxoSmithKline, Menarini Group, Lallemand, Mundipharma, Novartis, Pfizer, Verona Pharma, and Zambon, and has been a consultant to ABC Farmaceutici, Recipharm, Chiesi Farmaceutici, Lallemand, Novartis, Verona Pharma, and Zambon. His department was funded by Almirall, Boehringer Ingelheim, Novartis, and Zambon.

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Table 1. Characteristics of the studies included in the systematic review.

Study, year, and reference	Study characteristics	Number identifier	Treatment duration (weeks)	Number of analyzed patients	Investigated LAMAs as monotherapy or in combination, with doses and type of inhaler	Non-LAMA Comparator	Patients characteristics	Age (years)	Male (%)	Evaluated outcome
Ciciliani et al., 2021 [41]	In vitro/in silico study combining the Alberta throat model (in vitro) with a computational fluid dynamic model of the lungs (in silico)	NA	NA	NA	TIO 2.5 µg/actuation, SMI (Spiriva Respimat®); TIO/OLO 2.5/2.5 µg/actuation, SMI (Spiolto Respimat®)	OLO 2.5 µg/actuation, SMI (Striverdi Respimat®)	In vitro simulation of breathing patterns from patients with moderate COPD (FEV_1≥50% and <80% of predicted) or very severe COPD (FEV_1<30% of predicted)	NA	NA	Lung deposition
De Backer et al., 2020 [48]	Multicentre, Phase IIIB, randomized, double-blind, two-period, crossover study	NCT02937584	2	23	GLY 18 µg BID, MDI (NA)	FOR 9.6 µg BID, MDI	Moderate-to-severe COPD (FEV_1/FVC<0.70; post-bronchodilator FEV_1 >30% and <80% predicted; smoking history of ≥10 pack-years)	64.6	73.9	Body plethysmography
Ohar et al., 2020 [58]	In vitro study under simulated conditions of different breathing patterns	NA	NA	NA	GLY 25 µg, vibrating membrane nebulizer (eFlow® CS); TIO 18 µg, DPI (HandiHaler®)	NA	NA	NA	NA	Lung deposition
Dean et al., 2020 (TRIFLOW study) [55]	Single centre, Phase IV, randomized, open-label, two-way, crossover study	NCT03842904	1.4	23	BDP/FOR/GLY 200/12/20 µg BID, pMDI (Trimbow®)	BDP/FOR 200/12 µg BID, pMDI (Fostair®)	COPD (FEV_1/FVC<0.7; post-bronchodilator FEV_1>30% and <80% of predicted; RV>120% predicted; smoking history of ≥10 pack-years)	64.0	40.9	IOS and body plethysmography
Ke et al., 2020 [32]	In vitro study using a model of adult mechanical ventilation	NA	NA	NA	TIO 2.5 µg/actuation, SMI (NA)	SAL 100 µg/actuation, pMDI (NA)	NA	NA	NA	Lung deposition
Rogliani et al., 2020 [44]	Ex vivo study on passively sensitized human small airways pre-contracted by His (asthma model) and small airways from COPD donors (COPD model)	NA	NA	32	GLY (cumulative concentrations); BDP/FOR/GLY at 100:6:12.5 concentration-ratio (cumulative concentrations)	NA	Patients undergoing surgery for lung cancer with either normal lung function and without history of chronic airway disease (for ex vivo model of asthma) or with a lung function in agreement with spirometric diagnosis of COPD: FEV_1/FVC<0.7 (for ex vivo model of COPD)	50.4	53.1	Bronchorelaxant effect and pharmacological interaction in small airways
Israel et al. 2020 [54]	Single centre, Phase I, single-dose, randomized, two-period, crossover study	NCT03740373	2 days of testing	10	BUD/GLY/FOR 320/14.4/10 µg, pMDI (Aerosphere®)	NA	Healthy subjects	28.0–50.0	100.0	Lung deposition
Usmani et al., 2020 [53]	In silico study using FRI combined HRCT scans of COPD patients	NA	NA	20	BDP/FOR/GLY, pMDI (Trimbow®)	BDP/FOR, pMDI (Foster®)	Moderate-to-severe COPD (post-bronchodilator FEV_1 of 42.3%)	64.0	75.0	Lung deposition
Usmani et al., 2020 [60]	In silico study using FRI combined HRCT scans of COPD patients	NA	NA	20	BDP/FOR/GLY, pMDI (NA); FF/VI/UMEC, DPI (NA)	NA	Moderate-to-severe COPD (post-bronchodilator FEV_1 of 42.3%)	64.0	75.0	Lung deposition
Calzetta et al., 2019 [33]	Ex vivo study on human small airways pre-contracted by CCh	NA	NA	25	TIO 0.1 nM – 100 µM; TIO/OLO 1.5 – 19 nM/1.5 nM – 1.9 µM	NA	Patients undergoing surgery for lung cancer, without history of chronic airway disease	65.8	56.0	Bronchorelaxant effect and pharmacological interaction in small airways
Jabbal et al., 2019 [42]	Single-centre, Phase IV, randomized, open-label, active-controlled, crossover study	NCT02682862	2 – 4	16	HFA-BDP, pMDI (Clenil®) + TIO/OLO 5/5 µg QD, SMI (Respimat®); HFA-BDP, pMDI (Clenil®) + OLO 5 µg QD, SMI (Respimat®)	NA	Current smokers with persistent asthma, taking ≥400 µg/day of ICS (as HFA-BDP Clenil® equivalent dose), with FEV_1 of 84.0%	44.0	NA	IOS

Biddiscombe et al., 2018 [39]	Pragmatic clinical study	NA	28	44	TIO 18 μg QD, DPI (HandiHaler®); TIO 5 μg QD, SMI (Respimat®)	NA	Mild-to-moderate COPD (FEV_1 67.0% of predicted)	NA	NA	IOS
De Backer et al., 2018 [49]	Single-centre, Phase IIIB, randomized, double-blind, crossover study	NCT02643082	1	20	GLY/FOR 18/9.6 μg BID, MDI formulated using co-suspension delivery technology (NA)	PCB	Moderate-to-severe COPD (pre-bronchodilator FEV_1/FVC<0.7; post-bronchodilator FEV_1>30% and <80% of predicted; smoking history of ≥10 pack-years)	64.8	75.0	Body plethysmography and lung deposition
Pham et al., 2018 [47]	In vitro study under simulated breathing conditions	NA	8.6	NA	GLY 25 μg, vibrating membrane nebulizer (eFlow® CS)	NA	NA	NA	NA	Lung deposition
Taylor et al., 2018 [50]	Single-centre, Phase I, randomized, single-blind, crossover, PCB-controlled study	PT003020	4 days of testing	10	GLY/FOR 14.4/10 μg, pMDI formulated using co-suspension delivery technology (NA)	PCB	Healthy subjects	28.0–50-0	100.0	Lung deposition
Ciciliani et al., 2017 [59]	In vitro/in silico study combining the Alberta throat model (in vitro) with a computational fluid dynamic model of the lungs (in silico)	NA	NA	NA	TIO 5 μg QD, SMI (Respimat®); GLY 44 μg QD, DPI (Breezhaler®); ACL 322 BID, MDPI (Genuair®)	FF/VI 92/22 μg QD, DPI (Ellipta®)	In vitro simulation of breathing patterns from patients with moderate COPD or very severe COPD	NA	NA	Lung deposition
Iwanaga et al., 2017 [34]	Additional FRI analysis to the ongoing pragmatic in vitro study conducted to assess drug deposition of ICS/LABA combinations in small airways	UMIN000022840	NA	6	TIO 5 μg QD, SMI (Respimat®)	FOR, pMDI (Flutiform®), DPI (Symbicort® or Relvar®)	Mild to moderate asthma	NA	NA	Lung deposition
Molino et al., 2017 [51]	Single-centre, randomized, open-label, parallel group study	NA	52	40	GLY/IND 110/50 μg QD, DPI (Breezhaler®); TIO 2.5 μg QD, SMI (Respimat®)	NA	Moderate-to-severe COPD (FEV_1/FVC<0.7; post-bronchodilator FEV_1≥30% and <80% of predicted; smoking history of >10 pack-years)	71.1	87.5	IOS
Pecchiari et al., 2017 [37]	Single-centre, randomized, single-blind, parallel group study	NA	1 day of testing	51	TIO 18 μg, DPI (HandiHaler®); IND 150 μg, DPI (Breezhaler®)	NA	Moderate-to-severe COPD (FEV_1/FVC<0.7; FEV_1<80% of predicted; smoking history of >20 pack-years)	70.0	80.4	Body plethysmography and N_2 SBW test
Cazzola et al., 2016 [43]	Ex vivo study on passively sensitized human small airways pre-contracted by His	NA	NA	14	GLY (cumulative concentrations); GLY/BDP at concentrations inducing EC_{30}	NA	Patients undergoing surgery for lung cancer, without history of chronic airway disease	63.3	57.1	Bronchorelaxant effect and pharmacological interaction
Cazzola et al., 2016 [45]	Ex vivo study on human small airways pre-contracted by ACh	NA	NA	23	GLY (cumulative concentrations); GLY/IND (cumulative concentrations)	IND (cumulative concentrations)	Patients undergoing surgery for lung cancer, without history of chronic airway disease	63.2	56.5	Bronchorelaxant effect and pharmacological interaction
Manoharan et al., 2016 [61]	Single-centre, randomized, open-label, active-controlled, crossover study	NA	4 – 6	13	TIO 18 μg QD, DPI (Handihaler®) + ICS/LABA; ACL 322 μg BID, MDPI (Genuair®) + ICS/LABA	NA	Moderate-to-severe COPD (post-bronchodilator FEV_1≥30% and ≤80%; smoking history of ≥10 pack-years)	69.0	76.9	IOS
Usmani et al., 2016 [40]	Single-centre, Phase III, non-randomized, open-label, sequential assignment study	NCT02683668	26.1	44	TIO 18 μg QD, DPI (HandiHaler®); TIO 5 μg QD, SMI (Respimat®)	NA	Mild-to-moderate COPD (FEV_1 67.8% of predicted)	69.1	52.3	IOS and MBW test
Calzetta et al., 2015 [46]	Ex vivo study on human small airways pre-contracted by CCh	NA	NA	12	GLY (cumulative concentrations); GLY/ensifentrine (cumulative concentrations)	Ensifentrine (cumulative concentrations)	Patients undergoing surgery for lung cancer, without history of chronic airway disease	64.0	58.3	Bronchorelaxant effect and pharmacological interaction
Beeh et al., 2014 [52], BRIGHT study	Multicentre, Phase III, randomized, double-blind, double-dummy, PCB-controlled, three-period, crossover study	NCT01294787	3	84	GLY/IND 50/110 μg QD, DPI (Breezhaler®); TIO 18 μg QD, DPI (Handihaler®)	PCB	Moderate-to-severe COPD (FEV_1/FVC<0.7; post-bronchodilator FEV_1≥40% and <70% of predicted; smoking history of≥10 pack-years)	62.1	63.1	Body plethysmography

Cazzola et al., 2014 [56]	Ex vivo study on passively sensitized human small airways pre-contracted by ACh	NA	NA	23	ACL (cumulative concentrations); ACL/FOR (cumulative concentrations)	FOR (cumulative concentrations)	NA	63.1	60.9	Bronchorelaxant effect and pharmacological interaction
Brown et al., 2013 [31]	In vitro study on rat and human small airways pre-contracted by CCh	NA	NA	NA	TIO 0.3 nM, 30 pM – 1 nM; 4-DAMP 30 nM, 0.3 nM – 30 nM; AF-DX116 1 μM, 0.1 μM – 10 μM	NA	NA	NA	NA	Bronchorelaxant effect
Newman et al., 2009 [57]	Single-centre, Phase I, single-dose, open-label study	NA	1 day of testing	12	ACL 200 μg, DPI (Genuair®)	NA	Non-smoking with normal lung function	37.0	100.0	Lung deposition
Brand et al., 2007 [38]	Single-centre, repeated-dose, open-label study	Study code: 205.238	2	20	TIO 18 μg QD, DPI (HandiHaler®)	NA	Healthy subjects; patients with mild COPD (pre-bronchodilator FEV_1 ≥50% and <70% of predicted, FEV_1/FVC <0.7); moderate COPD (pre-bronchodilator FEV_1 ≥35% and <50% of predicted, FEV_1/FVC <0.7); severe COPD (FEV_1 <35% of predicted, FEV_1/FVC <0.7)	55.3	60.0	Lung deposition
Verbanck et al., 2007 [36]	Clinical study	NA	6	40	TIO 18 μg QD, DPI (HandiHaler®)	NA	COPD (post-bronchodilator FEV_1 <80% of predicted; FEV_1/FVC <0.7; smoking history of ≥15 pack-years)	66.5	90.0	Body plethysmography and MBW test
Incorvaia et al., 2007 [35]	Clinical randomized study	NA	3 days of testing	80	TIO 18 μg QD, DPI (NA); oxitropium bromide 800 μg QD, (NA)	NA	COPD (GOLD stage 2–4 according to FEV_1 values, with no exacerbations in the last 2 months)	73.4	63.2	Body plethysmography

ACh: acetylcholine; ACL: aclidinium bromide; BDP: beclomethasone dipropionate; BID: bis in die; twice daily; CCh: carbachol; COPD: chronic obstructive pulmonary disease; CS: closed system; DPI: dry powder inhaler; EC_{30}: concentrations inducing 30% of the maximal effect; FEV_1: forced expiratory volume in the 1st second; FF: fluticasone furoate; FOR: formoterol; FVC: forced vital capacity; FRI: functional respiratory imaging; GLY: glycopyrronium or glycopyrrolate; GOLD: Global Initiative for Chronic Obstructive Lung Disease; HFA: hydrofluoroalkane; HRCT: high-resolution computed tomography; IND: indacaterol; ICS: inhaled corticosteroid; IOS: impulse oscillometry; LABA: long-acting β_2-receptor agonist; LAMA: long-acting muscarinic antagonist; MBW: multiple-breath nitrogen washout; MDI: metered-dose inhaler; NA: not available; N_2 SBW: single-breath nitrogen washout; OLO: olodaterol; PCB: placebo; pMDI: pressurized metered dose inhaler; QD: quaque die, once daily; RV: residual volume; SMI: soft mist inhaler; TIO: tiotropium bromide; UMEC: umeclidinium; VI: vilanterol.

Figure 1. PRISMA flow diagram for the identification of the studies included in the systematic review. LAMA: long-acting muscarinic antagonist; PRISMA, Preferred Reporting Items for Systematic Review and Meta-Analysis.

Figure 2. Detrimental effect of SAD on lung hyperinflation leading to clinical deterioration in COPD and clinical benefits due to lung deflation when targeting small airways with LAMA administered via inhaler devices effective at delivering the drug into the small airway compartment.