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REVIEW

β_2-adrenoceptor agonists: current and future direction

Mario Cazzola[1], Luigino Calzetta[2] and Maria Gabriella Matera[3]

[1]*Unit of Respiratory Clinical Pharmacology, Department of Internal Medicine, University of Rome 'Tor Vergata', Rome, Italy,* [2]*IRCCS SanRaffaele Pisana, Rome, Italy, and* [3]*Unit of Pharmacology, Department of Experimental Medicine, Second University of Naples, Naples, Italy*

Correspondence
Mario Cazzola, Dipartimento di Medicina Interna, Università di Roma 'Tor Vergata', Via Montpellier 1, 00133, Rome, Italy. E-mail: mario.cazzola@uniroma2.it

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Despite the passionate debate over the use of β_2-adrenoceptor agonists in the treatment of airway disorders, these agents are still central in the symptomatic management of asthma and COPD. A variety of β_2-adrenoceptor agonists with long half-lives, also called ultra long-acting β_2-adrenoceptor agonists (ultra-LABAs; indacaterol, olodaterol, vilanterol, carmoterol, LAS100977 and PF-610355) are currently under development with the hopes of achieving once-daily dosing. It is likely that the once-daily dosing of a bronchodilator would be a significant convenience and probably a compliance-enhancing advantage, leading to improved overall clinical outcomes. As combination therapy with an inhaled corticosteroid (ICS) and a LABA is important for treating patients suffering from asthma, and a combination with an inhaled long-acting antimuscarinic agent (LAMA) is important for treating COPD patients whose conditions are not sufficiently controlled by monotherapy with a β_2-adrenoceptor agonist, some novel once-daily combinations of LABAs and ICSs or LAMAs are under development.

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Abbreviations
ACh, acetylcholine; cAMP, cyclic adenosine monophosphate; COPD, chronic obstructive pulmonary disease; EC_{50}, median effective concentration required to induce a 50% effect; ECG, electrocardiogram; EFS, electric field stimulation; E_{max}, the maximum possible effect for the agonist; FDA, Food and Drug Administration; FEV_1, Forced Expiratory Volume in One Second; HFA, hydrofluoroalkane; ICS, inhaled corticosteroid; IFN, interferon; IL, interleukin; LABA, long-acting β_2-adrenoceptor agonist; LAMA, long-acting antimuscarinic agent; mL, milliliter; pEC_{50}, negative logarithm of EC_{50}; PK, pharmacokinetics; QTc, corrected QT interval

Current direction

β_2-adrenoceptor agonists, mainly long-acting β_2-adrenoceptor agonists (LABAs) such as formoterol and salmeterol, are an important pharmacological approach to induce bronchodilation in patients suffering from chronic obstructive pulmonary disease (COPD) (Celli and MacNee, 2004; Rabe *et al.*, 2007) and asthma (National Asthma Education and Prevention Program, 2007; Bateman *et al.*, 2008a), although controversy has reigned over their regular use prescribed as monotherapy, at least in the treatment of asthma (Wijesinghe *et al.*, 2008). In effect, with chronic or high-dose exposure, β_2-adrenoceptor agonists demonstrate proinflammatory effects. *In vitro*, they can enhance the Th2 inflammatory pathway by inhibiting interleukin (IL)-12 and interferon (IFN)-γ (Panina-Bordignon *et al.*, 1997; Agarwal and Marshall, 2000). *In vivo*, pretreatment with β_2-adrenoceptor agonists increases the severity of the late asthmatic reaction (Lai *et al.*, 1989), continuous treatment is associated with an increase in sputum eosinophils, notably when the patient is not taking concomitant inhaled corticosteroids (ICSs) (Aldridge *et al.*, 2000; Lazarus *et al.*, 2001), and most studies demonstrate that

β_2-adrenoceptor agonists increase airway hyperresponsiveness (Taylor, 2009). Intriguingly, in mice harbouring overexpressed β_2-adrenoceptors (equivalent to continuous receptor stimulation), there is enhanced airway smooth muscle contractility (Taylor *et al.*, 1996).

Nonetheless, there is solid evidence suggesting that asthmatic patients in clinical practice treated with a single inhaler containing an ICS plus a LABA experience fewer asthma exacerbations than similar patients treated with ICSs alone (Hirst *et al.*, 2010). It is intriguing that, while the use of LABAs (with or without ICS) increased over the last decade, asthma mortality declined in major western European countries (Chatenoud *et al.*, 2009). Moreover, a rather recent analysis of more than 40 000 asthmatics (Rodrigo *et al.*, 2009) found that regular LABA use as monotherapy reduced acute exacerbations requiring oral corticosteroids by 20%, and withdrawals due to acute exacerbations by 32%. Additionally, this analysis did not identify any detrimental effect of LABAs on acute exacerbations requiring hospitalization or on life-threatening episode. However, the Food and Drug Administration (FDA) still recommends that LABAs be reserved for patients whose asthma cannot be adequately managed with asthma-controller medication such as an ICS, and long-term use of LABAs should be limited to patients who require prolonged use of ICSs (Chowdhury and Dal Pan, 2010).

Although regular monotherapy with β_2-adrenoceptor agonists should clearly be avoided, and the concomitant use of ICS does not mean that adverse effects of β_2-adrenoceptor agonists are a nonissue (Taylor, 2009), β_2-adrenoceptor agonists remain the most effective bronchodilators available for the immediate relief of asthma symptoms and, as such, they are still an important component of asthma treatment (Cazzola and Matera, 2007), however, taking into account that in acute asthma, a full agonist (high intrinsic efficacy) offers a clinical advantage over a partial agonist (low intrinsic efficacy) but with the potential of inducing dose-dependent adverse effects (Hanania *et al.*, 2010a). In any case, LABAs in combination with ICSs will continue to remain the main focus of treatment of asthma (Chung *et al.*, 2009).

A volume of published evidence supports the role of LABAs in the treatment of stable COPD (Cazzola *et al.*, 1997; Rossi *et al.*, 2008). Physiological studies have shown that β_2-adrenoceptor agonists dilate the airways and reduce air trapping, and this leads to improved lung function and improved exercise tolerance for patients (Di Marco *et al.*, 2003; O'Donnell *et al.*, 2004). Clinical trials clearly show that short- and long-acting β_2-adrenoceptor agonists improve dyspnoea and quality of life and reduce respiratory exacerbations in patients with COPD (Appleton *et al.*, 2006). Interestingly, LABAs, rather than short-acting β_2-adrenoceptor agonists, have also the potential to improve the mucociliary component of COPD (Rogers, 2005).

For patients whose conditions are not sufficiently controlled by monotherapy, combining bronchodilators of different classes, in particular an inhaled β_2-adrenoceptor agonist with an inhaled antimuscarinic agent, seems a convenient way of delivering treatment and obtaining superior results (Cazzola and Molimard, 2010). It is reasonable to postulate that targeting bronchoconstriction through two distinct mechanisms (sympathomimetic and anticholinergic) should maximize the bronchodilator response and help to overcome inter- and intra-patient variability in bronchomotor tone associated with COPD (Cazzola and Molimard, 2010). The nature of interaction between the two systems is not yet fully understood, but there is enough evidence to suggest that combining β_2-adrenoceptor agonists and antimuscarinic agents is pharmacologically useful for two reasons (Cazzola and Molimard, 2010). Firstly, the addition of a β_2-adrenoceptor agonist decreases the release of acetylcholine (ACh) through the modulation of cholinergic neurotransmission by prejunctional β_2-adrenoceptors, and thereby amplifies the bronchial smooth muscle relaxation induced by the muscarinic antagonist. Secondly, the addition of a muscarinic antagonist can reduce bronchoconstrictor effects of ACh, whose release has been modified by the β_2-adrenoceptor agonist, and thereby amplify the bronchodilation elicited by the β_2-agonist through the direct stimulation of smooth muscle β_2-adrenoceptors. Another possibility is the fact that the antimuscarinic agent and not the β_2-adrenoceptor agonist can suppress mucus/fluid secretions; hence, surface tension changes which would collapse the airways do not occur.

The LABA/long-acting antimuscarinic agent (LAMA) combination appears to play an important role in maximizing bronchodilation, with studies to date indicating that combining different classes of bronchodilators results in significantly greater improvements in lung function and other outcomes compared with individual drugs used alone, and that these combinations are well tolerated in patients with moderate to severe COPD (Cazzola and Tashkin, 2009).

For patients with more severe COPD and a history of exacerbations, current guidelines recommend the addition of an ICS to a LABA (Celli and MacNee, 2004; Rabe *et al.*, 2007), but evidence from clinical trials indicates that combining a LABA with an ICS and an antimuscarinic agent may provide clinical benefits additional to those associated with these treatments alone in patients with COPD (Cazzola *et al.*, 2007; Singh *et al.*, 2008).

It has been suggested that the use of β_2-adrenoceptor agonists might lead to an increased risk for adverse events in patients suffering from COPD (Salpeter *et al.*, 2004). However, TORCH trial has unequivocally demonstrated that long-term use of LABAs over a period of 3 years is safe and is associated with a slightly lower risk of mortality compared with placebo (Calverley *et al.*, 2007). Moreover, a review and meta-analysis of five trials that compared salmeterol against placebo and four trials that compared formoterol against placebo documented that LABAs by themselves did not significantly alter total mortality in COPD (Kliber *et al.*, 2010). Nonetheless, LABAs may have adverse cardiovascular effects, which are amplified in COPD patients with concomitant cardiac disorders (Cazzola *et al.*, 1998b). In these patients, LABAs should be used with some cautions (Cazzola *et al.*, 2005a). At the present level of our knowledge, the use of LABAs in COPD patients, even patients with cardiac disease comorbidities, does not put 'caveats' similar to the use of LABAs in asthma (Rossi *et al.*, 2008). This might be considered surprising to some extent, because COPD patients are older than patients with asthma and have a larger prevalence of cardiac disorders.

The central role of LABAs as bronchodilators in the treatment of asthma and COPD indicates important marketing opportunities. On the other hand, non-adherence to

medication plans is a major obstacle to successful management of asthma and COPD (Bender, 2002). In general, adherence to treatment with inhalants is poor because of the complex procedures required to use them, as well as the tedious frequent dosing (Jones *et al.*, 2003). Being able to increase adherence to treatment is, therefore, a main medical need. Since there is a well-established belief that the only limits set for the development of a long-lasting bronchodilator with a new product profile are medical needs and marketing opportunities (Cazzola and Matera, 2009), the great interest within the pharmaceutical industry in the discovery of a third-generation once-daily β_2-adrenoceptor agonist to be used as a part of a combination therapy for the treatment of asthma or also as single agent for the therapy of COPD is not surprising.

Future direction

Once-daily LABAs

Anyone planning to develop a once-daily LABA, which can also be called ultra-LABA (Cazzola *et al.*, 2005b), must very carefully consider the pharmacological characteristics of the β_2-adrenoceptor agonist component to understand how it will fit into treatment strategies, and whether it should only be used in combination with other drugs. Evidence shows that current LABAs can be improved upon. Ideally, there is a need for a new LABA that is fast acting and has true 24 h duration of action, providing consistent improvements in the symptoms that matter to patients (Table 1) (Cazzola and Matera, 2008). Such an ultra-LABA would provide flexibility to prescribers and could be used alone or in combination with a once-daily long-acting muscarinic antagonist. Obviously, an ideal ultra-LABA should be well tolerated with a favourable safety profile. Thus, a new entry to the market must ensure that potential cardiac effects are minimized, especially taking into account that mainly COPD patients are often older and may have cardiovascular comorbidities. Currently available LABAs have been associated with a potential antagonism to the bronchodilator effect of a rescue medication (fast-acting β_2-adrenoceptor agonists), due to competition for the same receptors (Cockcroft and Swystun, 1996; Cazzola *et al.*, 1998a). While the clinical relevance of this phenomenon in COPD is unclear, it is important that there should be minimal risk of this occurring with a new ultra-LABA. Further, patient compliance and treatment persistence could be improved with a once-daily treatment that provides immediate and sustained bronchodilation.

A variety of β_2-adrenoceptor agonists with longer half-lives are currently undergoing development, with the hope of achieving once-daily dosing. These agents include indacaterol – which received European regulatory approval in November 2009 and has already been launched in several countries – olodaterol, vilanterol, carmoterol, LAS100977 and PF-610355 (Figure 1; Table 2). It must be mentioned that only indacaterol has been studied extensively, while for the other ultra-LABAs, information is still scarce, often limited to conference abstracts.

Table 1

Designing a new LABA for COPD

Criteria for a new β_2-adrenoceptor agonist could include:

- Longer duration of action (compared with existing LABAs)
  - True 24 h sustained bronchodilator efficacy
  - Allowing once-daily dosing
- Fast onset of action
- Superior efficacy compared with existing LABAs
- Favourable safety and tolerability profile
- Efficient and convenient device
  - Breath actuated
  - With effective feedback to indicate successful inhalation

LABA, long-acting β_2-adrenoceptor agonist.

Indacaterol

Indacaterol, also known as QAB149, is a novel, chirally pure inhaled ultra-LABA. Within a series of 8-hydroxyquinoline 2-aminoindan derived β_2-adrenoceptor agonists, lipophilicity was used as the basis for the design and rationalization of their onset and duration of action profiles, as assessed by a guinea pig tracheal-strip assay. In addition to lipophilicity, potency and intrinsic efficacy have also been shown to be contributing factors in regulating these *in vitro* time course

Table 2

Functional properties of emerging β_2-adrenoceptor agonists against the three human β-adrenoceptor subtypes

Agonist	β_1 pEC_{50}	IA	β_2 pEC_{50}	IA	β_3 pEC_{50}	IA	Selectivity for β_2 over β_1	Reference
Indacaterol	6.60 ± 0.24	16 ± 2	8.06 ± 0.02	73 ± 1	6.72 ± 0.13	113 ± 7	1.46	Battram *et al.*, 2006
Olodaterol	7.55 ± 0.08	52 ± 8	9.93 ± 0.07	88 ± 2	6.57 ± 0.08	81 ± 2	2.38	Bouyssou *et al.*, 2010a
Vilanterol	6.4 ± 0.1		9.4 ± 0.0		6.1 ± 0.2		3.0	Procopiou *et al.*, 2010
Carmoterol			10.19 ± 0.15	88.6 ± 4.1				Rosethorne *et al.*, 2010

pEC_{50} is the negative logarithm of the molar drug concentration that produces a cAMP response equal to 50% of its maximal response. IA is the percentage of isoprenaline-induced maximal response. Selectivity for β_2 over β_1 expressed as pEC_{50} at β_2-adrenoceptor – pEC_{50} at β_1-adrenoceptor.

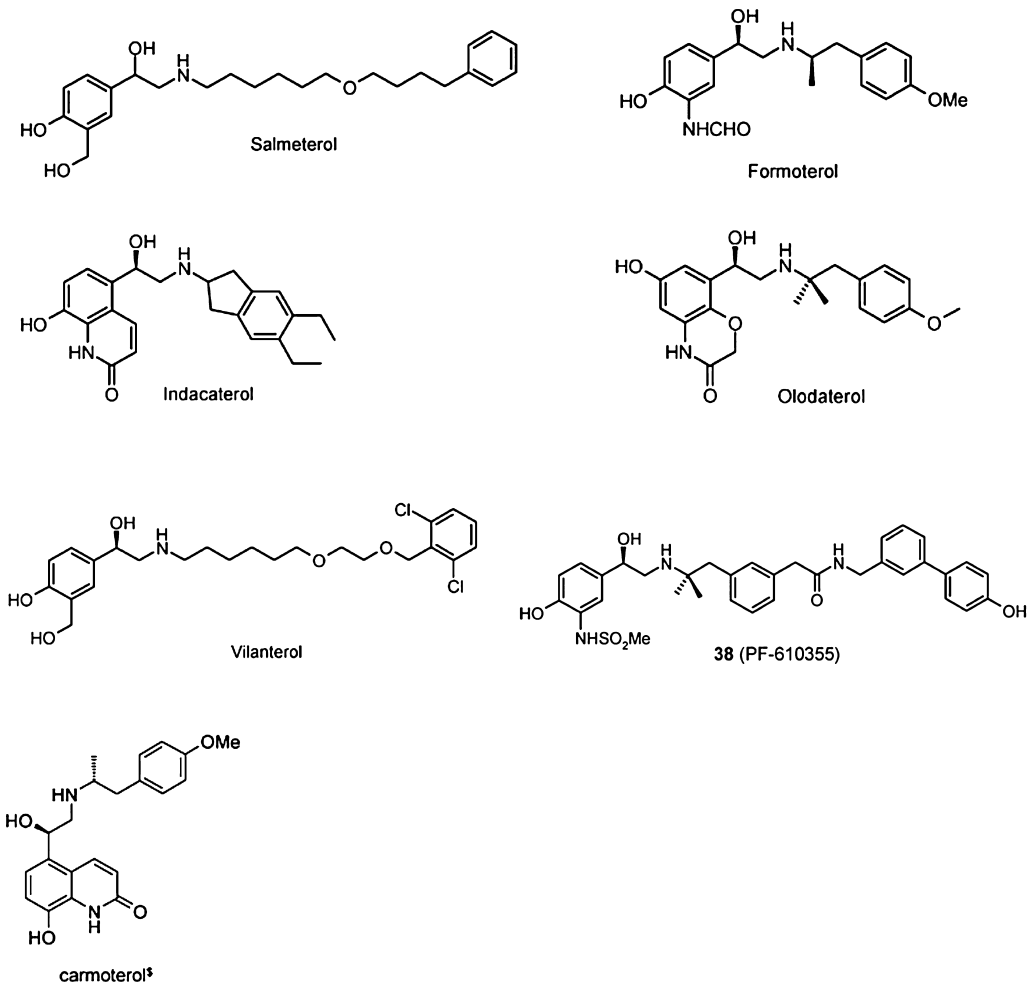

Figure 1

Chemical structures of salmeterol, formoterol and emerging ultra-LABAs.

profiles (Baur *et al.*, 2010). The 5,6-diethyl substituted indan analogue indacaterol was selected from these studies.

Extensive preclinical studies involving indacaterol have been performed both *in vitro* and *in vivo*, and have documented that it offers unique rapid onset of action and true 24 h bronchodilating effect (Cazzola *et al.*, 2010a).

Indacaterol appears to have a high intrinsic activity at human β_2-adrenoceptors *in vitro*. The mean maximum effect (E_{max}) for indacaterol was 73% of the maximum effect of isoprenaline, compared to 90%, 38% and 47% for formoterol, salmeterol and salbutamol respectively (Battram *et al.*, 2006). Similar to formoterol, indacaterol was a very weak agonist at the β_1-adrenoceptor (mean E_{max} = 16% of the maximal effect of isoprenaline) and a full agonist at the β_3-adrenoceptor (mean E_{max} = 113%) (Battram *et al.*, 2006). Studies with isolated human bronchi and small airway lung slices showed that indacaterol behaves as a high efficacy β_2-adrenoceptor agonist, with an onset of action that is not significantly different from that of formoterol and salbutamol but significantly faster than that of salmeterol, and a significantly longer duration of action than both formoterol and salmeterol (Naline *et al.*, 2007; Sturton *et al.*, 2008). In particular, a study that compared the properties of indacaterol with salmeterol, formoterol and salbutamol on small airways in precision-cut lung slices from human contracted with carbachol (Sturton *et al.*, 2008) confirmed that the onset of action is fast for salbutamol, formoterol and indacaterol, whereas it is significantly slower for salmeterol, and showed that indacaterol and formoterol had a higher intrinsic efficacy than salbutamol and salmeterol. It was also shown that indacaterol, in contrast with salmeterol, does not antagonize the bronchorelaxant effect of a fast-acting β_2-adrenoceptor agonist (Naline *et al.*, 2007).

Interestingly, no tachyphylaxis has been demonstrated for indacaterol, although significant improvement in protection against 5-hydroxytryptamine-induced bronchoconstriction has been documented after 5 day dosing of indacaterol and formoterol (compared with a single treatment), but not with salmeterol, at least in guinea pig (Battram *et al.*, 2006). The fact that indacaterol behaves as a nearly full β_2-adrenoceptor agonist could explain why indacaterol does not induce tachyphylaxis and also does not antagonize the bronchorelaxant effect of a fast-acting β_2-adrenoceptor agonist. Although low-efficacy agonists may cause less receptor desensitization at equal occupancy, they require more

receptors to generate a subsequent response so they will be more sensitive to loss of functional receptors (Charlton, 2009). High-efficacy agonists, in contrast, may cause a greater loss of receptors, but are more tolerant to this, as they have 'spare receptors', resulting in a loss in potency but not necessarily maximal effect and are, therefore, less sensitive to loss of receptors through desensitization (Charlton, 2009).

Preclinical data also suggest that, for a given degree of bronchodilator activity, indacaterol has a greater cardiovascular safety margin than formoterol or salmeterol (Battram *et al.*, 2006).

The faster onset of action and longer duration of action of indacaterol compared with some other β_2-adrenoceptor agonists may be related to lipid membrane interactions (Lombardi *et al.*, 2009). Thus, indacaterol and salmeterol show no major but several minor differences in their steady state and kinetic interactions with lipid membranes. The sum of these small differences, including higher partitioning of indacaterol into the microenvironment of the receptor and its faster membrane permeation, is likely to contribute to its faster onset and longer duration of therapeutic action. A striking difference was observed in the effect of the two compounds on membrane fluidity. While indacaterol did not alter membrane fluidity, salmeterol drastically increased membrane fluidity. This may affect the function of the receptor reducing the intrinsic efficacy of the compound (Lombardi *et al.*, 2009). It has also been suggested that lipid rafts, which are areas of cell membranes where β_2-adrenoceptors are held together in close contact with signalling molecules and effectors, and calveolae, which are a special type of lipid raft being small (50–100 nm) invaginations of the plasma membrane, in airway smooth muscle might play a role in long duration of action of indacaterol (Lombardi *et al.*, 2009). Indacaterol has twofold higher affinity for raft microdomains compared to salmeterol, and this might contribute to the difference in duration of action. Recently, it has also be suggested that indacaterol utilizes higher intrinsic efficacy to offset the high lipophilicity that is important for achieving long duration of action (Rosethorne *et al.*, 2010). In fact, in primary human bronchial smooth muscle cells, indacaterol displays a similar intrinsic activity to formoterol which, combined with comparable lipophilicity, translates to a fast rate of cyclic adenosine monophosphate (cAMP) accumulation, which plays a key role in β_2-adrenoceptor-induced smooth muscle relaxation in the airways.

After inhalation, indacaterol is rapidly absorbed into the systemic circulation with a median T_{max} of 15 min. It has linear and dose-proportional pharmacokinetics (PK), and steady state is reached within 12 days of once-daily dosing at doses of 150, 300 and 600 μg (Perry *et al.*, 2010).

Although the already mentioned issue about the long-term safety of LABAs in asthma mainly when used as monotherapy, several short-term studies have explored the effect of indacaterol in asthmatic patients (Cazzola *et al.*, 2010b). In particular, two large 7 day dose finding trials examined the effect of indacaterol 100, 200, 300 400 or 600 μg once daily, via single-dose dry powder inhaler (SDDPI) (Kanniess *et al.*, 2008a), indacaterol 50, 100, 200 or 400 μg via multiple-dose dry powder inhaler (MDDPI) and 400 μg via SDDPI once daily (LaForce *et al.*, 2008) in patients with persistent asthma respectively. Once-daily dosing with indacaterol provided sustained 24 h bronchodilation in patients with moderate to severe asthma, with a satisfactory overall safety profile. In the first study, mean forced expiratory volume in the 1st second (FEV_1) for indacaterol doses 200 μg or more on day 7 was higher than placebo predose and at all postdose time points (Kanniess *et al.*, 2008a). In the second study, all doses of indacaterol provided rapid onset, sustained 24 h bronchodilator efficacy on once-daily dosing from day 1, with no loss of efficacy after 7 days of treatment, although indacaterol 200 μg appeared to be the optimum dose, offering the best efficacy/safety balance (LaForce *et al.*, 2008).

In COPD, single doses of indacaterol 150 and 300 μg demonstrate a fast onset of action similar to that for salbutamol and faster than that for salmeterol-fluticasone (Balint *et al.*, 2010). Moreover, once-daily indacaterol 150 μg is at least as effective as tiotropium, with a faster onset of action (within 5 min) on the first day of dosing (Vogelmeier *et al.*, 2010).

The efficacy of indacaterol in the maintenance treatment of COPD in adults has been assessed in large, randomized, double-blind, parallel-group, placebo-controlled, multicentre phase III trials [Chapman *et al.*, (2011); Dahl *et al.*, 2010; Donohue *et al.*, 2010; Feldman *et al.*, 2010; Kornmann *et al.*, 2010; Laforce *et al.*, 2010]. The analysis of these trials (Moen, 2010) shows that indacaterol 150 and/or 300 μg once daily was more effective than tiotropium, formoterol or salmeterol for improving trough FEV_1 values versus placebo. COPD exacerbations were significantly reduced versus placebo for indacaterol 150 μg or 300 μg once daily. In a 52 week study (Dahl *et al.*, 2010), once-daily treatment with indacaterol prolonged the time to first COPD exacerbation and was effective in reducing incidence and frequency of COPD exacerbations, with no significant difference between indacaterol and formoterol. Patients treated with indacaterol had a significantly higher percentage of days with no use of as-needed rescue salbutamol than placebo recipients in all large studies. Moreover, the percentages of days with no rescue medication were significantly ($P < 0.05$) higher in the indacaterol groups than the active comparator groups in all studies. In general, indacaterol appeared to have greater effects on most COPD symptoms than tiotropium, formoterol or salmeterol, although differences between indacaterol and active comparators were not consistently statistically significant. Indacaterol also provided significant and clinically relevant better health-related quality of life. In all studies designed to investigate whether indacaterol has the same tolerability of LABAs already in the market, indacaterol was well tolerated at all doses and with a good overall safety profile. The most common treatment-emergent adverse events were COPD worsening and nasopharyngitis. Study investigators also recorded the incidence of cough. Cough was, however, mild in severity, transient in nature and duration, and tended to decline with the duration of treatment (Cazzola *et al.*, 2010a). Decreases in serum potassium levels to <3.0 mM were rare (≤0.5% of patients) in all treatment groups including placebo, and also increases in corrected QT (QTc) interval values of greater than 60 ms were unusual (≤0.7% of patients).

Olodaterol

Olodaterol, previously known as BI 1744 CL, is a novel, enantiopure inhaled β_2-adrenoceptor agonist. It was identified from a series of 6-hydroxy-4*H*-benzo[1,4]oxazin-3-ones

as potent agonist of the human β_2-adrenoceptor with a high β_1/β_2-selectivity (Bouyssou *et al.*, 2010b). *In vitro*, olodaterol showed a potent, nearly full agonistic response at the human β_2-adrenoceptor [median effective concentration required to induce a 50% effect (EC_{50}) = 0.1 nM; intrinsic activity = 88% compared with isoprenaline] and, unlike formoterol and salmeterol that exerted either a full-agonistic or a partial agonistic profile for all β-adrenoceptors, a significant selectivity profile (219- and 1622-fold against the human β_1- and human β_3-adrenoceptors respectively) (Bouyssou *et al.*, 2010a). On isolated human bronchi, olodaterol dose-dependently reversed the constriction induced by different stimuli, such as histamine, ACh, and EFS with an efficacy not statistically different from the nearly full agonist formoterol under all conditions (Bouyssou *et al.*, 2010a). Olodaterol and formoterol exhibited similar potencies and E_{max} at resting tone and in the presence of contracting stimuli. Formoterol induced significant β_2-adrenoceptor desensitization *in vitro*, whereas olodaterol preserved the β_2-adrenoceptor signalling capacity even after long-term pre-incubation (Naline *et al.*, 2010).

In vivo, antagonistic effects of single doses of olodaterol and formoterol were measured against ACh challenges in anesthetized guinea pigs and dogs for up to 24 h by using the Respimat® Soft Mist™ inhaler (Boehringer Ingelheim, Ingelheim, Germany). In both models, olodaterol provided bronchoprotection over 24 h. Formoterol applied at an equally effective dose did not retain efficacy over 24 h. In both models, olodaterol showed a rapid onset of action comparable with formoterol (Bouyssou *et al.*, 2010a). Interestingly, olodaterol has a biphasic dissociation profile from the human β_2-adrenoceptors, with the slow component (approx. 30–40% of the total β_2-adrenoceptor pool) showing a half life of dissociation of more than 12 h, providing a rationale for its long duration of action in human therapy (Schnapp *et al.*, 2009).

Initial studies achieved their objective by providing proof of concept of the 24 h bronchoprotective effect of olodaterol in patients with intermittent asthma (O'Byrne *et al.*, 2009) or COPD (van Noord *et al.*, 2008) and the 24 h bronchodilation following 4 weeks once-daily administration in COPD (van Noord *et al.*, 2009).

Vilanterol

Vilanterol, also known as GSK-642444, is the triphenylacetate salt of the 2,6-dichlorobenzyl analogue of a series of novel β_2-adrenoceptor agonists obtained by the incorporation of an oxygen atom at the homobenzylic position of the right-hand side phenyl ring of (R)-salmeterol (Procopiou *et al.*, 2010). The triphenylacetate salt was found to have suitable properties for inhaled administration.

Vilanterol is a potent, selective, β_2-adrenoceptor agonist in human functional cellular assays. Vilanterol has a greater intrinsic efficacy than salmeterol and a greater potency than indacaterol and salbutamol. In addition, it has been shown using human recombinant $\beta_{1/2/3}$-adrenoceptor cAMP assays that it has significantly greater β_2-adrenoceptor selectivity than formoterol, indacaterol and salbutamol (Procopiou *et al.*, 2010; Barrett *et al.*, 2010a). In isolated, electrically stimulated, guinea pig trachea strips, vilanterol [negative logarithm of EC_{50} (pEC_{50}) = 8.6] was equipotent with formoterol (pEC_{50} = 8.6) and more potent than salmeterol (pEC_{50} = 6.7) or indacaterol (pEC_{50} = 7.0) and was shown to be antagonized in a competitive manner by propranolol (Ford *et al.*, 2010a). Vilanterol, formoterol and indacaterol had a more rapid onset of activity when compared with salmeterol. On removal of vilanterol, the tissue showed no recovery after 1 h, suggesting a long duration of action in contrast with isoprenaline (rapid and full recovery) or formoterol (slow but continuous recovery) (Ford *et al.*, 2010a). In human tissue pre-constricted with 0.1 μM histamine, 1 nM vilanterol was shown to have a significantly faster onset of action (3.1 min) than 1 nM salmeterol (8.3 min) (Barrett *et al.*, 2010b). Vilanterol had a significantly longer duration of action compared to salmeterol as vilanterol still demonstrated a significant bronchodilator effect at 22 h, but salmeterol did not.

Vilanterol inhibited histamine-induced bronchoconstriction when administered to conscious guinea pigs as a nebulized solution, and was equipotent to salmeterol (Ford *et al.*, 2010a). At equieffective doses, the duration of action was similar to salmeterol (10 h) and longer than formoterol (<3 h); however unlike salmeterol, vilanterol duration increased in a dose-dependent manner.

Interestingly, vilanterol is a metabolically labile LABA, which undergoes conversion in human microsomes to metabolites with significantly lower β_2-adrenoceptor activity and exhibits low systemic exposure *in vivo* after inhaled dosing (Ford *et al.*, 2010b).

Vilanterol has been tested both in asthmatic and COPD patients. In mild to moderate persistent asthma patients, single doses of inhaled vilanterol (25–100 μg) produced a rapid and prolonged bronchodilation over 24 h, suggesting the potential for once-daily administration (Kempsford *et al.*, 2010a). All doses were well tolerated with no clinically significant unwanted systemic effects. Vilanterol (25–100 μg) produced rapid bronchodilation in COPD patients, which was maintained over 24 h at all doses (Kempsford *et al.*, 2010b). Following dosing with vilanterol, there were no serious adverse events or withdrawals due to adverse events and no clinically significant laboratory, vital sign or 12-lead electrocardiogram (ECG), QTc or Holter ECG abnormalities. Vilanterol was rapidly absorbed into plasma (median T_{max} at 10 min) with systemic exposure increasing in an approximately dose proportional manner across the vilanterol dose range (25–100 μg). A 28 day study in patients ≥12 years with persistent asthma on maintenance ICS showed that once-daily vilanterol was well tolerated and resulted in a prolonged duration of action of at least 24 h at doses ≥12.5 μg (Lötvall *et al.*, 2010a).

A 28 day dose-ranging (3, 6.25, 12.5, 25 or 50 μg) study of vilanterol in patients with COPD demonstrated statistically significant improvements in trough FEV_1 for all doses compared with placebo ($P < 0.001$) (92 mL, 98 mL, 110 mL, 137 mL and 165 mL respectively) (Hanania *et al.*, 2010b). The time to an increase of ≥100 mL in FEV_1 on Day 1 was significantly shorter for all vilanterol arms compared with placebo ($P < 0.001$) (median time of 6 min in the 25 and 50 μg groups). The same study documented that vilanterol was safe (Hanania *et al.*, 2010c). The incidence of adverse events was low (≤3%) with no apparent treatment or dose relationship. There was no clinically relevant effect on systolic or diastolic blood pressure, pulse rate or blood glucose or potassium levels.

Carmoterol

Carmoterol {CHF 4226, TA 2005; 8-hydroxy-5-[(1*R*)-1-hydroxy-2-[*N*-[(1*R*)-2-(*p*-methoxy-phenyl)-1-methylethyl]-amino]-ethyl]-carbostyril hydrochloride}, a pure (*R,R*)-isomer, is a non-catechol β_2-adrenoceptor agonist with a *p*-methoxyphenyl group on the amine side chain and a 8-hydroxyl group on the carbostyril aromatic ring (Kikkawa *et al.*, 1991), possessing structural elements from both formoterol and procaterol. It binds very firmly to β_2-adrenoceptors (Voss *et al.*, 1992), a property shared by some other agonists which, like carmoterol, are based on a carbostyril skeleton (Standifer *et al.*, 1989). In studies using chimeric β_2-adrenoceptors, the methoxyphenyl group in carmoterol has been found to be critical to the β_2-adrenoceptor selectivity of the molecule (Kikkawa *et al.*, 1998).

Carmoterol has been demonstrated to be a highly potent and selective β_2-adrenoceptor agonist [it has 53 times higher affinity for the β_2-adrenoceptors than for the β_1-adrenoceptors (Voss *et al.*, 1994), and is about five times more selective for the β_2-adrenoceptors present in the tracheal preparation than those mediating chronotropic response in the right atrium (Kikkawa *et al.*, 1998)]. Moreover, it displays a fast onset and long duration of activity both in *in vitro* and *in vivo* experimental conditions (Kikkawa *et al.*, 1991; Voss *et al.*, 1992; Kikkawa *et al.*, 1994). Interestingly, the persistence of action of carmoterol at the human recombinant β_2-adrenoceptors is similar to that of salmeterol, and longer than that of indacaterol, which is longer than that of formoterol (Summerhill *et al.*, 2008). In a study that measured the rate of accumulation of cAMP in primary human bronchial smooth muscle cells and compared this with measures of intrinsic activity in the same systems, carmoterol displayed a similar intrinsic activity to formoterol which, combined with comparable lipohilicity, translated to a fast rate of cAMP accumulation (Rosethorne *et al.*, 2010).

Carmoterol is more potent than other LABAs in methacholine precontracted guinea-pig tracheal smooth muscle (Kikkawa *et al.*, 1991; Voss *et al.*, 1992; Voss, 1994). Carmoterol has a similar onset of action compared to salbutamol and formoterol, and a faster onset of action compared to salmeterol. Furthermore, the duration of tracheal smooth muscle relaxation is longer for carmoterol compared to both formoterol and salmeterol (Voss, 1994).

In asthmatic patients, the PK of carmoterol is proportional to the dose, and nonlinear accumulation of the drug after repeated dosing treatments is negligible (Cazzola *et al.*, 2010b). Interestingly, using Modulite™ technology (Chiesi Farmaceutici, Parma, Italy) that utilizes hydrofluoroalkane (HFA) 134a as propellant, a lung deposition of carmoterol as high as 41% of the nominal dose can be reached (Haeussermann *et al.*, 2006).

In patients with persistent asthma carmoterol 2 μg administered once daily is as effective as formoterol 12 μg twice daily (Kottakis *et al.*, 2006). Safety and tolerability results are similar between carmoterol and formoterol (Nandeuil *et al.*, 2006).

In COPD, a single 4 μg – but not 1 or 2 μg – dose of carmoterol had an effect on 24 h trough FEV_1 that was better than that of two 50 μg doses of salmeterol given 12 h apart (Kanniess *et al.*, 2008b). After a 2 week treatment, once-daily doses of 2 and 4 μg carmoterol resulted in placebo-adjusted improvements compared to baseline in trough FEV_1 of 94 and 112 mL, respectively, whereas 50 μg salmeterol twice-daily resulted in an increase of 78 mL (Make *et al.*, 2008). There were no significant changes in ECG results, blood pressure or serum potassium or glucose levels compared with salmeterol or placebo (Bateman *et al.*, 2008b). No tolerance to the bronchodilatory effects of carmoterol or salmeterol was observed over the 2 weeks of treatment (Rossing *et al.*, 2008).

LAS100977

LAS100977 is a novel once-daily LABA. Its *in vitro* pharmacological profile has recently been reported (Aparici *et al.*, 2010). In radioligand displacement assays by using human β_1-, β_2- and β_3-adrenoceptors expressed in cell lines, it showed the highest β_2-adrenoceptor affinity (0.6 nM) in comparison to reference compounds. LAS100977 was 10 times more potent than salmeterol and similar to formoterol and indacaterol. LAS100977 onset of action (10 min) was faster than salmeterol and indacaterol (19 min and 14 min, respectively) and slower than formoterol (6 min), whereas its duration of action (670 min) was longer than formoterol and salmeterol (77 min and 230 min, respectively) and comparable to indacaterol (450 min). LAS100977 demonstrated higher β_2/β_1-adrenoceptor selectivity than formoterol and indacaterol in both binding (64-fold) and tissue functional studies (10 750-fold). In anaesthetized dogs, LAS100977 inhibited ACh-induced bronchoconstriction more potently and with a longer duration of action than salmeterol (Miralpeix *et al.*, 2010). LAS100977 also had a higher therapeutic index than salmeterol, suggesting a reduced potential for cardiac side effects in man.

In healthy subjects, LAS100977 at doses of 5, 10, 25 and 50 μg produced an increase in specific airway conductance (sGaw) at the 24 h post dose time point compared to the respective pre-dose value in contrast to placebo (Timmer *et al.*, 2010). In addition, airways resistance (Raw) decreased for LAS100977 at the 24 h post dose time point compared to the pre-dose value. The most frequent drug-related adverse events were palpitations and tremors, which were both of mild to moderate in intensity. Two preliminary clinical studies have documented 24 h duration of action. In the first study, single different once-daily doses (5, 10 and 25 μg) of LAS100977 induced a significant increase in FEV_1 compared with both placebo and salmeterol 50 μg twice-daily with a rapid onset of effect with improvements in lung function at 5 min post-dose in patients with mild to moderate asthma (Beier *et al.*, 2010). Tachycardia and tremor were seen in higher doses (Beier *et al.*, 2010). In another study, LAS100977 also demonstrated sustained efficacy during multiple-dose administrations with no significant increase in cardiovascular adverse events (Cazzola *et al.*, 2010b).

PF-610355

PF-610355 is a member of a novel series of potent and selective sulfonamide derived β_2-adrenoceptor agonists (Glossop *et al.*, 2010). The sulfonamide agonist headgroup confers high levels of intrinsic crystallinity that could relate to the acidic sulfonamide motif supporting a zwitterionic form in the solid state. Optimization of PK properties was achieved

through targeted introduction of a phenolic moiety to support rapid phase II clearance, thereby minimizing systemic exposure following inhalation and reducing systemically mediated adverse events. It is intriguing that plasma PK of orally inhaled PF-610355 are consistent and exhibit a sustained plateau after single/multiple doses, but plasma exposure is reduced in asthmatic patients, compared to healthy volunteers (Li *et al.*, 2009). In healthy subjects, duration of action of PF-610355 450 μg on airways determined by plethysmography was superior to salmeterol 50 μg by 9.77 h indicating the potential for sustained pharmacological effect in the lung (Macintyre *et al.*, 2009). A preliminary trial has documented that in asthmatic patients, PF-610355 elicits a clear dose-response for peak and trough (32 h post-dose) FEV_1. At doses of 368, 736 and 1472 μg, it produced higher peak FEV_1 than salmeterol 50 μg (Ward *et al.*, 2009).

Novel combinations with ultra-LABAs

A range of once-daily LABAs (ultra-LABAs) and LAMAs fixed-dose combinations, including QVA149 [the combination of indacaterol and the LAMA NVA237 (glycopyrronium)], olodaterol + tiotropium, and vilanterol + GSK-573719, are in clinical development as fixed combinations (Cazzola and Matera, 2008; 2009). There is documentation that a 7-day treatment with QVA149 (indacaterol 300 μg/glycopyrronium 50 μg) once daily via single dose dry powder inhaler is more effective than indacaterol 300 and 600 μg (van Noord *et al.*, 2010). Moreover, QVA149 at the dosage of 600/100 μg, 300/100 μg or 150/100 μg has a safe cardiovascular profile, with no different 24-h mean heart rate at day 14 between QVA149 and placebo, nor between QVA149 and indacaterol, and no clinically relevant differences in QTc intervals observed among treatment groups on days 1, 7 and 14 (van de Maele *et al.*, 2010). Addition of olodaterol enhanced the beneficial effect of the tiotropium monotherapy on ACh-induced bronchoconstriction with a longer duration of action in anaesthetized dogs (Bouyssou *et al.*, 2010c). It has also been shown that olodaterol/tiotropium (10/5 μg) fixed-dose combination administered using the Respimat® Soft Mist™ inhaler is more effective than tiotropium 5 μg in COPD patients, with superior bronchodilation over 24 h following 4 weeks once-daily dosing (Maltais *et al.*, 2010).

Drugs with a bifunctional mechanism of action, combining both muscarinic antagonist and β_2-adrenoceptor agonist pharmacology in a single molecule, which are known as dual-acting muscarinic antagonist/β_2-adrenoceptor agonist (MABA) bronchodilators, are a different interesting approach (Norman, 2006). MABAs have the advantage of delivering a fixed ratio into every region of the lung, reducing the complexity of combination inhalers (Cazzola and Matera, 2009).

TEI3252 is a novel bifunctional bronchodilator that, in experimental setting, showed bronchoprotective activities against acethylcholine and histamine stimulation in a dose-dependent manner at the dose range of 1–5 $\mu g \cdot kg^{-1}$ (Sugiyama *et al.*, 2010). Its efficacy was long lasting compared with existing bronchodilators, such as tiotropium and indacaterol. On the other hand, the inhibitory effect on salivation was not observed even at the dose up to 100 $\mu g \cdot kg^{-1}$. This finding suggests that TEI3252 has a reduced side effect.

Evaluation of THRX-200495, a single bifunctional molecule that possesses both muscarinic antagonist and β_2-adrenoceptor agonist pharmacology, in a guinea pig model of bronchoconstriction revealed a matched muscarinic antagonist and β_2-adrenoceptor agonist potency [median dose that causes 50% inhibition (ID_{50}) = 11.4 and 11.2 $\mu g \cdot mL^{-1}$, respectively], with similar onset of action and potent dual pharmacology (MABA ID_{50} = 3.5 $\mu g \cdot mL^{-1}$) lasting for >24 h (McNamara *et al.*, 2009).

GSK-961081 (formerly TD-5959) is a further novel bifunctional molecule. It conferred potent 24 h bronchoprotection in guinea pigs through a dual mechanism involving antagonism of muscarinic receptors and agonism of β_2-adrenoceptors. Dual pharmacology yielded bronchoprotection that was two- to fivefold more potent (MABA ID_{50} = 6.4 $mg \cdot mL^{-1}$) than either ipratropium or salbutamol alone (Pulido-Rios *et al.*, 2009). In phase I randomized double-blind placebo-controlled single and multiple-dose studies that enrolled healthy volunteers, GSK-961081 was generally well tolerated and demonstrated evidence of bronchodilation over 24 h after a single dose and after seven consecutive daily doses and, consequently, has entered into phase II (Cazzola and Matera, 2009). In a phase II study, GSK-961081 dosed at both 400 and 1200 μg once daily showed bronchoprotection at day 14 that was at least equivalent to that of 50 μg salmeterol twice-daily plus 18 μg tiotropium once-daily, as measured by changes in FEV_1 (Cazzola and Matera, 2009). Both the time to peak effect and maximum bronchodilation of GSK-961081 were numerically better than salmeterol plus tiotropium, although the study was not powered to compare the results to the salmeterol plus tiotropium control.

PF-3429281 is another inhaled dual antimuscarinic/β_2-adrenoceptor agonist. In anaesthetized guinea pigs, it caused a dose-related inhibition of ACh-induced bronchoconstriction (Philip *et al.*, 2010). This was 26-fold weaker than tiotropium and equipotent with salmeterol. Infusion of propranolol throughout the experiment blocked the effects of salmeterol on ACh-induced bronchoconstriction, whereas both tiotropium and PF-3429281 were unaffected. Data generated *in vitro* using guinea pig isolated trachea suggest that the duration of action of the β_2 component is longer than the M_3 one (Patel *et al.*, 2010). In an anaesthetized dog model of bronchoconstriction, PF-3429281 had an equivalent potency to ipratropium bromide and a superior therapeutic index and duration of action compared to salmeterol (Wright *et al.*, 2010).

Novel combinations of LABAs and ICSs under development

As combination therapy with an ICS and a LABA is considered an important approach for treating patients suffering from asthma and also severe COPD patients with frequent exacerbations (Celli and MacNee, 2004; National Asthma Education and Prevention Program, 2007; Rabe *et al.*, 2007; Bateman *et al.*, 2008a), there is a strong interest in developing a once-daily combination therapy, again in an attempt to simplify the treatment, and also to overcome the loss of patent protection. The awareness that new ICSs, such as ciclesonide, fluticasone furoate and mometasone furoate, which can be used as a once-daily dosing, have been developed or are in development, have further supported the development of new ultra-LABAs that can be used on a

once-a-day basis (Cazzola and Matera, 2009), although a combination of formoterol and mometasone furoate administered on a twice-daily basis has been developed and successfully tested in asthma (Maspero *et al.*, 2010).

A new inhaled therapy will combine indacaterol with mometasone (QMF-149) or indacaterol and QAE-397, a novel corticosteroid in phase II development for the treatment of asthma (Cazzola and Matera, 2009). In particular, two trials have investigated the safety and tolerability of QMF-149. The first was designed to evaluate the bronchodilatory efficacy of QMF-149 delivered via a MDDPI (Twisthaler) in adult patients with persistent asthma using open-label salmeterol/fluticasone (50/250 µg twice daily) as an active control (Cazzola and Matera, 2009), whereas the second one investigated the safety and tolerability of 14 days treatment with QMF-149 500/800 µg in patients with mild to moderate asthma (Cazzola and Matera, 2009). The results of these trials have not been released yet.

A next-generation, once-daily combination consisting of vilanterol and fluticasone furoate is another combination under development. A small study that enrolled 60 COPD (GOLD Stage II–III) patients documented that this combination had greater improvements than placebo in trough FEV_1 after a 4 week treatment with a good safety and tolerability profile (Lötvall *et al.*, 2010b).

A positive interaction of carmoterol with budesonide in the control of bronchoconstriction induced by acetaldehyde in the guinea pigs has been documented (Rossoni *et al.*, 2005). Intriguingly, carmoterol/budesonide was twofold more effective than the formoterol/budesonide combination. The finding suggests that carmoterol/budesonide, by optimizing each other's beneficial pharmacological potential, may represent a new fixed combination in asthma. In effect, in mild or moderate asthmatic patients, the systemic exposure to carmoterol 2 µg and budesonide 400 µg was not increased with the combination in comparison with each component administered alone (Poli *et al.*, 2009). Moreover, a prolonged bronchodilatation was observed with carmoterol/budesonide combination (Poli *et al.*, 2009). The fixed combination carmoterol/budesonide formulated as HFA 134a pMDI (Chiesi Modulite™ HFA technology) administered once a day in asthmatic patients maintained the bronchodilator effect over 24 h and was as effective as formoterol/budesonide Turbuhaler twice a day in patients with moderate or severe persistent asthma (Woodcock *et al.*, 2009).

Intravenous β_2-adrenoceptor agonist

An interesting new option is the development of a β_2-adrenoceptor agonist to be administered intravenously. Bedoradrine (MN-221) is a novel, highly selective β_2-adrenoceptor agonist under development for the treatment of acute exacerbation of asthma and COPD. Bedoradrine was calculated to be 832- and 126-fold more selective for β_2-adrenoceptors than for β_1- and β_3-adrenoceptors, respectively, which indicates that it is very highly selective for β_2-adrenoceptor in this assay system (Inoue *et al.*, 2009). However, it was initially considered for the treatment of preterm labour because it is 1590-fold more specific for the uterus than for the trachea (Inoue *et al.*, 2009).

The PK and pharmacodynamics of bedoradrine were investigated using data from a single i.v. dose study in stable moderate to severe COPD patients (Sadler *et al.*, 2010). Patients receiving doses of 600 and 1200 µg showed superior response to those receiving 300 µg. Patients receiving doses of 600 and 1200 µg showed superior response to those receiving 300 µg. At 1200 µg, the mean peak FEV_1 increase was about 55% of maximal lending support to this dose.

A basic study provided evidence that, while both salbutamol and bedoradrine induced an increase in heart rate independently, adverse effects on heart rate were not observed upon combination in dogs. Other cardiovascular parameters (QTc and monophasic action potential) were not adversely affected in the salbutamol + bedoradrine combination. These findings are consistent with some other data implicating bedoradrine as a partial agonist at β_1-adrenoceptors (Johnson *et al.*, 2010).

A study that evaluated the safety and tolerability of bedoradrine at doses of 150 to 900 µg via intravenous infusion in patients with mild to moderate stable asthma documented that it was safe and well tolerated, with dose-dependent improvements in FEV_1 (Matsuda *et al.*, 2010). A preliminary small trial showed that bedoradrine added to standard therapy for severe acute asthma exacerbations was safe and appeared to provide additional clinical benefit (Nowak *et al.*, 2010).

In a small group of COPD patients, bedoradrine, at doses of 300, 600 or 1200 µg i.v., appeared to improve lung function at all dose levels and reached statistical significance at both 600 and 1200 µg as compared to placebo (Pearle *et al.*, 2010). FEV_1 (L) increased as compared to baseline by an average of 21.5% ($P = 0.0025$) for the 1200 µg dose, 16.2% ($P = 0.02$) for the 600 µg dose, and 9.2% (P = NS) for the 300 µg dose compared to a decrease of 4.0% for placebo. Bedoradrine was generally well tolerated by all patients.

Conclusion

This review clearly indicates that, despite the passionate and sometimes harsh debate over the use of β_2-adrenoceptor agonists in the treatment of airway disorders, the interest of pharmaceutical companies in the field is still high. However, it is difficult to determine whether this is due to the success that this class of bronchodilators still has, or just the fact that we are not yet able to identify a new class of bronchodilators that can control the bronchial muscle tone without being burdened by the risks of β_2-adrenoceptor agonists. Whatever the case may be, pending new true bronchodilators, we believe that the possibility of administering β_2-adrenoceptor agonists on the once-daily basis is an advantage because it improves convenience and compliance, controls airflow over a complete 24 h period and allows the combination with other classes of drugs, such as antimuscarinic agents and ICSs, that are fundamental for treating asthma and/or COPD and now are administered on the once-daily basis. In any case, it is still too early to indicate which of the new ultra-LABAs will meet with greater success. In fact, excluding indacaterol, which has now entered the market having completed the

pivotal phases, we have too little information on other drugs to make a prediction based on the pharmacological and clinical evidence.

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References

Agarwal SK, Marshall GD Jr (2000). Beta-adrenergic modulation of human type-1/type-2 cytokine balance. J Allergy Clin Immunol 105: 91–98.

Aldridge RE, Hancox RJ, Taylor DR, Cowan JO, Winn MC, Frampton CM *et al*. (2000). Effects of terbutaline and budesonide on sputum cells and bronchial hyperresponsiveness in asthma. Am J Respir Crit Care Med 161: 1459–1464.

Aparici M, Gómez-Angelats M, Vilella D, Cortijo J, Morcillo EJ, Carcasona C *et al*. (2010). The *in vitro* pharmacological profile of LAS100977 – a potent, selective and long-acting beta-2 receptor agonist [abstract]. Am J Respir Crit Care Med 181: A5675.

Appleton S, Poole P, Smith B, Veale A, Lasserson TJ, Chan MM (2006). Long-acting β_2-agonists for poorly reversible chronic obstructive pulmonary disease. Cochrane Database Syst Rev 3: CD001104.

Balint B, Watz H, Amos C, Owen R, Higgins M, Kramer B (2010). Onset of action of indacaterol in patients with COPD: comparison with salbutamol and salmeterol-fluticasone. Int J Chron Obstruct Pulmon Dis 5: 311–318.

Barrett VJ, Emmons A, Ford AJ, Knowles R (2010a). *In vitro* pharmacological characterisation of GW642444, a novel long acting β_2-agonist (LABA) using human recombinant $\beta_{1/2/3}$ adrenoceptor cAMP assays [abstract]. Am J Respir Crit Care Med 181: A4451.

Barrett VJ, Morrison V, Sturton RG, Ford AJ, Knowles R (2010b). Pharmacological characterisation of GW642444, a long-acting β_2-agonist (LABA) with rapid onset and long duration, on isolated large and small human airways [abstract]. Am J Respir Crit Care Med 181: A4453.

Bateman ED, Hurd SS, Barnes PJ, Bousquet J, Drazen JM, FitzGerald M *et al*. (2008a). Global strategy for asthma management and prevention: GINA executive summary. Eur Respir J 31: 143–178.

Bateman ED, Make BJ, Nandeuil MA (2008b). Carmoterol – safety and tolerability of a long-acting β_2 agonist in patients with COPD [abstract]. Proc Am Thorac Soc 5: A653.

Battram C, Charlton SJ, Cuenoud B, Dowling MR, Fairhurst RA, Farr D *et al*. (2006). *In vitro* and *in vivo* pharmacological characterization of 5-[(R)-2-(5,6-diethyl-indan-2-ylamino)-1-hydroxy-ethyl]-8-hydroxy-1H-quinolin-2-one (indacaterol), a novel inhaled β_2 adrenoceptor agonist with a 24-h duration of action. J Pharmacol Exp Ther 317: 762–770.

Baur F, Beattie D, Beer D, Bentley D, Bradley M, Bruce I *et al*. (2010). The identification of indacaterol as an ultralong-acting inhaled β_2-adrenoceptor agonist. J Med Chem 53: 3675–3684.

Beier J, Fuhr R, Massana E, Jiménez E, Seoane B, de Miquel G *et al*. (2010). Efficacy and safety of single doses of inhaled LAS100977 in patients with persistent asthma [abstract]. Am J Respir Crit Care Med 181: A5414.

Bender BG (2002). Overcoming barriers to nonadherence in asthma treatment. J Allergy Clin Immunol 109: S554–S559.

Bouyssou T, Casarosa P, Naline E, Pestel S, Konetzki I, Devillier P *et al*. (2010a). Pharmacological characterization of olodaterol, a novel inhaled β_2-adrenoceptor agonist exerting a 24-hour-long duration of action in preclinical models. J Pharmacol Exp Ther 334: 53–62.

Bouyssou T, Hoenke C, Rudolf K, Lustenberger P, Pestel S, Sieger P *et al*. (2010b). Discovery of olodaterol, a novel inhaled β_2-adrenoceptor agonist with a 24 h bronchodilatory efficacy. Bioorg Med Chem Lett 20: 1410–1414.

Bouyssou T, Schnapp A, Casarosa P, Pieper MP (2010c). Addition of the new once-daily LABA BI 1744 to tiotropium results in superior bronchoprotection in pre-clinical models [abstract]. Am J Respir Crit Care Med 181: A4445.

Calverley PM, Anderson JA, Celli B, Ferguson GT, Jenkins C, Jones PW *et al*. (2007). Salmeterol and fluticasone propionate and survival in chronic obstructive pulmonary disease. N Engl J Med 356: 775–789.

Cazzola M, Matera MG (2007). Safety of long-acting β_2-agonists in the treatment of asthma. Ther Adv Respir Dis 1: 35–46.

Cazzola M, Matera MG (2008). Novel long-acting bronchodilators for COPD and asthma. Br J Pharmacol 155: 291–299.

Cazzola M, Matera MG (2009). Emerging inhaled bronchodilators: an update. Eur Respir J 34: 757–769.

Cazzola M, Molimard M (2010). The scientific rationale for combining long-acting β_2-agonists and muscarinic antagonists in COPD. Pulm Pharmacol Ther 23: 257–267.

Cazzola M, Tashkin DP (2009). Combination of formoterol and tiotropium in the treatment of COPD: effects on lung function. COPD 6: 404–415.

Cazzola M, Spina D, Matera MG (1997). The use of bronchodilators in stable chronic obstructive pulmonary disease. Pulm Pharmacol Ther 10: 129–144.

Cazzola M, Di Perna F, Noschese P, Vinciguerra A, Calderaro F, Girbino G *et al*. (1998a). Effects of formoterol, salmeterol or oxitropium bromide on airway responses to salbutamol in COPD. Eur Respir J 11: 1337–1341.

Cazzola M, Imperatore F, Salzillo A, Di Perna F, Calderaro F, Imperatore A *et al*. (1998b). Cardiac effects of formoterol and salmeterol in patients suffering from COPD with pre-existing cardiac arrhythmias and hypoxemia. Chest 114: 411–415.

Cazzola M, Matera MG, Donner CF (2005a). Inhaled β_2-adrenoceptor agonists. cardiovascular safety in patients with obstructive lung disease. Drugs 65: 1595–1610.

Cazzola M, Matera MG, Lötvall J (2005b). Ultra long-acting β_2-agonists in development for asthma and chronic obstructive pulmonary disease. Expert Opin Investig Drugs 14: 775–783.

Cazzola M, Andò F, Santus P, Ruggeri P, Di Marco F, Sanduzzi A *et al*. (2007). A pilot study to assess the effects of combining fluticasone propionate/salmeterol and tiotropium on the airflow obstruction of patients with severe-to-very severe COPD. Pulm Pharmacol Ther 20: 556–561.

Cazzola M, Proietto A, Matera MG (2010a). Indacaterol for chronic obstructive pulmonary disease (COPD). Drugs Today (Barc) 46: 139–150.

Cazzola M, Segreti A, Matera MG (2010b). Novel bronchodilators in asthma. Curr Opin Pulm Med 16: 6–12.

Celli BR, MacNee W (2004). Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper. Eur Respir J 23: 932–946.

Chapman K, Rennard S, Dogra A, Owen R, Lassen C, Kramer B (2011). Long-term safety and efficacy of indacaterol, a novel long-acting β_2-agonist, in subjects with COPD: a randomized, placebo-controlled study. Chest doi: 10.1378/chest.10-1830 [Epub ahead of print].

Charlton SJ (2009). Agonist efficacy and receptor desensitization: from partial truths to a fuller picture. Br J Pharmacol 158: 165–168.

Chatenoud L, Malvezzi M, Pitrelli A, La Vecchia C, Bamfi F (2009). Asthma mortality and long-acting β_2-agonists in five major European countries, 1994–2004. J Asthma 46: 546–551.

Chowdhury BA, Dal Pan G (2010). The FDA and safe use of long-acting beta-agonists in the treatment of asthma. N Engl J Med 362: 1169–1171.

Chung KF, Caramori G, Adcock IM (2009). Inhaled corticosteroids as combination therapy with β-adrenergic agonists in airways disease: present and future. Eur J Clin Pharmacol 65: 853–871.

Cockcroft DW, Swystun VA (1996). Functional antagonism: tolerance produced by inhaled β_2-agonists. Thorax 51: 1051–1056.

Dahl R, Chung KF, Buhl R, Magnussen H, Nonikov V, Jack D *et al*. (2010). Efficacy of a new once-daily long-acting inhaled β_2-agonist indacaterol versus twice-daily formoterol in COPD. Thorax 65: 473–479.

Di Marco F, Milic-Emili J, Boveri B, Carlucci P, Santus P, Casanova F *et al*. (2003). Effect of inhaled bronchodilators on inspiratory capacity and dysnoea at rest in COPD. Eur Respir J 21: 86–94.

Donohue JF, Fogarty C, Lötvall J, Mahler DA, Worth H, Yorgancioglu A *et al*. (2010). Once-daily bronchodilators for chronic obstructive pulmonary disease: indacaterol versus tiotropium. Am J Respir Crit Care Med 182: 155–162.

Feldman G, Siler T, Prasad N, Jack D, Piggott S, Owen R *et al*. (2010). Efficacy and safety of indacaterol 150 µg once-daily in COPD: a double-blind, randomised, 12-week study. BMC Pulm Med 10: 11.

Ford AJ, Hughes S, Morrison V, Barrett VJ, Knowles R (2010a). *In vitro* and *in vivo* pharmacological characterisation of GW642444, a novel long-acting β_2 agonist (LABA), with fast onset and long duration in the guinea-pig [abstract]. Am J Respir Crit Care Med 181: A5677.

Ford AJ, Hughes S, Smith C, Somers G, Ranshaw L (2010b). The therapeutic index of vilanterol trifenatate [abstract]. Eur Respir J 24: 208s.

Glossop PA, Lane CA, Price DA, Bunnage ME, Lewthwaite RA, James K *et al*. (2010). Inhalation by design: novel ultra-long-acting β_2-adrenoreceptor agonists for inhaled once-daily treatment of asthma and chronic obstructive pulmonary disease that utilize a sulfonamide agonist headgroup. J Med Chem 53: 6640–6652.

Haeussermann S, Acerbi A, Brand P, Poli G, Meyer T (2006). Lung deposition of carmoterol in healthy subjects, patients with asthma and patients with COPD [abstract]. Eur Respir J 28: 211s.

Hanania NA, Dickey BF, Bond RA (2010a). Clinical implications of the intrinsic efficacy of beta-adrenoceptor drugs in asthma: full, partial and inverse agonism. Curr Opin Pulm Med 16: 1–5.

Hanania N, Feldman G, Zachgo W, Shim JJ, Crim C, Sanford L *et al*. (2010b). Dose-related efficacy of vilanterol trifenatate in COPD [abstract]. Eur Respir J 24: 217s.

Hanania N, Feldman G, Zachgo W, Shim JJ, Crim C, Sanford L *et al*. (2010c). Safety of vilanterol trifenatate in a COPD dose-ranging study [abstract]. Eur Respir J 24: 208s.

Hirst C, Calingaert B, Stanford R, Castellsague J (2010). Use of long-acting β-agonists and inhaled steroids in asthma: meta-analysis of observational studies. J Asthma 47: 439–446.

Inoue Y, Yoshizato T, Kawarabayashi T (2009). Investigation of β_2-adrenoceptor subtype selectivity and organ specificity for bedoradrine (KUR-1246), a novel tocolytic β-adrenergic receptor stimulant. J Obstet Gynaecol Res 35: 405–413.

Johnson K, Iwaki Y, Feldman M, Matsuda K, Dunton AW (2010). Cardiovascular effects of MN-221 (bedoradrine) administered with albuterol in dogs [abstract]. Chest 138: 174A.

Jones C, Santanello NC, Boccuzzi SJ, Wogen J, Strub P, Nelsen LM (2003). Adherence to prescribed treatment for asthma: evidence from pharmacy benefits data. J Asthma 40: 93–101.

Kanniess F, Boulet LP, Pierzchala W, Cameron R, Owen R, Higgins M (2008a). Efficacy and safety of indacaterol, a new 24-h β_2-agonist, in patients with asthma: a dose-ranging study. J Asthma 45: 887–892.

Kanniess F, Make BJ, Petruzzelli S (2008b). Acute effect of carmoterol, a long-acting β2-agonist, in patients with COPD [abstract]. Proc Am Thorac Soc 5: A655.

Kempsford R, Norris V, Siederer S (2010a). GW642444, a novel inhaled long-acting Beta$_2$ adrenoceptor agonist (LABA), at single doses of 25, 50 and 100mcg, is well tolerated and demonstrates prolonged bronchodilation in asthmatic patients [abstract]. Am J Respir Crit Care Med 181: A5413.

Kempsford R, Norris V, Siederer S (2010b). GW642444, a novel inhaled long-acting Beta$_2$ adrenoceptor agonist (LABA), at single doses of 25, 50 and 100mcg, is well tolerated and demonstrates prolonged bronchodilation in COPD patients [abstract]. Am J Respir Crit Care Med 181: A4447.

Kikkawa H, Naito K, Ikezawa K (1991). Tracheal relaxing effects and β_2-selectivity of TA-2005, a newly developed bronchodilating agent, in isolated guinea-pig tissues. Jpn J Pharmacol 57: 175–185.

Kikkawa H, Kanno K, Ikezawa K (1994). TA-2005, a novel, long-acting and selective β_2-adrenocepter agonist: characterization of its *in vivo* bronchodilating action in guinea pigs and cats in comparison with other β_2-agonists. Biol Pharm Bull 17: 1047–1052.

Kikkawa H, Isogaya M, Nagao T, Kurose H (1998). The role of the seventh transmembrane region in high affinity binding of a β_2-selective agonist TA-2005. Mol Pharmacol 53: 128–134.

Kliber A, Lynd LD, Sin DD (2010). The effects of long-acting bronchodilators on total mortality in patients with stable chronic obstructive pulmonary disease. Respir Res 11: 56.

Kornmann O, Dahl R, Centanni S, Dogra A, Owen R, Lassen C *et al.* (2010). Once-daily indacaterol vs twice-daily salmeterol for COPD: a placebo-controlled comparison. Eur Respir J doi: 10.1183/09031936.00045810 [Epub ahead of print].

Kottakis I, Nandeuil A, Raptis H, Savu A, Linberg SE, Woodcock AA (2006). Efficacy of the novel very long-acting β_2-agonist carmoterol following 7 days once daily dosing: comparison with twice daily formoterol in patient with persistent asthma [abstract]. Eur Respir J 28: 665s.

LaForce C, Alexander M, Deckelmann R, Fabbri LM, Aisanov Z, Cameron R *et al.* (2008). Indacaterol provides sustained 24 h bronchodilation on once-daily dosing in asthma: a 7-day dose-ranging study. Allergy 63: 103–111.

Laforce C, Aumann J, de Teresa Parreño L, Iqbal A, Young D, Owen R *et al.* (2010). Sustained 24-hour efficacy of once daily indacaterol (300 μg) in patients with chronic obstructive pulmonary disease: a randomized, crossover study. Pulm Pharmacol Ther doi:10.1016/j.pupt.2010.06.005 [Epub ahead of print].

Lai CK, Twentyman OP, Holgate ST (1989). The effect of an increase in inhaled allergen dose after rimiterol hydrobromide on the occurrence and magnitude of the late asthmatic response and the associated change in nonspecific bronchial responsiveness. Am Rev Respir Dis 140: 917–923.

Lazarus SC, Boushey HA, Fahy JV, Chinchilli VM, Lemanske RF Jr, Sorkness CA *et al.* (2001). Long-acting β_2-agonist monotherapy vs continued therapy with inhaled corticosteroids in patients with persistent asthma: a randomized controlled trial. JAMA 285: 2583–2593.

Li GL, Mac Intyre F, Surujbally B, Chong CL, Davis J (2009). Pharmacokinetics of PF-00610355, a novel inhaled long-acting β_2-adrenoreceptor agonist [abstract]. Eur Respir J 34: 777s.

Lombardi D, Cuenoud B, Krämer SD (2009). Lipid membrane interactions of indacaterol and salmeterol: do they influence their pharmacological properties? Eur J Pharm Sci 38: 533–547.

Lötvall J, Bateman ED, Bleecker ER, Busse W, Woodcock A, Follows R *et al.* (2010a). Dose-related efficacy of vilanterol trifenatate, a long-acting beta$_2$ agonist with inherent 24-hour activity, in patients with persistent asthma [abstract]. Eur Respir J 24: 1013s.

Lötvall J, Bakke P, Bjermer L, Steinshamn S, Crim C, Sanford L *et al.* (2010b). Safety and efficacy of fluticasone furoate/vilanterol trifenatate in COPD patients [abstract]. Eur Respir J 24: 1013s.

Macintyre F, Jones I, Surujbally B (2009). A randomised, double-blind study to determine the duration of action of lung pharmacodynamics by plethysmography (sGaw) of a β_2 adrenoreceptor agonist, PF-00610355 [abstract]. Eur Respir J 34: 344s.

McNamara A, Kwan K, Martin WJ, Stangeland E, Hegde S, Mammen M *et al.* (2009). Comparative preclinical efficacy of combivent and a novel, bifunctional muscarinic antagonist and β2-adrenergic agonist [abstract]. Am J Respir Crit Care Med 179: A4573.

van de Maele B, Fabbri LM, Martin C, Horton R, Dolker M, Overend T (2010). Cardiovascular safety of QVA149, a combination of indacaterol and NVA237, in COPD patients. COPD 7: 418–427.

Make BJ, Kanniess F, Bateman ED, Linberg SE (2008). Efficacy of 3 different doses of carmoterol, a long-acting β_2-agonist in patients with COPD [abstract]. Proc Am Thorac Soc 5: A961.

Maltais F, Beck E, Webster D, Maleki-Yazdi MR, Seibt JV, Arnoux A *et al.* (2010). Four weeks once daily treatment with tiotropium+ olodaterol (BI 1744) fixed dose combination compared with tiotropium in COPD patients [abstract]. Eur Respir J 24: 1014s.

Maspero JF, Nolte H, Chérrez-Ojeda I (2010). Long-term safety of mometasone furoate/formoterol combination for treatment of patients with persistent asthma. J Asthma 47: 1106–1115.

Matsuda K, Iwaki Y, Feldman M, Dunton AW (2010). Evaluation of MN-221 (bedoradrine), a novel, highly selective beta2-adrenergic receptor agonist in mild to moderate asthma via intravenous infusion [abstract]. Chest 138: 167A.

Miralpeix M, Gómez-Angelats M, Aparici M, Viñals M, Beleta J, Gavaldà A *et al.* (2010). LAS100977, a novel β_2-agonist, has a longer duration of action and a more favourable therapeutic index than salmeterol in anaesthetised dogs [abstract]. Eur Respir J 24: 218s.

Moen MD (2010). Indacaterol in chronic obstructive pulmonary disease. Drugs 70: 2269–2280.

Naline E, Ostermann A, Devillier P, Casarosa P (2010). β_2 agonist intrinsic activity: comparison of BI 1744 and formoterol in different functional settings [abstract]. Am J Respir Crit Care Med 181: A4443.

Naline E, Trifilieff A, Fairhurst RA, Advenier C, Molimard M (2007). Effect of indacaterol, a novel long acting β_2-agonist, on isolated human bronchi. Eur Respir J 29: 575–581.

Nandeuil A, Kottakis I, Raptis H, Roslan H, Ivanov Y, Woodcock A (2006). Safety and tolerability of the novel very long acting β_2-agonist carmoterol given as a 2 μg qd dose; 8 days comparison with formoterol and placebo in patients with persistent asthma [abstract]. Eur Respir J 28: 665s.

National Asthma Education and Prevention Program (2007). Expert Panel Report 3 (EPR-3): guidelines for the diagnosis and management of asthma-summary report 2007. J Allergy Clin Immunol 120 (Suppl.): S94–S138.

van Noord JA, Smeets JJ, Drenth BM, Pivovarova A, Hamilton AL, Cornelissen PJG (2008). Single doses of BI 1744 CL, a novel long-acting β_2-agonist, are effective for up to 24 hrs in COPD patients [abstract]. Proc Am Thorac Soc 5: A961.

van Noord JA, Korducki L, Hamilton AL, Koker P (2009). Four weeks once daily treatment with BI 1744 CL, a novel long-acting β_2-agonist, is effective in COPD patients [abstract]. Am J Respir Crit Care Med 179: A6183.

van Noord JA, Buhl R, Laforce C, Martin C, Jones F, Dolker M *et al.* (2010). QVA149 demonstrates superior bronchodilation compared with indacaterol or placebo in patients with chronic obstructive pulmonary disease. Thorax 65: 1086–1091.

Norman P (2006). Dual-acting β_2 agonists/muscarinic antagonists. Expert Opin Ther Pat 16: 1327–1331.

Nowak R, Iwaki Y, Matsuda K, Johnson K, Dunton AW (2010). Reduced hospital admission and improved pulmonary function following intravenous MN-221 (bedoradrine), a novel, highly selective beta2-adrenergic receptor agonist, adjunctive to standard of care in severe acute exacerbation of asthma [abstract]. Chest 138: 66A.

O'Byrne P, Van der Linde J, Cockroft DW, Brannan JD, Fitzgerald M, Watson RM *et al.* (2009). Prolonged bronchoprotection against inhaled methacholine by inhaled BI 1744, a long-acting β_2-agonist, in patients with mild asthma. J Allergy Clin Immunol 124: 1217–1221.

O'Donnell DE, Voduc N, Fitzpatrick M, Webb KA (2004). Effect of salmeterol on the ventilatory response to exercise in chronic obstructive pulmonary disease. Eur Respir J 24: 86–94.

Panina-Bordignon P, Mazzeo D, Lucia PD, D'Ambrosio D, Lang R, Fabbri L *et al.* (1997). β_2-agonists prevent Th1 development by selective inhibition of interleukin 12. J Clin Invest 100: 1513–1519.

Patel S, Marshall S, Summerhill S, Strawbridge M, Stanley M, Stuart E *et al.* (2010). The *in-vitro* pharmacology of PF-3429281 – A novel inhaled dual antimuscarinic/β_2 adrenoceptor agonist [abstract]. Eur Respir J 24: 218s.

Pearle J, Iwaki Y, Dunton AW, Kitt E, Ruby K (2010). Intravenous MN-221, a novel, highly selective beta2-adrenergic receptor agonist, improves lung function in stable moderate to severe chronic obstructive pulmonary disease patients [abstract]. Chest 138: 487A.

Perry S, Woessner R, Kaiser G, Campestrini J, Picard F, Khindri S *et al.* (2010). Pharmacokinetics of indacaterol after single and multiple inhaled doses [abstract]. Am J Respir Crit Care Med 181: A4420.

Philip J, Gray A, Clarke N, Yeadon M, Perros-Huguet C (2010). Demonstration of dual pharmacology *in vivo* of PF-3429281: a novel inhaled dual antimuscarinic/β_2 agonist [abstract]. Eur Respir J 24: 218s.

Poli G, Devolder A, De Backer W, Acerbi D, Nandeuil A, De Decker M *et al.* (2009). Pharmacokinetic and pharmacodynamic interaction of carmoterol and budesonide in asthmatic patients [abstract]. Am J Respir Crit Care Med 179: A2789.

Procopiou PA, Barrett VJ, Bevan NJ, Biggadike K, Box PC, Butchers PR *et al.* (2010). Synthesis and structure-activity relationships of long-acting β_2 adrenergic receptor agonists incorporating metabolic inactivation: an antedrug approach. J Med Chem 53: 4522–4530.

Pulido-Rios MT, McNamara A, Kwan K, Martin W, Thomas R, Mammen M *et al.* (2009). TD–5959: a novel bifunctional muscarinic antagonist – β_2–adrenergic agonist with potent and sustained *in vivo* bronchodilator activity in guinea pigs [abstract]. Am J Respir Crit Care Med 179: A6195.

Rabe KF, Hurd S, Anzueto A, Barnes PJ, Buist SA, Calverley P *et al.* (2007). Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. Am J Respir Crit Care Med 176: 532–555.

Rodrigo GJ, Moral VP, Marcos LG, Castro-Rodriguez JA (2009). Safety of regular use of long-acting β-agonists as monotherapy or added to inhaled corticosteroids in asthma. A systematic review. Pulm Pharmacol Ther 22: 9–19.

Rogers DF (2005). Mucociliary dysfunction in COPD: effect of current pharmacotherapeutic options. Pulm Pharmacol Ther 18: 1–8.

Rosethorne EM, Turner RJ, Fairhurst RA, Charlton SJ (2010). Efficacy is a contributing factor to the clinical onset of bronchodilation of inhaled β_2-adrenoceptor agonists. Naunyn Schmiedebergs Arch Pharmacol 382: 255–263.

Rossi A, Khirani S, Cazzola M (2008). Long-acting β_2-agonists (LABA) in chronic obstructive pulmonary disease: efficacy and safety. Int J Chron Obstruct Pulmon Dis 3: 521–529.

Rossing TH, Make BJ, Heyman ER (2008). Carmoterol does not induce tolerance in COPD [abstract]. Proc Am Thorac Soc 5: A962.

Rossoni G, Manfredi B, Razzetti R, Civelli M, Bongrani S, Berti F (2005). Positive interaction of the β_2-agonist CHF 4226.01 with budesonide in the control of bronchoconstriction induced by acetaldehyde in the guinea-pigs. Br J Pharmacol 144: 422–429.

Sadler BM, Dunton AW, Kitt E, Bosley J, Beaver R (2010). Pharmacokinetics and pharmacodynamics of MN-221, a novel, highly selective beta2-adrenergic agonist for treatment of acute chronic obstructive pulmonary disease [abstract]. Chest 138: 471A.

Salpeter SR, Ormiston TM, Salpeter EE (2004). Cardiovascular effects of β-agonists in patients with asthma and COPD: a meta-analysis. Chest 125: 2309–2321.

Schnapp A, Pieper MP, Bouyssou T, Gantner F, Casarosa P (2009). In vitro characterization of BI 1744 CL, a novel long acting β_2 agonist: investigations to understand its long duration of action [abstract]. Am J Respir Crit Care Med 179: A1919.

Singh D, Brooks J, Hagan G, Cahn A, O'Connor BJ (2008). Superiority of 'triple' therapy with salmeterol/fluticasone propionate and tiotropium bromide versus individual components in moderate to severe COPD. Thorax 63: 592–598.

Standifer KM, Pitha J, Baker SP (1989). Carbostyril-based β-adrenergic agonists: evidence for long lasting or apparent irreversible receptor binding and activation of adenylate cyclase activity in vitro. Naunyn-Schmiedebergs Arch Pharmacol 339: 129–137.

Sturton RG, Trifilieff A, Nicholson AG, Barnes PJ (2008). Pharmacological characterization of indacaterol, a novel once daily inhaled β_2 adrenoceptor agonist, on small airways in human and rat precision-cut lung slices. J Pharmacol Exp Ther 324: 70–75.

Sugiyama H, Nomura J, Hara T, Mitsuyama E, Igarashi J, Yamanaka Y (2010). Pharmacological profile of a novel bronchodilator, TEI3252, as bifunctional M3 antagonist and beta2 agonist [abstract]. Am J Respir Crit Care Med 181: A4436.

Summerhill S, Stroud T, Nagendra R, Perros-Huguet C, Trevethick M (2008). A cell-based assay to assess the persistence of action of agonists acting at recombinant human β_2 adrenoceptors. Pharmacol Toxicol Methods 58: 189–197.

Taylor DR (2009). The β-agonist saga and its clinical relevance: on and on it goes. Am J Respir Crit Care Med 179: 976–978.

Taylor DR, Sears MR, Cockcroft DW (1996). The β-agonist controversy. Med Clin North Am 80: 719–748.

Timmer W, Massana È, Jiménez E, Seoane B, de Miquel G, Ruiz S (2010). Single doses of LAS100977, a novel long acting β2-agonist, show high activity and long duration in healthy subjects [abstract]. Am J Respir Crit Care Med 181: A5663.

Vogelmeier C, Ramos-Barbon D, Jack D, Piggott S, Owen R, Higgins M *et al.* (2010). Indacaterol provides 24-hour bronchodilation in COPD: a placebo-controlled blinded comparison with tiotropium. Respir Res 11: 135.

Voss H-P (1994a). Long-acting β_2-adrenoceptor agonists in asthma: molecular pharmacological aspects. Thesis, Vrije Universiteit, Amsterdam.

Voss H-P, Donnell D, Bast A (1992). Atypical molecular pharmacology of a new long-acting β_2-adrenoceptor agonist, TA-2005. Eur J Pharmacol 227: 403–409.

Voss HP, Shukrula S, Wu TS, Donnell D, Bast A (1994b). A functional β_2 adrenoceptor-mediated chronotropic response in isolated guinea pig heart tissue: selectivity of the potent β_2 adrenoceptor agonist TA 2005. J Pharmacol Exp Ther 271: 386–389.

Ward J, Macintyre F, Jones I, Surujbally B, Scholl D, Bateman E (2009). A randomised double-blind, study to determine the efficacy & safety of a once-daily inhaled β_2 adrenoreceptor agonist, PF-00610355 in asthmatic patients [abstract]. Eur Respir J 34: 778s.

Wijesinghe M, Perrin K, Harwood M, Weatherall M, Beasley R (2008). The risk of asthma mortality with inhaled long acting β-agonists. Postgrad Med J 84: 467–472.

Woodcock A, Nandeuil MA, Raptus H, Costantini M, Petruzzelli S, Singh D (2009). Single dose budesonide/carmoterol fixed combination improves lung function over 24 hours in patients with moderate to severe persistent asthma [abstract]. Eur Respir J 34: 776s.

Wright K, Clarke N, Yeadon M, Perros-Huguet C (2010). PF-3429281 – A novel inhaled dual antimuscarinic/β_2 adrenoceptor agonist in the anaesthetised dog model of bronchoconstriction [abstract]. Eur Respir J 24: 1014s.