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REVIEW

Novel long-acting bronchodilators for COPD and asthma

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An important step in simplifying asthma and chronic obstructive pulmonary disease (COPD) management and improving adherence with prescribed therapy is to reduce the dose frequency to the minimum necessary to maintain disease control. Therefore, the incorporation of once-daily dose administration is an important strategy to improve adherence and is a regimen preferred by most patients, which may also lead to enhancement of compliance, and may have advantages leading to improved overall clinical outcomes. Once-daily β_2-agonists or ultra long-acting β_2-agonists (LABAs) such as carmoterol, indacaterol, GSK-159797, GSK-597901, GSK-159802, GSK-642444 and GSK-678007 are under development for the treatment of asthma and COPD. Also some new long-acting antimuscarinic agents (LAMAs) such as aclidinium, LAS-35201, GSK656398, GSK233705, NVA-237 (glycopyrrolate) and OrM3 are under development. In any case, the current opinion is that it will be advantageous to develop inhalers containing combination of several classes of long-acting bronchodilator drugs in an attempt to simplify treatment regimens as much as possible. Consequently, several options for once-daily dual-action ultra LABA + LAMA combination products are currently being evaluated. A different approach is to have a dimer molecule in which both pharmacologies are present (these molecules are known as M_3 antagonist-β_2 agonist (MABA) bronchodilators). The advent of a successful MABA product will revolutionize the field and open the door for a new range of combination products.
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Keywords: aclidinium; asthma; bronchodilators; carmoterol; chronic obstructive pulmonary disease; combination therapy; indacaterol; long-acting antimuscarinic agents; NVA237; ultra-long-acting β-agonists

Abbreviations: ACh, acetylcholine; COPD, chronic obstructive pulmonary disease; FEF_{25-75}, forced expiratory flow at 25–75% of FVC; FEV_1, forced expiry volume in 1 s; FVC, forced vital capacity; HFA, hydrofluoroalkane; ICS, inhaled corticosteroid; LABA, long-acting β_2-adrenoceptor agonist; LAMA, long-acting antimuscarinic agent; MABA, dual-acting muscarinic antagonist-β_2 agonist; MDDPI, multidose dry-powder inhaler; MDI, metered dose inhaler; PDE, phosphodiesterase; SDDPI, single-dose dry-powder inhaler

The role of long-acting bronchodilators in the treatment of COPD and asthma

International guidelines (Celli and MacNee, 2004; National Asthma Education and Prevention Program, 2007; Rabe *et al*., 2007; Bateman *et al*., 2008) suggest that long-acting bronchodilator therapy should always be considered in both the treatments of chronic obstructive pulmonary disease (COPD) and asthma. In effect, bronchodilators are the mainstay of COPD therapy (Celli and MacNee, 2004; Rabe *et al*., 2007). However, in asthma their role should be considered more limited because they must always be combined with an antiinflammatory controller medication, also considering that monotherapy with long-acting β_2-adrenoceptor agonists (LABAs) alone is associated with deterioration in asthma control and more frequent exacerbations (National Asthma Education and Prevention Program, 2007; Bateman *et al*., 2008). Nonetheless, treatment of asthma with a long-acting bronchodilator is important because it is able to reduce symptoms that occur at night or in the early morning, impacting sleep patterns and so reducing overall quality of life (Bateman *et al*., 2008).

If there is no doubt that long-acting bronchodilator therapy should always be considered when patients with COPD are symptomatic, no distinction is made as to which class of drug should be considered first, although anticholinergic agents are of noteworthy value as parasympathetic cholinergic pathways arising from the vagus nerve are implicated in the pathophysiology of airflow obstruction in COPD (Barnes, 2004; Cazzola and Matera, 2004; Rodrigo and

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Nannini, 2007). The choice of a β_2-adrenoceptor agonist or an anticholinergic agent depends on availability, as well as the symptom relief and side effects experienced by the individual patient (Celli and MacNee, 2004; Rabe *et al*., 2007). For patients whose conditions are not sufficiently controlled by monotherapy, combining medications of different classes, in particular an inhaled anticholinergic agent with a β_2-adrenoceptor agonist, seems a convenient way of delivering treatment and obtaining better results (Celli and MacNee, 2004; Rabe *et al*., 2007). This includes better lung function and improved symptoms. Specifically, as airflow obstruction becomes more severe, both a long-acting antimuscarinic agent (LAMA) plus a LABAs are advocated, although data supporting this therapeutic approach are still scarce (Cazzola and Matera, 2006; Wise and Tashkin, 2007) and a long-term study has questioned the values of such a combination (Aaron *et al*., 2007).

In asthma, LABAs, including formoterol and salmeterol, are most effective when combined with inhaled corticosteroids (ICSs), and this combination therapy is the preferred treatment when a medium dose of ICS alone fails to achieve control of asthma (National Asthma Education and Prevention Program, 2007; Bateman *et al*., 2008). There is evidence both *in vitro* and *in vivo* to suggest that there is a favorable interaction between LABAs and ICSs at the receptor level, leading to enhanced steroid effect (Sin and Man, 2006). The addition of a LABA to an ICS also increases patient adherence to ICS therapy, both by providing a feeling of immediate symptom improvement and by allowing the use of a lower dose of ICS (National Asthma Education and Prevention Program, 2007; Bateman *et al*., 2008). Although concern regarding the safety of use of LABAs has arisen from prospective studies (Martinez, 2005), a large Cochrane systematic review provides evidence that LABAs are safe and beneficial in control of asthma, subgroup analyses indicating that this is true when ICSs are used and in their absence (Walters *et al*., 2005). The role of LAMAs in the treatment of asthma is limited, but they seem to be useful in COPD patients with concomitant asthma (Magnussen *et al*., 2008).

The need for novel long-acting bronchodilators

With chronic diseases such as COPD and asthma, patient adherence to medication plans is a major obstacle to successful management (Bender, 2002). One factor contributing to poor adherence is a complicated or multiple treatment regimen, and simplified dosing regimens are known to improve compliance (Claxton *et al*., 2001; Bender, 2002). Therefore, a main step in simplifying COPD and asthma management is to reduce the dose frequency to the minimum necessary to maintain disease control (Tamura and Ohta, 2007). In particular, the incorporation of once-daily dosing seems to be an important strategy to improve compliance, and is a regimen preferred by most patients (Campbell, 1999).

Novel long-acting β_2-adrenoceptor agonists

The interest in the pharmaceutical industry to develop LABAs with improved duration of action over salmeterol and formoterol is intense. The coming years will be pivotal as data for the most promising compounds will become available from clinical studies. A variety of β-adrenoceptor agonists with longer half-lives are currently under development with the hopes of achieving once-daily dosing (Table 1) (Cazzola *et al*., 2005; Matera and Cazzola, 2007). These agents, called ultra-LABAs, carmoterol and indacaterol, GlaxoSmithKline (GSK)159797, GSK597901, GSK159802, GSK642444, and GSK678007. A series of saligenin containing LABAs has also been prepared. These compounds have significantly longer durations of action than salmeterol in the well-validated guinea pig trachea model and have the potential for a once-daily profile in human (Brown *et al*., 2007).

In the evaluation of new agents under development, it needs to be taken into consideration if they are partial β_2-receptor agonists or full β-agonists. Partial β_2-adrenoceptor agonists in the presence of a full β-agonist may act as a β_2-antagonist (Lipworth and Grove, 1997). In fact, a partial β-adrenoceptor agonist exhibits opposite agonist and antagonist activity depending on the prevailing degree of adrenergic tone or the presence of a β-adrenoceptor agonist with higher intrinsic activity.

Carmoterol (CHF-4226, TA-2005)

Carmoterol is a non-catechol β_2-adrenoceptor agonist with a *p*-methoxyphenyl group on the amine side chain and an 8-hydroxyl group on the carbostyril aromatic ring. Compared with other β_1- and β_2-adrenoceptor agonists, it has a high selectivity as well as a high affinity for the β_2-adrenoceptor in pharmacological and radioligand-binding studies using isolated guinea pig tissues (Kikkawa *et al*., 1991). On the basis of *in vivo* studies, it was reported that carmoterol has long-lasting bronchodilating effects (Kikkawa *et al*., 1994). Voss *et al*. (1992) have also reported that carmoterol shows a high potency for the β_2-adrenoceptor and a long duration of action after removal of the drug using both guinea pig tracheal muscle relaxation and bovine trapezium muscle-binding experiments.

The results obtained in healthy volunteers and asthmatic patients provided evidence that the pharmacokinetics of carmoterol are proportional to the dose, and nonlinear accumulation of the drug after repeated dosing treatments is negligible (Chiesi Farmaceutici, 2004). Interestingly, using Modulite technology that utilizes a hydrofluoroalkane (HFA) propellant, a lung deposition of carmoterol as high as 41% of the nominal dose can be reached, with no significant differences in lung deposition of carmoterol between healthy subjects, patients with asthma and patients with COPD likely because of the small particle size of the HFA metered dose inhaler aerosol (0.8 μm) (Haeussermann *et al*., 2006). This finding might justify the effectiveness and the safety profile of carmoterol. In fact, inhalation using Modulite technology provides a very high therapeutic ratio because a relatively low dose achieves high local concentrations (Acerbi *et al*., 2007).

A randomized, double-blind parallel group trial in 124 patient with persistent asthma documented that carmoterol 2 μg administered once daily was as effective as formoterol

Table 1 Ultra long-acting β-agonists under development

	Pros	*Latest developments*	*Company working on this strategy*
Indacaterol	It offers a quick onset of action and true 24-h control. It behaves as a potent β_2-AR[a] agonist with high intrinsic efficacy that, in contrast to salmeterol, does not antagonize the bronchorelaxant effect of a short-acting β_2-AR[a] agonist	Phase III	Novartis
Carmoterol	It displays a fast onset and long duration (30 h) of activity	Phase III	Chiesi
GSK159797	It produces clinically significant increases in FEV_1[b] through 24 h, with little change in heart rate	Phase IIb	Theravance/ GlaxoSmithKline
GSK642444	Apparently, it presents a potentially greater therapeutic index than GSK159797	Phase IIa	Theravance/ GlaxoSmithKline

[a]β_2-AR, β_2-adrenoceptor.
[b]FEV_1, forced expiratory volume in 1 s.

12 μg twice daily (Kottakis *et al*., 2006). The study, conducted over an 8-day treatment period, showed that carmoterol and formoterol both provided significant improvements in lung function. The trough forced expiry volume in 1 s (FEV_1) values on the morning of day 8 were clinically and significantly greater in both active treatment groups, compared with placebo and the effect of carmoterol on trough FEV_1 was comparable to formoterol. Safety and tolerability results were similar between carmoterol and formoterol (Nandeuil *et al*., 2006).

Indacaterol (QAB-149)

Also indacaterol (QAB-149) offers a quick onset of action and true 24-h control (Battram *et al*., 2006). On the electrically stimulated guinea pig tracheal strip preparation, the onset of action was fast for indacaterol (30 ± 4 min), formoterol (32 ± 1 min) and salbutamol (28 ± 3 min), whereas it was much slower for salmeterol (169 ± 32 min) (Battram *et al*., 2006). The durations of action of indacaterol (529 ± 99 min) and salmeterol (475 ± 130 min) were greater than they were for formoterol (158 ± 30 min) or salbutamol (22 ± 9 min) (Battram *et al*., 2006). 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) showed that indacaterol and formoterol had the highest intrinsic efficacy (73 ± 27 and 67 ± 11%, respectively), followed by salbutamol (48 ± 4%) and salmeterol (35 ± 5%). Formoterol and salmeterol had subnanomolar potency with IC_{50} values of 0.3 ± 0.1 and 0.7 ± 0.2 nM, respectively. Indacaterol had an IC_{50} value of 37 ± 8 nM, and salbutamol was of lower potency (125 ± 11 nM). The onset of action was fast for salbutamol (1.6 ± 0.3 min), formoterol (2.0 ± 0.3 min) and indacaterol (3.0 ± 0.2 min), whereas it was significantly slower for salmeterol (6.6 ± 0.3 min, $P<0.05$).

Functional data indicate that indacaterol is a partial agonist although, relative to isoprenaline, it has more of a full agonist profile (73%) than the partial agonist salmeterol (38%) (Naline *et al*., 2007). Moreover, it has twofold higher intrinsic activity than salbutamol or salmeterol (Battram *et al*., 2006). This profile could explain why indacaterol does not induce tachyphylaxis (Battram *et al*., 2006) and also does not antagonize the bronchorelaxant effect of a short-acting β_2-adrenoceptor agonist (Sturton *et al*., 2008). 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). In the isolated guinea pig left atrium (a β_1 adrenoceptor-containing preparation), all of the compounds induced a concentration-dependent inotropic effect. The maximal efficacy observed at the highest concentration tested (1 μM) in percentage increase from baseline was 163 ± 28, 75 ± 25 and 46 ± 4% for formoterol, indacaterol and salmeterol, respectively. However, at their respective pEC_{50} values in the tracheal preparation, the inotropic effect was 17 ± 3, 4 ± 1 and 10 ± 2% for formoterol, indacaterol and salmeterol, respectively.

Several trials have evaluated the efficacy and safety of indacaterol in patients with asthma and COPD. In 42 patients with intermittent or mild-to-moderate persistent asthma, single 200 and 400 μg doses of indacaterol provided effective and sustained 24-h bronchodilator control with a rapid onset of action (<5 min) and a good tolerability and safety profile (Beeh *et al*., 2007). In another trial, 436 patients with persistent asthma on a stable regimen of ICSs were randomized to treatment for 7 days with once-daily indacaterol 50, 100, 200 or 400 μg by multidose dry-powder inhaler (MDDPI, Certihaler), indacaterol 400 μg by single-dose dry-powder inhaler (SDDPI) or placebo LaForce *et al*., 2008). 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. A randomized, open-label crossover study in adult subjects with asthma ($FEV_1 \geqslant 60\%$ predicted) confirmed that indacaterol 200 μg provides effective 24-h bronchodilation, with a longer duration than salmeterol 50 μg and a good overall safety profile (Brookman *et al*., 2007).

The safety and tolerability of indacaterol were assessed in 156 asthma patients in a multicentre, randomized, double-blind, placebo-controlled study (Chuchalin *et al*., 2007). Patients received indacaterol 200, 400 or 600 μg or placebo once daily for 28 days. The results of this study suggested that indacaterol has a wide therapeutic index—it is well tolerated and is neither associated with adverse cardiac effects nor clinically significant changes in β_2-mediated

systemic effects. This may be because doses used were simply not high enough to have an impact on these safety variables despite all indacaterol doses achieving clinically relevant differences in FEV_1 of >200 mL versus placebo at most post-dose time points. In a subsequent study, higher doses of indacaterol (800 μg) demonstrated effects on serum potassium and blood glucose; however, these changes were considered to be not clinically meaningful (Yang *et al.*, 2007). At a higher single dose of 1000 μg, indacaterol had a good safety profile and was not associated with sustained systemic adverse effects; mean heart rate and QTc interval remained within normal ranges following administration (Brookman *et al.*, 2007).

Indacaterol was also investigated in COPD patients in whom it caused 24-h bronchodilation, with a clinically meaningful bronchodilator effect by at least 1 h post-dose and no evidence of tachyphylaxis (Aubier *et al.*, 2005). In the Rennard's study (Rennard *et al.*, 2008), 635 patients with moderate-to-severe COPD received indacaterol 50, 100, 200 or 400 μg once daily by MDDPI, indacaterol 400 μg once daily by SDDPI or placebo. All doses of indacaterol were associated with statistically significant dose-dependent improvements in FEV_1 compared with placebo, starting from 5 min after the first dose on day 1 of the 7-day treatment period. Dose-dependent FEV_1 increases were seen for indacaterol by the first time point (5 min) and at all time points on days 1 and 7 ($P<0.05$ vs placebo, all doses). The treatment effect persisted throughout the 24-h dosing interval, and increases in trough FEV_1 with the 200 and 400 μg per day dosages were classified as clinically relevant. Forced vital capacity (FVC) and forced expiratory flow at 25–75% of FVC were also significantly improved by indacaterol *vs* placebo, and rescue medication use was reduced. During an open-label extension period involving 263 patients, the effects of indacaterol on FEV_1 were similar to those of tiotropium bromide.

The safety and tolerability of once-daily administration of two doses of indacaterol (400 and 800 μg) with that of placebo, over a 28-day period, has been compared in patients with moderate COPD (Beier *et al.*, 2007). Once-daily indacaterol was well tolerated at doses up to 800 μg with a good overall safety profile. There was no statistical difference at any dose between the safety of indacaterol and placebo. As 800 μg represents 2–4 times the therapeutic dose suggested by earlier studies, these results imply that the therapeutic window for indacaterol may be wide.

GSK159797 (TD3327) and GSK642444

LABA compounds from GlaxoSmithKline and Theravance have been put into a pool for potential development for clinical use. Unfortunately, the available information is scarce and none of the compounds in development has been officially presented at scientific congresses or is present in the literature yet. GSK159797 (TD3327), a Theravance-discovered compound, and GSK642444, a GlaxoSmithKline-discovered compound, seem to be the most promising agents.

The compound GSK159797 (TD3327) is an ultra-LABA for the potential once-daily treatment for asthma and COPD, but its structure has not yet been disclosed. Recently, a novel, highly stable, crystalline form of a formoterol derivative as a novel LABA, with processes for its preparation, compositions containing the β_2-agonist and its use for the treatment of airway diseases, has been claimed. The claimed compound is believed to be GSK159797 (Matera and Cazzola, 2007).

GSK159797 achieved the target increase in FEV_1 throughout the 25-h evaluation period in a study of 38 patients with mild asthma following single-dose inhalation. It was well tolerated, with no increase in heart rate (Cazzola *et al.*, 2005). A placebo-controlled crossover study tested the bronchodilatory effect, safety and tolerability of multiple dose levels of GSK159797 administered by a dry powder inhaler in 20 patients with mild asthma (Matera and Cazzola, 2007). Doses in the anticipated clinical range produced clinically significant increases in FEV_1 through 24 h, with little change in heart rate. At 24 h, 10 and 20 μg doses of GSK159797 produced adjusted mean changes from baseline FEV_1 of 460 and 540 mL, respectively, compared to a change of 130 mL for placebo. The placebo-corrected mean maximum heart rate increase over the 26-h period of measurement was 1.0 b.p.m. for the 10 μg dose and 2.7 b.p.m. for the 20 μg dose.

GSK642444 is another ultra-LABA in development by GlaxoSmithKline. All doses studied of GSK642444 (25, 100 and 400 μg), dosed once a day over 14 days in patients with asthma, showed greater bronchodilator activity than salmeterol dosed twice a day and produced placebo-adjusted, dose-dependent mean changes from baseline FEV_1 of over 200 mL at trough on the fourteenth day of treatment. The two lower doses also produced smaller changes than salmeterol in placebo-adjusted weighted mean heart rate over the first 4 h after dosing on day 14 (Anonymous, 2007). Apparently, GSK642444 presents a potentially greater therapeutic index than GSK159797 (Anonymous, 2007). It will progress as the lead compound into 28-day studies.

Novel long-acting antimuscarinic agents

Also some new LAMAs, such as aclidinium (formerly known as LAS34273), LAS35201, GSK656398 (formerly known as TD5742), GSK233705, NVA237 (glycopyrrolate), OrM3, CHF 5407 and QAT370 are under development (Table 2).

Preclinical studies have shown aclidinium's selectivity, long duration of action and rapid clearance from the plasma (Gavalda *et al.*, 2007; Miralpeix *et al.*, 2007). When compared to other bronchodilatory agents *in vitro*, aclidinium demonstrated potent anticholinergic activity comparable to both tiotropium and ipratropium, but with a faster onset of action than tiotropium and a significantly longer duration of action versus ipratropium, allowing for 24-h duration of action (Miralpeix *et al.*, 2007). Findings of a phase I single-dose study demonstrated the bronchodilatory effects of aclidinium (Schelfhout *et al.*, 2007). In the phase I study, in 12 healthy volunteers, bronchoconstriction was induced with methacholine challenge and then treated with one of three doses of aclidinium. Aclidinium proved superior to placebo in improving specific airway conductance. Aclidinium also provided statistically significant and sustained protection against methacholine-induced airway constriction over 24 h.

Table 2 Long-acting antimuscarinic agents under development

	Pros	*Latest developments*	*Company working on this strategy*
NVA237 (glycopyrrolate)	At doses showing similar efficacy, NVA237 demonstrated a significantly lower effect on cardiovascular parameters than tiotropium	Phase III	Vectura/Novartis
OrM3	It has been formulated as an oral tablet for less compliant patients and those who have difficulty using aerosol therapy	Phase IIb	Merck Research Laboratories
GSK233705	Its long duration of action when administered via inhalation in animal models supports the potential for use as a once-daily bronchodilator for COPD	Phase II	GlaxoSmithKline
Aclidinium	Potent anticholinergic activity comparable to both tiotropium and ipratropium, but with a faster onset of action than tiotropium and a significantly longer duration of action versus ipratropium, allowing for 24-h duration of action	Phase III	Almirall
CHF 5407	It is an antagonist as potent and long-acting as tiotropium at M_3 receptors, but significantly short-acting at M_2 receptors	Phase I	Chiesi

Abbreviation: COPD, chronic obstructive pulmonary disease.

Aclidinium was well tolerated throughout the trial. Headache was reported by two subjects and one subject experienced a serious adverse event, which was not considered to be related to study drug. Single doses of inhaled aclidinium produced a significant bronchodilatory response in 17 patients with COPD according to results of a phase IIa trial (Joos *et al.*, 2007). Results of the study showed that mean FEV_1 and FVC values—important measures of lung function—were significantly increased with all studied doses of aclidinium over a 24-h time period, as compared to placebo. Onset of significant bronchodilation was observed as early as 15 min after aclidinium treatment and this effect was sustained for at least 24 h.

NVA237 is at a later stage of drug development than aclidinium. It has been documented in an experimental setting that at doses showing similar efficacy, NVA237 demonstrated a significantly lower effect on cardiovascular parameters than tiotropium, which may indicate a potential clinical benefit in man (Cooper *et al.*, 2006). In effect, inhaled NVA237 has low systemic absorption, and therefore should not be expected to be associated with typical systemic antimuscarinic adverse effects. This is supported by the observed lack of dry mouth (a classic antimuscarinic adverse effect) with inhaled NVA237, and suggests a favorable safety profile for this once-daily antimuscarinic bronchodilator (Thomas *et al.*, 2006). A single 480 μg dose of NVA237 demonstrated bronchodilatory efficacy up to 32 h post-dose in patients with reversible obstructive airways disease, supporting the potential for once-daily dosing, and exhibited a rapid onset of action (Gunawardena *et al.*, 2006). In particular, single doses of NVA237 provided a similar degree of bronchodilation to the short-acting β_2-agonist salbutamol over the first 40 min post-dose (Singh *et al.*, 2006). Results of a large, phase III study showed that this bronchodilator has rapid onset of effect (by 5 min post-dose) (Kuna *et al.*, 2007). Comparisons with baseline values showed that all doses of NVA237 were associated with sustained increases in trough FEV_1 on days 2, 7, 14 and 28, which was the primary end point. Compared with placebo, however, only peak FEV_1 improved significantly ($P<0.05$) with all doses of NVA237 at most time points; in contrast, trough FEV_1 was better than placebo on day 28 with only the 120 μg dose ($P=0.0048$). Overall, improvements in lung function with NVA237 appeared comparable with those of tiotropium.

OrM3 is a 4-acetamidopiperidine derivative with a high degree of selectivity (120-fold) for the M_3 receptor over M_2 receptors (Lu *et al.*, 2006). It has been formulated as an oral tablet, a potentially more convenient formulation, particularly for less compliant patients and those who have difficulty using aerosol therapy. Dosed orally, pharmacokinetic data demonstrated that OrM3 has a long half-life ($t_{1/2}=14.20$ h), which would potentially allow for a once-daily dosing regimen. In effect, OrM3 demonstrated a significant dose-related improvement in serial FEV_1 and a trend for dose-related improvement in patient-reported symptoms compared with placebo (Lu *et al.*, 2006). However, at a dose that provided efficacy less than that of ipratropium, the incidence of dose-related, mechanism-based side effects for OrM3 exceeded those observed for ipratropium (Lu *et al.*, 2006).

CHF 5407 is an antagonist as potent and long-acting as tiotropium at human M_3 receptors ($t_{1/2}=166$ min; 54% radioligand bound at 32 h) as did [^{3}H]-tiotropium ($t_{1/2}=163$ min; 65% radioligand bound at 32 h), but significantly short-acting at M_2 receptors ($t_{1/2}=31$ min; 0% radioligand bound at 2 h) whereas [^{3}H]-tiotropium dissociated slowly also from human M_2 receptor ($t_{1/2}=297$ min; 14% radioligand bound at 32 h) (Patacchini *et al.*, 2007). In anaesthetized guinea pigs, acetylcholine (ACh)-induced bronchospasm was dose dependently reduced by intratracheal administration of CHF5407 that was ~2–3 times more potent than tiotropium and ipratropium (Patacchini *et al.*, 2007). Studies performed in conscious guinea pigs using whole body plethysmography showed that aerosolized CHF5407 prevented ACh-induced bronchospasm for at least 24 h as did tiotropium, whereas ipratropium showed duration of action of 5 h (Villetti *et al.*, 2007).

Novel combinations of long-acting β-agonists and long-acting antimuscarinics agent under development

Bronchodilators are still central in the symptomatic management of COPD and asthma (Celli and MacNee, 2004;

National Asthma Education and Prevention Program, 2007; Rabe *et al*., 2007; Bateman *et al*., 2008). For this reason, the current opinion is that it will be advantageous to develop inhalers containing combination of several classes of long-acting bronchodilator drugs in an attempt to simplify treatment regimens as much as possible.

Several recent clinical trials have shown that concomitant therapy with a LABA and tiotropium provides statistically significant and clinically relevant improvements in bronchodilation and COPD symptoms over each individual bronchodilator (Cazzola *et al*., 2004a, b; van Noord *et al*., 2005, 2006; Vogelmeier *et al*., 2006; Tashkin *et al*., 2008), or a LABA/ICS combination (Rabe *et al*., 2008). Moreover, a combination of a LABA and a LAMA is more effective than treatment with either bronchodilator alone in reducing the rate of exacerbations (Arievich *et al*., 2006). Nonetheless, a longer-duration trial, the Canadian Optimal Management Trial, has shown that there is no clinical advantage of combining tiotropium with salmeterol (Aaron *et al*., 2007). Therefore, further long-term studies are required to determine whether the combination of a LABA and a LAMA has a real clinically relevant effect.

Looking at the aforementioned trials, one might argue that it is possible that the type of LABA included in combination with tiotropium can make a difference in the result and that, apparently, the presence of formoterol rather than salmeterol might ensure better outcomes. This could be the likely reason for which several companies are proceeding in attempts to create fixed combinations with a LAMA and formoterol (Cazzola and Matera, 2006) (Table 3). Nonetheless, trials that are exploring the effect of inhalation of a combination of tiotropium and salmeterol in COPD patients are still in progress (Cazzola and Matera, 2006) (Table 3).

Recently, Rossoni *et al*. (2007) provided, in an experimental setting, a clear evidence of a positive interaction between tiotropium and carmoterol in controlling the bronchoconstriction elicited in guinea pigs by different challenges, anaphylactic reaction included. In particular, in the presence of doses of tiotropium ineffective *per se*, the ED_{50} values of carmoterol were significantly reduced by 5 to over 30 times, depending on the challenge. Interestingly, even parameters not directly linked to airway smooth muscle relaxation such as thromboxane A release and survival time were significantly modified by the combination. These findings suggest that carmoterol and tiotropium combination represents a new therapeutic option for patients affected by increase in airway resistance.

Also indacaterol is being developed as a fixed-dose combination (QVA149) with NVA237 (Matera and Cazzola, 2007). A combination of GSK159797 with GSK233705, a new LAMA, that is being developed for once-daily treatment of COPD, is another possible option (Cazzola and Matera, 2006).

Several companies are also adopting a different approach and have a dimer molecule in which a bifunctional mechanism of action, combining both muscarinic antagonist and β_2 agonist pharmacology, is present in a single molecule, which is known as a dual-acting muscarinic antagonist-β_2 agonist (MABA) bronchodilator (Fitzgerald and Fox, 2007). These molecules use different linker radicals for covalently linking the M_3 antagonist to the β_2 agonist, indicating that the structure of the linker radical is not critical to preserve both activities. This is not surprising as the molecule is not required to interact with the M_3 and β_2 receptors simultaneously. In phase I randomized double-blind, placebo-controlled, single- and multiple-dose studies that enrolled healthy volunteers, GSK961081 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. Bicyclo[2.2.1]hept-7-ylamine derivatives are other potential MABAs under development.

Table 3 Combination of LABA[a] + LAMA[b] under development

	Pros	*Latest developments*	*Who is working on this strategy*
Formoterol + tiotropium	The combination of a LABA[a] with tiotropium is superior to either single agent alone	Development of unit dose oral inhalation products for nebulization	Novartis, Boehringer Ingelheim, Dey
Salmeterol + tiotropium	The combination of a LABA[a] with tiotropium is superior to either single agent alone	Phase III	Boehringer Ingelheim, GlaxoSmithKline
Carmoterol + tiotropium	In the presence of doses of tiotropium ineffective *per se*, the ED_{50} values of carmoterol were significantly reduced by 5 to over 30 times, depending on the challenge	Preclinical phase	Chiesi
Indacaterol + NVA237 (QVA149)	Detailed status not disclosed	Phase II	Novartis/Sosei/Vectura
GSK159797 + GSK233705	Detailed status not disclosed.	Preclinical phase	Theravance/GlaxoSmithKline
GSK961081	It is both a muscarinic antagonist and a β_2-AR[c] agonist	Phase II	GlaxoSmithKline

ED_{50}, effective dose 50%.
[a]LABA, long-acting β-agonist.
[b]LAMA, long-acting antimuscarinic agent.
[c]β_2-AR, β_2-adrenoceptor.

Table 4 Combination therapy with an ICS[a] and an ultra LABA[b] under development

	Pros	*Latest developments*	*Who is working on this strategy*
Carmoterol + budesonide	It is twofold more effective than the formoterol/budesonide combination	Preclinical phase	Chiesi
Indacaterol + mometasone (QMF149)	It has a superior delivery profile than formoterol/budesonide owing to its once-daily dosing	Phase III	Novartis

[a]ICS, inhaled corticosteroid.
[b]LABA, long-acting β-agonist.

Novel combinations of long-acting β-agonists and ICSs under development

As combination therapy with an ICS and a LABA is now considered a therapeutic option for treating patients suffering from severe to very severe COPD or asthma, there is a factual 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 ICS such as ciclesonide and mometasone, 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, 2006) (Table 4).

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 and COPD.

Combinations containing formoterol and the once-daily ICS mometasone or formoterol and ciclesonide in a single inhalation device are under development (Fitzgerald and Fox, 2007). A next-generation, once-daily combination consisting of GSK685698, a new long-acting ICS, and GSK159797, was stated to be in phase II studies. GSK685698 has shown evidence of greater potency and the potential for once-daily dosing, compared to existing treatments (Matera and Cazzola, 2007). Another new inhaled therapy will combine indacaterol with mometasone or indacaterol and QAE397, a novel corticosteroid in phase II development for the treatment of asthma (Matera and Cazzola, 2007).

Combinations of long-acting β-agonists, LAMAs and ICSs +

It is likely that the development of once-daily dual-action ultra-LABA + LAMA combination products may serve as a basis for improved 'triple therapy' combinations through co-formulation with novel ICSs (Cazzola and Matera, 2006). The potential for these therapeutic strategies to be administered once daily simplifies patient treatment regimens and therefore increases the likelihood of compliance with therapy.

Three studies have explored the impact of this approach (Aaron *et al*., 2007; Cazzola *et al*., 2007; Singh *et al*., 2008). Apparently, the triple combination provided greater improvements in lung function, quality of life and hospitalization rates without further influencing exacerbation frequency, symptom control and/or use of rescue medication. These two studies have combined salmeterol and fluticasone, which are twice daily drugs, with tiotropium. It will be interesting to explore whether a true once-daily triple combination with and an ultra-LABA, a LAMA and a once-daily ICS will have a better clinically relevant effect on patient-centered outcomes.

A combination of indacaterol, NVA237 and mometasone seems to be a possibility (Fitzgerald and Fox, 2007).

Combinations of long-acting β-agonists, LAMAs and novel antiinflammatory compounds

The development of once-daily dual-action ultra-LABA + LAMA combination products may also serve as a basis for improved triple therapy combinations through co-formulation with novel antiinflammatory compounds, such as inhaled phosphodiesterase (PDE)4 inhibitors, that could deliver three complementary therapeutic effects for patients with asthma and, mainly, COPD (Cazzola and Matera, 2006; Fitzgerald and Fox, 2007).

It is anticipated that the first of these combinations will feature the inhaled PDE4 inhibitor tofimilast (phase II) with a LAMA (potentially tiotropium) and then, potentially, with a MABA (Fitzgerald and Fox, 2007).

Intriguingly, a recent study has explored the additive effect of titrated oral theophylline in patients with stable COPD who received both tiotropium, and formoterol (Cazzola and Matera, 2007). Its results question the importance of adding theophylline in stable COPD patients already treated with two long-acting bronchodilators, but also indicate the possibility that some of them can benefit from theophylline because of a symptomatic improvement.

Conclusion

Bronchodilators are still central in the symptomatic management of COPD and asthma. We believe 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 in patients with asthma and COPD. The only limits set for the development of a long-lasting bronchodilator with a new product profile are medical needs and marketing opportunities. The current opinion is that it will be advantageous to develop inhalers containing combination of several classes of long-acting bronchodilator drugs in an attempt to simplify treatment regimens as much as possible. The investigational therapies for COPD and asthma discussed above have shown promising results. It is likely that the development of once-daily dual-action ultra-LABA + LAMA combination products may serve as a basis for improved triple therapy combinations through co-formulation with novel ICS that could deliver three complementary therapeutic effects for patients with COPD and asthma. In any case, the development of once-daily dual-action ultra-LABA + LAMA combination products may also serve as a basis for improved triple therapy combinations through co-formulation with novel antiinflammatory compounds such as inhaled PDE4 inhibitors.

Conflict of interest

Mario Cazzola has received honoraria for speaking and consulting and/or financial support for attending meetings from AstraZeneca, Boehringer Ingelheim, Chiesi Farmaceutici, Dey, GSK, Menarini Farmaceutici, Novartis, Nycomed (former Altana), Pfizer and Sanovel. Maria Gabriella Matera has received honoraria for consulting and/or financial support for attending meetings from Altana, AstraZeneca, Boehringer Ingelheim, GSK, Novartis and Pfizer. No funding has been provided for this article.

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