Optimizing the Development Strategy of Combination Therapy in Respiratory Medicine: From Isolated Airways to Patients

Luigino Calzetta · Maria Gabriella Matera · Mario Cazzola · Paola Rogliani

Received: September 13, 2019 / Published online: October 25, 2019 © The Author(s) 2019

ABSTRACT

The current recommendations for the treatment of chronic obstructive pulmonary disease (COPD) are pushing towards triple combination therapy based on the combination of an inhaled corticosteroid (ICS) associated with two bronchodilator agents. However, dual bronchodilation remains the cornerstone for the treatment of most COPD patients. Combining a long-acting β2 adrenoceptor agonist (LABA) with a long-acting muscarinic antagonist (LAMA) induces appreciable synergistic bronchorelaxant effect in human airways, especially when the medications are combined at isoeffective concentrations. Thus, each LABA/LAMA combination is characterized by a specific range of concentration-ratio at which the drug mixture may induce sustained synergistic interaction. Results of a recent randomized controlled trial (RCT, NCT00696020) and evidences from pre-clinical studies in human isolated airways poses the question whether combining tiotropium 5 µg with olodaterol 5 µg is the best combination option: tiotropium/olodaterol 5/5 µg has the same efficacy profile of tiotropium/olodaterol 5/2 µg, and it is less effective than tiotropium/olodaterol 5/10 µg. Furthermore, tiotropium/olodaterol 5/2 µg, 5/5 µg, and 5/10 µg combinations are generally characterized by the same safety profile. Indeed tiotropium/olodaterol 5/5 µg is effective and safe in COPD, but a different development strategy based on solid data obtained from human isolated airways would have driven towards a better-balanced FDC to be tested in Phase III RCTs. Accurate bench-to-bedside plans are needed also in the development of triple combination therapies for asthma and COPD, in which the presence of an ICS in the formulation may further modulate the beneficial interaction between the LABA and the LAMA.

Enhanced Digital Features To view enhanced digital features for this article go to https://doi.org/10.6084/m9.figshare.9944756.

L. Calzetta · M. Cazzola · P. Rogliani
Unit of Respiratory Medicine, Department of Experimental Medicine, University of Rome “Tor Vergata”, Rome, Italy
e-mail: luigino.calzetta@uniroma2.it

M. G. Matera
Unit of Pharmacology, Department of Experimental Medicine, University of Campania “Luigi Vanvitelli”, Naples, Italy

Keywords: Asthma; COPD; Drug development; Human isolated airways; LABA/LAMA combination; Respiratory; Synergy; Triple therapy
Key Summary Points

Dual bronchodilation therapy is the cornerstone for the treatment of most COPD patients.

LABAs and LAMAs should be balanced at isoeffective concentrations to elicit synergistic effect.

Not all the currently marketed LABA/LAMA FDCs are correctly balanced.

Tiotropium/olodaterol 5/5 µg FDC is effective and safe, but a development strategy based on data from human isolated airways would have driven towards a better-balanced combination to be tested in Phase III RCTs.

COMMENTARY

The current recommendations for the pharmacological treatment of chronic obstructive pulmonary disease (COPD) are pushing towards the use of triple combination therapy based on the combination of an inhaled corticosteroid (ICS) associated with two bronchodilator agents characterized by different mechanisms of action [1]. However, to date the dual bronchodilation therapy remains the cornerstone for the treatment of most COPD patients [2].

Several evidences resulting from ex vivo studies [3–8] indicate that combining a long-acting β2 adrenoceptor agonist (LABA) with a long-acting muscarinic antagonist (LAMA) induces appreciable synergistic bronchorelaxant effect at the level of both medium and small bronchi, by potentiating the airway smooth muscle (ASM) relaxation ≥25% compared to the additive effect and ≥45% compared to the effect induced by the monocomponents [9].

Interestingly, such a significant synergistic interaction is prevalent when the medications are administered at low and isoeffective concentrations. These two pivotal conditions put the rationale to reduce and balance the doses of each single bronchodilator agent included into the currently marketed LABA/LAMA fixed-dose combination (FDC) formulations in order to increase the safety profile of dual bronchodilation therapy by maintaining an effective level of bronchorelaxation in COPD patients [10].

In order to better elucidate the importance of the concept that different classes of bronchodilators must be combined at “low and isoeffective concentrations”, we have performed a post hoc analysis by calculating the Combination Index, the core of the Unified Theory in drug combination studies [11], from data of four pre-clinical studies [3–6] that assessed the interaction between LABAs and LAMAs. Briefly, in these experiments the relaxant effect of LABAs and LAMAs was tested on the contractile tone induced by cholinergic activation in human isolated bronchi. The bronchodilator agents were administered alone and in combination at different concentrations eliciting the same level of ASM relaxation, the isoeffective concentrations. The graphical representation of the Combination Index indicates that at low, nanomolar concentrations the pharmacological interaction between the LABA/LAMA combinations aclidinium/formoterol (Fig. 1a), glycopyrronium/indacaterol (Fig. 1b), tiotropium/olodaterol (Fig. 1c), and umeclidinium/vilanterol (Fig. 1d), elicited strong to very strong synergistic bronchorelaxant effect.

Since 1990s it is well known that the optimal condition to induce pharmacological synergy is to administer the drugs at isoeffective concentrations [12]. However, although the investigated bronchodilator agents were administered at concentrations eliciting the same bronchorelaxant effect, it is evident from the graphs shown in Fig. 1 that the most favourable concentration-ratio between the LABAs and LAMAs necessary to optimize the synergistic bronchorelaxant effect may vary considerably across the different LABA/LAMA combinations. In other words, each LABA/LAMA combination is characterized by a specific range of concentration-ratio at which the drug mixture may induce appreciable synergistic interaction.

This is an important assumption that supports the use of preclinical studies in isolated
airways to correctly identify the best concentration-ratio between the monocomponents, which only then should be further investigated in randomized controlled trials (RCTs). Indeed, such an approach represents the most rationale bench-to-bedside way to correctly develop LABA/LAMA FDCs that can provide real clinical benefits compared to the effects of single bronchodilator therapy.

In the last years there has been increasing interest in producing solid pre-clinical data on the bronchorelaxant synergy between LABAs and LAMAs [3–6], between different bronchodilator agents and ICSs [13, 14], and between mixed phosphodiesterase inhibitors and specific bronchodilators [15, 16]. Paradoxically, most of the pre-clinical investigations on LABA/LAMA combinations have been carried out, either independently [6, 17, 18] or under the support of the Drug Companies [3–5], after the regulatory approval of the FDCs. As a result, it has been demonstrated that in some of the isolated airways in which ASM contractile tone was induced by the activation of cholinergic pathway. The fraction affected (Fa) indicates the percentage of bronchorelaxant effect, where 0.5 is 50% and 1 is 100%. ASM: airway smooth muscle

---

**Fig. 1** Graphical representation of Unified Theory analysis via logarithmic combination index plot for aclindinium/formoterol (a), glycopyrronium/indacaterol (b), triotropium/olodaterol (c), and umeclidinium/vilanterol (d) combinations. The drugs were administered in combinations at isoeffective concentrations in human medium airways in which ASM contractile tone was induced by the activation of cholinergic pathway. The fraction affected (Fa) indicates the percentage of bronchorelaxant effect, where 0.5 is 50% and 1 is 100%. ASM: airway smooth muscle
currently marketed LABA/LAMA FDCs the monocomponents were not adequately balanced, with the bronchorelaxant effect mainly due to the action of the LAMA, with the lack of any synergistic interaction detected not only in ex vivo studies but also in RCTs [6, 19].

Recently, Maltais and colleagues [20] published a Phase II dose-finding RCT to determine the dose-ratio of tiotropium/olodaterol combination vs. tiotropium alone in moderate-to-severe COPD patients. Correctly, the authors stated that during the clinical development of a FDC of drugs, the best practice suggests to perform dose-finding investigations to determine the optimal dose of each component [20]. Making available to the scientific community just in 2019 the results of a Phase II study of the LABA/LAMA FDC tiotropium/olodaterol 5/5 μg approved in both EU and US since 2015 appears at least peculiar. However, data reported by Maltais and colleagues [20] are of interest and worth of considerations that go beyond those discussed in their paper.

First of all, tiotropium plus olodaterol administered at 5/2 μg and at the currently approved dose 5/5 μg produced a numerical, but not statistically significant, increase in trough forced expiratory volume in 1 s (FEV₁) compared to tiotropium 5 μg. Only adding olodaterol 10 μg to tiotropium 5 μg elicited a significant improvement in trough FEV₁ vs. tiotropium 5 μg. After 4 weeks of treatment, tiotropium/olodaterol 5/10 μg enhanced 57 mL trough FEV₁ compared to tiotropium 5 μg, a borderline value when considering the minimal clinically important difference (MCID) with respect to active comparators (MCID: > 60 mL) [21]. Conversely, the effects of tiotropium/olodaterol 5/2 μg and 5/5 μg on trough FEV₁ were far from the MCID (27 mL and 33 mL, respectively).

Furthermore, contrary to what the authors stated [20], there was no dose–response effect when increasing doses of olodaterol were combined with tiotropium. In fact, the impact of tiotropium/olodaterol 5/2 μg on trough FEV₁, peak FEV₁, and FEV₁(10–3 h) was generally equivalent to that induced by tiotropium/olodaterol 5/5 μg. Conversely, adding olodaterol 10 μg to tiotropium 5 μg induced a substantial improvement in trough FEV₁, peak FEV₁, and FEV₁(10–3 h) with respect to tiotropium/olodaterol 5/2 μg and 5/5 μg.

Although these findings appear unexpected by a functional point of view, they can be explained by considering the data on the pharmacological characterization of the interaction between tiotropium and olodaterol in human isolated airways [3]. In fact, looking at the concentration–response curves generated by ex vivo investigations, tiotropium was characterized by greater potency and more inclined hill-slope than olodaterol [3]. Moreover, while tiotropium completely relaxed human ASM, olodaterol produced ≤ 75% relaxant effect [3]. These pharmacological evidences clearly indicate that, in order to adequately balance the concentration-ratio between tiotropium and olodaterol and satisfy the concept of isoefficiveness, the amount of olodaterol included in the FDC should have been greater than that of tiotropium.

Effectively, the RCT of Maltais and colleagues [20] provides the strong evidence that the best combination is that in which tiotropium 5 μg is combined with olodaterol 10 μg, and not with olodaterol 5 μg. Therefore, along with data from pre-clinical studies, it seems that tiotropium should have been combined with olodaterol at a ≈ 1:2 concentration ratio.

The findings of Maltais and colleagues [20] are consistent with those of a previous unpublished study (NCT00720499), in which 4 weeks of treatment with tiotropium/olodaterol 5/2 μg produced the same improvement in trough FEV₁ when compared with of tiotropium/olodaterol 5/5 μg (57 mL and 55 mL, respectively). Another RCT [22] indicated that there was little difference in trough FEV₁ when tiotropium 5 μg is combined with either olodaterol 5 μg or 10 μg (155 mL and 163 mL, respectively), but we cannot omit that tiotropium/olodaterol 5/10 μg produced a substantial improvement in FEV₁(0–6) when compared with tiotropium/olodaterol 5/5 μg (342 mL and 307 mL, respectively) [22]. Overall, there is no doubt that tiotropium/olodaterol 5/10 μg guarantees a greater and more sustained bronchodilation than that induced by tiotropium/olodaterol 5/5 μg.
Considering the dual bronchodilator combinations included in this commentary, adding a LABA to a LAMA in patients with COPD induces an overall clinically relevant increase in FEV$_1$ of $\approx 65$ mL, when the drug mixtures were administered at the currently approved doses (Table 1). Nevertheless, and remarkably, when olodaterol 10 $\mu$g is added to tiotropium 5 $\mu$g the improvement in FEV$_1$ even doubled, regardless of any synergistic interaction proved for this combination in vivo in COPD patients (Table 1).

| Drug Combination                        | Change from baseline in FEV$_1$ ($\mu$L) | Delta effect vs. LAMA alone | Time-point | Proved synergy in vivo COPD patients | References |
|-----------------------------------------|------------------------------------------|-------------------------------|------------|--------------------------------------|------------|
| Aclidinium 400 $\mu$g                   | 67 $\pm$ 27                              | -                             | Peak effect at day 1: 240 min post dose | NA         | [17]                                 |
| Aclidinium/formoterol 400/12 $\mu$g     | 138 $\pm$ 16                             | + 70 $\pm$ 16                | Peak effect at day 1: 120 min post dose | Yes        |                                      |
| Glycopyrronium 50 $\mu$g                | 239 $\pm$ 33                             | -                             | Peak effect at day 1: 90 min post dose | NA         | [18]                                 |
| Glycopyrronium/indacaterol 50/150 $\mu$g| 255 $\pm$ 41                             | + 17 $\pm$ 41                | Peak effect at day 1: 90 min post dose | No at peak effect; yes at 15 min (± 75 ± 31 mL) |                        |
| Tiotropium 5 $\mu$g                     | 241 $\pm$ 22                             | -                             | Peak effect at week 4: 120 min post dose | NA         | [20]                                 |
| Tiotropium/olodaterol 5/2 $\mu$g        | 330 $\pm$ 27                             | + 89 $\pm$ 27                | Peak effect at week 4: 120 min post dose | Data not available |                        |
| Tiotropium/olodaterol 5/5 $\mu$g        | 327 $\pm$ 25                             | + 86 $\pm$ 25                | Peak effect at week 4: 180 min post dose | Data not available |                        |
| Tiotropium/olodaterol 5/10 $\mu$g       | 381 $\pm$ 24                             | + 140 $\pm$ 24               | Peak effect at week 4: 180 min post dose | Data not available |                        |
| Umeclidinium 62.5 $\mu$g                | 91 $\pm$ 92                              | -                             | Data on trough FEV$_1$ at day 15 | NA         | [6]                                  |
| Umeclidinium/vilanterol 62.5/25 $\mu$g  | 168 $\pm$ 91                             | + 77 $\pm$ 91                | Data on trough FEV$_1$ at day 15 | No         |                                      |

COPD chronic obstructive pulmonary disease, FEV$_1$ trough forced expiratory volume in 1 s, LABA long-acting $\beta_2$ adrenoceptor agonist, LAMA long-acting muscarinic antagonist, NA not applicable
Therefore, taken together the pre-clinical and clinical evidences, it is unclear what is the rational for combining tiotropium 5 μg with olodaterol 5 μg: tiotropium/olodaterol 5/5 μg has the same efficacy profile of tiotropium/olodaterol 5/2 μg, and it is less effective than tiotropium/olodaterol 5/10 μg. Finally, but not less important, looking at the frequency of adverse events (AEs) and serious AEs reported in the ClinicalTrials.gov database (i.e. NCT01040403, NCT00720499, and NCT00696020), tiotropium/olodaterol 5/2 μg, 5/5 μg, and 5/10 μg combinations are generally characterized by the same safety profile. Specifically, considering the European Medicine Agency [EMA] ranking, the pooled analysis of the frequency of all the AEs, both serious and not serious, was common (9%) by combining tiotropium 5 μg with olodaterol administered at 2 μg, or 5 μg or 10 μg (www.ema.europa.eu/docs/en_GB/document_library/Presentation/2013/01/WC500137021.pdf).

Considering that the study of Maltais and colleagues [20] was designed when tiotropium Respimat® was already licensed in several countries at the dose of 5 μg, and olodaterol was still in development, with the Phase III trials ongoing with 5 μg and 10 μg, in our opinion tiotropium/olodaterol 5/10 μg FDC should have been the best balanced formulation to be marketed, as it optimizes the synergistic interaction between the monocomponents leading to clinically appreciable improvement in lung function.

Indeed, several factors such as the pharmacokinetic (PK) characteristics, safety profile, and devices might be important for the choice of final doses in RCTs. In this respect, to date all the LABA/LAMA FDCs considered in this post hoc analysis where well characterized by a PK viewpoint (NCT02969317) [23–25], an in-depth meta-analysis is currently available concerning their safety profile [26], and data are also available on the role of inhaler devices in optimizing the dual bronchodilation therapy [27]. In any case, these factors are outside the topic of this commentary and require specific and extensive considerations.

Concluding, tiotropium/olodaterol 5/5 μg FDC remains an effective and safe pharmacological treatment for most COPD patients [28–30]. Perhaps a different development strategy based on solid data obtained from human isolated airways, and not from not-translational animal models of drugs interaction [20, 31], would have driven towards a better-balanced LABA/LAMA FDC to be tested in Phase III RCTs. Accurate bench-to-bedside plans are needed also in the development of triple combination therapies for asthma and COPD, in which the presence of an ICS in the formulation may further modulate the beneficial interaction between the LABA and the LAMA in the formulation [21, 32].

ACKNOWLEDGEMENTS

Funding. No funding or sponsorship was received for this study or publication of this article.

Authorship. All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Authorship contributions. All the authors provided substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND drafted the work or revising it critically for important intellectual content; AND approved the final version to be published; AND agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Disclosures. Luigino Calzetta has participated as an advisor in scientific meetings under the sponsorship of Boehringer Ingelheim and Novartis, received non-financial support from AstraZeneca, a research grant partially funded by Chiesi Farmaceutici, Boehringer Ingelheim, Novartis and Almirall, and is or has been a consultant to ABC Farmaceutici, Recipharm, Zambon, Verona Pharma and Ockham Biotech. His department was funded by Almirall, Boehringer Ingelheim, Chiesi Farmaceutici, Novartis and Zambon. Maria Gabriella Matera has
participated as a lecturer and advisor in scientific meetings and courses under the sponsorship of Almirall, AstraZeneca, Boehringer Ingelheim, Chiesi Farmaceutici, GlaxoSmithKline and Novartis, and has been a consultant to ABC Farmaceutici and Chiesi Farmaceutici. Her department was funded by Novartis. Mario Cazzola has participated as a faculty member 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, Recipharm, Verona Pharma and Zambon, and is or has been a consultant to ABC Farmaceutici, AstraZeneca, Chiesi Farmaceutici, Recipharm, Lallemand, Novartis, Ockham Biotech, Verona Pharma and Zambon. His department was funded by Almirall, Boehringer Ingelheim, Novartis and Zambon. Paola Rogliani has participated as a lecturer and advisor in scientific meetings and courses under the sponsorship of Almirall, AstraZeneca, Biofutura, Boehringer Ingelheim, Chiesi Farmaceutici, GlaxoSmithKline, Menarini Group, Mundipharma and Novartis. Her department was funded by Almirall, Boehringer Ingelheim, Chiesi Farmaceutici, Novartis and Zambon.

**Compliance with Ethics Guidelines.** This article is based on previously conducted studies and does not contain any studies with human participants or animals performed by any of the authors.

**Data Availability.** The datasets during and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Open Access.** This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (http://creativecommons.org/licenses/by-nc/4.0/), which permits any non-commercial use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

**REFERENCES**

1. GOLD. Global Initiative for Chronic Obstructive Lung Disease. Global strategy for diagnosis, management, and prevention of COPD—2019 Report. 2019. http://goldcopd.org/wp-content/uploads/2018/11/GOLD-2019-v1.5-FINAL-04Nov2018_WMS.pdf. Accessed 11 Mar 2019.

2. Calzetta L, Matera MG, Rogliani P, Cazzola M. Dual LABA/LAMA bronchodilators in chronic obstructive pulmonary disease: why, when, and how. Boca Raton: Taylor & Francis; 2018.

3. Calzetta L, Rogliani P, Page C, Rinaldi B, Cazzola M, Matera MG. Pharmacological characterization of the interaction between tiotropium bromide and olodaterol on human bronchi and small airways. Pulm Pharmacol Ther. 2019;56:39–50.

4. Cazzola M, Calzetta L, Puxeddu E, Ora J, Facciolo F, Rogliani P, et al. Pharmacological characterisation of the interaction between glycopyrronium bromide and indacaterol fumarate in human isolated bronchi, small airways and bronchial epithelial cells. Respir Res. 2016;17:70.

5. Cazzola M, Calzetta L, Page CP, Rogliani P, Facciolo F, Gavalda A, et al. Pharmacological characterization of the interaction between aclidinium bromide and formoterol fumarate in human isolated bronchi. Eur J Pharmacol. 2014;745:135–43.

6. Calzetta L, Matera MG, Cazzola M. Pharmacological mechanisms leading to synergy in fixed-dose dual bronchodilator therapy. Curr Opin Pharmacol. 2018;40:95–103.

7. Calzetta L, Matera MG, Cazzola M. Pharmacological mechanisms leading to synergy in fixed-dose dual bronchodilator therapy. Curr Opin Pharmacol. 2018;40:95–103.

8. Calzetta L, Matera MG, Cazzola M. Pharmacological mechanisms leading to synergy in fixed-dose dual bronchodilator therapy. Curr Opin Pharmacol. 2018;40:95–103.

9. Calzetta L, Matera MG, Cazzola M. Pharmacological mechanisms leading to synergy in fixed-dose dual bronchodilator therapy. Curr Opin Pharmacol. 2018;40:95–103.

10. Calzetta L, Matera MG, Cazzola M. Pharmacological interaction between LABAs and LAMAs in the airways: optimizing synergy. Eur J Pharmacol. 2015;761:168–73.
11. Chou TC. Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies. Pharmacol Rev. 2006;58:621–81.

12. Berenbaum MC. Isobolographic, algebraic, and search methods in the analysis of multiagent synergy. J Am Coll Toxicol. 1988;7:927–38.

13. Calzetta L, Matera MG, Facciolo F, Cazzola M, Rogliani P. Beclomethasone dipropionate and formoterol fumarate synergistically interact in hyperresponsive medium bronchi and small airways. Respir Res. 2018;19:65.

14. Cazzola M, Calzetta L, Rogliani P, Puxeddu E, Facciolo F, Matera MG. Interaction between corticosteroids and muscarinic antagonists in human airways. Pulm Pharmacol Ther. 2016;36:1–9.

15. Calzetta L, Cazzola M, Page CP, Rogliani P, Facciolo F, Matera MG. Pharmacological characterization of the interaction between the dual phosphodiesterase (PDE) 3/4 inhibitor RPL554 and glycopyrronium on human isolated bronchi and small airways. Pulm Pharmacol Ther. 2015;32:15–23.

16. Calzetta L, Page CP, Spina D, Cazzola M, Rogliani P, Facciolo F, et al. Effect of the mixed phosphodiesterase 3/4 inhibitor RPL554 on human isolated bronchial smooth muscle tone. J Pharmacol Exp Ther. 2013;346:414–23.

17. Cazzola M, Calzetta L, Ora J, Puxeddu E, Rogliani P, Matera MG. Searching for the synergistic effect between aclidinium and formoterol: from bench to bedside. Respir Med. 2015;109:1305–11.

18. Cazzola M, Calzetta L, Segreti A, Facciolo F, Rogliani P, Matera MG. Translational study searching for synergy between glycopyrronium and indacaterol. COPD. 2015;12:175–81.

19. Donohue JF, Singh D, Munzu C, Kilbride S, Church A. Magnitude of umeclidinium/vilanterol lung function effect depends on monotherapy responses: results from two randomised controlled trials. Respir Med. 2016;112:65–74.

20. Maltais F, Hamilton A, Voss F, Maleki-Yazdi MR. Dose determination for a fixed-dose drug combination: a phase II randomized controlled trial for tiotropium/olodaterol versus tiotropium in patients with COPD. Adv Ther. 2019;36:962–8.

21. Cazzola M, Rogliani P, Calzetta L, Matera MG. Triple therapy versus single and dual long-acting bronchodilator therapy in COPD: a systematic review and meta-analysis. Eur Respir J. 2018;52:1801586.

22. Aalbers R, Maleki-Yazdi MR, Hamilton A, Waitere-Wijker S, Zhao Y, Amatto VC, et al. Randomized, double-blind, dose-finding study for tiotropium when added to olodaterol, administered via the respimat® inhaler in patients with chronic obstructive pulmonary disease. Adv Ther. 2015;32:809–22.

23. Fuhr R, Leselbaum A, Aubets J. Pharmacokinetics of aclidinium bromide/formoterol fumarate fixed-dose combination compared with individual components: a phase I, open-label, single-dose study. Clin Pharmacol Drug Dev. 2016;5:109–17.

24. Demin I, Bartels C, Graham G, Bieth B, Gautier A, Tillmann HC, et al. Population pharmacokinetics of IND/GLY (indacaterol/glycopyrronium) in COPD patients. Int J Clin Pharmacol Ther. 2016;54:405–15.

25. Goyal N, Beerahee M, Kalberg C, Church A, Kilbride S, Mehta R. Population pharmacokinetics of inhaled umeclidinium and vilanterol in patients with chronic obstructive pulmonary disease. Clin Pharmacokinet. 2014;53:637–48.

26. Calzetta L, Rogliani P, Matera MG, Cazzola M. A systematic review with meta-analysis of dual bronchodilation with LAMA/LABA for the treatment of stable COPD. Chest. 2016;149:1181–96.

27. Rogliani P, Calzetta L, Coppola A, Cavalli F, Ora J, Puxeddu E, et al. Optimizing drug delivery in COPD: the role of inhaler devices. Respir Med. 2017;124:6–14.

28. Rogliani P, Matera MG, Ora J, Cazzola M, Calzetta L. The impact of dual bronchodilation on cardiovascular serious adverse events and mortality in COPD: a quantitative synthesis. Int J COPD. 2017;12:3469–85.

29. Calzetta L, Ora J, Cavalli F, Rogliani P, O’Donnell DE, Cazzola M. Impact of LABA/LAMA combination on exercise endurance and lung hyperinflation in COPD: a pair-wise and network meta-analysis. Respir Med. 2017;129:189–98.

30. Calzetta L, Rogliani P, Ora J, Puxeddu E, Cazzola M, Matera MG. LABA/LAMA combination in COPD: a meta-analysis on the duration of treatment. Eur Respir Rev. 2017;26:1–11.

31. Bouyssou T, Casarosa P, Pieper M, Schnapp A, Gantner F. Synergistic bronchoprotective activity of the long-acting beta 2-agonist olodaterol with tiotropium (long-acting M3 antagonist) and ciclesonide (inhaled steroid) on the ovalbumin-induced bronchoconstriction in anaesthetized guinea pigs. Eur Respir Soc. 2011;38:3451.

32. Calzetta L, Cazzola M, Matera MG, Rogliani P. Adding a LAMA to ICS/LABA therapy: a meta-analysis of triple combination therapy in COPD. Chest. 2019;155:758–70.