Long-acting muscarinic receptor antagonists for the treatment of chronic airway diseases

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Abstract: Acetylcholine (neuronal and non-neuronal origin) regulates bronchoconstriction, and mucus secretion. It has an inflammatory effect by inducing attraction, survival and cytokine release from inflammatory cells. Muscarinic receptors throughout the bronchial tree are mainly restricted to muscarinic M1, M2 and M3 receptors. Three long-acting muscarinic receptor antagonists (LAMAs) were approved for the treatment of chronic obstructive pulmonary disease (COPD) in Europe: once-daily tiotropium bromide; once-daily glycopyrronium bromide; and twice-daily aclidinium bromide. All have higher selectivity for M3 receptors than for M2 receptors, and dissociate more slowly from the M3 receptors than they do from the M2 receptors. Some LAMAs showed anti-inflammatory effects [inhibition of neutrophil chemotactic activity and migration of alveolar neutrophils, decrease of several cytokines in the bronchoalveolar lavage (BAL) including interleukin (IL)-6, tumor necrosis factor (TNF)- α and leukotriene (LT)B₄] and antiremodeling effects (inhibition of mucus gland hypertrophy and decrease in MUC5AC-positive goblet cell number, decrease in MUC5AC overexpression). In the clinic, LAMAs showed a significant improvement of forced expiratory volume in 1 second (FEV₁), quality of life, dyspnea and reduced the number of exacerbations in COPD and more recently in asthma. This review will focus on the three LAMAs approved in Europe in the treatment of chronic airway diseases.

Keywords: aclidinium bromide, asthma, chronic obstructive pulmonary disease, glycopyrronium bromide, long-acting muscarinic receptor antagonists, tiotropium bromide

Introduction

Asthma and chronic obstructive pulmonary disease (COPD) are both characterized by airflow limitation, chronic inflammation and structural changes to the airways. The patterns of inflammation and remodeling have different effects on the bronchial tree and the alveoli in both diseases. Clinical heterogeneity is associated with various mechanisms recently recognized as endotypes. Innate immunity and remodeling of the bronchial epithelium and smooth muscle cells may have different effects on asthma and COPD. Overall, in the future we should better discriminate those entities according to their pathophysiology rather than their response to bronchodilators. Despite these descriptive definitions and still poor understanding of the mechanisms, most of the treatments used today have dramatically improved overall management.

However, none of the current therapies interfere with the natural history of the diseases. Inhaled corticosteroids (ICSs) are the cornerstone in the management of persistent asthma according to the current guidelines [GINA, 2012]; however, bronchodilators are the main recommended treatment in pharmacological therapy for COPD [GOLD, 2013], as they improve symptoms, lung function, quality of life, and reduce the frequency of exacerbations in COPD patients with a forced expiratory volume in 1 second (FEV₁) <60% predicted [GOLD, 2013].

Two classes of long-acting bronchodilators are now available: long-acting beta₂ agonists (LABAs) and long-acting muscarinic antagonists (LAMAs). At present, three LAMAs have been approved for the treatment of COPD in different countries within Europe: once-daily tiotropium bromide

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Figure 1. Muscarinic receptors.

(Spiriva®, Boehringer Ingelheim Pharmaceuticals Inc); once-daily glycopyrronium bromide (Seebri® Breezhaler, Novartis Pharma AG); and twice-daily aclidinium bromide (Eklira® Genuair, Almirall). Recently, several studies described the potential use of these treatments in the management of asthma where they are mainly used in the most severe form of the disease without formal health-authority approval. This review will focus on the profile of LAMAs in the treatment of chronic airway diseases.

Acetylcholine and muscarinic receptors

Acetylcholine is the primary parasympathetic neurotransmitter in the airways and a paracrine/autocrine hormone released from non-neuronal origins. Acetylcholine is synthesized from choline and acetyl-CoA mainly by the enzyme choline acetyltransferase (ChAT) [Wessler and Kirkpatrick, 2008]. Airway epithelial cells express ChAT and release acetylcholine [Proskocil *et al.* 2004]. Other cells (macrophages, mast cells, lymphocytes, granulocytes, fibroblasts, and smooth

muscle) have been suggested to express ChAT [Wessler and Kirkpatrick, 2008] but release of acetylcholine from these cells has not yet been demonstrated directly. The parasympathetic network penetrates deep into the airway wall, and regulates bronchoconstriction, the release of mucus from submucosal glands, and from goblet cells in the airway epithelium [Gosens *et al.* 2006]. The distribution of muscarinic receptors throughout the bronchial tree is mainly restricted to muscarinic M1, M2 and M3 receptors [Gosens *et al.* 2006; Belmonte, 2005] (Figure 1). Muscarinic M1 receptors are expressed by epithelial cells, where they play a modulatory role in electrolyte and water secretion, and in the ganglia, where they facilitate parasympathetic neurotransmission. Muscarinic M2 receptors are expressed by neurons, where they function as autoreceptors, inhibiting the release of acetylcholine from both preganglionic nerves and from parasympathetic nerve terminals. Muscarinic M2 autoreceptors are dysfunctional in allergic asthma due to eosinophil-derived release of major basic protein, which acts as an allosteric antagonist of the M2

receptor [Jacoby *et al.* 1993], augmenting acetylcholine release. Furthermore, M2 receptors are widely expressed by airway mesenchymal cells such as fibroblasts and smooth muscle cells [Gosens *et al.* 2006]. Muscarinic M3 receptors are probably the best characterized subtype and are the dominant receptor subtype in the regulation of mucus secretion from submucosal glands and airway smooth muscle contraction [Gosens *et al.* 2006]. As a result, muscarinic M3 receptors are the primary target for LAMAs.

LAMAs interactions with muscarinic receptor subtypes

Acridinium, glycopyrronium, and tiotropium bind to human M1–M5 receptors in a concentration-dependent manner, all of them have higher selectivity for M3 receptors than for M2 receptors, and dissociate more slowly from the M3 receptors than they do from the M2 receptors [Vogelmeier and Banerji, 2011; Gavalda *et al.* 2009].

The association rate of acridinium with the human M3 receptor subtype is 2.6 times faster than that of tiotropium, but both show slow dissociation from M3 receptors, with residence half-lives of approximately 29 and 62 h, respectively. The acridinium half-life for the M2 receptor is 3.22 times shorter than that obtained for tiotropium [Gavalda *et al.* 2009], whereas glycopyrronium bromide associates four to five times faster with the M3 receptor than tiotropium bromide and acridinium bromide [Sykes *et al.* 2009; Vestbo *et al.* 2010; Ulrik, 2012]. The more rapid association of LAMA with the M3 receptor is assumed to underlie its faster onset of action.

Inflammation and the cholinergic system

Acetylcholine has been shown to affect inflammatory cells involved in asthma and COPD, by inducing proliferation or cytokine release from these cells [Kistemaker *et al.* 2012]. The muscarinic agonist, carbachol, can induce proliferation of rodent macrophages *in vitro* [De la Torre *et al.* 2008]. Cigarette smoke extract upregulates the non-neuronal cholinergic system in bronchial epithelial cells, and increases the expression of muscarinic M2 and M3 receptors. Consequently, acetylcholine levels in cell extracts were significantly higher after this stimulation. This increase could be reduced by tiotropium [Profita *et al.* 2011]. On the other hand atropine suppresses the

proliferation of T cells [Razani-Boroujerdi *et al.* 2008]. Acetylcholine is known to induce eosinophil, monocyte and neutrophil chemotactic activity in bronchial epithelial cells [Koyama *et al.* 1992, 1998]. Neutrophil chemotactic activity induced by acetylcholine could be inhibited by tiotropium [Profita *et al.* 2008]. The acetylcholine-induced neutrophil chemotactic activity from epithelial cells is partially dependent on interleukin (IL)-8 release, since it is inhibited by an anti-IL-8 monoclonal antibody, and the increase in IL-8 release in response to acetylcholine could be partially inhibited by tiotropium [Profita *et al.* 2008].

Alveolar macrophages have granulocyte and monocyte chemotactic activity in response to acetylcholine, which is likely explained by cholinergic induction of leukotriene B₄ (LTB₄) release [Sato *et al.* 1998; Buhling *et al.* 2007]. Chemotactic activity induced by acetylcholine could be inhibited by tiotropium [Buhling *et al.* 2007]. Acetylcholine can also induce the release of LTB₄ from sputum cells of COPD patients [Profita *et al.* 2005]. Tiotropium inhibited alveolar macrophage mediated migration of neutrophils from COPD patients: it was demonstrated that this effect is mediated via the muscarinic M3 receptor [Vacca *et al.* 2011].

Stimulation of bovine airway smooth muscle strips with the muscarinic agonist carbachol induces pro-inflammatory gene expression, including IL-6, IL-8 and cyclo-oxygenase-2 [Kanefsky *et al.* 2006], and induces the protein release of IL-6 and IL-8 via muscarinic M3 receptors [Gosens *et al.* 2009].

Anti-inflammatory effects

Tiotropium treatment in an animal model of cigarette smoke-induced COPD showed a concentration-dependently decreased in cell numbers and neutrophils in the bronchoalveolar lavage (BAL). Furthermore, tiotropium decreased cytokine levels in the BAL supernatant, including IL-6, tumor necrosis factor (TNF)- α , and some proinflammatory mediators such as LTB₄ [Wollin and Pieper, 2010].

Tiotropium partially inhibits eosinophilia recruitment in a guinea-pig model of asthma [Bos *et al.* 2007; Buels *et al.* 2012]. IL-4, IL-5, and IL-13 levels measured in BAL supernatant were decreased [Ohta *et al.* 2010].

In a mouse model of *Aspergillus fumigatus*-induced asthma, acridinium treatment completely abrogated the methacholine-induced lung resistance, and reduced the numbers of eosinophils in BAL fluid with no significant changes in other cell types or in IL-4 or IL-6 levels. The treatment markedly decreased total protein levels in BAL fluid with a correlation with lung injury and capillary leakage indices [Damera *et al.* 2010].

Airway remodeling

Airway remodeling in asthma and COPD is characterized by abnormalities of the structure of the airways from the nose to the alveoli including the distal conducting airways. It affects the bronchial and alveolar epithelium with goblet cell hyperplasia and glands hypertrophy, causes profound changes to smooth muscle and extracellular matrix and, in COPD, pulmonary vascular remodeling occurs [Jeffery, 2001; Sköld, 2010].

In asthma, the epithelium and subepithelial layers are abnormal: a considerable thickening of the airway smooth muscle bundle is observed [An *et al.* 2007]. Airway remodeling correlates weakly with disease severity [Hogg *et al.* 2004; James *et al.* 2009]. It is considered as a marker of chronicity and as a potential major component of irreversible airflow limitation in these diseases [An *et al.* 2007], and may be involved in the faster decline of lung function that is observed [Pare *et al.* 1997].

Mucus secretion

Mucus hypersecretion is an important feature of most of chronic airway diseases such as COPD and asthma, and contributes to altered quality of life, morbidity, and mortality in affected patients [Voynow and Rubin, 2009]. MUC5AC is the predominant mucin gene expressed in healthy human airway epithelial cells, and its expression is markedly increased in smokers, COPD, and asthmatic patients [Morcillo and Cortijo, 2006].

Mucus secretion can be increased predominantly via muscarinic M3 receptors expressed on the submucosal glands [Rogers, 2001]. In addition, electrolytes and water secretion are regulated by muscarinic M1 and M3 receptors [Ishihara *et al.* 1992; Ramnarine *et al.* 1996]. In response to acetylcholine, goblet cells also produce mucus [Rogers, 2001].

Tiotropium reduces allergen-induced mucus gland hypertrophy and the numbers of MUC5AC-positive goblet cell in a guinea-pig model [Bos *et al.* 2007]. Furthermore, tiotropium inhibits neutrophil elastase-induced goblet cell metaplasia in mice [Arai *et al.* 2010] and was shown to inhibit the increased MUC5AC expression and mucus gland hypertrophy in a guinea-pig model of COPD [Pera *et al.* 2011]. *In vitro* studies have demonstrated that acridinium suppressed both carbachol-induced MUC5AC overexpression and cigarette smoke-induced MUC5AC expression in human bronchial tissue [Cortijo *et al.* 2011].

Fibroblasts and airway smooth muscle cells

Fibroblasts and airway smooth muscle cells contribute to airway remodeling by means of an increase of their proliferation and the release of various inflammatory mediators, including the matrix metalloproteinases (MMPs). MMPs play a key role in the dynamic of the extracellular matrix by affecting elastic and collagen fiber turnover. They have been shown to be associated with some single-nucleotide polymorphism (SNIP) in COPD genomic analysis and are key players in neutrophilic inflammation and emphysema [Lagente and Boichot, 2010]. In COPD patients, increased expression levels of MMP-1, MMP-2 and MMP-9 have been reported in the sputum [Cataldo *et al.* 2000; Imai *et al.* 2001]. In asthma, MMP-9 is the predominant proteinase and its expression may increase when patients have spontaneous acute exacerbations or in response to a local allergenic challenge [Kelly and Jarjour, 2009]. The biological activity of the MMPs can be inhibited by tissue inhibitor of matrix metalloproteinases (TIMPs) [Lagente and Boichot, 2010]. TIMPs bind MMPs in a 1:1 fashion; thus, an increase in the molar ratio of MMP/TIMP may favor tissue destruction, while the reverse could be associated with marked fibrosis [Kelly and Jarjour, 2009]. It was found that tiotropium inhibited transforming growth factor (TGF)- β -induced protein expression of both MMP-1 and MMP-2 in human lung fibroblasts, but had no effect on the TGF- β -induced TIMP-1 and TIMP-2 expression [Asano *et al.* 2008, 2010]. Therefore, these data suggest that treatment with tiotropium may improve the balance between MMPs and TIMPs, inhibiting some profibrotic responses [Kistemaker *et al.* 2012].

In vitro studies have provided evidence that the stimulation of muscarinic receptors from

fibroblasts induces cell proliferation and the synthesis of collagen through muscarinic M2 receptor activation [Haag *et al.* 2008; Matthiesen *et al.* 2006]. Recently, it was demonstrated that aclidinium can inhibit the transition from human fibroblast to myofibroblast. The cells were obtained from smokers and COPD patients, and the transition was induced *in vitro* by exposure to carbachol, TGF- β 1, and cigarette smoke extracts. On the other hand, this drug dose-dependently reduced fibroblast proliferation and migration [Milara *et al.* 2012, 2013].

In a guinea-pig model of asthma using repeated challenges with ovalbumin, treatment with tiotropium significantly inhibited airway smooth muscle remodeling and inhibited the overexpression of the contractile protein 'myosin' [Gosens *et al.* 2005]. Increased peribronchial collagen deposition was prevented in a guinea-pig model of COPD [Pera *et al.* 2011]. Tiotropium also significantly inhibited smooth muscle thickening and the release of TGF- β 1 in BAL fluid [Ohta *et al.* 2010] in a murine model of asthma

Clinical efficacy of LAMAs in chronic airway diseases

Effects on pulmonary function tests

Tiotropium. In COPD, The Understanding Potential Long-term Impacts on Function with Tiotropium (UPLIFT) study [Tashkin *et al.* 2008] demonstrated that in the tiotropium group, the mean values for FEV₁ and forced vital capacity (FVC) before and after bronchodilation showed significant improvements that were maintained at all timepoints after randomization. Mean improvements in FEV₁ in the tiotropium group, as compared with the placebo group, ranged from 87 to 103 ml before bronchodilation and from 47 to 65 ml after bronchodilation ($p < 0.001$). The rate of decline in the mean values for FEV₁ and FVC either before or after bronchodilation from day 30 to the end of study was not affected by the treatment.

In asthma, several studies have investigated the efficacy of tiotropium in asthma (*versus* placebo, salmeterol or doubling ICSs); see Table 1. We will present the results of two studies.

In the first study [Peters *et al.* 2010], the researchers examined the effects of adding

salmeterol 100 μ g daily or tiotropium 18 μ g daily (via a Handihaler®) *versus* doubling the dose of extra-fine hydrofluoroalkane (HFA)-beclometasone (160 μ g) over 14 weeks. Tiotropium showed significant superiority for the primary outcome (morning peak expiratory flow [PEF]) over doubling the dose of HFA-beclometasone, amounting to a mean difference of 26 l/min, as well as noninferiority *versus* salmeterol with a mean difference of 6 l/min. For the secondary endpoint of FEV₁, mean differences with the addition of tiotropium were significantly improved by 100 ml *versus* doubling the dose of HFA-belcometasone and by 110 ml *versus* adding salmeterol. There were no significant differences between tiotropium treatment and salmeterol treatment with respect to the evening PEF ($p = 0.05$), the proportion of asthma control days ($p = 0.78$), the score for daily symptoms ($p = 0.10$), and the score on the Asthma Control Questionnaire ($p = 0.18$).

In the second study [Kerstjens *et al.* 2012], the researchers randomized 912 patients (in two trials) with severe uncontrolled asthma despite treatment with inhaled glucocorticoids and LABAs, of whom 24% were exsmokers (COPD with asthmatic component), to receive tiotropium 5 μ g daily (via Respimat®) or placebo for 48 weeks as add on to pre-existing ICS/LABA. At 24 weeks, the mean [$\pm$ standard error (SE)] change in the peak FEV₁ from baseline was greater with tiotropium than with placebo in the two trials: a difference of 86 ± 34 ml in trial 1 ($p = 0.01$) and 154 ± 32 ml in trial 2 ($p < 0.001$). The predose (trough) FEV₁ also improved in trials 1 and 2 with tiotropium, as compared with placebo: a difference of 88 ± 31 ml ($p = 0.01$) and 111 ± 30 ml ($p < 0.001$), respectively.

Aclidinium. Two phase III studies (ACCLAIM I and II) [Jones *et al.* 2011] evaluated the efficacy and safety of aclidinium 200 μ g, showed a statistically significant improvement of FEV₁ compared with placebo at weeks 12 and 28, in ACCLAIM I (by 61 and 67 ml, respectively; both $p < 0.001$) and ACCLAIM II (by 63 and 59 ml, respectively; both $p < 0.001$). In both studies, this effect was maintained over the 52-week study period. A recent phase III study [Kerwin *et al.* 2012a] evaluated the efficacy and safety of aclidinium 200 and 400 μ g twice daily *versus* placebo (ACCORD COPD I). At week 12, aclidinium 200 and 400 μ g showed a significant improvement in FEV₁ by 86 (45–127) ml and 124 (83–164) ml, respectively, compared with placebo.

Table 1. Efficacy of tiotropium in asthma.

Studies	PFT	Design	Patients	Treatment period	Major findings
Fardon <i>et al.</i> [2007]	FEV ₁ : 51%	RCP. Half-dose of ICS+SM or half-dose of ICS+SM+TIO	18 nonsmoking severe asthmatics	4 weeks	Improvement in PEF and Raw in ICS+SM; improvement in PEF, Raw, FEV ₁ , FVC and eNO in ICS+SM+TIO; no difference in AQLQ
Magnussen <i>et al.</i> [2008]	FEV ₁ : 53%	RCP. Usual respiratory medications except for inhaled anticholinergics + TIO or placebo	472 patients with COPD and concomitant asthma	12 weeks	Increase in FEV ₁ , AUC, FVC, AUC from 0 to 6 h and in FEV ₁ in TIO; reduction in rescue medication in TIO <i>versus</i> placebo
Park <i>et al.</i> [2009]	FEV ₁ : 58%	Open label. Add TIO to usual treatment 'ICS+LABA'	138 severe asthmatics	12 weeks	33.3% of patients responded to TIO. The presence of Arg16Gly in ADRB2 was associated with response to TIO
Peters <i>et al.</i> [2010]	FEV ₁ : 71.5%	Double-blind. Double ICS, ICS+SM or ICS+TIO	210 asthmatics	14 weeks	Improvement in PEF and FEV ₁ in TIO <i>versus</i> double ICS. Improvement in FEV ₁ in TIO <i>versus</i> SM; improvement in asthma control days and reduction in symptom score and ACQ in TIO and SM <i>versus</i> double ICS
Kerstjens <i>et al.</i> [2011]	FEV ₁ : 65%	RCP. ICS+LABA+TIO 10/5 µg <i>versus</i> ICS+LABA+placebo	107 severe asthmatics	8 weeks	Improvement in FEV ₁ , FVC, PEF in both doses of TIO <i>versus</i> placebo; no differences in rescue medication, in symptom scores and in mini-AQLQ
Bateman <i>et al.</i> [2011]	FEV ₁ : 68% PEF: 358 l/min	RCP. ICS+ SM, TIO 5 µg or placebo	388 asthmatics with 'B16-Arg/Arg' genotype	16 weeks	TIO is as effective as SM in PEF improvement; difference 'non-statistically significant' in rescue medication, symptom scores, asthma-control days and mini-AQLQ
Kerstjens <i>et al.</i> [2012]	FEV ₁ : 62%	RCP. ICS+LABA+TIO 5 µg or placebo	912 severe asthmatics (24% exsmokers)	48 weeks	TIO improves FEV ₁ , reduces risk of exacerbation.

RPC, randomized placebo-controlled trial; SM, salmeterol; TIO, tiotropium; LABA, long-acting beta agonist; ICS, inhaled corticosteroid; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; PEF, peak expiratory flow; AUC, area under curve; ADRB2, beta₂ adrenoceptor; ACQ, asthma control questionnaire; AQLQ, asthma quality of life questionnaire; P/Y, packs/year; UNK, unknown; PFT, pulmonary function test.

Glycopyrronium bromide. In one of the largest published clinical trials of glycopyrronium bromide (GLOW1) [D'Urzu *et al.* 2011] the researchers randomized patients with stable moderate-to-severe COPD to either glycopyrronium bromide 50 µg once daily or placebo for 26 weeks. Least squares mean trough FEV₁ at week 12 was significantly higher in patients receiving glycopyrronium bromide (1.41 l) *versus* placebo (1.30 l), with a treatment difference of 108 ± 15 ml ($p = 0.001$). Significant improvements in trough FEV₁ were observed at the end of day 1 and sustained through week 26.

In the GLOW2 study [Kerwin *et al.* 2012b], patients with moderate-to-severe COPD were randomized to either glycopyrronium bromide 50 µg once daily, open-label tiotropium 18 µg once daily, or placebo for 52 weeks. Least squares mean trough FEV₁ at week 12 was significantly higher in patients receiving glycopyrronium bromide and tiotropium compared with that in patients receiving placebo, with a treatment difference of 97 ml ($p = 0.001$) and 83 ml ($p = 0.001$), respectively. Furthermore, trough FEV₁ at weeks 26 and 52 was significantly higher for patients treated with glycopyrronium bromide *versus* placebo and

Table 2. Clinical efficacy of long-acting muscarinic receptor antagonists in chronic obstructive pulmonary disease.

	Tiotropium		Aclidinium		Glycopyrronium	
	Statistically significant	Clinically significant	Statistically significant	Clinically significant	Statistically significant	Clinically significant
FEV ₁	+	–	+	–	+	+
SGRQ	+	+/-	+	+/-	+	–
Dyspnea	+	–	+	+/-	+	+

FEV₁, forced expiratory volume in 1 second; SGRQ, St George's Respiratory Questionnaire.

comparable with tiotropium. At these time points, the treatment difference, compared with placebo, in favor of glycopyrronium bromide, was 134 and 108 ml, respectively ($p < 0.001$), while the difference in favor of tiotropium was 84 ml and 89 ml, respectively ($p < 0.001$). On day 1, the least squares mean FEV₁ treatment difference for glycopyrronium bromide/placebo and tiotropium bromide/placebo was 87 and 45 ml at 5 minutes, respectively, and 143 ml and 78 ml at 15 minutes, respectively ($p < 0.001$). The latter finding further supports the notion that glycopyrronium bromide has a faster onset of action than other inhaled antimuscarinic agents, including tiotropium bromide [Ulrik, 2012].

Dyspnea

Tiotropium. Two studies examined the change in the Transition Dyspnea Index (TDI) for tiotropium compared with placebo in COPD patients [Brusasco *et al.* 2003; Casaburi *et al.* 2002]. They showed a statistically significant improvement in the dyspnea score in favor of tiotropium compared with placebo. These improvements have not reached clinically significant change (one-unit change).

Aclidinium. In ACCLAIM I, more patients receiving aclidinium *versus* placebo exceeded the minimal clinically important difference (1 unit) for TDI focal score at 52 weeks (56.4% *versus* 38.0%; $p < 0.0001$). In ACCLAIM II, the difference was significant only at week 12 (53.1% *versus* 42.7%; $p = 0.029$). The ACCORD study showed a significant improvement of dyspnea for the two doses 200 and 400 mg *versus* placebo with a TDI focal score (>1 unit) at 12 weeks for aclidinium 400 mg.

Glycopyrronium bromide. In the GLOW1 study [D'Urzu *et al.* 2011] patients receiving glycopyrronium bromide had a significantly greater TDI

focal score at week 26 (1.84) compared with patients receiving placebo (0.80). This difference was statistically and clinically significant; see Table 2. Furthermore, patients treated with glycopyrronium bromide were 1.7-fold more likely to have at least a one-point improvement in transition dyspnea index focal score compared with those on placebo ($p = 0.001$). Similar findings were reported from the GLOW2 trial [Kerwin *et al.* 2012b].

Exacerbations

Tiotropium. In COPD, tiotropium treatment significantly reduced the numbers of patients suffering from acute COPD exacerbations. In addition, tiotropium also reduced exacerbations leading to hospitalization but no significant difference was found for hospitalization of any cause or mortality [Karner *et al.* 2012]. These findings were confirmed by a pooled analysis [Halpin *et al.* 2009] examining nine studies, which found a significantly reduced risk of COPD exacerbation and the risk of exacerbation-associated hospitalization, in COPD (reduction by 21%). The TIOSPIR study [Wise *et al.* 2013] showed that tiotropium Respimat® at a dose of 5 µg is not superior to HaniHaler® with respect to the risk of the first exacerbation [hazard ratio 0.98; 95% confidence interval (CI) 0.93–1.03].

In asthma, tiotropium increased the time to the first severe exacerbation (282 *versus* 226 days) in patients with severe uncontrolled asthma despite treatment with inhaled glucocorticoids and LABAs, with an overall reduction of 21% in the risk of a severe exacerbation (hazard ratio 0.79; $p = 0.03$) [Kerstjens *et al.* 2012].

Aclidinium. The moderate or severe COPD exacerbation rate in ACCLAIM I is nearly the same *versus* placebo (26.6% *versus* 25.7%), and so is the

duration before the first moderate or severe exacerbation ($p = 0.9$ versus placebo). In ACCLAIM II, fewer patients in the aclidinium group experienced a moderate or severe exacerbation compared with those in the placebo group (33.2% versus 39.8%, respectively; $p = 0.0046$). The time to first moderate or severe exacerbation with aclidinium was significantly delayed compared with placebo ($p = 0.01$). In the ACCORD study, there was no significant difference of the exacerbation numbers but this study was of shorter duration and not powered for this outcome.

Glycopyrronium bromide. In the GLOW 1 trial [D'Urzu *et al.* 2011], during the 26-week study period, (17.5%) in the glycopyrronium bromide group had at least one moderate or severe COPD exacerbation, compared with 24.2% in the placebo group. Glycopyrronium bromide significantly reduced the risk of COPD exacerbations in terms of time to first moderate or severe COPD exacerbation by 31% compared with placebo ($p = 0.023$). Furthermore, a significant reduction was also observed in the future risk of a COPD exacerbation leading to hospitalization in the glycopyrronium bromide group versus placebo ($p = 0.022$).

In the GLOW2 trial [Kerwin *et al.* 2012b], glycopyrronium significantly reduced the risk of exacerbations in terms of time to first moderate or severe exacerbation by 34% compared with placebo ($p = 0.001$) over 52 weeks, thereby demonstrating similar results to those reported for tiotropium, which provided a 39% risk reduction versus placebo ($p = 0.001$). Further, a 35% reduction was observed in the rate of moderate-to-severe COPD exacerbations in the glycopyrronium group compared with placebo (0.54 versus 0.80 per year, $p = 0.003$).

Quality of life

Tiotropium. Two meta-regression analyses examined quality-of-life of studies that compared tiotropium with placebo. The first [Yohannes *et al.* 2011] examined 20 studies (22,468 COPD patients) and found that the proportion of COPD patients who obtained a clinically significant change (a four-unit change) in the St George's Respiratory Questionnaire (SGRQ) score was significantly higher in the tiotropium group compared with the placebo group. The second [Karner *et al.* 2012] examined 22 studies (23,309 COPD patients) and reported that patients treated with

tiotropium obtained a statistically significant difference in the SGRQ over placebo, but this difference was not clinically relevant. The UPLIFT study [Tashkin *et al.* 2008] demonstrated a statistically significant difference of the mean quality of life, the overall mean between-group difference in the SGRQ total score at any time point was 2.7, which was not clinically significant.

Aclidinium. In ACCLAIM I and II [Jones *et al.* 2011], significantly more patients receiving aclidinium had an improvement in SGRQ total score ≥ 4 units versus placebo; at week 52, the percentage of patients achieving this improvement was 48.1% versus 39.5% for aclidinium versus placebo, respectively ($p = 0.025$), in ACCLAIM I and 39.0% versus 32.8% for aclidinium versus placebo, respectively ($p = 0.074$), in ACCLAIM II. The difference observed between the two studies is probably due to the higher number of abortions in the placebo group of ACCLAIM II.

Glycopyrronium bromide. In GLOW 1 [D'Urzu *et al.* 2011] patients treated with glycopyrronium bromide had a significantly lower SGRQ total score at week 26 than patients treated with placebo. The treatment difference was -2.81 , which was statistically but not clinically significant. The percentage of patients achieving a >4 -point reduction in SGRQ score was significantly greater with glycopyrronium bromide than with placebo (56.8% and 46.3%, respectively, $p = 0.006$). Similar findings were reported from the GLOW2 trial [Kerwin *et al.* 2012b].

Safety issues

Tiotropium. The most commonly reported adverse event (AE) in a meta-analysis performed by Yohannes and colleagues [Yohannes *et al.* 2011] was dry mouth: 10 studies reported the incidence of dry mouth. The cumulative incidence was 7.4% with tiotropium and 2.0% with placebo. In this meta-analysis, the summary odds estimate for the number of patients who experienced a serious AE (SAE) was not statistically significant when tiotropium was compared with placebo.

A recent meta-analysis [Singh *et al.* 2011] investigated the safety profile of five randomized controlled trials ($n = 6522$) examining tiotropium delivered using the Respimat® inhaler in comparison with placebo. They found that using the tiotropium Respimat® inhaler was associated

with a significantly increased risk of mortality. Another recent meta-analysis examined 42 randomized controlled trials ($n = 52516$) of tiotropium Soft Mist Inhaler, tiotropium HandiHaler®, LABAs, ICSs, and LABA-ICS combination with at least a 6-month treatment duration [Dong *et al.* 2013]. Tiotropium Soft Mist Inhaler was associated with a universally increased risk of overall death compared with placebo [odds ratio (OR) 1.51; 95% CI 1.06–2.19], tiotropium HandiHaler® (OR 1.65; 95% CI 1.13–2.43), LABA (OR 1.63; 95% CI 1.10–2.44), and LABA-ICS (OR 1.90; 95% CI 1.28–2.86). The risk was obvious for cardiovascular death, in patients with severe COPD, and at a higher daily dose. However, the UPLIFT [Tashkin *et al.* 2008] study showed that tiotropium was superior in reducing all causes of mortality by 11% compared with placebo.

A more recent randomized, double-blind, parallel-group trial 'TIOspir' [Wise *et al.* 2013] involving 17,135 patients with COPD (mean FEV₁ after bronchodilation was 48%), 10% of them have a previous stable and non-life-threatening arrhythmia and 15% have a previous ischemic heart disease, treated with tiotropium HandiHaler® 18 µg once daily, tiotropium Respimat® 5 µg once daily, or tiotropium Respimat® 2.5 µg once daily, evaluated the risk of death (noninferiority study, Respimat® at a dose of 5 or 2.5 µg *versus* HandiHaler®). This study showed that Respimat® was noninferior to HandiHaler® with respect to the risk of death (Respimat® at a dose of 5 µg *versus* HandiHaler®: hazard ratio 0.96; 95% CI 0.84–1.09; Respimat® at a dose of 2.5 µg *versus* HandiHaler®: hazard ratio 1.00; 95% CI 0.87–1.14), Respimat® was not associated with higher mortality than HandiHaler® among patients with previous cardiac disease, including stable arrhythmias at baseline, and was also not associated with a higher incidence of arrhythmias. There were no significant differences among the three study groups in terms of SAEs and nonfatal and fatal major adverse cardiovascular events. The limitations of this study are the absence of a placebo group and the exclusion of patients with unstable cardiovascular conditions (myocardial infarction within the previous 6 months, hospitalization for class III or IV heart failure, or unstable or life-threatening arrhythmia) or moderate or severe renal impairment, so the study findings cannot be extended to these populations.

In conclusion, in light of these results, we can conclude that tiotropium Respimat® may be

prescribed to patients with COPD with a good tolerance, even in the case of stable ischemic heart disease or stable cardiac non-life-threatening arrhythmia. However, we do not actually recommend that tiotropium Respimat® be prescribed in cases of unstable ischemic heart disease, unstable or life-threatening cardiac arrhythmia.

Acclidinium. The rapid plasma hydrolysis of acclidinium results in minimal to no systemic exposure 15 minutes following a dose, suggesting a low potential for extrapulmonary side effects regardless of renal impairment [Prat *et al.* 2009; Sentellas *et al.* 2010].

In phase I studies in humans, acclidinium had no significant effect on heart rate or QT interval at doses up to 800 µg [Lasseter *et al.* 2011; Schelfhout *et al.* 2010]. In phase I–III studies in humans [Schelfhout *et al.* 2010; Chanez *et al.* 2010; Jansat *et al.* 2009; Jones *et al.* 2011; Joos *et al.* 2010; Maltais *et al.* 2011], non-SAEs were generally comparable in incidence to placebo, and included headache, dry mouth, cough, sore throat, upper respiratory tract infection, diarrhea, myalgias, and arthralgias. In particular, the incidence of anticholinergic side effects in the phase III study was very low and not significantly different from placebo [Jones *et al.* 2011]. None of the studies reported SAEs that were thought to be related to study drug.

Glycopyrronium bromide. In the GLOW1 trial [D'Urzu *et al.* 2011], the incidence of AEs was lower in patients receiving glycopyrronium bromide (57.5% of patients), compared with placebo (65.2%). Other AEs, including those typically associated with anticholinergics (gastrointestinal disturbances, urinary difficulty, urinary retention, dry mouth), occurred at low frequencies in the glycopyrronium bromide and placebo groups. SAEs and discontinuations due to AEs were less frequent in patients receiving glycopyrronium bromide than in those receiving placebo. The number of deaths was low in the glycopyrronium bromide group (3 patients, 0.5%) and in the placebo group (3 patients, 1.1%). Notable QTcF intervals were reported in 4.0% of patients treated with glycopyrronium bromide and 1.1% of those receiving placebo. Changes in QTcF interval from a baseline of 30–60 ms or >60 ms occurred in 11.8% of patients receiving glycopyrronium bromide and 8.2% of those receiving placebo.

In GLOW2 [Kerwin *et al.* 2012b], the overall incidence of AEs was similar across the three

treatment groups (glycopyrronium bromide 76.6%, placebo 76.5%, tiotropium 74.2%). Antimuscarinic side effects, such as dry mouth, constipation, urinary retention and urinary tract infections, occurred with a low frequency in the glycopyrronium bromide, placebo, and tiotropium treatment groups. SAEs occurred with a lower frequency in the glycopyrronium bromide group, compared with the tiotropium and placebo groups. The percentage of patients with newly occurring or worsening clinically notable QTcF values was low across treatment groups (glycopyrronium bromide 4.4%, tiotropium 5.3%, and placebo 6%). Seven deaths were reported during the treatment and the 30-day follow-up period; three in the glycopyrronium bromide group (0.6%) and two each in the placebo (0.7%) and tiotropium groups (0.7%). None of the deaths was suspected to be related to the study medication.

Conclusion

Three LAMAs have been approved for the treatment of COPD in Europe: tiotropium bromide, glycopyrronium bromide, and aclidinium bromide. All have higher selectivity for M3 receptors than for M2 receptors, and dissociate more slowly from the M3 receptors than they do from the M2 receptors.

Some LAMAs show anti-inflammatory effects and antiremodeling effects but most of these studies are *in vitro* on cellular models or in animal models.

Clinically, tiotropium and glycopyrronium have longer half-lives than aclidinium, which allows for a once-daily dose compared with twice daily for aclidinium. Can we use the term LAMA for aclidinium and 'ultra-LAMA' for tiotropium and glycopyrronium? Glycopyrronium has a rapid onset of action. In COPD, all three products produced a statistically significant improvement of FEV₁ but only glycopyrronium produced a clinically significant improvement. There was also improvement in the quality of life measured by the SGRQ, but this was not clinically significant. Only glycopyrronium produced statistically and clinically significant improvement of dyspnea. In asthma, only tiotropium has been tested: in patients with severe asthma (not well controlled on medications), it improved FEV₁ and the risk of exacerbations.

At present, there is no unique way to select the best safety and efficacy profile among the three

products for patients with COPD. In the future, and in the real clinical setting, the satisfaction of the patients according to different parameters including the persistence of night symptoms may favor one of these drugs over the other two. Combinations of LAMAs and LABAs may quickly change the use of these molecules in the treatment of COPD. At present, tiotropium has been found to be efficient in severe uncontrolled asthma.

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Conflict of interest statement

The author declares that there is no conflict of interest.

References

- An, S., Bai, T., Bates, J., Black, J., Brown, R., Brusasco, V. *et al.* (2007) Airway smooth muscle dynamics: a common pathway of airway obstruction in asthma. *Eur Respir J* 29: 834–860.
- Arai, N., Kondo, M., Izumo, T., Tamaoki, J. and Nagai, A. (2010) Inhibition of neutrophil elastase-induced goblet cell metaplasia by tiotropium in mice. *Eur Respir J* 35: 1164–1171.
- Asano, K., Shikama, Y., Shibuya, Y., Nakajima, H., Kanai, K., Yamada, N. *et al.* (2008) Suppressive activity of tiotropium bromide on matrix metalloproteinase production from lung fibroblasts in vitro. *Int J Chron Obstruct Pulmon Dis* 3: 781–789.
- Asano, K., Shikama, Y., Shoji, N., Hirano, K., Suzuki, H. and Nakajima, H. (2010) Tiotropium bromide inhibits TGF-beta-induced MMP production from lung fibroblasts by interfering with Smad and MAPK pathways in vitro. *Int J Chron Obstruct Pulmon Dis* 5: 277–286.
- Bateman, E., Kornmann, O., Schmidt, P., Pivovarov, A., Engel, M. and Fabbri, L. (2011) Tiotropium is noninferior to salmeterol in maintaining improved lung function in B16-Arg/Arg patients with asthma. *J Allergy Clin Immunol* 128: 315–322.
- Belmonte, K. (2005) Cholinergic pathways in the lungs and anticholinergic therapy for chronic obstructive pulmonary disease. *Proc Am Thorac Soc* 2: 297–304; discussion 311–312.
- Bos, I., Gosens, R., Zuidhof, A., Schaafsma, D., Halayko, A., Meurs, H. *et al.* (2007) Inhibition of allergen-induced airway remodelling by tiotropium

- and budesonide: a comparison. *Eur Respir J* 30: 653–661.
- Brusasco, V., Hodder, R., Miravittles, M., Korducki, L., Towse, L. and Kesten, S. (2003) Health outcomes following treatment for six months with once daily tiotropium compared with twice daily salmeterol in patients with COPD. *Thorax* 58: 399–404.
- Buels, K., Jacoby, D. and Fryer, A. (2012) Non-bronchodilating mechanisms of tiotropium prevent airway hyperreactivity in a guinea pig model of allergic asthma. *Br J Pharmacol* 165: 1501–1514.
- Buhling, F., Lieder, N., Kuhlmann, U., Waldburg, N. and Welte, T. (2007) Tiotropium suppresses acetylcholine-induced release of chemotactic mediators in vitro. *Respir Med* 101: 2386–2394.
- Casaburi, R., Mahler, D.A., Jones, P.W., Wanner, A., San, P.G., ZuWallack, R.L. *et al.* (2002) A long-term evaluation of once-daily inhaled tiotropium in chronic obstructive pulmonary disease. *Eur Respir J* 19: 217–224.
- Cataldo, D., Munaut, C., Noel, A., Frankenne, F., Bartsch, P., Foidart, J. *et al.* (2000) MMP-2- and MMP-9-linked gelatinolytic activity in the sputum from patients with asthma and chronic obstructive pulmonary disease. *Int Arch Allergy Immunol* 123: 259–267.
- Chanez, P., Burge, P., Dahl, R., Creemers, J., Chuchalin, A., Lamarca, R. *et al.* (2010) Acclidinium bromide provides long-acting bronchodilation in patients with COPD. *Pulm Pharmacol Ther* 23: 15–21.
- Cortijo, J., Mata, M., Milara, J., Donet, E., Gavalda, A., Miralpeix, M. *et al.* (2011) Acclidinium inhibits cholinergic and tobacco smoke-induced MUC5AC in human airways. *Eur Respir J* 37: 244–254.
- Damera, G., Jiang, M., Zhao, H., Fogle, H., Jester, W., Freire, J. *et al.* (2010) Acclidinium bromide abrogates allergen-induced hyperresponsiveness and reduces eosinophilia in murine model of airway inflammation. *Eur J Pharmacol* 649: 349–353.
- De la Torre, E., Genaro, A., Ribeiro, M., Pagotto, R., Pignataro, O. and Sales, M. (2008) Proliferative actions of muscarinic receptors expressed in macrophages derived from normal and tumor bearing mice. *Biochim Biophys Acta* 1782: 82–89.
- Dong, Y., Lin, H., Shau, W., Wu, Y., Chang, C. and Lai, M. (2013) Comparative safety of inhaled medications in patients with chronic obstructive pulmonary disease: systematic review and mixed treatment comparison meta-analysis of randomised controlled trials. *Thorax* 68: 48–56.
- D'Urzo, A., Ferguson, G., van Noord, J., Hirata, K., Martin, C., Horton, R. *et al.* (2011) Efficacy and safety of once-daily NVA237 in patients with moderate-to-severe COPD: the GLOW1 trial. *Respir Res* 12: 156.
- Fardon, T., Haggart, K., Lee, D. and Lipworth, B. (2007) A proof of concept study to evaluate stepping down the dose of fluticasone in combination with salmeterol and tiotropium in severe persistent asthma. *Respir Med* 101: 1218–1228.
- Gavaldà, A., Miralpeix, M., Ramos, I., Otal, R., Carreno, C., Vinals, M. *et al.* (2009) Characterization of acclidinium bromide, a novel inhaled muscarinic antagonist, with long duration of action and a favorable pharmacological profile. *J Pharmacol Exp Ther* 331: 740–751.
- GINA (2012) Global Initiative for Asthma (update 2012). Available at: <http://www.ginaasthma.org>.
- GOLD (2013) Global Initiative for Obstructive Lung Disease. Global strategy for the diagnosis, management and prevention of chronic obstructive pulmonary disease. Available at: <http://www.goldcopd.com>.
- Gosens, R., Bos, I., Zaagsma, J. and Meurs, H. (2005) Protective effects of tiotropium bromide in the progression of airway smooth muscle remodeling. *Am J Respir Crit Care Med* 171: 1096–1102.
- Gosens, R., Rieks, D., Meurs, H., Ninaber, D., Rabe, K., Nanninga, J. *et al.* (2009) Muscarinic M3 receptor stimulation increases cigarette smoke-induced IL-8 secretion by human airway smooth muscle cells. *Eur Respir J* 34: 1436–1443.
- Gosens, R., Zaagsma, J., Meurs, H. and Halayko, A. (2006) Muscarinic receptor signaling in the pathophysiology of asthma and COPD. *Respir Res* 7: 73.
- Haag, S., Matthiesen, S., Juergens, U. and Racke, K. (2008) Muscarinic receptors mediate stimulation of collagen synthesis in human lung fibroblasts. *Eur Respir J* 32: 555–562.
- Halpin, D., Menjoge, S. and Viel, K. (2009) Patient-level pooled analysis of the effect of tiotropium on COPD exacerbations and related hospitalisations. *Prim Care Respir J* 18: 106–113.
- Hogg, J., Chu, F., Utokaparch, S., Woods, R., Elliott, W., Buzatu, L. *et al.* (2004) The nature of small-airway obstruction in chronic obstructive pulmonary disease. *N Engl J Med* 350: 2645–2653.
- Imai, K., Dalal, S., Chen, E., Downey, R., Schulman, L., Ginsburg, M. *et al.* (2001) Human collagenase (matrix metalloproteinase-1) expression in the lungs of patients with emphysema. *Am J Respir Crit Care Med* 163: 786–791.
- Ishihara, H., Shimura, S., Satoh, M., Masuda, T., Nonaka, H., Kase, H. *et al.* (1992) Muscarinic receptor subtypes in feline tracheal submucosal gland secretion. *Am J Physiol* 262: L223–L228.

- Jacoby, D., Gleich, G. and Fryer, A. (1993) Human eosinophil major basic protein is an endogenous allosteric antagonist at the inhibitory muscarinic M2 receptor. *J Clin Invest* 91: 1314–1318.
- James, A., Bai, T., Mauad, T., Abramson, M., Dolhnikoff, M., McKay, K. *et al.* (2009) Airway smooth muscle thickness in asthma is related to severity but not duration of asthma. *Eur Respir J* 34: 1040–1045.
- Jansat, J., Lamarca, R., de Miquel, G., Schrodter, A., Miletzki, B. and Gurniak, M. (2009) Safety and pharmacokinetics of multiple doses of acridinium bromide, a novel long-acting muscarinic antagonist for the treatment of chronic obstructive pulmonary disease, in healthy participants. *J Clin Pharmacol* 49: 1239–1246.
- Jeffery, P. (2001) Remodeling in asthma and chronic obstructive lung disease. *Am J Respir Crit Care Med* 164: S28–S38.
- Jones, P., Rennard, S., Agusti, A., Chanez, P., Magnussen, H., Fabbri, L. *et al.* (2011) Efficacy and safety of once-daily acridinium in chronic obstructive pulmonary disease. *Respir Res* 12: 55.
- Joos, G., Schelfhout, V., Pauwels, R., Kanniess, F., Magnussen, H., Lamarca, R. *et al.* (2010) Bronchodilatory effects of acridinium bromide, a long-acting muscarinic antagonist, in COPD patients. *Respir Med* 104: 865–872.
- Karner, C., Chong, J. and Poole, P. (2012) Tiotropium *versus* placebo for chronic obstructive pulmonary disease. *Cochrane Database Syst Rev* 7: CD009285.
- Kanefsky, J., Lenburg, M. and Hai, C. (2006) Cholinergic receptor and cyclic stretch-mediated inflammatory gene expression in intact ASM. *Am J Respir Cell Mol Biol* 34: 417–425.
- Kelly, E. and Jarjour, N. (2003) Role of matrix metalloproteinases in asthma. *Curr Opin Pulm Med* 9: 28–33.
- Kerstjens, H., Disse, B., Schröder-Babo, W., Bantje, T., Gahlemann, M., Sigmund, R. *et al.* (2011) Tiotropium improves lung function in patients with severe uncontrolled asthma: a randomized controlled trial. *J Allergy Clin Immunol* 128: 308–314.
- Kerstjens, H., Engel, M., Dahl, R., Paggiaro, P., Beck, E., Vandewalker, M. *et al.* (2012) Tiotropium in asthma poorly controlled with standard combination therapy. *N Engl J Med* 367: 1198–1207.
- Kerwin, E., D'Urzo, A., Gelb, A., Lakkis, H., Garcia Gil, E., Caracta, C., for the ACCORD I study investigators (2012a) Efficacy and safety of a 12-week treatment with twice-daily acridinium bromide in COPD patients (ACCORD COPD I). *COPD* 9: 90–101.
- Kerwin, E., Hebert, J., Gallagher, N., Martin, C., Overend, T., Alagappan, V.K. *et al.* (2012b) Efficacy and safety of NVA237 *versus* placebo and tiotropium in patients with moderate-to-severe COPD over 52 weeks: The GLOW2 study. *Eur Respir J* 40: 1106–1114.
- Kistemaker, L., Oenema, T., Meurs, H. and Gosens, R. (2012) Regulation of airway inflammation and remodeling by muscarinic receptors: Perspectives on anticholinergic therapy in asthma and COPD. *Life Sci* 91: 1126–1133.
- Koyama, S., Rennard, S. and Robbins, R. (1992) Acetylcholine stimulates bronchial epithelial cells to release neutrophil and monocyte chemotactic activity. *Am J Physiol* 262: L466–L471.
- Koyama, S., Sato, E., Nomura, H., Kubo, K., Nagai, S. and Izumi, T. (1998) Acetylcholine and substance P stimulate bronchial epithelial cells to release eosinophil chemotactic activity. *J Appl Physiol* 84: 1528–1534.
- Lagente, V. and Boichot, E. (2010) Role of matrix metalloproteinases in the inflammatory process of respiratory diseases. *J Mol Cell Cardiol* 48: 440–444.
- Lasseter, K., Aubets, J., Chuecos, F. and Gil, E. (2011) Acridinium bromide, a long-acting antimuscarinic, does not affect QT interval in healthy subjects. *J Clin Pharmacol* 51: 923–932.
- Magnussen, H., Bugnas, B., van Noord, J., Schmidt, P., Gerken, F. and Kesten, S. (2008) Improvements with tiotropium in COPD patients with concomitant asthma. *Respir Med* 102: 50–56.
- Maltais, F., Celli, B., Casaburi, R., Porszasz, J., Jarreta, D., Seoane, B. *et al.* (2011) Acridinium bromide improves exercise endurance and lung hyperinflation in patients with moderate to severe COPD. *Respir Med* 105: 580–587.
- Matthiesen, S., Bahulayan, A., Kempkens, S., Haag, S., Fuhrmann, M., Stichnote, C. *et al.* (2006) Muscarinic receptors mediate stimulation of human lung fibroblast proliferation. *Am J Respir Cell Mol Biol* 35: 621–627.
- Milara, J., Serrano, A., Peiró, T., Artigues, E., Gavalda, A., Miralpeix, M. *et al.* (2013) Acridinium inhibits cigarette smoke-induced lung fibroblast to myofibroblast transition. *Eur Respir J* 41: 1264–1274.
- Milara, J., Serrano, A., Peiró, T., Gavalda, A., Miralpeix, M., Morcillo, E. *et al.* (2012) Acridinium inhibits human lung fibroblast to myofibroblast transition. *Thorax* 67: 229–237.

- Morcillo, E. and Cortijo, J. (2006) Mucus and MUC in asthma. *Curr Opin Pulm Med* 12: 1–6.
- Ohta, S., Oda, N., Yokoe, T., Tanaka, A., Yamamoto, Y., Watanabe, Y. *et al.* (2010) Effect of tiotropium bromide on airway inflammation and remodelling in a mouse model of asthma. *Clin Exp Allergy* 40: 1266–1275.
- Pare, P., Roberts, C., Bai, T. and Wiggs, B. (1997) The functional consequences of airway remodeling in asthma. *Monaldi Arch Chest Dis* 52: 589–596.
- Park, H., Yang, M., Park, C., Kim, T., Moon, H., Min, K. *et al.* (2009) Additive role of tiotropium in severe asthmatics and Arg16Gly in ADRB2 as a potential marker to predict response. *Allergy* 64: 778–783.
- Pera, T., Zuidhof, A., Valadas, J., Smit, M., Schoemaker, R., Gosens, R. *et al.* (2011) Tiotropium inhibits pulmonary inflammation and remodelling in a guinea pig model of COPD. *Eur Respir J* 38: 789–796.
- Peters, S., Kunselman, S., Icitovic, N., Moore, W., Pascual, R., Ameredes, B. *et al.* (2010) Tiotropium bromide step-up therapy for adults with uncontrolled asthma. *N Engl J Med* 363: 1715–1726.
- Prat, M., Fernandez, D., Buil, M., Crespo, M.I., Casals, G., Ferrer, M. *et al.* (2009) Discovery of novel quaternary ammonium derivatives of (3R)-quinuclidinol esters as potent and long-acting muscarinic antagonists with potential for minimal systemic exposure after inhaled administration: identification of (3R)-3-{[hydroxy(di-2-thienyl)acetyl]oxy}-1-(3-phenoxypropyl)-1-azoniabicyclo[2.2.2]octane bromide (aclidinium bromide). *J Med Chem* 52: 5076–5092.
- Profita, M., Bonanno, A., Montalbano, A., Ferraro, M., Siena, L., Bruno, A. *et al.* (2011) Cigarette smoke extract activates human bronchial epithelial cells affecting non-neuronal cholinergic system signalling in vitro. *Life Sci* 89: 36–43.
- Profita, M., Bonanno, A., Siena, L., Ferraro, M., Montalbano, A., Pompeo, F. *et al.* (2008) Acetylcholine mediates the release of IL-8 in human bronchial epithelial cells by a NFkB/ERK- dependent mechanism. *Eur J Pharmacol* 582: 145–153.
- Profita, M., Giorgi, R., Sala, A., Bonanno, A., Riccobono, L., Mirabella, F. *et al.* (2005) Muscarinic receptors, leukotriene B4 production and neutrophilic inflammation in COPD patients. *Allergy* 60: 1361–1369.
- Proskocil, B., Sekhon, H., Jia, Y., Savchenko, V., Blakely, R., Lindstrom, J. *et al.* (2004) Acetylcholine is an autocrine or paracrine hormone synthesized and secreted by airway bronchial epithelial cells. *Endocrinology* 145: 2498–2506.
- Ramnarine, S., Haddad, E., Khawaja, A., Mak, J. and Rogers, D. (1996) On muscarinic control of neurogenic mucus secretion in ferret trachea. *J Physiol* 494: 577–586.
- Razani-Boroujerdi, S., Behl, M., Hahn, F., Pena-Philippides, J., Hutt, J. and Sopori, M. (2008) Role of muscarinic receptors in the regulation of immune and inflammatory responses. *J Neuroimmunol* 194: 83–88.
- Rogers, D. (2001) Motor control of airway goblet cells and glands. *Respir Physiol* 125: 129–144.
- Sato, E., Koyama, S., Okubo, Y., Kubo, K. and Sekiguchi, M. (1998) Acetylcholine stimulates alveolar macrophages to release inflammatory cell chemotactic activity. *Am J Physiol* 274: L970–L979.
- Schellhout, V., Ferrer, P., Jansat, J., Peris, F., Gil, E.G., Pauwels, R.A. *et al.* (2010) Activity of aclidinium bromide, a new long-acting muscarinic antagonist: a phase I study. *Br J Clin Pharmacol* 69: 458–464.
- Sentellas, S., Ramos, I., Alberti, J., Salvà, M., Antón, F., Miralpeix, M. *et al.* (2010) Aclidinium bromide, a new, long-acting, inhaled muscarinic antagonist: in vitro plasma inactivation and pharmacological activity of its main metabolites. *Eur J Pharm Sci* 39: 283–290.
- Singh, S., Loke, Y., Enright, P. and Furberg, C. (2011) Mortality associated with tiotropium mist inhaler in patients with chronic obstructive pulmonary disease: systematic review and meta-analysis of randomised controlled trials. *BMJ* 342: d3215.
- Sköld, C. (2010) Remodeling in asthma and COPD – differences and similarities. *Clin Respir J* 4(Suppl. 1): 20–27.
- Sykes, D., Dowling, M. and Charlton, S. (2009) Exploring the mechanism of agonist efficacy: a relationship between efficacy and agonist dissociation rate at the muscarinic M3 receptor. *Mol Pharmacol* 76: 543–551.
- Tashkin, D., Celli, B., Senn, S., Burkhart, D., Kesten, S., Menjoge, S. *et al.* for the UPLIFT Study Investigators (2008) A 4-year trial of tiotropium in chronic obstructive pulmonary disease. *N Engl J Med* 359: 1543–1554.
- Ulrik, C. (2012) Once-daily glycopyrronium bromide, a long-acting muscarinic antagonist, for chronic obstructive pulmonary disease: a systematic review of clinical benefit. *Int J COPD* 7: 673–678.
- Vacca, G., Randerath, W. and Gillissen, A. (2011) Inhibition of granulocyte migration by tiotropium bromide. *Respir Res* 12: 24.
- Vestbo, J., Vogelmeier, C., Creemers, J., Faiques, M., Ribera, A. and Gil, E. (2010) Onset of effect

of aclidinium, a novel long-acting muscarinic antagonist, in patients with COPD. *COPD* 7: 331–336.

Vogelmeier, C. and Banerji, D. (2011) NVA237, a long-acting muscarinic antagonist, as an emerging therapy for chronic obstructive pulmonary disease. *Ther Adv Respir Dis* 5: 163–173.

Voynow, J. and Rubin, B. (2009) Mucins, mucus, and sputum. *Chest* 135: 505–512.

Wessler, I. and Kirkpatrick, C. (2008) Acetylcholine beyond neurons: the non-neuronal cholinergic system in humans. *Br J Pharmacol* 154: 1558–1571.

Wise, R., Anzueto, A., Cotton, D., Dahl, R., Devins, T., Disse, B. *et al.* for the TIOSPIR Investigators (2013) Tiotropium Respimat inhaler and the risk of death in COPD. *N Engl J Med* 369: 1491–1501.

Wollin, L. and Pieper, M. (2010) Tiotropium bromide exerts anti-inflammatory activity in a cigarette smoke mouse model of COPD. *Pulm Pharmacol Ther* 23: 345–354.

Yohannes, A., Willgoss, T. and Vestbo, J. (2011) Tiotropium for treatment of stable COPD: a meta-analysis of clinically relevant outcomes. *Respir Care* 56: 477–487.