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Pharmacology and Therapeutics of Bronchodilators Revisited

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Abstract——**Bronchodilators remain the cornerstone of the treatment of airway disorders such as asthma and chronic obstructive pulmonary disease (COPD). There is therefore considerable interest in understanding how to optimize the use of our existing classes of bronchodilator and in identifying novel classes of bronchodilator drugs. However, new classes of bronchodilator have proved challenging to develop because many of these have no better efficacy than existing classes of bronchodilator and often have unacceptable safety profiles. Recent research has shown that optimization of bronchodilation occurs when both arms of the autonomic nervous system are affected through antagonism of muscarinic receptors to reduce the influence of parasympathetic innervation of the lung and through stimulation of β2-adrenoceptors (β2-ARs) on** airway smooth muscle with β2-AR–selective agonists to mimic the sympathetic influence on the lung. This is currently achieved by use of fixed-dose combinations of inhaled long-acting β2-adrenoceptor agonists (LABAs) and long-acting muscarinic acetylcholine receptor antagonists (LAMAs). Due to the distinct mechanisms of action of LAMAs and LABAs, the additive/synergistic effects of using these drug classes together has been extensively investigated. More recently, so-called "triple inhalers" containing fixed-dose combinations of both classes of bronchodilator (dual bronchodilation) and an inhaled corticosteroid in the same inhaler have been developed. Furthermore, a number of so-called "bifunctional drugs" having two different primary pharmacological actions in the same molecule are under development. This review discusses recent

advancements in knowledge on bronchodilators and bifunctional drugs for the treatment of asthma and COPD.

Significance Statement——Since our last review in 2012, there has been considerable research to identify novel classes of bronchodilator drugs, to further understand how to optimize the use of the existing classes of bronchodilator, and to better understand the role of bifunctional drugs in the treatment of asthma and chronic obstructive pulmonary disease.

I. Introduction

Inhaled bronchodilators are critical to the optimal management of patients with chronic obstructive pulmonary disease (COPD) at all phases of the disease (https://www.goldcopd.org/) and are crucial in the management of patients with asthma (https://ginasthma.org).

In 2012 we published a comprehensive review on the pharmacology and therapeutics of bronchodilators (Cazzola et al., 2012b); since then, extensive research has been undertaken to identify novel classes of bronchodilator drugs (Cazzola and Matera, 2014) and to better understand how to optimize the use of the existing classes of bronchodilator. This review provides an update on the recent advances in this area of pharmacology since 2012.

II. Pharmacology of Bronchodilators

A. The Physiologic Rationale for Using Bronchodilators

Physiologically, airway smooth muscle (ASM) tone is predominantly controlled by the parasympathetic nervous system via cholinergic and, to a lesser degree, noncholinergic innervation (Cazzola et al., 2012b). It is now well established that increased vagus nerve activity plays a key role in eliciting airway obstruction in patients with COPD or asthma and seems to be a major reversible component of airway obstruction. Acetylcholine (ACh) is the neurotransmitter in the parasympathetic nervous system, both in the ganglia and at the neuroeffector junction. ACh activates muscarinic acetylcholine receptors (mAChRs) postsynaptically on ASM and mucous glands (reviewed by Cazzola et al., 2012b) to elicit bronchoconstriction and mucous secretion, respectively.

G_i-coupled M_2 mAChRs are highly expressed on ASM and activation of this receptor type inhibits adenylyl cyclase (AC) to reduce the intracellular concentration of cAMP. ACh also activates G_q-coupled M_3 mAChRs on ASM to activate phospholipase C (PLC) and elevate intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) through several pathways that involve Ca^{2+} influx and sarcoplasmic reticulum (SR) Ca^{2+} release. There is a nearly 4-fold higher expression of the M_2 subtype in the large airways, although ASM contraction is mediated primarily through activation of M_3 mAChRs (Cazzola et al., 2012b; Rydell-Törmänen et al., 2013).

The activation of M_3 mAChRs (reviewed by Meurs et al., 2013) by ACh and the subsequent activation of PLC lead to the hydrolysis of phosphatidylinositol 4,5-bisphosphate to generate inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol in ASM. IP_3 causes the mobilization of Ca^{2+} from the SR, an effect that induces a fast and temporary increase in $[Ca^{2+}]_i$, which initiates contraction of ASM by activation of Ca^{2+}-calmodulin–dependent myosin light chain (MLC) kinase that is followed by the phosphorylation of the 20-kDa MLC. It is commonly believed that the tonic phase of contraction of ASM is the consequence of a continuing influx of Ca^{2+} through receptor-operated channels and store-operated channels, with the involvement of canonical transient receptor potential channels and Stim1/Orai1 proteins. Furthermore, M_3 mAChRs activate CD38, also known as cyclic ADP ribose hydrolase, which leads to an increase in the production of cyclic ADP ribose, resulting in the release of Ca^{2+} from the SR via ryanodine receptor channels. Diacylglycerol also activates protein kinase C (PKC), which may be involved in ASM contraction via Ca^{2+}-dependent and Ca^{2+}-independent mechanisms. PKC activation contributes to agonist-induced Ca^{2+} mobilization from the SR with the subsequent influx of extracellular Ca^{2+} and also causes the phosphorylation of PKC-potentiated phosphoprotein phosphatase inhibitor 17 kDa (CPI-17), a phosphorylation-dependent inhibitory protein for myosin light chain phosphatase (MLCP) expressed in smooth muscle that, by inhibiting MLCP, induces Ca^{2+} sensitization. Ca^{2+} sensitization also regulates M_3 mAChR-mediated ASM contraction via activation of the RhoA/Rho kinase pathway that phosphorylates MLCP and CPI-17. Activation of M_3 mAChRs also influences β_2-adrenoceptor (β_2-AR)–mediated relaxation of ASM by activating PKC, which successively phosphorylates β_2-AR and the G_s subunits leading to desensitization of these proteins (Calzetta et al., 2015).

The vagus nerve innervates larger airways and when stimulated releases ACh that activates M_3 mAChRs located on ASM cells. In the small airways, M_3 mAChRs are present on ASM but there is no vagal innervation, and it is thought that the so-called non-neurogenic ACh is released locally from epithelial cells lining the airways known to contain choline acetyltransferase activity (Matera and Cazzola, 2017).

Direct adrenergic innervation of ASM is sparse in humans, but β_2-ARs are densely expressed on this cell type. Activation of β_2-ARs on ASM generates intracellular cAMP by AC activation, which in turn involves the activation of its effector molecules, cAMP-dependent

protein kinase A (PKA) and exchange protein activated directly by cAMP, which is a Rap1 guanine nucleotide exchange factor (reviewed by Billington et al., 2013; Cazzola et al., 2013b). PKA phosphorylates crucial regulatory proteins implicated in the control of ASM tone, whereas exchange protein activated directly by cAMP downregulates Rho and consequently induces ASM relaxation via a PKA-independent manner. The generation of intracellular cAMP results in sequestration of $[Ca^{2+}]_i$ that results in the relaxation of ASM (Billington and Hall, 2012). However, increasing evidence is emerging that signaling through AC-coupled pathways is not as simple as previously thought (Billington and Hall, 2012). In fact, many studies have questioned whether cAMP formation indeed is the main mechanism whereby activation of β_2-ARs elicits ASM relaxation (Cazzola et al., 2013b). Thus, it has been demonstrated that calcium-activated potassium (KCa^{++}) channel activation is important for β_2-AR–mediated ASM relaxation through either cAMP/PKA-mediated signaling or non-cAMP pathways (Dale et al., 2014).

B. The Pharmacological Optimization of Bronchodilation

There is now considerable evidence suggesting that combining mAChR antagonists and β_2-AR agonists, with the possible addition of inhaled corticosteroids (ICSs), is a method to optimize bronchodilation, at least with the currently approved drugs. These combinations offer both short- and long-term benefit to subjects suffering from asthma or COPD, without the need to increase the dose of the monocomponents, which thus reduces the risk of adverse events (Fig. 1).

1. Combining β_2-Adrenoceptor Agonists and Muscarinic Acetylcholine Receptor Antagonists (Dual Bronchodilation). It is now well documented that combinations of low and isoeffective concentrations of long-acting β_2-adrenoceptor agonists (LABAs) and long-acting muscarinic acetylcholine receptor antagonists (LAMAs) induce a greater relaxation of ASM than using the monocomponents, likely due to significant synergistic interaction between the two distinct pharmacological approaches (Cazzola et al., 2014, 2016a). This synergistic interaction has been confirmed in small clinical trials in patients with COPD (Cazzola et al., 2015b,c).

Several mechanisms have been suggested to explain the synergy between LABAs and LAMAs at the ASM level and presynaptically in parasympathetic nerve fibers (Cazzola and Molimard, 2010; Calzetta et al., 2015, 2018b,d). At the presynaptic level, prejunctional β_2-ARs activated by LABAs in turn stimulate KCa^{++} channels and thus modulate the release of ACh into the synaptic space. It is likely that these channels reduce the concentration of $[Ca^{2+}]_i$ by hyperpolarizing the cell membrane and, consequently, inhibit the release of ACh.

LAMAs can also inhibit ganglionic transmission because they antagonize M_1 mAChRs, which are facilitatory to $\alpha7$ nicotinic ACh receptors in the cell body of postganglionic parasympathetic neurons. LAMAs antagonize the activation of mAChRs by ACh on ASM to prevent contraction, whereas LABAs are agonists at β_2-ARs stimulating relaxation of ASM directly. β_2-AR activation of ASM induces intracellular signaling mechanisms described above that reduce IP_3 production mediated by M_3 mAChRs with the probable involvement of PKA. In contrast, M_3 mAChR antagonism can influence the relaxation of ASM induced by β_2-AR agonists, most likely because it blocks the activation of PKC and the consequent phosphorylation of β_2-AR and/or G_s protein. Thus, the coadministration of a LAMA with a LABA increases the synthesis of cAMP to result in increased ASM relaxation. Protein tyrosine kinases and KCa^{++} channels were also recently shown to be central to the synergistic interaction between LABAs and LAMAs. In small airways, the modulation of ASM tone is also under the control of non-neurogenic ACh that is synthetized in and released from the epithelium. LABAs and LAMAs also activate β_2-ARs and antagonize M_3 mAChRs expressed on the surface of bronchial epithelial cells, which leads to a reduction of the release of non-neurogenic ACh by modulating organic cationic transporter activity (Cazzola et al., 2016a).

A number of LABA/LAMA combinations in a single inhaler have now been approved for clinical use as treatments for patients with COPD (Table 1).

2. Combining β_2-Adrenoceptor Agonists and Inhaled Corticosteroids. Although it has been suggested that LABAs and ICSs each cause several independent, non-interacting responses that are associated in an additive way to induce clinical benefit (Newton and Giembycz, 2016), bidirectional molecular interactions between corticosteroids and β_2-agonists have also been suggested for the reciprocal potentiation of the pharmacological effects of ICS and LABAs (Pelaia et al., 2015). Such a cooperative cross-talk between these two drug classes is widely exploited in the treatment of patients with asthma and in some patients with COPD by providing complementary bronchodilator and anti-inflammatory actions when these two drug classes are administered concomitantly (Cazzola and Matera, 2017). Furthermore, recent in vitro evidence indicates that LABA/ICS combinations can produce a synergistic bronchorelaxant effect in both small and medium-size human airways, with a greater synergistic effect in hyperresponsive airways than in nonsensitized tissues (Calzetta et al., 2018c).

The expression of tumor necrosis factor α–induced protein 3 (TNFAIP3), a feedback regulator of nuclear factor-κB that negatively influences the repression of nuclear factor-κB–dependent transcription and also the repression of TNF expression, is induced by
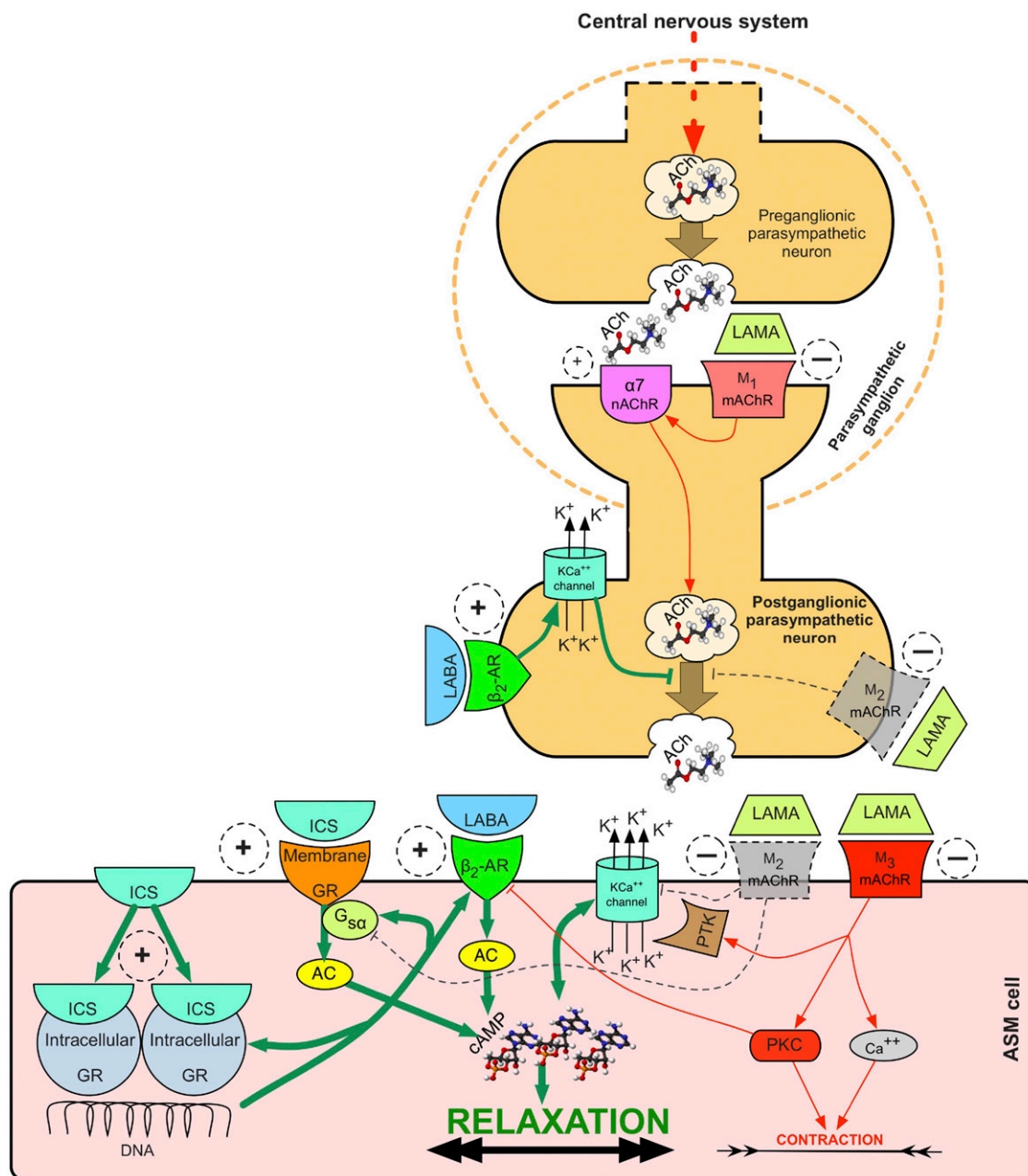
Fig. 1. The pharmacological optimization of bronchodilation. LABAs and LAMAs both modulate the bronchial tone via different pathways localized at the level of presynaptic parasympathetic fibers and ASM cells. The exact nature of the interactions between these pathways is not completely understood, but there is cross-talk at many levels in ASM cells that is also regulated by the activity of calcium-activated potassium channels and protein tyrosine kinases. ICSs freely diffuse from the circulation across cell membranes into cells, where they activate the GR. The activated ICS-GR also binds to the β_2-AR gene, leading to an increase in the number of β_2-ARs in the cell membrane. On the other side, LABA stimulates β_2-ARs, leading to the priming of the GR and increasing translocation of the receptor into the nucleus of the cell. GR, glucocorticoid receptor; PTK, protein tyrosine kinase.

a corticosteroid and is noticeably, although transiently, influenced by a LABA, which alone has only a minor effect on TNFAIP3 mRNA expression (Altonsy et al., 2017). Furthermore, treatment with an ICS plus a LABA enhances TNF-induced TNFAIP3 expression, a protein known to be reduced in patients with asthma (Rider et al., 2018).

Corticosteroids induce gene transcription via glucocorticoid receptor–mediated transactivation (Newton and Giembycz, 2016). LABAs can also increase the

expression of some glucocorticoid-inducible genes (Rider et al., 2018), although the function of individual genes of relevance to treating asthma or COPD is not fully understood.

It is important to note that LABAs are not identical and the differences, which can also be significant, need to be taken into account when choosing a LABA/ICS combination (Cazzola et al., 2013c). In particular, formoterol has a very rapid onset of action compared with the slower-acting salmeterol and this important

TABLE 1
Fixed-dose combination inhalers

Drug Class	Generic Name	Form	Dose
LABA/LAMA	Formoterol/aclidinium	Inhalation powder	12/400 μg twice a day
	Indacaterol/glycopyrronium	Inhalation powder	27.5/15.6 μg twice a day (United States)
	Formoterol/glycopyrronium	Inhalation aerosol	9.6/18 μg twice-a-day (United States); 10/14.4 μg twice-a-day (European Union)
	Indacaterol/glycopyrronium	Inhalation powder	85/43 μg once-a-day
	Olodaterol/tiotropium	Inhalation spray	5/5 μg once-a-day
	Vilanterol/umeclidinium	Inhalation powder	25/62.5 μg once-a-day
LABA/LAMA/ICS	Formoterol/tiotropium/ciclesonide	Inhalation powder	12/18/400 μg once-a-day
	Formoterol/glycopyrronium/beclomethasone dipropionate	Inhalation spray	5/11/87 μg twice-a-day
	Vilanterol/umeclidinium/fluticasone furoate	Inhalation powder	22/65/92 μg once-a-day
	Formoterol/glycopyrronium/budesonide	Inhalation aerosol	9.6/14.4/320 μg twice-a-day

difference is now being exploited in using formoterol with budesonide in so-called single maintenance and reliever therapy (SMART), whereby this combination can be used on demand and not just for maintenance treatment (Thomas and Pavord, 2012; Beasley et al., 2019). There is also evidence that corticosteroid insensitivity caused by oxidative stress is reversed by formoterol, but not salmeterol, and that formoterol is also able to inhibit phosphatidylinositol-3 kinase-δ signaling (Rossios et al., 2012). Moreover, it has been demonstrated that cytokines inhibit the tracheal relaxation and cAMP signaling induced by formoterol and salmeterol; however, budesonide is only able to prevent the inhibitory effects on the actions of formoterol (Adner et al., 2010). Formoterol has also been shown to increase corticosteroid sensitivity without receptor activation, but rather by activating the Ser/Thr protein phosphatase 2A, which results in the dephosphorylation of the glucocorticoid receptor at serine 226 (Kobayashi et al., 2012).

3. Combining Muscarinic Acetylcholine Receptor Antagonists and Inhaled Corticosteroids. There is increasing evidence that the LAMA, tiotropium bromide, at a low dose attenuates airway inflammation and remodeling in a guinea pig model of chronic asthma when chronically combined with the ICS ciclesonide. Such findings suggest that treatment with a LAMA and an ICS may also elicit anti-remodeling activity in addition to the well recognized bronchodilator and anti-inflammatory properties of these drugs, respectively (Kistemaker et al., 2016).

The pharmacological mechanisms of the interactions between mAChR antagonists and ICS are not yet clear. However, it has been shown that experimentally combining the mAChR antagonist, glycopyrronium, with the ICS, beclomethasone dipropionate, in isolated human ASM passively sensitized and precontracted with histamine is effective in relaxing both medium bronchi and bronchioles causing submaximal/maximal inhibition of the contractile tone, especially in small airways, where the combination is able to increase the relaxation of ASM by 2.5-fold compared with monocomponents (Cazzola et al., 2016b).

This synergist interaction between LAMAs with ICSs is detectable only in sensitized airways, likely because the challenge of airway tissue with high concentrations of IgE causes an increased release of endogenous ACh that activates M_3 mAChRs and LAMAs inhibit such activation (Cazzola et al., 2016b). It has also been hypothesized that in passively sensitized human ASM, airways can be protected against G_s protein dysfunction by the low affinity of the LAMA for the M_2 mAChR, whereas the response to $G_{s\alpha}$ stimulation is increased by the short-term treatment with the ICS (Cazzola et al., 2016b). Indeed, there is evidence that when airways are passively sensitized with serum obtained from atopic donors, M_2 mAChR/G_i-coupled expression increases, phosphorylation of G_s protein and inhibition of AC and reduced cAMP accumulation occur, and, consequently, this has a negative effect on ASM relaxation (Hakonarson et al., 1995; Song et al., 2000).

4. Combining β_2-Adrenergic Receptor Agonists, Muscarinic Receptor Antagonists, and Inhaled Corticosteroids (Triple Inhalers). The concept of triple therapy with LABAs, LAMAs, and ICSs has recently attracted considerable attention as a potentially improved way of delivering an ICS with dual bronchodilator therapy in one inhaler device rather than having to take these drugs in separate inhalers. A number of these triple inhaler combinations have now been approved for clinical use for the treatment of patients with COPD (Table 1).

C. Emerging Classes of Bronchodilator Drugs

There remains an interest in identifying new targets for drugs to induce bronchodilation or to improve the

effectiveness of existing drug classes. At least eight new classes of bronchodilator drugs are in development (Fig. 2), including bitter taste receptor agonists, E-prostanoid (EP) receptor 4 agonists, Rho kinase inhibitors, calcilytics, agonists of peroxisome proliferator–activated receptor-γ (PPARγ), relaxin family peptide receptor 1 (RXFP1) receptor agonists, soluble guanylyl cyclase (sGC) activators, and pepducins (Table 2). Furthermore, there is a considerable amount of research being undertaken to improve the existing classes of bronchodilators with the development of novel selective phosphodiesterase (PDE) inhibitors, novel xanthines, novel β_2-AR agonists, and novel mAChR antagonists (Table 3). Research is also ongoing to develop new options for combination therapy such as bifunctional bronchodilator drugs and bifunctional bronchodilator/anti-inflammatory drugs (Table 4).

1. Novel Classes of Bronchodilators.

a. Bitter Taste Receptor Agonists. Stimulation of type 2 taste receptors (TAS2Rs), which are members of a family of about 25 G protein–coupled receptors (GPCRs), expressed in respiratory epithelia and smooth muscle has been demonstrated to have effects on protective airway reflexes, to increase beat frequency of motile cilia of airway epithelial cells, and to induce bronchodilation (Deshpande et al., 2010). Three of the 25 TAS2Rs are highly expressed in human ASM, termed subtypes 10, 14, and 31. When activated, these receptors elicit relaxation through a calcium-dependent, Gα_i-coupled mechanism (Kim et al., 2017) and subsequent activation of PLC. Activation of TAS2Rs increases SR Ca^{2+} release via IP$_3$ receptor channels, leading to activation of large-conductance KCa^{++} channels, membrane hyperpolarization, and bronchodilation (reviewed by Prakash, 2016).

Recent findings have also demonstrated that activation of TAS2Rs can counteract mAChR enhancement of Ca^{2+} influx via voltage-gated calcium channels, suggesting that TAS2Rs may modestly enhance [Ca^{2+}]$_i$ without affecting the contraction under baseline conditions but they act to reduce [Ca^{2+}]$_i$ in the presence of an agent causing bronchoconstriction (reviewed by Prakash, 2016). Furthermore, TAS2Rs appear to have antimitogenic properties; as TAS2R stimulation activates intracellular signaling pathways that are distinct from those activated by β_2-ARs, TAS2R agonists appear to remain effective even when tachyphylaxis has been induced by prolonged β_2-AR activation (Devillier et al., 2015b).

However, there is currently very little clinical evidence supporting TAS2R agonists having significant bronchodilator activity, although several drugs currently marketed for a wide range of indications, including erythromycin, carisoprodol, flufenamic acid, dapsone, quinine, chloroquine, caffeine, azelastine, and colchicine, are known to activate TAS2Rs and improve lung function or decrease exercise- or histamine-induced
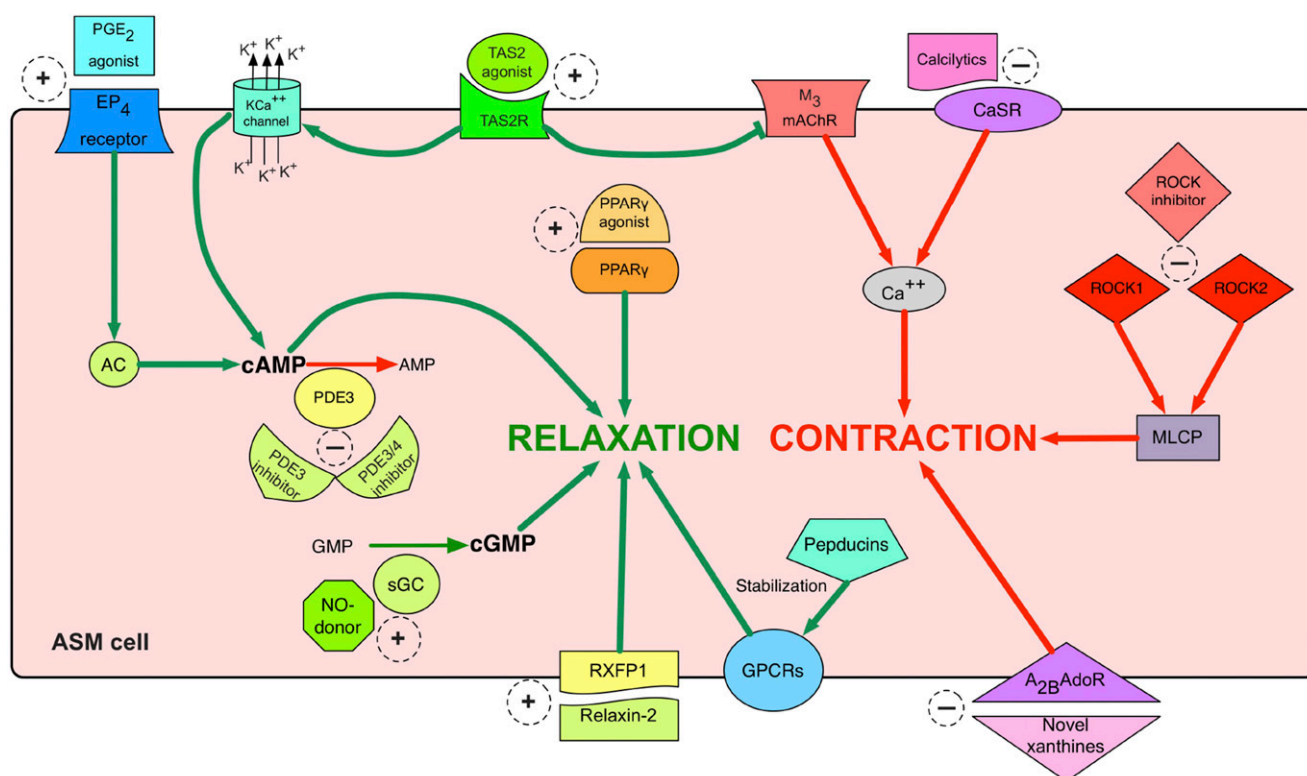
Fig. 2. Potential targets for new classes of bronchodilator.

TABLE 2
Novel classes of bronchodilators

Class	Molecule	Potential Therapeutic Effects
TAS2R agonists	Erythromycin	Bronchodilation, reduction in exercise- or histamine-induced bronchoconstriction in patients with asthma
	Arisoprodol	
	Flufenamic acid	
	Dapsone	
	Quinine	
	Chloroquine	
	Caffeine	
	Azelastine	
	Colchicine	
EP receptor 4 agonists	ONO-AE1-329	Relaxation of ASM, reversal of histamine-induced contraction of ASM, increase in glucocorticoid response element-dependent transcription when combined with ICS
	L-902688	
	TCS2510	
	Rivenprost	
Rho kinase inhibitors	Fasudil	Relaxation of ASM, relaxation of methacholine-constricted human small airways
	Ripasudil	
	Netarsudil	
	AMA0076/PHP-201	
	Y-39983	
Calcilytics	Calcityrol, B2.1-E1	Bronchodilator and anti-inflammatory actions
Agonists of PPARγ	Pioglitazone	Relaxation of ASM, inhibition of the initiation and development of a maximal contraction induced by methacholine, synergistic interaction with β_2-AR agonists on ASM cell proliferation
	Rosiglitazone	
	Troglitazone	
	Ciglitazone	
RXFP1 agonists	Serelaxin	Relaxation of ASM, increase in airway dilator responsiveness to β-AR agonists
	B-chain only agonists (B7-33)	
	Small molecule RXFP1 agonists (ML290)	
	Fatty acid conjugated relaxin-2 analogs (R9-13)	
sGC activators	BAY 41-2272	Bronchodilation, reduction of airway hyperresponsiveness
	BAY 60-2770	
	BAY 58-2667	
Pepducins	Pepducin ICL$_{3-9}$	Relaxation of ASM
	AT1-2341	
	P4pal-10	

bronchoconstriction in patients with asthma. In any case, most of these compounds have poor selectivity and weak potency for these receptors and many of them have pharmacological effects unrelated to the stimulation of TAS2Rs that cannot be ruled out as contributing to the beneficial effects seen on ASM (Grassin-Delyle et al., 2015). Furthermore, some TAS2Rs only respond to a limited number of bitter compounds, whereas other TAS2Rs can be activated by a broad range of agents; therefore, it is likely that drugs targeting TAS2Rs will have many off-target effects, and it is likely that these receptors play a role in the side effects of bitter-tasting drugs in clinical use (Lee et al., 2019). In addition, some TAS2Rs are highly polymorphic, which may affect the sensitivity of patients to bitter tastants (Lee et al., 2019).

b. E-Prostanoid Receptor 4 Agonists. The biologic actions of prostaglandin E$_2$ (PGE$_2$) are predominantly mediated by the activation of the four EP receptors, EP$_1$ to EP$_4$. The bronchodilator properties of PGE$_2$ in human airways are well documented and are mediated via activation of EP$_4$ receptors, whereas the undesirable triggering of airway sensory nerves, which causes airway irritation and cough, appears to be via activation

of EP$_3$ receptors (Buckley et al., 2011). Stimulation of the EP$_4$ receptor with PGE$_2$ induces a direct activation of AC via the Gα_s subunit that converts adenosine triphosphate to cAMP. Therefore, it remains plausible that EP subtype-selective receptor agonists could have therapeutic potential as bronchodilators (Buckley et al., 2011).

This conclusion is reinforced by the observation that the selective EP$_4$ receptor agonist ONO-AE1-329 (2-[3-[(1R,2S,3R)-3-hydroxy-2-[(E,3S)-3-hydroxy-5-[2-(methoxymethyl)phenyl]pent-1-enyl]-5-oxocyclopentyl]sulfanylpropylsulfanyl]acetic acid) can relax human airways and the selective EP$_4$ receptor antagonist ONO-AE3-208 (4-Cyano-2-[[2-(4-fluoro-1-naphthalenyl)-1-oxopropyl] amino]benzenebutanoic acid) is able to reverse the bronchodilatation induced with PGE$_2$ and ONO-AE1-329 (Buckley et al., 2011). In addition, the selective EP$_4$ agonists ONO-AE1-329 and L-902688 (5-[(1E,3R)-4,4-difluoro-3-hydroxy-4-phenyl-1-buten-1-yl]-1-[6-(2H-tetrazol-5R-yl)hexyl]-2-pyrrolidinone) have also been demonstrated to reverse histamine-induced contraction of ASM (Benyahia et al., 2012) and the specific EP$_4$ antagonist GW62768X ((N-{2-[4-(4,9-diethoxy-1-oxo-1,3-dihydro-2H-benzo[f]isoindol-2-yl)phenyl]acetyl} benzene

TABLE 3
Novel molecules in the existing classes of bronchodilators

Class	Subgroup	Molecule	Potential Therapeutic Effects
Selective PDE inhibitors	PDE3–PDE4 inhibitors	Ensifentrine	Bronchodilation, suppression of airway inflammatory responses
	PDE4–PDE7 inhibitors	YM-393059	Suppression of airway hyperresponsiveness, superior anti-inflammatory activity over PDE4 inhibition alone
		T-2585 ASB16165 BC54	
	PDE4–PDE5 inhibitors	LASSBio596	Decrease in arterial pulmonary hypertension, lung inflammation, and remodeling; improvement in lung function
		Compound A	
Novel β_2-AR agonists		Trantinterol	Bronchodilation
		8-Hydroxy-5-(2-hydroxy-1-((4-hydroxyphenethyl) amino)ethyl)quinolin-2(1H)-one	
		2-Amino-2-phenylethanol	
		Derivatives molecules with a 5-(2-amino-1-hydroxyethyl)-8-hydroxyquinolin-2(1H)-one moiety S1319	
		7-[(R)-2-((1R,2R)-2- benzyloxycyclopentylamino)-1-hydroxyethyl]-4-hydroxybenzothiazolone (C26)	
Novel mAChR antagonists		(R)-3-[2-hydroxy-2,2-di(thiophen-2-yl) acetoxy]-1,1-dimethylpyrrolidinium bromide	Bronchodilation
		(S)-3-[2-hydroxy-2,2-di(thiophen-2-yl) acetoxy]-1,1-dimethylpyrrolidinium bromide (revefenacin)	
		V0162	
		AZD8683	
		AZD9164	
		Bencycloquidium	
		CHF 5407	
		Allosteric modulators that selectively act on the M$_3$ mAChR	
Novel xanthines		Doxofylline	Improvement in lung function, anti-inflammatory effect, potential steroid-sparing effect
		Inhaled theophylline	

sulphonamide) was shown to prevent PGE$_2$-induced relaxation of human bronchial tissue precontracted with histamine (Benyahia et al., 2012). There is also evidence that activation of the EP$_4$ receptor in human airway epithelial cells augmented the glucocorticoids responsive element-dependent transcription in a manner that was qualitatively similar to that previously reported with a LABA (Yan et al., 2014). Thus, an ICS/EP$_4$ receptor

TABLE 4
Bifunctional bronchodilator drugs and bifunctional bronchodilator/anti-inflammatory drugs

Class	Subgroup	Molecules
Bifunctional bronchodilator drugs	MABAs	Batefenterol, AZD8871, LAS190792, CHF6366
Bifunctional bronchodilator/anti-inflammatory drugs	PDE3–PDE4 inhibitors	Ensifentrine
	PDE4–PDE5 inhibitors	LASSBio596
	NO–ICS	TPI 1020
	NO–β_2-AR agonist	NCX 950
	PDE4 inhibitor–β_2-AR agonist	GS-5759
	PDE4 inhibitor–mAChR antagonist	UCB-101333-3
	Vasoactive intestinal peptide–pituitary AC-activating polypeptide type 2 receptor	RO 50-24118

agonist combination may represent a novel approach to having a bronchodilator that can also suppress airway inflammation. However, none of the available EP$_4$ receptor agonists, such as TCS2510 ((5R)-5-[(3S)-3-Hydroxy-4-phenyl-1-buten-1-yl]-1-[6-(2H-tetrazol-5-yl) hexyl]-2-pyrrolidinone), L-902688, rivenprost, and ONO-AE1-329, have been tested as bronchodilators in humans to date (Lebender et al., 2018).

c. Rho Kinase Inhibitors. Rho-associated protein kinases (ROCK1 and ROCK2) are serine/threonine kinases that can regulate the contraction of ASM and other smooth muscles. After being activated by the small GTPase RhoA, ROCKs phosphorylate and inactivate the constitutively active MLCP, an effect that maintains the contraction of human ASM by increasing and sustaining MLC phosphorylation (Chiba and Misawa, 2004; Chiba et al., 2010). Inhibition of RhoA has also been shown to induce relaxation of methacholine-constricted human small airways (Yoo et al., 2017). There are already two ROCK inhibitors approved for clinical use in Japan and China, but they are not approved for treating airway diseases. Fasudil was approved for the treatment of cerebral vasospasm and ripasudil for the treatment of glaucoma. There are also a number of novel ROCK inhibitors under development for the treatment of glaucoma, including netarsudil and AMA0076/PHP-201 (3-[2-(Aminomethyl)-5-[(pyridin-4-yl)

carbamoyl]phenyl] benzoate), but to date there are no clinical data available using this class of drug for treating patients with asthma or COPD. Nonetheless, it has been suggested that ROCK inhibitors may act synergistically with other classes of bronchodilator and therefore provide additional benefit beyond existing classes of bronchodilator (Barnes, 2016).

However, a major concern with ROCK inhibitors is the potential for unwanted side effects, particularly in the cardiovascular system, given the ubiquitous expression of these enzymes across a range of tissues that may limit their use in patients with asthma or COPD. For this reason, a number of compounds have been described that intentionally include a metabolically labile aspect to their chemical structure, such that the drug undergoes rapid metabolism in plasma to produce nontoxic metabolites. Thus, development of such compounds by inhalation may be one approach to reduce unwanted systemic side effects with ROCK inhibitors (Defert and Boland, 2017).

d. Calcilytics. Calcilytics are small molecules that block the G protein (heterotrimeric guanine nucleotide-binding protein)–coupled calcium-sensing receptor (CaSR) that can exert beneficial effects in the context of airway diseases (Yarova et al., 2015). CaSR activation leads to $[Ca^{2+}]_i$ mobilization, cAMP breakdown, and p38 mitogen-activated protein kinase phosphorylation in ASM cells inducing airway hyperresponsiveness and airway remodeling (Penn, 2015). CaSRs are upregulated and constitutively activated in airways obtained from subjects with asthma (Yarova et al., 2015). Calcilytics (e.g., calcityrol) have been developed as drugs to affect calcium levels systemically and several have been evaluated for non-pulmonary indications clinically that have subsequently been stopped. However, calcilytics can also act as bronchodilators and anti-inflammatory agents and are therefore under development for local administration to the lung to reduce their effects on blood calcium concentrations because it has been documented in vivo using a mouse model of allergic asthma that inhaled calcilytics do not significantly modify plasma $[Ca^{2+}]_i$ levels up to 24 hours after inhalation, signifying that calcilytic delivered directly to the lung in humans should not negatively influence mineral ion homeostasis (Yarova et al., 2015).

e. Agonists of Peroxisome Proliferator–Activated Receptor-γ. PPARγ ligands are another class of drug developed for a different therapeutic use that have also been shown to relax ASM in vitro (Bourke et al., 2014), particularly under conditions of impaired or limited β-AR responsiveness (Donovan et al., 2015). Rosiglitazone, a PPARγ agonist, and chloroquine, a TAS2R agonist, but not β-AR agonists, were able to cause complete relaxation of mouse small airways, showing similar potency when maintained under conditions of homologous β2-AR desensitization (Donovan et al., 2014). Although both rosiglitazone and chloroquine

are able to counteract contraction due to calcium sensitivity alone, only rosiglitazone prevented the initiation and development of a maximal methacholine-induced contraction of ASM. Interestingly, it has been documented that PPARγ agonists and β2-AR agonists interact in a synergistic manner on ASM cell proliferation (Fogli et al., 2013). These experimental observations have been extended to beneficial effects in steroid-naïve asthmatics where rosiglitazone has been demonstrated to improve both airway hyperresponsiveness and forced expiratory volume in 1 second (FEV_1) in a dose- and time-dependent manner (Sandhu et al., 2010).

f. Relaxin Family Peptide Receptor 1 Receptor Agonists. Relaxin-2 is a member of the relaxin peptide family and is the major stored and circulating form of relaxin. Relaxin-2 contains two chains (A and B) that are linked by a disulfide bond (Lam et al., 2018). The B-chain has two arginine residues at positions 13 and 17 that allow the binding of relaxin to its cognate GPCR, RXFP1. Both relaxin and RXFP1 are detectable in epithelial cells as well as in fibroblasts and ASM, although in smaller quantities (Lam et al., 2016).

It has been shown that in methacholine-precontracted rat trachea, the recombinant human form of relaxin-2, serelaxin, elicited a partial relaxation relative to isoprenaline, although with a slower onset of action (Lam et al., 2016). This observation may suggest that this class of drug may not be appropriate to develop as a monotherapy, although the onset of relaxation of rat trachea induced by serelaxin was improved when combined with rosiglitazone compared with either treatment alone (Lam et al., 2016).

Serelaxin has been investigated in a large phase III clinical trial as a potential treatment of acute decompensated heart failure but with disappointing results, likely because of its short half-life (Teneggi et al., 2018). Nonetheless, serelaxin has been shown to increase β-AR agonist–induced ASM relaxation in multiple species (Bourke et al., 2018) using signaling pathways that seem to be independent of cAMP-dependent pathways downstream of β2-AR signaling and due in part to the production of epithelial-derived relaxing factors (Lam et al., 2018).

Relaxin-2 analogs, single chain relaxin (B-chain), and small molecule RXFP1 agonists have been reported, but to date none of these drugs has been evaluated in the clinical setting apart from serelaxin. Recently, a series of potent fatty acid conjugated relaxin-2 analogs that have longer half-lives in vivo have been synthesized by means of a semisynthetic methodology and they are effective as bronchodilators (Muppidi et al., 2019).

g. Soluble Guanylyl Cyclase Activators. Nitric oxide (NO)-donor compounds are another class of drugs that have been shown to relax human airways in vitro, raising the probability that they may be useful as a novel class of bronchodilator (Cazzola et al., 2012b). NO binds to the heme moieties on the α/β heterodimer

sGC, which catalyzes the conversion of guanosine triphosphate to cGMP that subsequently activates cGMP-dependent protein kinases leading to ASM relaxation (Papapetropoulos et al., 2006). However, inflammation has been shown to desensitize sGC toward its natural activator NO (Gladwin, 2006), so it is of interest that recent evidence has shown that drugs targeting the NO-sGC-cGMP pathway that bypass the need for NO, and instead activate sGC directly, cause bronchodilation (Ghosh et al., 2016). Thus, the sGC stimulator BAY 41-2272 ((3-(4-Amino-5-cyclopropylpyrimidin-2-yl)-1-(2-fluorobenzyl)-1H-pyrazolo[3,4-b]pyridine) and activator BAY 60-2770 4-[((4-Carboxy-butyl)-{2-[5-fluoro-2-(4′-trifluoromethyl-biphenyl-4-ylmethoxy)-phenyl]-ethyl}-amino)-methyl]-benzoic acid, 4-[[(4-Carboxybutyl)[2-[5-fluoro-2-[[4′-(trifluoromethyl)[1,1′-biphenyl]-4-yl]methoxy]phenyl]ethyl]amino]methyl]benzoic acid) reduced airway hyperresponsiveness in mice with allergic airway inflammation and were able to restore lung function (Ghosh et al., 2016). Furthermore, activating sGC induced significant bronchodilation in human lung slices similar to the bronchodilating effects observed with isoprenaline or formoterol (Stuehr et al., 2017).

h. Pepducins. Pepducins are cell-penetrating palmitoylated peptides corresponding to amino acid sequences found on the inside surface of certain GPCRs that can allosterically stabilize the active state to induce receptor–G protein signaling (Carr and Benovic, 2016).

β-arrestin-2–induced GPCR desensitization, an effect that manifests itself with the appearance of a steric modification that prevents the interaction between the receptor and G protein and also causes receptor internalization and a decrease in the amount of cAMP generated by G protein signaling due to recruitment of PDEs, is induced when a GPCR kinase phosphorylates the GPCR and increases its affinity for β-arrestin-2 (Matera et al., 2013). Thus, it is plausible that tachyphylaxis may be reduced by using G protein–biased ligands that are able to avoid β-arrestin recruitment while inducing an effective relaxation of ASM (Matera et al., 2013). By accessing effectors from subcellular compartments, internalized receptors can form active ternary complexes that also contribute to cellular signaling. An incessant signaling from internalized compartments contributes to the overall extent of intracellular signaling as well as to the duration of cellular responses and can cause signal distortion by directing receptors toward alternative pathways/compartments or degradation (Rosethorne et al., 2016), so-called biased agonism.

It is now recognized that β_2-ARs can signal not only via activation of G proteins but also via β-arrestins. Therefore, binding of β_2-AR agonists to β_2-AR is pleiotropically coupled to many intracellular pathways, and a subset of different intracellular responses can be triggered depending on the state of the β_2-AR

when activated (Matera et al., 2018a). Biased agonism essentially describes the capacity of ligands to stabilize different conformations of a GPCR linked to distinct intracellular signaling pathways that in turn cause specific functional outcomes in the cell. This concept has led to the search for pathway-specific drugs that may reduce the incidence of on-target side effects (Klein Herenbrink et al., 2016). For example, it has been suggested that differences between epinephrine and salmeterol in the hydrogen-bond network involving residues Ser[204] and Asn[293] are responsible for a dissimilar active-state conformation that causes the partial efficacy of G protein activation and the limited β-arrestin recruitment observed with salmeterol, meaning that this drug is a partial agonist (Masureel et al., 2018).

Pepducins from the third intracellular loop (ICL3) of the β_2-AR mediate G_s-biased signaling (Carr et al., 2014), and those from ICL1 mediate arrestin-biased signaling (Carr et al., 2016). ICL3 pepducins can stabilize a conformation of the β_2-AR that interacts selectively with G_s or, alternatively, directly activate G_s (Panettieri et al., 2018). Because of the capacity of pepducins to bias G_s signaling, there is strong interest in developing small compounds that would have a similar bias. Initial efforts have been focused on screening molecules using the β_2-AR as a target receptor population to find G_s-biased agonists, G_s-biased positive allosteric modulators, and arrestin-biased negative allosteric modulators (Panettieri et al., 2018).

2. Improvements to Existing Classes of Bronchodilators.

a. Selective Phosphodiesterase Inhibitors. PDE3 is the predominant PDE isoenzyme in ASM and its inhibition produces smooth muscle relaxation, whereas the PDE4 isoenzyme is predominant in inflammatory cells involved in the pathogenesis of asthma and COPD (Page and Cazzola, 2014, 2017). Furthermore, PDE3 inhibition leads to the enhancement of relaxation evoked by β_2-AR stimulation (Mokry and Mokra, 2013).

PDE3 inhibitors have also been demonstrated to inhibit allergen-induced early and late airway responses in patients with allergic asthma (Bardin et al., 1998). Moreover, PDE3 inhibitors have been shown to induce bronchodilation in patients with asthma (Matera et al., 2014a), although there is concern about the inhibition of PDE3 to treat pulmonary diseases because PDE3 also participates in the regulation of vascular smooth muscle and cardiac muscle function and PDE3 inhibition thus has the potential to elicit adverse events, particularly in the cardiovascular system. This concern has limited the amount of drug development in this field and to date no PDE3 inhibitor has been approved for the treatment of asthma or COPD, despite systemically active PDE3 inhibitors being approved for other clinical indications (Matera et al., 2014a,b). Nonetheless, studies initiated because of enoximone's known bronchodilatory activity

(Leeman et al., 1987) have reported beneficial and life-saving effects of this PDE3 inhibitor in patients with status asthmaticus (Beute, 2014).

Evidence from preclinical studies suggests that concomitant inhibition of PDE3 and PDE4 suppresses spasmogen-induced contraction of human and bovine ASM in a synergistic manner (reviewed by Abbott-Banner and Page, 2014). This observation has been demonstrated by combining a PDE3 with a PDE4 inhibitor; more recently, these observations have been extended through experiments using a dual inhibitor of PDE3 and PDE4, ensifentrine (formerly called RPL 554), that is able to induce significant relaxation of human bronchi in vitro (see the section on bifunctional bronchodilator/anti-inflammatory drugs for further details) (Calzetta et al., 2013) and provides rapid and sustained bronchodilation in patients with asthma or COPD (Franciosi et al., 2013).

b. Novel β_2-Adrenoceptor Agonists. Despite the failure of some novel β_2 agonists such as carmoterol, milveterol, PF-610355 (N-[(4'-Hydroxy-3-biphenylyl) methyl]-2-[3-(2-{[(2R)-2-hydroxy-2-{4-hydroxy-3-[(methylsulfonyl)amino]phenyl}ethyl]amino}-2-methylpropyl) phenyl]acetamide), and AZD3199 (N-(2-(diethylamino) ethyl)-N-(2-((2-(4-hydroxy-2-oxo-2,3-dihydrobenzo[d] thiazol-7-yl)ethyl)amino)ethyl)-3-(2-(naphthalen-1-yl) ethoxy)propanamide), which suggests that challenges remain in identifying β_2-AR agonists having a better profile than those currently available, there is still interest in identifying novel β_2-AR agonists with a core structure different from the drugs in this class currently in clinical use. However, while it may be possible to find better β_2-AR agonists, the costs involved in their research and development may outweigh any incremental improvement such drugs might have (Van Ly and Oliver, 2015).

Nonetheless, trantinterol [SPFF; 2-(4-amino-3-chloro-5-trifluomethyl-phenyl)-2-*tert*-butylamino-ethanol hydrochloride] bears a novel scaffold of 2-amino-2-phenylethanol instead of the classic scaffold of 2-amino-1-phenylethanol and is an example of a novel β_2-AR agonist under development (Gan et al., 2003). In guinea pigs, (S)-trantinterol was more effective than (R)-trantinterol at causing ASM relaxation, despite the observation that there was no significant difference observed for the receptor selectivity of (S)-trantinterol and (R)-trantinterol (Pan et al., 2014). This finding contrasts with the documentation that other (S)-enantiomers are effectively inert at the β_2-AR (Jacobson et al., 2018). However, it was found that the concentration of (S)-trantinterol was higher than that of (R)-trantinterol, implying the stereoselective disposition of trantinterol enantiomers in humans (Qin et al., 2015). This drug is currently being evaluated in phase III clinical trials in China for the treatment of patients with asthma.

A series of novel β_2-AR agonists based on the previously identified nonclassic 2-amino-2-phenylethanol scaffold have been synthesized and evaluated in β_2-AR–expressing cells (Ge et al., 2018a). 8-Hydroxy-5-(2-hydroxy-1-((4-hydroxyphenethyl)amino)ethyl)quinolin-2(1H)-one (compound 5j) demonstrated the best pharmacological profile among all of the evaluated compounds. Another series of 2-amino-2-phenylethanol derivatives have also been developed (Ge et al., 2018b), and these compounds are effective β_2-AR agonists with high selectivity and with the (S)-configuration again being more active. The binding of these compounds is reminiscent of β_2-AR agonists as predicted by molecular modeling (Ge et al., 2018b).

Furthermore, a series of novel β_2-AR agonists with a 5-(2-amino-1-hydroxyethyl)-8-hydroxyquinolin-2(1H)-one moiety have been developed (Xing et al., 2019). Compounds 9g and (R)-18c produced potent relaxant effects on ASM with a rapid onset and sustained duration of action in guinea pig trachea in vitro. Furthermore, the disclosure of the marine natural product S1319 as a β_2-AR agonist with equivalent activity to formoterol, due to the presence of the 4-hydroxybenzothiazolone as a phenol-containing bicyclic catechol mimetic, has led to the exploration of a 4-hydroxybenzothiazolone series of β_2-AR agonists (Beattie et al., 2010). 7-[(R)-2-((1R,2R)-2-benzyloxycyclopentylamino)-1-hydroxyethyl]-4-hydroxy-benzothiazolone (C26) was originally identified as a high-affinity β_2-AR agonist with a predicted long duration of action and this compound exhibited greater intrinsic activity than endogenous adrenaline in producing increases in cAMP and moreover it recruited β-arrestin-2, and internalized the β_2-AR slower than isoprenaline and adrenaline (Rosethorne et al., 2016). Compound C26 caused β-arrestin-2 recruitment with levels that increased up to 4 hours, when the intrinsic activity was 126% $\pm$ 3.52% of the maximal response to isoprenaline. The effect of isoprenaline and adrenaline on the levels of internalized β_2-ARs began to decline around 15 minutes after agonist washout and became increasingly less efficient until baseline levels were achieved within 2 hours after agonist washout. In contrast, the effects of compound C26 persisted over time; at 4 hours after agonist washout, C26 held approximately 80% of internalized β_2-ARs inside the cell, with a very slow rate of decline of internalized vesicles. Compound C26 also displayed very slow receptor kinetics, with a receptor residence half-life of 32.7 minutes at 37°C, which is the slowest dissociation rate detected for any β_2-AR agonist examined to date. Because of these characteristics, it was proposed that compound C26 acts as a superagonist (Rosethorne et al., 2016). Superagonists at GPCRs may have relevance in therapy, specifically compensating for the reduction in the effectiveness of drugs as a result of desensitization that can sometimes occur after continuous therapy with some agonists (Schrage et al., 2016).

c. Novel Muscarinic Acetylcholine Receptor Antagonists. A series of chiral quaternary ammonium

salts of pyrrolidinol esters designed by structural recombination of the privileged scaffolds of aclidinium bromide and glycopyrronium bromide have been developed as novel M_3 mAChR antagonists (Xiang et al., 2017). Among them, (R)-3-[2-hydroxy-2,2-di(thiophen-2-yl)acetoxy]-1,1-dimethylpyrrolidinium bromide and (S)-3-[2-hydroxy-2,2-di(thiophen-2-yl)acetoxy]-1,1-dimethylpyrrolidinium bromide appeared to be the most active M_3 mAChR antagonists. These novel M_3 mAChR antagonists also possessed longer stability in human plasma (half-life = 9.34, 19.2 minutes), particularly (R)-3-[2-hydroxy-2,2-di(thiophen-2-yl)acetoxy]-1,1-dimethylpyrrolidinium bromide, which potentially could lead to more unwanted anticholinergic effects if the drugs entered the systemic circulation. In addition, structure–activity relationship studies showed that the target compounds with a (3R)-pyrrolidine moiety were undoubtedly more effective than agents with the (3S)-pyrrolidine moiety.

There is a considerable amount of ongoing research investigating the development of mAChR antagonists formulated differently from the currently available LAMAs. In particular, nebulizers are increasingly seen as the best delivery device for patients suffering from chronic airway diseases, particularly if the disease is severe or there are frequent exacerbations, in the presence of physical and/or cognitive limitations (Dhand et al., 2012) and when peak inspiratory flow rates are very compromised (Mahler et al., 2014).

One example is revefenacin (TD-4208; biphenyl-2-ylcarbamic acid 1-(2-{[4-(4-carbamoylpiperidin-1-ylmethyl)benzoyl]methylamino}ethyl)piperidin-4-yl ester), which is a LAMA under development as a nebulized inhalation solution that exhibits selective antagonism with high affinity at human recombinant mAChRs and kinetic functional selectivity for M_3 over M_2 mAChRs. Revefenacin is a potent, slowly reversible antagonist in rat, guinea pig, and human airway tissues expressing native mAChRs (Hegde et al., 2018). Single doses of revefenacin were more active than glycopyrronium, and repeat dosing regimens with this LAMA were superior to glycopyrronium and also tiotropium when the lung selectivity index (i.e., ratio of antisialagogue and bronchoprotective potency) was calculated (Pulido-Rios et al., 2013). In patients with COPD, revefenacin demonstrates a fast onset of action and a bronchodilator effect that lasts over 24 hours after once-a-day administration, with a pharmacokinetic profile that is commensurate with low systemic exposure (Quinn et al., 2018). In patients with COPD with a FEV_1 of <50% predicted and a suboptimal peak inspiratory flow rate of <60 l/min, nebulized revefenacin provided greater improvement in lung function compared with tiotropium bromide delivered by a dry powder inhaler (DPI) (Mahler et al., 2018), which may provide evidence of the type of patient who could benefit from nebulized therapy versus a DPI.

Many other new LAMAs are also under investigation with the hope that they are more effective than currently available M_3 mAChR antagonists, or at least equally effective but with a better safety profile (Mastrodicasa et al., 2017). For example, V0162 (10-[(3R)-1-azabicyclo[2.2.2]oct-3-ylmethyl]-10H-phenothiazine) is the R-isomer of mequitazine, an existing oral racemic H1 receptor antagonist with one of the highest affinities for mAChRs being in the nanomolar range. V0162 exhibits both rapid and slow kinetic components with respect to the interaction with human M_3 mAChRs; this drug exhibits considerable resistance to washout (Heusler et al., 2015). It has been suggested that V0162 has a "salmeterol-like" mechanism of action and a long period of receptor interaction may be relevant for its sustained activity. At least preclinically, this drug reversed bronchoconstriction induced either by ACh or histamine and also prevented ovalbumin-induced bronchoconstriction and lung inflammation (Devillier et al., 2015a).

Several other LAMAs (AZD8683 (R)-1-(2-oxo-2-(pyridin-2-ylamino)ethyl)-3-((1-phenylcycloheptane-1-carbonyl)oxy)quinuclidin-1-ium bromide, AZD9164 (R)-1-(4-fluorophenethyl)-3-(((S)-2-phenyl-2-(piperidin-1-yl)propanoyl)oxy)quinuclidin-1-ium, bencycloquidium, CHF 5407 (3R)-3-[[[(3-fluorophenyl)](3,4,5-trifluorophenyl)methyl]amino]carbonyl]oxy]-1-[2-oxo-2-(2-thienyl)ethyl]-1-azoniabicyclo[2.2.2]octane bromide) have also been described, but to date there are no published results and no recent trials with these drugs are registered on ClinicalTrial.gov.

However, in recent years, as a consequence of substantial technological advances, there has been a renewed interest in broadening the knowledge on M_3 mAChR physiology and structure (Kruse et al., 2014). Starting from the well known M_3 mAChR structure, it is now possible to create drugs capable of modulating the M_3 mAChR in an allosterically selective way, activating or blocking it. Such allosteric agents can influence the potency and efficacy of orthosteric ligands, may possess agonist or inverse agonist activity in their own right, and reduce the risk of adverse events compared with orthosteric muscarinic ligands.

Beginning with the crystal structures of M_2 mAChR and M_3 mAChR, a single amino acid difference in their orthosteric binding pockets using molecular docking and structure-based design has been exploited (Liu et al., 2018). The resulting M_3 mAChR antagonists had up to 100-fold selectivity over M_2 mAChR in terms of affinity and over 1000-fold selectivity in vivo. The crystal structure of a M_3 mAChR-selective antagonist in complex with M_3 mAChRs corresponded closely to the docking-predicted geometry, providing a template for further optimization of novel drugs in this class.

d. Novel Xanthines. Currently, the use of xanthines for the treatment of asthma or COPD is decreasing because xanthines can have numerous side effects or

drug–drug interactions and often require the measurement of drug levels in plasma to prevent "toxicity," all of which serve to limit the use of this drug. Moreover, there is evidence generated by a meta-analysis of seven observational studies that theophylline slightly increases all-cause death in patients with COPD (Horita et al., 2016).

Nonetheless, although theophylline is a weak bronchodilator compared with other inhaled classes of bronchodilator, it can still be effective in the treatment of respiratory diseases, not only because it offers an advantageous oral bioavailability but also because it possesses anti-inflammatory effects of relevance to asthma and COPD (Barnes, 2013). For instance, theophylline reduces neutrophil-associated inflammation in the airways of patients with COPD (Kobayashi et al., 2004) and the numbers of eosinophils in the airways of patients with mild asthma (Sullivan et al., 1994; Lim et al., 2001). Interestingly, these anti-inflammatory effects occur at plasma concentrations much lower than those necessary for its bronchodilator action (Barnes, 2013). Furthermore, xanthines have been reported to affect diaphragm contractility, a pharmacological effect that may explain some of the clinical benefit of this class of drug in patients with COPD.

Therefore, other xanthines have been developed for treating these diseases (Basu et al., 2017). Doxofylline [7-(1,3-dioxolan-2-ylmethyl)-3,7-dihydro-l,3 dimethyl-lH-purine-2,6-dione] is a newer-generation xanthine with both bronchodilating and anti-inflammatory activities (Page, 2010). The presence of the dioxolane group at position 7 as well as a distinct pharmacological profile differentiate doxofylline from theophylline, and doxofylline thus should not be considered as just a modified theophylline (Matera et al., 2017a). In effect, doxofylline does not interact significantly with any type of adenosine receptor or any of the known PDE isoforms, except for PDE2A1, and its pharmacological action is not influenced by any of the known histone deacetylase enzymes (van Mastbergen et al., 2012). Doxofylline can also interact with β_2-ARs, eliciting relaxation of vascular smooth muscle and ASM (Zhang et al., 2016), inhibits lipopolysaccharide-induced inflammatory response in the lung, and regulates leukocyte migration in vivo and in vitro (Riffo-Vasquez et al., 2014). Furthermore, doxofylline elicits a substantial steroid-sparing effect in both an allergic and a nonallergic model of lung inflammation (Riffo-Vasquez et al., 2018).

A fundamental pharmacokinetic difference between doxofylline and theophylline lies in the fact that doxofylline does not interfere with the cytochrome P450 enzymes CYP1A2, CYP2E1, and CYP3A4, which allows it to not interact with other drugs metabolized via these pathways in the liver (Lazzaroni et al., 1990).

In a recent network meta-analysis, doxofylline was shown to be more effective than aminophylline and theophylline in patients with COPD (Cazzola et al.,

2018a). Doxofylline was not more efficacious than aminophylline or theophylline in improving basal FEV_1, but it was more effective than theophylline in improving dyspnea and, above all, significantly safer than both aminophylline and theophylline. In patients with asthma, doxofylline was not significantly different from theophylline in increasing FEV_1, in reducing the occurrence of asthma episodes, or in the use of salbutamol as a rescue medication to relieve acute asthma symptoms (Calzetta et al., 2018a). However, the safety profile of doxofylline was much better than that of theophylline. This would suggest that of the currently available xanthines, doxophylline has the best therapeutic window.

Recent studies have reported the development of inhaled formulations of theophylline for delivery by a propellant metered dose inhaler (Zhu et al., 2015a) and by DPI formulations containing 1.0% (w/w) sodium stearate as a lipophilic adjunct (Zhu et al., 2015b) for possible administration to patients with COPD. It was suggested in these studies that methyl xanthines could potentially be repurposed successfully for delivery by inhalation if given by appropriate aerosol delivery systems (Zhu et al., 2015a,b) to try and improve the therapeutic window compared with administering xanthines systemically. Another approach is to combine theophylline with polyamines such as spermine to exploit the uptake system for polyamines from the systemic circulation into the lung (Benaouda et al., 2018).

e. Fixed-Dose Combination Therapies. The combination of two or more drugs with dissimilar but complementary mechanisms of action, of which at least one is a bronchodilator, has become standard in the treatment of asthma, COPD, and asthma–chronic obstructive pulmonary disease overlap (ACO) syndrome (Cazzola et al., 2012b). Thus, there is now accruing evidence that fixed-dose combination (FDC) therapies in a single inhaler (LABA/LAMA and LABA/ICS) seem to be more active in controlling symptoms than single-agent therapies (LAMA and LABA) (Oba et al., 2018).

Many LABA/LAMA, LABA/ICS, and LAMA/ICS FDCs as well as "triple inhaler" FDCs containing LABA/LAMA/ICS have been approved or are in clinical development as treatments for asthma and COPD. There are currently five LABA/LAMA FDCs licensed for maintenance treatment of COPD. Some are once-a-day FDCs (110/50 μg indacaterol/glycopyrronium; 5/5 μg olodaterol/tiotropium; 25/62.5 μg vilanterol/umeclidinium), whereas others are twice-a-day FDCs (12/400 μg formoterol/aclidinium; 27.5/15.6 μg indacaterol/glycopyrronium approved in the United States; 9.6/18 μg formoterol/glycopyrronium in the United States and 10/14.4 μg in the European Union) (Table 3). With the use of appropriate doses, 12- to 24-hour bronchodilation can be achieved with these FDCs.

There are also several LABA/ICS FDCs (salmeterol/fluticasone propionate, formoterol/budesonide, formoterol/beclomethasone, formoterol/fluticasone propionate,

formoterol/mometasone, vilanterol/fluticasone furoate, indacaterol/mometasone, formoterol/ciclesonide) that are already available or will be marketed within the next few years for the treatment of asthma and/or COPD. No LAMA/ICS FDC has yet been approved, but umeclidinium/fluticasone furoate is under development. The first triple combination to enter the market in India was 12/18/400 μg formoterol/tiotropium/ciclesonide and there are now three other LABA/LAMA/ICS FDCs (formoterol/glycopyrronium/beclomethasone dipropionate, vilanterol/umeclidinium/fluticasone furoate, and formoterol/glycopyrronium/budesonide) available, with others still under development.

i. Bifunctional Bronchodilator Drugs. The use of combinations containing drugs with complementary pharmacological actions in the treatment of patients with asthma or COPD suggested the need to develop single molecules able to elicit two different primary pharmacological actions. These molecules have been called "bifunctional drugs" (Page and Cazzola, 2014, 2017). A number of new drugs that have both mAChR antagonism and β_2-AR agonist activity in the same molecule and are referred to as MABAs have now reached clinical development or are at a preclinical stage.

In a phase IIb trial, the MABA, batefenterol (GSK961081), was numerically better than salmeterol in improving trough FEV_1, the primary endpoint, after 4-week treatment in patients with moderate to severe COPD (Wielders et al., 2013). Another MABA, AZD8871 (LAS191351; 2-Thiopheneacetic acid, alpha-hydroxy-alpha-2-thienyl-, trans-4-((3-(5-((((2R)-2-(1,2-dihydro-8-hydroxy-2-oxo-5-quinolinyl)-2-hydroxyethyl)amino)methyl)-1H-benzotriazol-1-yl)propyl)methylamino)cyclohexyl ester), has been shown to be safe, well tolerated, and able to elicit sustained bronchodilation at doses of 200–2100 μg in patients with mild asthma (Jimenez et al., 2017); at a 400 or 1800 μg dose, this drug delivered significant and sustained bronchodilation, superior to placebo, and at the highest dose was superior to 150 μg indacaterol or 18 μg tiotropium, with no obvious safety concerns (Singh et al., 2017). LAS190792 (((trans)-4-((2-(((2-chloro-4-((((R)-2-hydroxy-2-(8-hydroxy-2-oxo-1,2-dihydroquinolin-5-yl)ethyl)amino)methyl)-5-methoxyphenyl)carbamoyl)oxy)ethyl)(methyl)amino)cyclohexyl 2-hydroxy-2,2-di(thiophen-2-yl)acetate), another MABA, is able to induce relaxation of electrically stimulated airway tissue with a similar efficacy to batefenterol, with muscarinic receptor antagonism in the presence of propranolol being slightly higher than batefenterol, and with a longer duration of action (Aparici et al., 2017). In dogs, CHF6366 ((S)-3,5-dichloro-4-(2-(3-(cyclopropylmethoxy)-4-(difluoromethoxy)phenyl)-2-(3-(cyclopropylmethoxy)-4-(methylsulfonamido)benzoyloxy)ethyl)pyridine 1-oxide), but not batefenterol or formoterol, provided full, 24-hour bronchoprotection without systemic side effects (Carnini et al., 2017).

MABAs have some advantages compared with combination therapy, mainly the possibility of delivering a fixed ratio of mAChR antagonism and β_2-AR agonist activity at the cellular level. However, the development of MABAs is also associated with some major challenges in optimizing the LABA and LAMA activities in the molecule because there are often different pharmacodynamic half-lives for the mAChR antagonism and β_2-AR agonist activity in these drugs, with most MABAs having a predominance of either LAMA or LABA activity and the ratio of the two different pharmacological activities cannot be adjusted as needed, which obviously limits dosing flexibility (Cazzola et al., 2013a).

Nonetheless, MABA compounds are believed to be a better opportunity than LABA/LAMA combinations to be coformulated in "triple therapy" combinations that could elicit three complementary therapeutic actions in patients with asthma or COPD (Page and Cazzola, 2014, 2017). In fact, MABAs combined with an ICS are already in development and administration of a single high dose of batefenterol/fluticasone furoate resulted in a reduction of the fluticasone furoate exposure compared with administering fluticasone furoate alone (Ambery et al., 2019).

ii. Bifunctional Bronchodilator/Anti-Inflammatory Drugs. Since PDE4 inhibitors are mainly anti-inflammatory agents and PDE3 inhibitors are bronchodilators, molecules able to induce dual inhibitory activity for both PDE3 and PDE4 enzymes have been developed to obtain simultaneously both bronchodilator and anti-inflammatory activity (Page and Cazzola, 2014, 2017). Ensifentrine, previously known as RPL554, is an inhaled PDE3/4 inhibitor that has been shown to induce bronchodilation with peak bronchodilator effect in patients with asthma or COPD comparable to that seen after administration of an inhaled β_2-AR agonist, and also bronchoprotective and anti-inflammatory activities at the same dose, with negligible adverse events (Franciosi et al., 2013). Ensifentrine combined with standard bronchodilators (salbutamol, ipratropium, or tiotropium) was recently shown to cause additional bronchodilation and, importantly, a significant reduction of hyperinflation in patients with stable moderate-to-severe COPD (Singh et al., 2018). Unfortunately, studies demonstrating an anti-inflammatory effect in patients with COPD are still lacking (Cazzola and Page, 2018; Cazzola et al., 2018c), although this drug has been demonstrated to reduce lipopolysaccharide-induced neutrophilia in the airways of healthy subjects (Franciosi et al., 2013) at doses that induce bronchodilation.

LASSBio596 (2-[4-(1,4-tiazinan-4-ylsulfonyl) phenylcarbamoyl] benzoic acid), which originates from thalidomide and an aryl sulphonamide, is a molecule that powerfully blocks both PDE4 and PDE5 (Rocco et al., 2003). In preclinical studies that used a murine model of elastase-induced emphysema, this drug decreased lung inflammation and remodeling with consequent

improvement of lung mechanics (Guimaraes et al., 2014).

The combination of the bronchodilator effects of NO with the anti-inflammatory actions of an ICS is another approach to induce anti-inflammatory and bronchodilator actions with a single molecule. NO-budesonide (TPI 1020) was the first agent with these characteristics. In guinea pigs, it potentiated the bronchoprotection induced by formoterol or tiotropium (Turner et al., 2012), but the development of this drug has been discontinued because of limited clinical efficacy. Furthermore, NO was combined with salbutamol into a single molecule (NCX 950, (α'-[[(1,1-dimethylethy)amino]methyl]-4-hydroxy-1,3-benzenedimethanol nitrate) to simultaneously induce bronchodilation and anti-inflammatory activity (Lagente et al., 2004), but again this drug showed limited efficacy in comparison with salbutamol alone.

Another approach has covalently linked a PDE4 inhibitor to a LABA in an attempt to develop a drug with both anti-inflammatory and bronchodilator activity in a single molecule. Since both PDE4 inhibitors and LABAs increase intracellular levels of cAMP in ASM, such an approach is attractive because it is anticipated that this type of drug could induce additive or synergistic bronchodilator and anti-inflammatory activity in the lung (Matera et al., 2014b), especially in target tissues in which β_2-AR numbers are a limiting factor (Giembycz and Newton, 2015). An example of this approach is GS-5759 ((R)-6-[(3-{[4-(5-{[2-hydroxy-2-(8-hydroxy-2-oxo-1,2-dihydroquinolin-5-yl)ethyl]amino}pent-1-yn-1-yl) phenyl] carbamoyl}phenyl)sulphonyl]-4-[(3-methoxyphenyl)amino]-8-methylquinoline-3-carboxamide trifluoroacetic acid), a novel, bifunctional ligand, in which the quinolinone-containing pharmacophore that causes β_2-AR agonism in several LABAs has been conjugated to a PDE4 inhibitor (Joshi et al., 2017). These two ligands can interact on target tissues in a synergistic manner (Joshi et al., 2017) and the inhibition of PDE4 with this drug was shown to be similar to that induced by roflumilast, although the β_2-AR agonism is not substantially different from that elicited by indacaterol (Tannheimer et al., 2014).

The possibility of combining PDE4 inhibition with mAChR antagonism in a single molecule has also been investigated. UCB-101333-3 (6-(azepan-1-yl)-N,2-dicyclopropyl-5-methylpyrimidin-4-amine) was the first such compound described (Provins et al., 2007). However, other compounds that use a pyrazolopyridine pharmacophore as the PDE4 inhibitor and a biaryl-containing pharmacore to achieve mAChR antagonism have also been described (Phillips and Salmon, 2012).

RO 50-24118 is a stable synthetic peptide analog of vasoactive intestinal peptide that is highly selective for the vasoactive intestinal peptide/pituitary AC-activating polypeptide type 2 receptor and is able to elicit both bronchodilation and anti-inflammatory activity. Thus, RO 50-24118 relaxes ASM cells, prevents bronchoconstriction, and reduces the influx of neutrophils and CD8$^+$ T cells, at least in a preclinical model of inflammatory lung disease (Tannu et al., 2010).

III. What Is the Optimal Use of Bronchodilators?

Bronchodilators are fundamental in the management of patients with airway obstruction, but there are still questions about their optimal use that need to be clarified.

A. Is It Appropriate to Treat All Patients Suffering from Airway Obstruction with Long-Acting Bronchodilators?

There is still no convergence of opinion on the use of long-acting bronchodilators in all patients with airway obstruction. This is a critical issue because many patients suffering from airway obstruction do not complain about the classic disturbing symptoms such as cough, sputum, wheezing, and dyspnea. Although the use of bronchodilators is considered a key element in the treatment of COPD, scientific support for their use in asymptomatic patients with COPD is still very poor (Qaseem et al., 2011). However, in patients who remain asymptomatic in early-stage COPD, there is often a rapid decline in lung function with a clear association between FEV_1 decline and the appearance of functional small airway disease, even before the development of spirometrically detected airflow obstruction (Bhatt et al., 2016). There is documentation that a treatment with long-acting bronchodilators in early-stage disease could slow the disease progression by influencing both the decline in lung function and health status (Welte et al., 2015). Unquestionably, as far as possible, patients should not be allowed to become symptomatic, because once symptomatic, they have a faster decline in lung function and a lower health-related quality of life (HRQL) with increased health utilization compared with asymptomatic individuals (Decramer et al., 2008).

However, this opinion contrasts with the U.S. Preventive Services Task Force recommendation against screening for COPD in asymptomatic adults because there is a lack of evidence showing the benefit of early detection and treatment (Siu et al., 2016). Nevertheless, in a large cohort of patients who had Global Initiative for Chronic Obstructive Lung Disease (GOLD) strategy stage 1 or stage 2 COPD, tiotropium bromide resulted in a higher FEV_1 than placebo at 24 months and decelerated the annual decline in the postbronchodilator FEV_1 in patients with a COPD assessment test (CAT) score of <10 (milder disease), but not in those with a CAT score of ≥10 (more severe disease) (Zhou et al., 2017). Furthermore, tiotropium bromide was more effective than placebo in improving other parameters of lung function and quality of life and reducing the rate of acute COPD exacerbations.

These findings suggest that, due to the current inability to identify patients who are fast decliners, it would seem appropriate to treat all patients with COPD with long-acting bronchodilators, including those at the early stages of the disease (Cazzola and Page, 2014). However, it has not yet been determined whether early intervention with a long-acting bronchodilator modifies the long-term course of COPD. Therefore, much larger and longer studies are needed to address this challenge (Cazzola and Rogliani, 2018).

In contrast, it is not appropriate to treat all asthmatic patients with long-acting bronchodilators. In patients with asthma, ICSs are central to fully control symptoms for many patients, but LABAs should be considered as an add-on long-term controller medication when this pharmacological approach does not control asthma when administered at a medium dose (Ducharme et al., 2010). However, LABAs must not be used as monotherapy because patients are at risk of exacerbating (Cazzola and Matera, 2007), although combination inhalers containing a LABA/ICS have been shown to be superior to ICS alone, with no such safety concerns (Bateman et al., 2014). Given that fast-acting inhaled β_2-AR agonists are the most active bronchodilators for rapid relief of asthma symptoms and therefore form a central component of asthma management, a SMART strategy that contains formoterol, which produces a very fast onset of action that is similar to that of short-acting β_2-adrenoceptor agonists (SABAs) and also induces the long-acting bronchodilator benefits of LABAs, combined with ICSs in a single inhaler is fundamental because it can control symptoms during acute exacerbations of asthma and prevent further exacerbations (Sobieraj et al., 2018b). In addition, there is also evidence that LAMAs added to ICSs lower the risk of asthma exacerbations compared with placebo (Cazzola et al., 2017d).

B. Is It Better to Start with a β_2-Adrenergic Receptor Agonist or with a Muscarinic Acetylcholine Receptor Antagonist?

There is currently not much evidence that can help us differentiate the effectiveness of LABAs from that of LAMAs, or that of different agents within each class of long-acting bronchodilator used as maintenance therapy in patients suffering from COPD.

The choice of bronchodilator in a patient with COPD depends mainly on the outcome of interest (Cazzola and Page, 2014). A recent meta-analysis showed that patients treated with a LAMA were at a lower risk of suffering from acute exacerbations and reporting adverse events than those treated with a LABA. However, in terms of lung function (trough FEV_1), symptom score (transition dyspnea index), and health status (Saint George's Respiratory Questionnaire score), there were no significant differences between treatment with a LAMA or a LABA (Chen et al., 2017).

Conversely, LAMAs seem more effective than LABAs in the treatment of exacerbations, and this is regardless of whether LABAs are dosed once- or twice-a-day. LAMAs are able to significantly reduce the number of hospitalizations due to COPD exacerbations, and they also decrease the risk of severe adverse effects, but do not alter mortality (Maia et al., 2017). Therefore, if a single bronchodilator must be chosen for treating a patient with COPD, a LAMA seems to be a better option than a LABA, mainly for patients who are frequent exacerbators.

In patients with asthma, there is a weak signal in favor of LAMAs compared with LABAa as add-on therapy when measures of lung function are the goal of treatment, whereas LABAs are significantly better if quality of life is the goal, but the differences are all small (Kew et al., 2015). Given the much larger evidence base for LABAs versus placebo for people whose asthma is not well controlled on ICSs, the current evidence is not strong enough to say that a LAMA can be substituted for a LABA as add-on therapy. However, the choice of LAMAs makes most sense when asthma has a psychogenic origin or is characterized by chronic bronchial obstruction, when patients suffer from bronchospasm triggered by β-AR blockers, or in the presence of arginine/glycine variation in codon 16 of the *ADRB2* gene, which predisposes to a reduced response to β-AR agonists (Cazzola et al., 2017d).

C. Is It Useful to Use a Bronchodilator with a Rapid Onset of Action?

Generally, a rapid bronchodilation is accompanied by an almost immediate relief of dyspnea; in fact, salbutamol is used as a rescue medication in this setting (Cazzola et al., 2015a). There is evidence showing that fast-acting LABAs and LAMAs may also have an additional role in the control of symptoms that many patients experience during the morning and/or nighttime and that impact daily activities and HRQL, thus improving HRQL (Lopez-Campos et al., 2013). Such observations suggest that in the symptomatic patient, formoterol, indacaterol, and olodaterol are preferred over salmeterol and vilanterol, and glycopyrronium or aclidinium to tiotropium or umeclidinium.

As discussed above, acute bronchodilation correlates significantly with symptom improvement in patients with COPD, a correlation that is strongest in patients with baseline hyperinflation (Di Marco et al., 2006), although some evidence suggests that the ability to reduce dyspnea at rest may be better associated with reduction in airway resistance rather than lung deflation (Santus et al., 2014). However, fast onset does not seem to be particularly useful in patients with relatively stable symptoms who receive regular treatment with bronchodilators (Cazzola et al., 2015a). Conversely, fast onset of bronchodilation seems to be helpful when adherence to treatment is suboptimal,

since compliance increases when the patient perceives rapid improvement of symptoms. Fast onset could also be convenient in patients with a large variability of symptoms.

Two recent studies have explored the advantage of the fast onset of action of the LABA formoterol combined with the ICS budesonide in patients with mild asthma (Bateman et al., 2018; O'Byrne et al., 2018). Both studies showed that formoterol/budesonide used as needed was not inferior to using budesonide alone in reducing exacerbations, while allowing the metered daily dose of ICS to be reduced. Furthermore, formoterol/budesonide was better than terbutaline, a SABA, used on demand to both control asthma symptoms and decrease the risk of asthma exacerbations (O'Byrne et al., 2018); maintenance therapy with budesonide plus as-needed SABA was marginally better for symptom control, with differences in lung function and patient-reported outcomes such as Asthma Control Questionnaire-5 score well below the clinically relevant and minimal clinically important differences (Bateman et al., 2018).

D. Is Once-a-Day or Twice-a-Day Dosing Preferable?

When once-a-day LAMAs or LABAs have been compared with twice-a-day agents (LABAs) in clinical studies, it was observed that the former induced better results in terms of standard clinical results (lung function, symptoms, and state of health) (Cazzola et al., 2015a). Once-a-day dosing is relatively simple and convenient compared with multiple daily dosing and, consequently, may increase patient adherence when such drugs are required chronically (Bjerg et al., 2012). Thus, it has been demonstrated that persistence of using inhaled medications in patients with COPD is directly associated with the daily frequency of administration (Cramer et al., 2007). However, it has also been suggested that because of the diurnal nature of lung function, night-time symptoms might be better controlled with twice-a-day dosing ensuring bronchodilation through the night (Celli, 2018).

In patients with COPD, a high self-perceived need for controller medication is associated with once-a-day preference (Price et al., 2013). In patients with asthma, good control and low self-perceived controller medication need were also associated with once-a-day preference. However, in patients with asthma, the observed bronchodilator efficacy over 24 hours was similar when using 5 μg tiotropium once-a-day or 2.5 μg twice-a-day as an add-on to ICS therapy (Beeh et al., 2016).

E. When Should a Second Bronchodilator with a Different Mechanism of Action Be Added?

Meta-analyses of LABA/LAMA FDC studies in patients with COPD have documented that LABA/LAMA FDC therapy gave superior improvements overall in lung function (trough FEV_1), symptoms (transition

dyspnea index score), and quality of life (Saint George's Respiratory Questionnaire score) compared with the constituent monocomponents (Calzetta et al., 2016). This evident superiority of LABA/LAMA FDCs appeared in the first 3 months of treatment and then decreased, although modestly, after 6 and 12 months of treatment (Calzetta et al., 2017c). These studies suggest that improvements in FEV_1 induced by LABA/LAMA FDCs emerge in the first 3 months of treatment and then remain stable. Furthermore, LABA/LAMA combinations constantly meet the assumed clinically important differences for both endurance time and inspiratory capacity and, in this respect, are superior to their monocomponents (Calzetta et al., 2017b). Notably, the evaluation of the safety profile of different LABA/LAMA FDCs in the pivotal studies involving patients with COPD showed that dual bronchodilation is a safe therapeutic approach and, above all, it does not increase the risk of the adverse events that characterize LAMAs and LABAs when used as monotherapy (Matera et al., 2016).

It has been suggested that it is sensible to start treatment of patients with COPD with dual bronchodilation soon after a diagnosis of COPD is made to optimize bronchodilation by using the maximal doses currently approved (Cazzola and Matera, 2018a). However, given the well described pharmacological synergistic interaction between LAMAs and LABAs, at least in modulating bronchial tone and improving lung function (Cazzola et al., 2014, 2015b,c, 2016a), the possibility that lowering the doses of the single drugs in any inhaled LABA/LAMA FDC should reduce the risk of unwanted systemic exposure and consequently decrease the risk of cardiovascular adverse events while preserving an adequate level of clinical effectiveness should be explored. It was recently shown that 27.55/15.6 μg indacaterol/glycopyrronium twice-a-day was better than 110/50 μg indacaterol/glycopyrronium once-a-day when efficacy and safety profiles were combined using the improved bidimensional surface under the cumulative ranking curve score (Rogliani et al., 2019).

Although the clinical benefits of LABA/LAMA FDCs are now extensively established, very few head-to-head studies between all of the available LABA/LAMA FDCs have been carried out, making choosing the most appropriate FDC difficult (Rogliani et al., 2018a). There is therefore a need for such evidence to clarify which FDC is best from both an efficacy and safety standpoint (Rogliani et al., 2018b).

In asthma, a second bronchodilator with a different mechanism of action, usually a LAMA, is added for patients with poorly controlled disease despite receiving gold standard regular treatment with a LABA/ICS combination (Kerstjens et al., 2012) and regardless of age, sex, cigarette use, ACO, initial FEV_1, serum eosinophil count, and body mass index (Kerstjens et al., 2016). However, adding a LAMA must not be

considered the first choice in symptomatic asthmatics with serum total IgE levels > 430 μg/l (Cheng et al., 2018).

F. When Must an Inhaled Corticosteroid Be Added?

1. Combination Therapy of β_2-Adrenergic Receptor Agonists and Inhaled Corticosteroids. Most LABAs used in clinical practice are now dispensed as an FDC with an ICS (LABA/ICS), including those prescribed as initial treatment, because there is compelling evidence that LABA/ICS combinations are superior compared with ICSs and LABAs administered as monotherapy or versus placebo in reducing the risk of exacerbations in patients with stable moderate-to-very severe COPD (Criner et al., 2015). However, in an observational study conducted in routine daily practice, starting to treat COPD with LABA/ICS inhalers appeared more effective than LAMAs only in patients who had high concentrations ($>4\%$) or counts (>300 cells/μl) of eosinophils in the blood and possibly in frequent exacerbators (Suissa et al., 2018).

However, LABA/ICS combinations can increase the risk of pneumonia in patients with COPD compared with LABA/LAMA combinations or LAMA and LABA monotherapies (Oba et al., 2018). Salmeterol/fluticasone propionate use tends to show a higher risk of pneumonia than does use of formoterol/budesonide (Janson et al., 2013). It has been suggested that when an ICS has a longer retention in the airways/lung because of its very low water solubility and relative lipophilicity, there is the risk of a greater local immunosuppression that may explain the intraclass difference in risk of pneumonia. In fact, the immunosuppressive effect can make patients more susceptible to respiratory infectious risk and potentially increases the risk of pneumonia mainly in the presence of impaired mucociliary clearance and altered lung microbiome, which are common in COPD (Janson et al., 2017). However, no difference in COPD-related exacerbations or pneumonia events was observed in real-world practice between salmeterol/fluticasone propionate and formoterol/budesonide when patients were new to LABA/ICS treatment (Kern et al., 2015).

Nevertheless, patients with COPD with elevated circulating eosinophils have more exacerbations and ICSs are beneficial for decreasing the occurrence of exacerbations in patients with high eosinophil counts; in addition, the clinical response to ICSs is enhanced with increasing eosinophil counts (Bafadhel et al., 2017). Therefore, it has been suggested that adding an ICS to a LABA should be considered only in patients with a history of asthma when blood eosinophil counts are <100 eosinophils/μl, and always in patients who still experience exacerbations of COPD despite appropriate bronchodilator treatment when blood eosinophil counts are >300 eosinophils/μl (Agusti et al., 2018). In patients with 100–300 eosinophils/μl, the choice of

adding an ICS is individual for each patient, considering the potential benefits and risks of this approach.

In patients with asthma, adding a LABA must be always considered when asthma is not adequately controlled with a low-to-moderate dose of ICS alone (https://ginasthma.org). However, by using a quasi-cohort design, a recent study concluded that subjects with asthma were also at greater risk for pneumonia if they used ICS therapy (Qian et al., 2017). The relative risk was dose related, and the risk was higher for budesonide and fluticasone compared with other agents. For this reason, in mild asthma, patient-adjusted symptom-driven treatment with low-dose ICS/fast-acting LABA on an intermittent or regular basis should be considered (Cho and Oh, 2019).

2. Combining Muscarinic Acetylcholine Receptor Antagonists and Inhaled Corticosteroids. There are only a few studies specifically designed to evaluate the effect of LAMA/ICS combinations in patients with COPD or asthma, and this is an area that deserves further investigation (Matera and Cazzola, 2017). There are some data that suggest there is no advantage in combining a LAMA with an ICS in patients with COPD (Cazzola et al., 2016b). Furthermore, a small clinical study has shown that adding ciclesonide to regular glycopyrronium does not influence lung volumes or exercise tolerance in patients with severe COPD (Rogliani et al., 2018c).

Nevertheless, in patients with COPD, the combination of a LAMA with an ICS elicits better spirometric responses than those induced by a LABA/ICS combination and, moreover, it is also better than LABA/ICS in affecting clinically important endpoints such as dyspnea, health status, and frequency of exacerbations (Hodder et al., 2007). Furthermore, there is documentation that among patients who in real life take only long-acting bronchodilators, alone or in combination, LAMA/ICS, but not LABA/ICS, is able to reduce all-cause mortality (Manoharan et al., 2014). These findings, and also the indirect evidence that adding an ICS to tiotropium can reduce the risk of COPD exacerbations (Calverley et al., 2018), suggest that a LAMA/ICS combination is an effective therapeutic option for patients with COPD and a history of exacerbations (Rogliani et al., 2018d).

In asthma, adding tiotropium to background treatment with an ICS induces advantageous effects that are not substantially different from those obtained with adding a LABA in terms of lung function measurements, exacerbations, asthma control, and other clinically relevant endpoints (Buhl et al., 2018). These benefits can be seen not only in patients with severe asthma, but also in patients with less severe disease and in those who experience adverse events from LABA treatment or in those where LABAs are ineffective. However, evidence that LAMAs are more effective than LABAs on trough FEV_1 in adults whose asthma is not

well controlled on ICS alone is of moderate quality, whereas there is some high-quality evidence suggesting that LABAs are a little better at improving quality of life, although the differences in the Asthma Quality of Life Questionnaire are small (Kew et al., 2015).

Certainly, the possibility of adding a LAMA instead of the classic controllers to low or moderate doses of ICSs in patients with moderate symptomatic asthma is supported by increasing evidence (Chung, 2015), mainly in patients suffering from fixed airflow obstruction (Lee et al., 2015). Tiotropium bromide as a once-a-day add-on to a low- to medium-dose ICS may also be efficacious and well tolerated in adults with moderate uncontrolled asthma (Wang et al., 2019). However, the results of a recent systematic review and meta-analysis confirmed the usefulness of combining a LAMA with an ICS as maintenance therapy in patients aged ≥12 years with persistent uncontrolled asthma (Sobieraj et al., 2018a). In these patients, LAMA/ICS combination reduced exacerbation risk and improved spirometric values compared with patients treated with ICS alone. However, there is no evidence that adding a LAMA to an ICS in this population is clinically preferable to adding a LABA.

3. Combining Dual Bronchodilation and Inhaled Corticosteroids. The key question when treating a patient with COPD is no longer whether and/or when it is appropriate to switch the patient from a LABA/ICS regimen to a LABA/LAMA one, but rather what is the type of patient for which we can add an ICS to dual bronchodilation and, above all, when (Cazzola et al., 2018e)?

In general, adding an ICS to a LABA/LAMA combination provides some clinical advantage in patients with COPD, but the presence of a high blood eosinophil count greatly affects the clinical response (Cazzola et al., 2018d). The protective effect of triple therapy compared with dual bronchodilation, relative to the risk of moderate or severe acute exacerbation of COPD, seems to be superior in patients with higher blood eosinophil counts. When blood eosinophil counts are ≥300 cells/μl, the number needed to treat (NNT) for LABA/LAMA/ICS therapy versus LABA/LAMA therapy, to prevent one exacerbation of COPD in 1 year, is approximately nine patients, whereas when blood eosinophil counts are <300 cells/μl, the NNT is approximately 46 (Cazzola et al., 2018d).

However, although dual bronchodilation therapy with a LABA plus a LAMA is the basis of the therapy of stable COPD (https://goldcopd.org), a LABA/ICS FDC is still among the most prescribed treatments for patients with COPD. This suggests that triple therapy is usually approached by adding a LAMA to the LABA/ICS FDC, which is of interest because there is some evidence that adding a LAMA to a LABA/ICS combination elicited relevant clinical benefit in patients with COPD regardless of the blood eosinophil count (Calzetta

et al., 2019), with a NNT of approximately four patients treated to have a patient with a ≥100-ml increase from baseline in trough FEV$_1$, and of approximately 23 patients to be treated for 1 year to prevent one moderate or severe exacerbation of COPD when triple therapy was compared with the LABA/ICS combination.

In patients with asthma, there is high-quality evidence demonstrating a benefit to lung function with the addition of tiotropium bromide (vs. placebo) to LABA/ICS (Kew and Dahri, 2016). However, the studies reporting scores from the Asthma Quality of Life Questionnaire did not show any significant and clinically relevant benefit of tiotropium bromide over LABA/ICS alone, but the effect estimate favored adding tiotropium bromide and, furthermore, the addition of tiotropium bromide (studied over 48–52 weeks) led to fewer exacerbations. Nevertheless, it was impossible to rule out a lack of difference (Kew and Dahri, 2016).

IV. Therapeutics of Using Bronchodilators

A. The Role of Bronchodilators in the Management of Asthma

The current view is to never begin asthma treatment using a bronchodilator in the absence of an ICS. Patients should not be given a SABA alone, except for the treatment of infrequent short-lived wheeze (White et al., 2018). According to the Global Initiative for Asthma (GINA) recommendations published in 2019 (https://ginasthma.org), SABA-only treatment should be reserved for patients with "occasional daytime symptoms (e.g., less than twice-a-month) of short duration (a few hours), with no night waking and normal lung function," as asthma death can occur in patients who are treated only with a SABA (Selroos, 2008). The use of more than three SABA inhalers a year seems to be associated with the risk of hospital admission (Hull et al., 2016), and morbidity and mortality increase progressively with the increased use of SABAs administered per year (Suissa et al., 1994). An alternative strategy for these patients would be the use of an inhaled combination of a SABA plus an ICS or a LABA plus an ICS to be taken when symptoms are present, used as a reliever rather than for regular maintenance use (Beasley et al., 2014). However, this strategy only works if the β_2-AR agonist component has a fast onset of action for symptom relief (Papi et al., 2007) and some evidence suggests that this approach seems to have advantages over regular ICS therapy (Beasley et al., 2014).

As discussed above, a LABA should always be combined with an ICS, either separately or in an FDC, and is considered first-line maintenance treatment of patients whose symptoms are poorly controlled despite a low-to-moderate dose of ICS alone (https://ginasthma.org). In addition, the already-mentioned option of prescribing a formoterol/ICS FDC for both maintenance and reliever therapy, rather than using

a fast-acting β_2-AR agonist as monotherapy for rapid symptom relief (rescue use), so-called SMART therapy, is recommended in the GINA strategy (https://ginasthma.org) as per the license for this product. However, few physicians currently use this approved strategy, instead prescribing additional SABAs or other bronchodilators on top of maintenance doses of ICS (Chapman et al., 2017).

There have been ongoing safety concerns with medicines containing LABAs, causing the U.S. Food and Drug Administration to call for large postmarketing surveillance studies with these medicines and to introduce black box warnings for these drugs (Chowdhury and Dal Pan, 2010). However, in December 2017, the U.S. Food and Drug Administration eliminated the black box warning on LABA/ICS combinations because of the results of recently completed large safety trials that manufacturers have conducted at the request of the agency (Seymour et al., 2018). In fact, the combined analysis of four large, harmonized, postmarketing safety trials did not find a greater risk of the most severe asthma-related outcomes (endotracheal intubation or death) among patients who received LABA/ICS combination therapy than among those receiving an ICS alone (Busse et al., 2018).

Short-acting muscarinic acetylcholine receptor antagonists (SAMAs) do not seem to be very efficient in controlling asthma, although there is consistent evidence from clinical trials that combinations of inhaled therapy with SAMAs and SABAs could play a role in asthmatic patients who develop fixed airway obstruction despite receiving regular treatment and also in the presence of severe asthma exacerbations, because these bronchodilators when combined have the potential to lead to greater and more rapid improvement in lung function and can reduce the need for prolonged emergency room treatments and hospitalization (Cazzola et al., 2017d). This clinical evidence has been incorporated into many clinical guidelines for asthma management (https://www.sign.ac.uk/assets/sign158.pdf; https://ginasthma.org). However, a population-based nested case-control study documented that the use of ipratropium bromide was associated with an increased risk of arrhythmias in asthmatic patients aged 12–24 years (Adimadhyam et al., 2014).

According to the GINA recommendations, tiotropium bromide should be added at steps 4 and 5 in patients aged ≥12 years who still exacerbate despite treatment with ICS and LABAs, before stepping up to biologics (https://ginasthma.org)). Similarly, German (Buhl et al., 2017), Spanish (Plaza Moral et al., 2016), and British asthma guidelines (https://www.sign.ac.uk/assets/sign158.pdf) recommend using tiotropium bromide as add-on therapy in patients with severe asthma when high-dose LABA/ICS therapies are unable to control asthma. However, this recommendation is for adults only. The use of tiotropium bromide in

place of a LABA as an addition to an ICS should be considered in patients at GINA step 4 who are susceptible to the adverse effects of LABAs (e.g., palpitations, tremor) (https://ginasthma.org; Oppenheimer and Borish, 2018). Furthermore, it seems correct to treat with triple therapy asthmatic patients who have smoked and remain symptomatic or suffer from frequent exacerbations despite initial inhaler therapy with an ICS/LABA (Cazzola et al., 2019).

Other LAMAs may also be advantageous in the treatment of patients with severe asthma, but robust proof is still lacking (Cazzola et al., 2017d).

For patients who are well controlled on triple therapy, the step-down strategy should begin with discontinuation of the LAMA and maintenance of the LABA/ICS therapy (Chipps et al., 2019). For patients well controlled on LAMA/ICS therapy, the recommended first step is to discontinue the LAMA. However, as there is still limited availability of data, it may also be necessary to reduce the dose of ICS before discontinuing tiotropium bromide, particularly if the patient is sensitive to the adverse effects of ICS.

Current international guidelines do not recommend the use of theophylline in the treatment of asthma because an ICS is much more effective when combined with a LABA than with sustained-release theophylline (Adachi et al., 2008). Nevertheless, theophylline can induce additional bronchodilation even when maximally effective doses of a β_2-AR agonist have been administered (Bonini and Usmani, 2016). Thus, the two can be usefully combined in specific circumstances. Furthermore, theophylline can help in the treatment of nocturnal asthma because slow-release preparations ensure the maintenance of therapeutic concentrations throughout the night, although they are less effective than a LABA. A number of studies have demonstrated that theophylline elicits corticosteroid-sparing effects (Markham and Faulds, 1998; Spina and Page, 2017).

B. The Role of Bronchodilators in the Management of Chronic Obstructive Pulmonary Disease

According to the GOLD 2020 strategy document (https://goldcopd.org), a trial of short-acting bronchodilator treatment of intermittent symptoms and a long-acting bronchodilator for low-grade persistent symptoms is recommended, with provision for stopping or switching medications on the basis of response in patients with lower symptom burden who are not frequent exacerbators. For patients with a high symptom burden but not frequent or severe exacerbations, long-acting bronchodilator monotherapy is recommended with escalation to dual bronchodilator therapy for persistent symptoms. However, for patients with severe dyspnea, initial therapy with two bronchodilators is also a possibility to be considered. Treatment can be stepped down to a single bronchodilator if the addition of a second bronchodilator does not improve symptoms.

The choice of LABA/LAMA should be individualized for each patient's preferences and degree of disease burden, as there are currently no recommendations that prioritize any specific LABA/LAMA therapy over another and no LABA/LAMA therapy is approved for specific subgroups of patients with COPD (Anzueto and Miravitlles, 2018). According to the 2017 Spanish COPD guidelines (Miravitlles et al., 2017), in patients with a low risk, those with mild or moderate airflow obstruction and low-grade dyspnea in the absence of therapy, and patients who are of the nonexacerbator phenotype, a LAMA should be the initial treatment choice. In symptomatic patients or in patients with any limitation to perform exercise despite LAMA monotherapy, therapy with LABA/LAMA should be tried because it offers a further functional advantage compared with the LAMA, reducing the need for rescue medications and improving symptoms and quality of life.

For frequent exacerbators with lower symptom burden, recommendations are for use of a LAMA as preferred monotherapy (https://goldcopd.org). For escalation of treatment, the use of two bronchodilators is recommended for those patients with persistent dyspnea or exercise limitation on LAMA monotherapy. LABA/LAMA or LABA/ICS are recommended for those patients with recurrent exacerbations on LAMA monotherapy. LABA/ICS may be preferred for patients with a history or findings suggestive of asthma. For patients with persistent dyspnea or exercise limitation on LABA/ICS treatment, it is advisable to switch to triple therapy by adding a LAMA. However, if maximization of therapy with the addition of an ICS in patients with a degree of clinical instability is useful to control the disease, then such an approach may not be necessary during periods of clinical stability (Cazzola et al., 2015d). Generally, if the patient is strongly symptomatic and is at high risk of exacerbation, therapy can be started with a LAMA that affects both dyspnea and exacerbations (https://goldcopd.org). For patients with more severe symptoms, mainly dyspnea and/or exercise limitation, LABA/LAMA may be considered as the preferable initial therapy. In some patients, LABA/ICS may be the first choice because it is the treatment that is the most effective in reducing exacerbations in patients with blood eosinophil counts ≥300 cells/μl.

If LABA/ICS therapy does not reduce the risk of exacerbation, the use of triple therapy by adding a LAMA is recommended. Triple therapy can further improve outcomes in patients with COPD on LABA/ICS combination without increasing the risk of severe cardiovascular adverse events (Calzetta et al., 2019).

In the event that treatment with an ICS is ineffective or causes too many side effects, it is possible to switch the therapy to LABA/LAMA, discontinuing the ICS. In patients with persistent exacerbations despite triple therapy, ICS may be discontinued, although it has been

demonstrated that ICS withdrawal can significantly increase the risk of severe exacerbations and also impairs both lung function and quality of life, but in a nonclinically important manner (Calzetta et al., 2017a).

The 2017 Spanish COPD guidelines recommend that dual bronchodilation should be tried in symptomatic patients or those with clear exercise limitation despite bronchodilator monotherapy, and in any case it is the initial choice in patients with severe or very severe spirometric impairment (Miravitlles et al., 2017). Furthermore, it is preferred over treatment with LABA/ICS in patients who are symptomatic despite a long-acting bronchodilator, especially if they present exacerbations. Only patients with ACO should be initially treated with a LABA/ICS combination. If there is not a good control of exacerbations with two drugs, triple therapy with LABA/LAMA/ICS should be considered.

The use of xanthines for treating patients with COPD remains controversial. According to the 2017 Spanish COPD guidelines (Miravitlles et al., 2017), xanthines are a third-line treatment because of the limited clinical efficacy and narrow therapeutic window. Xanthines should be used mainly in high-risk patients who continue to be dyspneic while receiving regular dual bronchodilator therapy. Furthermore, adding theophylline to an ICS and a LABA might be associated with an increased risk for overall exacerbation (Wilairat et al., 2019). A large study in patients with COPD treated with ICSs because they were considered at high risk of exacerbation demonstrated that adding low-dose theophylline did not decrease the episodes of COPD exacerbations over a 1-year period compared with placebo (Devereux et al., 2018). In its last report, GOLD strategy does not recommend the use of theophylline because its small therapeutic index and most of benefits occurs only when near-toxic doses are given an also because there are more efficient medications (https://goldcopd.org).

Bronchodilators are also critical in the treatment of acute exacerbations of COPD, together with systemic corticosteroids and appropriate antibiotics. The GOLD 2020 strategy document (https://goldcopd.org) suggests using a SABA alone at high dose or combined with a SAMA, while starting treatment with a long-acting bronchodilator as soon as possible before hospital discharge if the patient was hospitalized, and avoiding the use of xanthines. Some small studies suggest the possibility of using formoterol (Cazzola et al., 2003) instead of salbutamol (Cazzola et al., 2002) to overcome the short pharmacodynamic half-life of this SABA and thus reduce the need for rescue medication. Interestingly, there is a signal in favor of the use of the formoterol/tiotropium combination in patients with mild-to-moderate acute exacerbations of COPD (Di Marco et al., 2006). In particular, it has been reported that hospital stays were shorter when tiotropium

bromide was added to the respiratory care protocol including formoterol (Drescher et al., 2008).

In addition, indacaterol, a once-a-day (ultra)-LABA, was found to be effective in improving lung function during an acute exacerbation of COPD at the dose of 300 μg, with an effect that was similar to that obtained with high doses of nebulized salbutamol (1250 μg three times a day) (Segreti et al., 2013), although this caused transient episodes of oxygen desaturation (Rogliani et al., 2014b).

Systemic corticosteroids play a key role in treating severe exacerbations of COPD, but their use is limited to a few days of treatment because of unwanted side effects. It is of interest therefore that adding budesonide influenced the fast onset of action of formoterol (Cazzola et al., 2004) and reduced the potential for acute effects of formoterol on arterial blood gases (Cazzola et al., 2006).

It is anticipated that bifunctional drugs such as ensifentrine or GS-5759 could also be useful in the treatment of acute exacerbations of COPD, but to date there is no evidence supporting this approach.

C. Use of Bronchodilators for Treatable Traits in Airway Diseases

Overlapping characteristics of asthma and COPD, such as airway inflammation, atopy, hyperresponsiveness, and a smoking history, can coexist in some patients (Rogliani et al., 2016). ACO has been defined as a presence of fixed airflow obstruction and bronchodilator reversibility and/or bronchial hyperresponsiveness, in addition to eosinophilic inflammation, a history of allergic disease, and cigarette smoke exposure. However, ACO is not a disease entity (Cazzola and Rogliani, 2016), just as asthma and COPD are not, all being defined merely by a set of symptoms or changes in lung function (Fingleton et al., 2018).

However, it has recently been proposed that patients with airway diseases should be treated based on treatable traits such as airflow obstruction, exacerbations, and eosinophilic inflammation that may be differently present in each patient (both asthma and COPD together with ACO share these treatable traits) (Agusti et al., 2016). This would involve the abandonment of the traditional diagnostic labels, but thus far this approach has not been recommended in any guidelines.

Traditionally, airflow limitation is subclassified as variable or fixed, with the former often assumed to be due to airway hyperresponsiveness. However, there could be other potentially treatable factors contributing to variable airflow limitation, including but not limited to sensitization of airway nerves and release of airway inflammatory mediators that affect ASM contractility, intrinsic abnormality of ASM, and structural changes to the airway architecture (Shrimanker et al., 2017).

Bronchial hyperresponsiveness can be targeted symptomatically by treatment including inhaled β_2-AR agonists. mAChR antagonists are potentially less able to prevent bronchoconstriction mediated by inflammatory mechanisms such as mast cell mediator release, although in practice seem to be as effective as β_2-AR agonists in most patients with established airflow limitation. In this population, the combination of LABAs and LAMAs produced additive benefits and this is increasingly regarded as the optimum treatment (Shrimanker et al., 2017).

A small study recently suggested that traits associated with the treatment response to LABAs in patients with stable COPD are postbronchodilator FEV$_1$, bronchodilator reversibility to salbutamol, expression of three genes (*CLN8*, *PCSK5*, and *SKP2*), and damaged-gene scores of four genes (*EPG5*, *FNBP4*, *SCN10A*, and *SPTBN5*) (Kang et al., 2017). Traits associated with the treatment response to a LAMA were CAT score, bronchodilator reversibility to salbutamol, expression of four genes (*C1orf115*, *KIAA1618*, *PRKX*, and *RHOQ*), and damaged-gene scores of three genes (*FBN3*, *FDFT1*, and *ZBED6*). However, this approach is very complicated and is not suitable for most levels of care.

A simpler approach that could be adapted to different levels of care has been proposed (Shrimanker et al., 2017), which focuses on just two of the major treatable traits in patients with airway disease, eosinophilic inflammation and airflow limitation, with the risk of exacerbations as a result of eosinophilic inflammation and symptoms due to airflow limitation. When symptoms predominate and inflammation is absent or modest, bronchodilators (LABA and LAMA alone or in combination) are the cornerstone of treatment. When both symptoms and inflammation are predominant, triple therapy (LABA/LAMA/high-dose ICS) is needed.

Obviously, this is an oversimplification that does not take into account, for example, that it is necessary to establish at the beginning of the therapy which of the three main phenotypes of ACO—eosinophilic COPD (high T 2 inflammation), neutrophilic asthma (low T 2 inflammation), and also paucigranulocytic ACO—is the one that must be treated (Barnes, 2015). For those patients with ACO without a distinct cellular inflammatory response, bronchodilators are the cornerstone of treatment (Leung and Sin, 2017). In paucigranulocytic ACO, inflammatory cell counts do not increase. Therefore, this phenotype can be treated advantageously with long-acting bronchodilators, including LABAs, LAMAs, or their combination, which are important because they reduce hyperinflation, or triple combination with an ICS (Cazzola et al., 2017b).

V. Challenges with the Use of Bronchodilators

A. Bronchodilators in Children and Elderly Patients

1. In Children. In the acute care setting, SABAs are the most effective bronchodilators, with a significant bronchodilator effect documented in children aged

≥1 year (Grigg and Ducharme, 2019). No specific SABA is favored in terms of efficacy or safety. The addition of ipratropium bromide to β_2-AR agonists has been shown to be superior to β_2-AR agonists alone (Griffiths and Ducharme, 2013).

Currently, there are very limited data on the pharmacokinetic behavior of LABAs in the pediatric population and, consequently, pediatric LABA use is based primarily on data from adults, despite children not being small adults, and the magnitude of changes in dose exposure and/or exposure response may not be solely reflected by differences in body weight (Matera et al., 2017b). This is an explanation for why the use of LABAs is unlicensed in children aged <4 years for salmeterol and 6 years for formoterol.

In effect, the systemic exposure to formoterol for children versus adults is almost doubled and that for adolescents is about 30% higher compared with adults; both correlate inversely with age and body size (Chawes et al., 2014). Nevertheless, the lack of relationship between once-a-day vilanterol exposure and age or sex in pediatric asthma subjects aged 5–11 years inadequately controlled on an ICS has been documented, suggesting that a single dose may be suitable for this population; however, the study did not show any significant increase in trough FEV_1 from baseline with any of the vilanterol doses examined (Oliver et al., 2014) and there are very few studies investigating the use of vilanterol in pediatric patients (Dwan et al., 2016).

Interestingly, an increased responsiveness to β_2-AR stimulation in children compared with adults has been documented (Bisgaard and Szefler, 2006), which may be the reason why children who receive a LABA present a higher rate of serious adverse effects compared with adults (Rodrigo et al., 2009). Nevertheless, the risk of a serious asthma-related event induced by salmeterol/ fluticasone FDC in children aged between 4 and 11 years who needed daily asthma treatment and had a history of asthma exacerbations in the previous year was similar to that caused by fluticasone alone (Stempel et al., 2016). Although it was not a primary safety endpoint, staying on an FDC containing an ICS reduced the risk of having a severe exacerbation of asthma by 25% compared with switching to ICS monotherapy. In addition, an FDC of formoterol/budesonide was effective in children, being able to induce larger lung function improvements than budesonide alone with no remarkable safety differences between treatments (Pearlman et al., 2017). These findings show that it is an appropriate therapeutic option for asthmatic children aged 6–11 years who remain symptomatic on ICS alone.

In children and adolescents taking ICSs, LAMAs are capable of inducing a further benefit compared with placebo but have a minimal effect on exacerbation rate and asthma worsening in children who are responsive to bronchodilators (Blake and Raissy, 2018). It has been shown that tiotropium bromide delivered via the Respimat inhaler was well tolerated as an add-on controller option to ICSs regardless of the presence of additional maintenance therapy in children aged 1–5 years with persistent asthmatic symptoms, with a safety and tolerability profile similar to that of placebo (Vrijlandt et al., 2018). However, several questions remain unanswered, including evidence on the long-term safety, the efficacy of tiotropium bromide compared with LABAs and leukotriene receptor antagonists individually as add-on therapy, and biomarkers to predict treatment response (Anderson et al., 2017).

Almost all children achieve a serum concentration of 5–15 mg/l and 70% achieve levels <10 mg/l using the current aminophylline intravenous loading dosage recommendation (5 mg/kg infused over 20–30 minutes; Morris et al., 2015; Cooney et al., 2017). Obviously, serum concentrations must be maintained in the therapeutic range using an aminophylline infusion and the British National Formulary for Children recommends choosing the dose of intravenous aminophylline infusion taking into account the patient's age (Paediatric Formulary Committee, 2016). Children aged <11 years must receive 1 mg/kg per hour and adolescents aged 12–18 years must receive 0.5–0.7 mg/kg per hour, to achieve the therapeutic range (10–20 mg/l). However, the therapeutic range is often not reached or can be exceeded due to high interindividual variation that is seen with this approach and so many children will be over- or underdosed (Saint et al., 2018).

A recent review of population pharmacokinetic analyses concluded that body weight and age (postconceptional age and postnatal age) were the most important factors associated with the clearance of theophylline in pediatric patients (Ma et al., 2016). In these patients, the volume of distribution was influenced only by body weight. The median values of volume of distribution in pediatric patients (0.897 l/kg) were larger than those in children (0.57 l/kg) and in adults (0.485 l/kg). This difference may be due to the larger volume of extracellular fluids in newborns and the increased total body water in neonates.

2. In Elderly Patients. Aging disturbs the absorption of several drugs (including inhaled ones), which may influence their action and safety profile (Matera et al., 2018b). It can affect the pharmacokinetics of a drug because of factors such as renal and hepatic impairment, weight reduction, body fat increase, and a decrease in body water that can characterize elderly patients. In particular, the pharmacokinetics of inhaled drugs is influenced by aging also because of the progressive reduction in respiratory function (Wallin et al., 2018).

However, solid information on the role that age can play on responses induced by inhaled bronchodilators is still lacking. Subgroup analysis of the TORCH study has shown that increased age is a risk factor for future exacerbations (Jenkins et al., 2012). However, in a pooled analysis of 11 studies that included 8445

patients with moderate to severe COPD, indacaterol once-a-day was equally effective in improving lung function, dyspnea, and HRQL and in reducing the use of rescue medication in patients aged ≥65 years and in those aged <65 years. Although older patients frequently suffer from comorbidities, indacaterol was also well tolerated in subjects aged ≥75 years (Girodet et al., 2017).

Nevertheless, the cardiovascular safety of LABAs, LAMAs, and their combination, particularly in elderly individuals, is still debated. A large observational population-based study focused on patients with a mean age of 79.0 ± 7.1 years documented that both LABAs and LAMAs equally increase the cardiovascular-related hospitalization rate (Gershon et al., 2013). However, in a group of patients aged ≥80 years with COPD with several comorbidities, a single dose of indacaterol/glycopyrronium (110/50 μg) FDC did not increase short-term proarrhythmic risk and was associated with a good cardiac safety profile (Spannella et al., 2018).

In a COPD population-based study in which patients were older than 65 years, starting a treatment with a LABA/ICS combination induced a modest but significant reduction in deaths and hospitalizations for COPD compared with starting therapy with LABA alone, an effect that was even more significant in those who suffered from asthma or did not take LAMAs (Gershon et al., 2014).

In a nested case-control study, use of mAChR antagonists by older adults was significantly associated with a greater risk of developing community-acquired pneumonia compared with no use (Chatterjee et al., 2016). The mechanisms that explain the onset of community-acquired pneumonia are still unclear. It is possible that blockage of peripheral mAChRs causing dry mouth compromises the transport of the oropharyngeal bolus, and eventually induces aspiration. However, it cannot be excluded that the scarce dense secretions that follow the use of mAChR antagonists act as pabulum increasing the risk of bacterial growth and depress the mucociliary transport, which facilitates bacterial residence in the lungs and can eventually cause respiration infection. Furthermore, it also cannot be excluded that these pharmacological agents can cause acid reflux and subsequent aspiration by relaxing lower esophageal sphincter muscles(Chatterjee et al., 2016).

Older men, but not women, with COPD initiated on LAMAs are at increased risk of urinary tract infection (Gershon et al., 2017) because LAMAs can inhibit contraction of the detrusor muscle, which causes partial urinary obstruction and urinary stasis predisposing to urinary tract infection. Although this complication is more likely with oral mAChR antagonists, it can occur even with inhaled preparations; therefore, there is a need for careful monitoring of older patients prescribed inhaled LAMAs because these drugs can also cause cognitive impairment, falls, and closed angle glaucoma (Dunn et al., 2018).

At least as far as we can understand from studies with vilanterol/umeclidinium, the LABA/LAMA combinations are effective in elderly patients, improving lung function in a clinically important manner regardless of age (Ray et al., 2018). Furthermore, age does not influence the safety profile of vilanterol/umeclidinium.

B. Bronchodilators and Comorbidities

Bronchodilators can impact comorbidities of asthma and COPD, sometimes favorably and sometimes negatively.

1. Cardiovascular Diseases. Patients with COPD or asthma often also suffer from cardiovascular diseases (Cazzola et al., 2012a). This is an important issue when using bronchodilators because both LABAs and LAMAs can potentially affect cardiac function (Matera et al., 2016). In patients with COPD who start to use LABAs or LAMAs, there is a 1.5-fold increased cardiovascular risk within 30 days of therapy initiation (Wang et al., 2018).

Nevertheless, both salbutamol and indacaterol have been demonstrated to induce a rapid reduction in B-type natriuretic peptide levels in patients with an acute exacerbation of COPD (Segreti et al., 2013). A plausible explanation of this effect is that the capacity of β_2-AR agonists in reducing air trapping causes a reduction in intrathoracic pressure, including pressure on the whole heart and, consequently, right-ventricular overload and left-ventricular diastolic dysfunction improve. LABA/LAMA combinations can also reduce lung hyperinflation in a significant manner, and this improvement in lung-function parameters translates into an approximately 10% increase in left-ventricular end-diastolic volume, with an improved cardiac index mediated by increased stroke volume (Hohlfeld et al., 2018).

Besides, a large trial that enrolled >16,000 patients showed that treatment of patients with moderate COPD with the inhaled ultra-LABA, vilanterol, alone or combined with an ICS, appeared to be safe for patients with heightened cardiovascular risk (Brook et al., 2017).

Often, bronchoconstriction is present in patients with heart failure. Fortunately, it can be treated with inhaled β_2-AR agonists, which then decrease the work of breathing that otherwise could possibly further decrease cardiac workload (Minasian et al., 2013). This means that inhaled bronchodilators are useful in the management of these patients if they suffer from airway obstruction.

Furthermore, when a patient suffers from COPD and also from chronic heart failure at the same time, concurrent administration of a LABA and a β-AR blocker is an option that must always be taken into consideration (Cazzola et al., 2017a; Cazzola and Matera, 2018b) because there is experimental evidence that the coadministration of a β_2-AR agonist and a β_1-AR blocker can influence cardiac remodeling (Rinaldi et al., 2015). In this study in rats with heart failure, both

indacaterol and metoprolol alone significantly diminished the infarct size compared with the untreated group. However, when the two drugs were combined, the infarct size was further reduced, both blood pressure and heart rate decreased, and the ejection fraction and left-ventricular systolic and diastolic internal diameters were normalized. Furthermore, the decreased β_1-AR mRNA and cardiac GPCR kinase 2 expression returned to normal, and cAMP levels and catecholamine, atrial natriuretic peptide, B-type natriuretic peptide, and collagen 1 mRNA levels were also normalized. Interestingly, a post hoc analysis of two large clinical trials that investigated the efficacy and safety of olodaterol/tiotropium FDC documented that the addition of a β-AR blocker for treating a concomitant cardiovascular disease in patients with COPD was safe (Maltais et al., 2018).

SAMAs may slightly increase the risk of heart failure, whereas there seems to be no additional risk of incident heart failure due to LAMAs or with a LABA/LAMA combination (Roversi et al., 2016).

Cardiac arrhythmias are common in patients with COPD or asthma (Cazzola et al., 2012a). Although bronchodilators are potentially arrhythmogenic agents (Cazzola et al., 2012b), available evidence suggests an overall satisfactory safety profile when using a LABA, LAMA, or dual bronchodilation (Matera et al., 2016; Rogliani et al., 2018b). Thus, the overall risk of cardiac arrhythmias does not increase with tiotropium bromide (Cazzola et al., 2017c), whereas glycopyrronium bromide causes a slightly higher incidence of atrial fibrillation compared with placebo, despite an overall good safety profile (D'Urzo et al., 2015). In contrast, SABAs and theophylline may precipitate atrial fibrillation and worsen ventricular rate control and caution is thus needed when using them (Roversi et al., 2016).

2. Diabetes Mellitus. Insulin and glucagon secretion, hepatic glucose production, and uptake of glucose into skeletal muscle can be modulated by β-agonists that can in turn influence glucose homeostasis and increase blood sugar levels, although this effect seems to be important only when patients are near glucose intolerance (Philipson, 2002).

Conversely, β_2-agonists can protect against the vascular complications of diabetes (Galvan and Danesh, 2017), an effect that is probably related to the β-arrestin2/inhibitor of κB/nuclear factor-κB signaling pathway. When activated, β_2-ARs increase the levels of β-arrestin2 and its interaction with inhibitor of κB, causing a reduction in the effects of inflammatory stimuli and allowing tissue protection. There is also experimental evidence that β_2-AR agonists increase the survival of pericytes, which are central to preserving the quiescence of endothelial cells and the integrity of their tight junctions, thereby preventing diabetes-induced pericyte loss in the retina (Yun et al., 2018). Furthermore, it has been suggested that chronic administration

of salbutamol could ameliorate nerve dysfunction, as well as exert an antinociceptive effect in diabetic peripheral neuropathy, an effect that is most likely mediated by β_2-ARs (Baraka et al., 2015).

At least in patients with COPD, the combination of an ICS with a β_2-AR agonist apparently reduces, and that with a mAChR antagonist increases, the association between COPD and diabetes (Rogliani et al., 2014a). However, there is no evidence to date that this happens in patients with asthma, but hyperinsulinemia associated with obesity increases vagally induced bronchoconstriction as this inhibits neuronal M_2 mAChRs and facilitates ACh release from airway parasympathetic nerves (Nie et al., 2014).

3. Anxiety/Depression. Anxiety and depression are associated with β-adrenergic and cholinergic initiated signaling (Drevets et al., 2013; Naka et al., 2013). An experimental study in rats documented that β-AR agonists, by activating β-ARs in the bed nucleus of the stria terminalis, elicited an anxiety-like behavior in a dose-dependent manner (Naka et al., 2013). It has also been suggested that depression and related structural and functional changes in certain brain areas are associated with cholinergic dysfunction (Drevets et al., 2013).

In some adult asthmatics, there is an association between major depression and a reduced bronchodilator response that is independent of anxiety symptoms (Han et al., 2016). In patients with COPD, SABAs are associated with a considerably increased risk of onset of bipolar disorder, a risk that is absent with LABAs, SAMAs, and LAMA (Su et al., 2017). There is experimental and clinical evidence that peripheral inflammation is associated with the upregulation of inflammation in the central nervous system and SABAs, when used regularly, increase airway inflammation and bronchial hyperresponsiveness and reduce lung function, whereas LABAs, SAMAs, and LAMAs are capable of exerting a clinically relevant anti-inflammatory activity (Su et al., 2017). Nevertheless, it has also been documented that bronchodilator therapy for COPD has no significant direct effects on depression or anxiety (Hyun et al., 2016), although others have suggested that the valuable effects on dyspnea are likely to relieve associated emotional and mood symptoms (Yohannes et al., 2018).

Intriguingly, in some adult asthmatics, theophylline, but not LABAs, can induce an increased risk of suicidal ideation (Favreau et al., 2012). This effect might be caused by a theophylline-induced increase in cerebral blood resistance, with decreased cerebral blood flow and cerebral oxygen, or by increased blood levels of catecholamines in a pathophysiological manner similar to that observed in patients with panic disorder (Favreau et al., 2012). It would seem sensible therefore to exert caution when prescribing theophylline or similar agents to asthmatics at risk of suicide and ensure adequate follow-up.

4. Gastroesophageal Reflux Disease. β-AR agonists decrease the tone of the lower esophageal sphincter in

patients with COPD or asthma, reducing the function of the antireflux barrier, whereas theophylline increases reflux, although the exact mechanisms that cause these effects are still unknown (Shimizu et al., 2012; Del Grande et al., 2016). There is evidence suggesting that β_2-AR agonists, mainly nebulized salbutamol, can worsen reflux in some patients (Babu and Morjaria, 2018). However, the use of salbutamol is less problematic than theophylline in this context (Mungan and Pınarbaşı Şimşek, 2017).

Inhaled mAChR antagonists may decrease or, at least, not increase the risk of gastroesophageal reflux disease (Kim et al., 2013). However, the reduction of the lower esophageal sphincter pressure induced by central and peripherally acting mAChR antagonists is balanced by their antitussive effect that can suppress cough and thereby minimize the occurrence of changes in intra-abdominal pressure, which may facilitate the onset of gastroesophageal reflux disease (Kim et al., 2013).

5. Cough. Since it is often difficult to differentiate chronic cough derived from asthma from that accompanying rhinosinusitis or gastroesophageal reflux disease, it has been proposed to consider chronic cough as a single independent disease entity referred to as "hypersensitivity of the cough reflex" (Morice et al., 2011).

Experimental studies suggest that modifications in airway caliber increase the sensitivity of the afferents involved in the cough reflex, although there are difficulties in proving this effect in the clinical setting. This finding suggests that bronchodilators might have a rather minor role in the treatment of cough (Matera et al., 2017d). However, the mechanism(s) by which these agents alleviate cough still need to be clarified (Dicpinigaitis et al., 2014).

There is evidence that mAChR antagonists reduce experimental cough (Dicpinigaitis et al., 2014). In naive guinea pigs, tiotropium bromide and ipratropium bromide improved cough reflex sensitivity by modulating transient receptor potential cation channel subfamily V member 1 (Birrell et al., 2014). A study in rabbits showed that acid-sensing ion channels and mechanoreceptors might mediate LAMA-induced downregulation of cough (Mutolo et al., 2016). Furthermore, modulation of cough reflex sensitivity, but not bronchodilation, is the mechanism by which tiotropium bromide may alleviate asthmatic cough refractory to LABA/ICS (Fukumitsu et al., 2018), although mAChR antagonists do not inhibit ozone-induced hypertussive responses at doses that are bronchodilatory (Clay et al., 2016). Results from phase III studies suggest that aclidinium bromide (400 μg) taken twice-a-day may improve cough by reducing, in particular, night-time cough frequency and severity (McGarvey et al., 2016). LAMAs are also used in tertiary care to dampen down laryngeal nerve irritability as well as treat (dry) airway secretions that may be aggravating the cough reflex pathways (Wang et al., 2017).

The antitussive properties of β_2-AR agonists, if any, are mediated by acting directly on β_2-ARs on sensory nerve endings in the lung (Maher et al., 2011). However, the use of β_2-AR agonists in children with acute cough, but without bronchoconstriction, is not supported by any evidence and the evidence supporting the routine use of β_2-AR agonists in adults with acute cough is also very modest (Becker et al., 2015).

Nonetheless, postviral cough is effectively improved by a combination of inhaled ipratropium bromide and salbutamol (Zanasi et al., 2014). There is also documentation that the combination of aclidinium/formoterol significantly improves night-time and early-morning cough severity in patients with moderate to severe COPD (Bateman et al., 2015).

Interestingly, in guinea pig models of allergic inflammation, budesonide and salmeterol combined at half-dose show comparable antitussive, bronchodilatory, and anti-inflammatory effects to a full-dose therapy with budesonide alone but are more effective in stimulating the ciliary beat frequency (Pappová et al., 2016). Indeed, cough symptoms in patients with cough-variant asthma improve significantly when treated with a combination of a β_2-AR agonist plus an ICS (Bao et al., 2013).

C. Use of Bronchodilators in Pregnancy

Within the LABA class, formoterol and salmeterol seem to be associated with similar prevalences of low birth weight, preterm birth, and neonates being small for gestational age (Cossette et al., 2014). Importantly, however, there is no evidence of a higher risk of major congenital malformations among asthmatic pregnant women treated with a LABA plus ICS combination than that seen in women receiving ICS monotherapy at a higher dose during the first trimester (Eltonsy et al., 2015).

Nevertheless, data from a database of congenital anomaly registries documented the risk of cleft palate and gastroschisis when pregnant women are exposed to inhaled β_2-AR agonists in the first trimester (Garne et al., 2015). Additionally, a meta-analysis of aggregated data from three cohort studies performed in Norway, Wales, and Denmark found associations between the use of SABAs in the first trimester of pregnancy and onset of renal dysplasia (Garne et al., 2016). However, because these outcomes are uncommon and the relative risks are very low, they represent an absolute risk that is truly minimal (Namazy and Schatz, 2017). In addition, these increased relative risks may be the result of asthma exacerbations that could be associated with a risk of congenital malformations, and SABAs are only a confounding factor in that they are used for the treatment of asthma exacerbations (Namazy and Schatz, 2017). However, in a Swedish study, the risk of cleft palate was lower for SABAs than for ICSs and other bronchodilators and

no increased risk of gastroschisis linked to the use of SABAs was reported (Källén, 2014).

It has been suggested that exposure to β_2-AR agonists during the prenatal period is associated with an increased risk for autism spectrum disorders (Gidaya et al., 2016). Furthermore, there is an increased risk of cerebral palsy associated with maternal use of β_2-AR agonists during pregnancy in female offspring born at term (Li et al., 2018). Nonetheless, no association between tocolytic exposure and subsequent development of autistic disorders or behavioral or developmental disabilities among term and preterm born infants has been found (Altay et al., 2017). Neither the time of treatment initiation nor the duration and dosage of β-AR agonist exposure is correlated with an increased risk of autism spectrum disorders.

There have been no adequate well-controlled clinical studies of tiotropium bromide or other LAMAs specifically performed in pregnant women. Therefore, LAMAs should be used with caution in this population (Kerstjens and O'Byrne, 2016).

D. Pharmacogenetics of Airway Obstruction and Bronchodilators

Genetic polymorphisms can modify responses to inhaled bronchodilators (Kersten and Koppelman, 2017); therefore, the discovery, or confirmation, of a genetic marker that could accurately predict responses to LABA and/or LAMA treatment would have high clinical value.

Pharmacogenetic studies of β_2-AR agonists have mainly focused on the gene encoding the β_2-AR (ADRB2). There is evidence for functional relevance of Gly[16]Arg (rs1042713), Gln[27]Glu (rs1042714), and Thr[164]Ile (rs1800888). These three single nucleotide polymorphisms (SNPs) lessen the level of agonist-promoted downregulation of β_2-AR expression and stimulate AC activity (Slob et al., 2018). Variants that alter ADRB2 function could increase disease risk or reduce the response to endogenous and inhaled β_2-AR agonists in asthma (Zuurhout et al., 2013) and COPD (Nielsen et al., 2017), but there are contradictory reports as to whether functional genetic variants in ADRB2 are associated with response to bronchodilator treatment. ADRB2 Gly[16]Arg variant is consistently associated with response to LABA; however, it increases the risk of exacerbation only in children, but not adults (Slob et al., 2018). Rare variants can also influence responses to SABAs (Drake et al., 2014) or LABAs (Ortega et al., 2014), with a large effect size.

A systematic review and meta-analysis found neither a significant association between Gly[16]Arg, Gln[27]Glu, and Thr[164]Ile genotypes and the risk of COPD nor consistent differences in treatment response to β_2-AR agonists in patients with COPD as a function of ADRB2 (Nielsen et al., 2017). However, a large pharmacogenetics genome-wide association study of patients with COPD showed that responses to inhaled SABAs are associated with variants upstream from the gene KCNJ2 (Hardin et al., 2016). Furthermore, it has been documented that patients with COPD with the Arg[16]Arg genotype had better exacerbation outcomes in response to salmeterol than those with Gly[16]Gly and Arg[16]Gly genotypes (Rabe et al., 2014). In addition, there is a significant association between bronchodilator responsiveness and several SNPs in the genes CDH13 and SGCD in African Americans (Hardin et al., 2016).

There are significant variations between ethnic groups in genetics of ADRB2 (Cazzola et al., 2018b). Several uncommon variants with a frequency of <5%, most of which were unique to a particular ethnic group, have been identified by resequencing ADRB2 in multiethnic groups of asthmatics (Ortega et al., 2015). Thus, the infrequent coding variant Thr[164]Ile was identified mainly in non-Hispanic whites, whereas it was possible to detect a 24-basepair promoter insertion variant at nucleotide position -376 relative to the start codon (i.e., -376 In-Del) only in African Americans and Puerto Ricans (Ortega et al., 2015).

A meta-analysis that investigated the link between childhood asthma exacerbations and the Arg[16] polymorphism using data from five White Northern European and Hispanic/Latin pediatric populations recruited in five cross-sectional studies documented the association between the variant rs1042713 and an increased risk of asthma exacerbation in children treated with ICS/LABAs (Turner et al., 2016). However, a large pharmacogenetic study, in which the entire genome of 1441 asthmatic children belonging to different racial and ethnic groups (Puerto Ricans, Mexicans, and African Americans) with extreme responses to salbutamol was sequenced, failed to identify any association between the response of bronchodilator and specific variants from β_2-AR signaling pathways (Mak et al., 2018).

Several polymorphisms in the M_2 mAChR and also, although less frequently, in the M_3 mAChR subtype genes have been described (Matera et al., 2017c). The possible existence of an increased risk to develop a more severe disease in patients with COPD carrying the SNP of the M_2 mAChR (rs1824024) has been suggested (Cherubini et al., 2016). This risk is linked to lower lung function parameters and symptoms and increased susceptibility to exacerbations in these individuals than in patients not having this SNP. Despite the key function of CHRM3, which codes for the M_3 mAChR and consequently is important in the physiology of human airways, and the number of its polymorphisms, there is not solid documentation that these variations have pharmacological relevance (Matera et al., 2017c).

Theophylline mainly undergoes 8-hydroxylation to 1,3-dimethyl uric acid by CYP1A2, a subtype of cytochrome P450, and also N-demethylation to 1-methylxanthine or to 3-methylxanthine by CYP2E1 and CYP1A2. It has been

documented that increased theophylline clearance was significantly related to the -3860G>A polymorphism in the *CYP1A2* gene, and this polymorphism could influence the enzyme inducibility of CYP1A2, at least in Korean nonsmoking asthmatics (Yim et al., 2013). In a Chinese population, the rate of theophylline clearance was not related to the genotypes of the *CYP1A2* gene, G-3860A; in contrast, that of the two groups of *CYP1A2* G-3113A gene loci A allele carriers (AA + GA genotype) appeared to be significantly lower than that of the G allele carriers (GG genotype) (Xiong and Li, 2018). The frequency of G-3113A mutation was much higher in Chinese and ethnic minority groups (approximately 10% and 11%, respectively) than in a Korean population (2.7%) and a Swedish population (2.3%) (Hatta et al., 2015). CYP1A2 enzyme activity is intensified by polymorphism of *CYP1A2*1F* allele (*CYP1A2*1F* (-163C>A) allele) and conversely, is decreased by five SNPs of *CYP2E1* (-1055C>T, -1027T>C, -807T>C, -1566T>A, and -1295 G>C) that also reduce 1,3- dimethyl uric acid/theophylline ratio (Sutrisna, 2016).

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Matera, Page, Calzetta, Rogliani, Cazzola.

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