Emerging role of long acting muscarinic antagonists for asthma

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Keywords

asthma, muscarinic antagonist, tiotropium, β -adrenoceptor

Received

16 January 2013

Accepted

18 March 2013

Accepted Article

Published Online

28 March 2013

Acetylcholine is involved in the control of airway smooth muscle constriction and in recruitment of inflammatory cells via neuronal and paracrine effects on muscarinic type 3 receptors. Long acting muscarinic antagonists (LAMA) are well established in guidelines for COPD but are not currently licensed for use in asthma. There are emerging data from key clinical trials to show that LAMA may confer bronchodilator effects and improved control when used in addition to inhaled corticosteroid (ICS) alone or in conjunction with long acting β -adrenoceptor agonists (LABA). Further studies in persistent asthmatic patients are required to evaluate ICS sparing effects of LAMA looking particularly at airway hyper-responsiveness and surrogate inflammatory markers, in addition to evaluation of possible synergy between LAMA and LABA when given together. Future possible development of combination inhalers comprising ICS/LAMA or ICS/LAMA/LABA will require long term studies looking at asthma control and exacerbations in both adult and paediatric patients.

The unmet need in asthma management

Current guidelines for asthma management advocate the use of inhaled corticosteroids (ICS) as first line anti-inflammatory therapy at step 2, followed at step 3 by either increasing the dose of ICS or the addition of a second line controller with a long acting β -adrenoceptor agonist (LABA) to achieve sustained bronchodilatation, usually as single ICS/LABA combination inhaler [1]. Short acting muscarinic antagonists (SAMA), such as ipratropium, are presently used as high dose nebulized reliever therapy in acute severe asthma, taken in conjunction with short acting β -adrenoceptor agonists (SABA), such as salbutamol. A Cochrane review found no evidence to support the routine use of SAMA for patients whose asthma is not well controlled on standard therapy [2].

Long acting muscarinic antagonists (LAMA) while well established in COPD guidelines are not currently licensed for asthma [3]. Currently available LAMAs include the long established tiotropium, as well as the newer drugs, namely

aclidinium and glycopyrronium, all of which are presently licensed for use in COPD.

The aim of using regular LABA as a second line controller is to stabilize airway smooth muscle and hence improve asthma control and reduce exacerbations, while at the same time allowing the use of lower doses of ICS. However in one study, only 41% of patients achieved total asthma control and 71% became well controlled when treated with ICS/LABA combination, suggesting an unmet therapeutic need [4]. Chronic use of ICS/LABA is accompanied by pharmacological adaptation involving down-regulation and uncoupling of β_2 -adrenoceptors (β_2 ADR), in turn producing sub-sensitivity of response, also known as tachyphylaxis [5]. This manifests as loss of protection against a variety bronchoconstrictor stimuli as well as cross sub-sensitivity to acute reliever therapy with salbutamol [6, 7]. Indeed clinical studies have shown that airway inflammation and associated asthma control may become worse when adding LABA to ICS [8–11]. In approximately 15% of genetically susceptible individuals who possess the homozygous arginine-16 β_2 ADR

polymorphism, the degree of sub-sensitivity of response is more pronounced, such that regular ICS/LABA may result in increased airway hyper-responsiveness (AHR) and associated worsening of control [12–16], although in such patients the long term bronchodilator response appears to remain preserved [17, 18]. The United States Food and Drug Administration (FDA) in turn after carefully reviewing the available evidence recommended that LABA should not be given for long term therapy even in combination with ICS, and that where possible patients should step down to ICS alone [19, 20]. In particular the role of LABA in asthmatic children has come under close scrutiny by the FDA given the paucity of evidence for their efficacy. Against this background of concern regarding LABA, there is an unmet need for an alternative long acting bronchodilator agent for use as add on therapy to ICS alone or ICS/LABA to improve control at steps 3/4 of current guidelines.

The purpose of this article is therefore to review the emerging clinical evidence for use of LAMA in asthma when used in addition to ICS and ICS/LABA.

Pharmacology of long acting muscarinic antagonists

A detailed review of the basic pharmacology of muscarinic antagonists is given elsewhere [21]. In brief the normal airway tone is maintained as a balance of dilatation by stimulation of airway smooth muscle β_2 ADR by adrenaline and constriction by stimulation of muscarinic receptors by acetylcholine (Figure 1). The effects of acetylcholine are mediated via post-junctional M_3 receptors resulting in smooth muscle constriction, as well as via pre-junctional M_2 auto-receptors which results in reduced release of acetylcholine, thus effectively acting as a brake to cholinergic

Figure 1

In humans there is no direct sympathetic innervation of the smooth muscle, but β_2 ADR (β_2) are available to circulating adrenaline. Pre-synaptically the sympathetic nerves lie close to the parasympathetic system and are thought to influence β_2 ADR here. It is thought both β_2 ADR and M_2 receptors are inhibitory to the release of acetylcholine (ACh) and that there is crosstalk between these receptor types (broken line). The M_2 receptor is stimulated by ACh to reduce further ACh secretion. This M_2 autoreceptor is thought to be defective in asthma and in response to viruses, hence this negative feedback is lost and there is increased airway hyperreactivity (AHR). Post-synaptically the muscarinic M_3 receptor is the main parasympathetic bronchoconstrictor mechanism. Although post-synaptic M_2 receptors are present their role is to prolong M_3 initiated bronchoconstriction by opposing the actions of β_2 ADR. Hence the same receptors may have different actions pre- and post-synaptically exerting independent effects on AHR and airway tone respectively

transmission, i.e. a negative feedback loop preventing over-stimulation of post-junctional M_3 receptors [22]. Hence LAMA such as tiotropium which preferentially block M_3 receptors, avoid the potential problem of increased pre-junctional acetylcholine release associated with SAMA such as ipratropium, which is less selective blocking both M_2 and M_3 receptors. Other LAMA such as aclidinium and glycopyrronium also exhibit prolonged exhibit M_3 selective blockade. Moreover there is cross talk between M_2 receptors and pre-junctional β_2 ADR, with the latter also acting as a brake towards acetylcholine release. This explains why blockade of β_2 ADR results in unopposed increased cholinergic transmission, airway constriction and associated increased AHR. Hence muscarinic antagonists may be used prevent acute bronchoconstriction due to β_2 ADR antagonism with propranolol in asthma [23, 24]. It is also perhaps conceivable that muscarinic antagonists might in a similar fashion also prevent the adverse adaptive response to chronic exposure with LABA, where down regulation of pre-junctional β_2 ADR could augment cholinergic transmission, i.e. a potential synergistic effect between LAMA and LABA in asthma.

In addition *in vitro* studies have shown that acetylcholine plays an important role in mediating inflammatory cell chemotaxis and activation, as discussed in detail elsewhere [25]. In brief, acetylcholine may be released neurally or secreted in paracrine fashion from inflammatory cells as well as airway epithelium, in response to a variety of trigger factors such as cigarette smoke, viruses or allergens. A number of inflammatory cytokines including interleukin 6 and 8, as well as leukotriene B_4 are produced which may produce chemotaxis and inflammatory cell activation. Activation of signalling pathways such as

extracellular-signal-regulated kinase 1/2 and nuclear factor kappa B, mediates these effects via M_3 receptors expressed on airway smooth muscle and epithelial cells. This in turn suggests a potential role in asthma for LAMA such as tiotropium as novel anti-inflammatory agents in addition to their known bronchodilator actions by relaxing airway smooth muscle.

Key clinical trials

This section will focus on key chronic dosing studies with LAMA where the primary diagnosis is asthma rather than COPD. To date the key clinical trials with LAMA (as tiotropium) have focused at step 3/4 of current guidelines for use as add on therapy to ICS alone or ICS/LABA, as summarized in Table 1. These trials were primarily driven by requirements for regulatory submission and as such were powered on bronchodilator sensitive outcomes such as forced expiratory volume in 1 s (FEV_1) or peak flow (PEF).

LAMA or LABA as add on to ICS

In a three way crossover study in 210 poorly controlled asthmatics aged 42 years, Peters *et al.* [26] examined the effects of adding in salmeterol 100 μ g daily or tiotropium 18 μ g daily (via Handihaler) vs. doubling the dose of extra fine HFA-beclometasone over 14 weeks, after an initial run-in period on 160 μ g daily, in asthmatic patients with a mean FEV_1 of 71.5% and mean salbutamol reversibility to salbutamol and ipratropium of 14.9% and 12.4%, respectively. For the primary outcome of morning PEF there was significant superiority with adding tiotropium over

Table 1

Key clinical trials in asthma with LAMA as add on to ICS or ICS/LABA

Design	Comparison	FEV_1	Reversibility	Tiotropium FEV_1 difference	AQLQ
Fardon <i>et al.</i> 2007 [30] XO, 4 weeks	ICS/LABA/TIO18	51%	23%	0.17 I (0.03, 0.31)*	0.30, NS
	ICS/LABA (vs. ICS $\times$ 2)			0.11 (–0.03, 0.25) NS	0.30, NS
Peters <i>et al.</i> 2010 [26] XO, 14 weeks	ICS/TIO18	71%	15%	0.10 I (0.03, 0.17)**	0.10, NS
	ICS/LABA (vs. ICS $\times$ 2)			0 I (–0.08, 0.07) NS	0.23**
Bateman <i>et al.</i> 2011 [27] PG, 16 weeks	ICS/TIO5	68%	26%	0.15 I (0.07, 0.23)***	0.09, NS
	ICS/LABA (vs. ICS)			0.17 I (0.09, 0.24)***	0.24**
Kerstjens <i>et al.</i> 2011 [32] XO, 8 weeks	ICS/LABA/TIO5	51%	14%	0.14 I (0.10, 0.18)***	0.10, NS
	ICS/LABA/TIO10 (vs. ICS/LABA)			0.17 I (0.13, 0.21)***	0.10, NS
Kerstjens <i>et al.</i> 2012 [33] XO, 48 weeks	ICS/LABA/TIO5:	55%	15%		
	Trial 1			0.09 I (0.02, 0.15)*	0.04 NS
	Trial 2 (vs. ICS/LABA)			0.15 I (0.09, 0.22)***	0.18*

The effect of adding tiotropium (TIO) on FEV_1 in all studies was less than the minimal important difference (MID) of 0.23 I, and on asthma quality of life questionnaire (AQLQ) was less than MID of 0.5. XO, crossover; PG, parallel group. Duration of treatment shown in weeks. TIO5/10 μ g refers to dose via fine mist RespiMat device, while TIO18 μ g refers to dose via dry powder Handihaler device. Reversibility is the % change in FEV_1 at baseline after salbutamol/ipratropium. The tiotropium effect is shown as the difference in FEV_1 compared with either inhaled corticosteroid (ICS) or inhaled corticosteroid with long acting β -adrenoceptor agonist (ICS/LABA). Values for FEV_1 are shown as means (95% CI):

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS: not significant.

doubling the dose of HFA-beclometasone, amounting to a mean difference of 26 l min⁻¹, as well as non-inferiority vs. salmeterol with a mean difference of 6 l min⁻¹. For the secondary end point of FEV₁, mean differences with the addition of tiotropium were significantly improved by 0.10 l vs. twice the dose of HFA-beclometasone and by 0.11 l vs. adding salmeterol. Similar results were observed for secondary outcomes including asthma control questionnaire and asthma quality of life score. One could argue that the primary outcome of PEF is more sensitive to effects on airway smooth muscle rather than inflammation, and since patients were bronchodilator reversible at baseline, it is perhaps hardly surprising that both salmeterol and tiotropium were superior to doubling the dose of HFA-beclometasone. Inflammatory surrogates including exhaled breath nitric oxide and sputum eosinophils were both low at baseline and thereafter with randomized treatments.

Bateman and coworkers [27] took this concept one step further by evaluating add on therapy in parallel groups with salmeterol 100 µg or tiotropium 5 µg daily (via Respimat) or placebo for 16 weeks in a cohort of 388 genetically susceptible asthmatics possessing the homozygous arginine-16 β₂ADR polymorphism who were not controlled on ICS alone, aged 43 years, with mean FEV₁ of 68% and reversibility of 26% after ipratropium 80 µg and salbutamol 400 µg. Essentially the primary outcome of PEF deteriorated on placebo but was maintained with both active treatments, with the difference being significant. There was non-inferiority for morning PEF with a mean difference of 21 l min⁻¹ when comparing salmeterol or tiotropium vs. placebo, along with 0.11 l and 0.09 l mean differences in FEV₁ respectively vs. placebo. Asthma symptom free days and asthma quality of life score were also significantly improved by salmeterol but not tiotropium vs. placebo, although differences between treatments were not significant.

The only study to have evaluated add on effects of tiotropium on AHR was in 18 mild to moderate persistent asthmatics taking ICS at a beclometasone equivalent dose of 440 µg daily. Histamine challenge was performed after 4 weeks of tiotropium plus ICS and then after a further 2 weeks of ICS alone, analyzed as part of the placebo arm of a study looking at effects of propranolol on AHR [24]. There was no significant bronchoprotection conferred by the addition of tiotropium to ICS, amounting to a mean difference of 0.12 doubling dilution in the histamine provocation concentration to produce a 20% fall in FEV₁ (PC₂₀). There was a small but significant improvement in pre-challenge FEV₁% when comparing tiotropium plus ICS vs. ICS alone (4.8% difference), but not for post-challenge FEV₁ (2.3% difference). This is also in keeping with an acute dose ranging study with ipratropium where the bronchodilator effects were not accompanied by commensurate improvements in histamine PC₂₀ [28]. However in the antigen sensitized rodent model, tiotropium has been shown to

attenuate the late phase allergen response, suggesting a possible role of M₃ receptors in mediating sensory nerve inhibition [29].

LAMA as add on to ICS/LABA

The first proof of concept study by Fardon *et al.* [30] was in 18 non-smoking severe persistent asthmatics aged 54 years, FEV₁ 51%, salbutamol reversibility 22.6% and ipratropium reversibility 17.6%. After an initial run-in period on fluticasone 1000 µg daily, patients were then treated in randomized crossover fashion for 4 weeks having stepped down to half the dose of fluticasone (500 µg daily), with either the addition of both salmeterol and tiotropium (i.e. triple therapy with fluticasone/salmeterol combination 500/100 µg daily + tiotropium 18 µg daily) or the addition of salmeterol alone (i.e. double therapy with fluticasone/salmeterol combination 500/100 µg daily + placebo). There was a small but significant improvement in FEV₁ of 0.17 l (6.8% predicted) and significantly reduced exhaled breath nitric oxide in response to triple therapy but not double therapy, as compared with baseline with twice the dose of fluticasone alone. Domiciliary morning PEF significantly increased on both randomized treatments compared with baseline, with no difference between the improvements seen with double (41 l min⁻¹) and triple (55 l min⁻¹) therapy, while there were no significant differences between treatments in asthma quality of life scores. While the change in FEV₁ was less than the minimal important difference (MID) of 0.23 l, the change in PEF exceeded the MID of 19 l min⁻¹ [31]. In addition at baseline acute sequential reversibility to salbutamol/ipratropium was evaluated while taking fluticasone 1000 µg, such that the FEV₁ after salbutamol 400 µg was greater than while taking fluticasone/salmeterol 500/100 µg, and after salbutamol 400 µg/ipratropium 80 µg was significantly greater than while taking fluticasone/salmeterol 500 µg/100 µg plus tiotropium 18 µg, amounting to mean differences of 9% predicted and 13.7% predicted, respectively.

A further two larger pivotal step 4 studies have been performed looking at the concept of triple therapy. In the first of these by Kerstjens *et al.* [32], 107 patients aged 55 years of whom 36% were ex-smokers, were evaluated with severe uncontrolled asthma despite ICS/LABA therapy, being randomized in crossover fashion to receive add on with either placebo, tiotropium 5 or 10 µg daily (via Respimat) for 8 weeks each. Mean FEV₁ was 51% predicted with mean salbutamol reversibility of 14%. For the primary end point of peak FEV₁ there was no difference between 5 µg and 10 µg doses of tiotropium, which were both significantly superior to placebo, with respective mean differences of 0.14 l and 0.17 l, while mean values for trough morning PEF were significantly higher with tiotropium 10 µg than 5 µg at 15 l min⁻¹ vs. 8 l min⁻¹, respectively. Moreover there were no significant improvements at either dose in asthma quality of life score or asthma diary cards.

The second study by Kerstjens *et al.* [33] involved a composite analysis of two parallel group trials in 912 patients with severe uncontrolled asthma aged 53 years, FEV₁ 55%, 15% salbutamol reversibility, of whom 24% were ex-smokers, who were randomized to receive tiotropium 5 µg daily (via Respimat) or placebo for 48 weeks as add on to pre-existing ICS/LABA. For the two co-primary pulmonary function end points after 24 weeks, there was significant superiority of peak and trough FEV₁ amounting to respective mean overall differences (i.e. mean values for trials 1 and 2) of 0.12 l and 0.10 l, along with a 22 l min⁻¹ improvement in morning PEF. For the third co-primary end point there was also a significant 21% overall reduction in risk of a severe exacerbation over the 48 weeks, while changes in asthma control questionnaire and quality of life score were both less than the minimal important difference of 0.5 units. One could argue that the subgroup of smokers may have had COPD with an asthmatic component, although in practice treatment would effectively be the same irrespective of the diagnosis. The management of asthmatic smokers poses particular therapeutic challenges, as like patients with COPD they tend to be relatively resistant to ICS even at high doses, but are more responsive to LABA [34, 35]. Thus the use of triple therapy with ICS/LABA/LAMA in asthmatics who smoke may be a logical option and warrants further investigation of this particular phenotype.

Adverse effects

In terms of safety the largest study used tiotropium Respimat 5 µg over 48 weeks [33], where drug related anticholinergic adverse effects were seen in 25/456 patients (5.7%) in the tiotropium group compared with 21/456 (4.6%) in the placebo group, with dry mouth in eight (1.8%) and three (1.7%) patients, and cardiac events in two (0.4%) and one (0.2%), respectively. There were no differences in heart rate, blood pressure or electrocardiographic abnormalities between groups. Some concerns have been raised regarding a possible increase in cardiac toxicity with tiotropium via the Respimat device, mostly on the basis of data trawling from meta-analysis in COPD patients where mortality was not the primary outcome [36].

Moreover in terms of biological plausibility there appears to be no rationale for the disconnect between the apparent increased mortality with tiotropium via the fine mist Respimat on the one hand but reduced mortality with tiotropium via the dry powder handihaler on the other, since the Respimat 5 µg actually has 24% lower systemic bioavailability (as AUC_{0-6h}) than the Handihaler 18 µg at steady state in patients with COPD, when comparing the devices at commonly used therapeutic doses [37]. If tiotropium were inherently cardiotoxic at current therapeutic doses, then one would expect to see some increase in mortality signal in real life even with the dry powder inhaler due to systemic exposure, as in susceptible hypoxic COPD patients with concomitant cardiovascular disease due to

smoking. For example in a real life study of 2853 patients with COPD aged 68 years, FEV₁ 51% who were followed up over a mean period of 4.65 years, the addition of tiotropium mostly via Handihaler (as ICS/LABA/LAMA) actually produced a 35% reduction in all cause mortality, pointedly with 47% of patients exposed to tiotropium having concomitant cardiovascular disease along with a reduced mean oxygen saturation of 91% indicating hypoxaemia [38]. No such long term follow up data on mortality are currently available on the other newer LAMA in COPD, such as with aclidinium or glycopyrronium. It is conceivable that aclidinium might confer a superior systemic safety profile since it is rapidly metabolized in plasma resulting in a short half-life [39]. However in the setting of asthma where patients tend to be younger non-smokers without cardiovascular disease, any concerns about potential cardiotoxicity from tiotropium are much less relevant.

Conclusions and the way forward

To appraise properly the clinical relevance of these pivotal studies with tiotropium, one has to consider the results for the main outcomes in the context of the minimal important difference (Table 1). All of the trials have shown small improvements in FEV₁ which were less than the MID of 0.23 l [31], inferring that the bronchodilator effects while statistically significant were not particularly clinically relevant. Furthermore these studies all showed changes in quality of life or asthma control questionnaire scores which were less than the minimal important difference of 0.5 units for both outcomes [40, 41]. Thus it is hard to explain the 21% reduction in severe exacerbations in the study of Kerstjens *et al.* [33] purely on the basis of such small improvements in airway caliber *per se*, perhaps invoking anti-inflammatory activity with tiotropium [42].

Assessment of AHR was not performed in any of the key studies in severe asthma presumably because it was precluded on safety grounds by the baseline FEV₁ being less than 60% in a large proportion of enrolled patients. In one study no significant bronchoprotection against histamine challenge was observed with chronic dosing of tiotropium in ICS treated mild to moderate asthmatics, despite a significant improvement in pre challenge FEV₁ [24]. This needs to be put in the context of previous studies which have shown that chronic dosing with ICS/LABA produces loss of bronchoprotection due to sub-sensitivity of response [43, 44], or even a worsening in AHR in susceptible asthmatics possessing the arginine-16 genotype [12, 13]. Perhaps in future trials using a lower provocation threshold such as a 10% fall in FEV₁ might be possible, for example using an abbreviated mannitol bronchial challenge, which might be applicable in trials with more severe asthmatics [45, 46]. The apparent lack of effect of SAMA or LAMA on AHR may merely reflect the type of challenge as histamine acts directly on airway smooth muscle, while indirect acting

agents such as mannitol and AMP act via release of inflammatory mediators. In other words using a challenge agent which more closely reflects the underlying asthmatic inflammatory process may be more likely to detect a signal with LAMA therapy. Further placebo controlled studies are therefore indicated to evaluate effects of LAMA on AHR, for example using mannitol challenge when compared with LABA as add on therapy to ICS or ICS/LABA. Indeed such airway stabilization activity of LAMA might confer protection against exacerbations, as has been already been shown with LABA.

Only two clinical studies have to date evaluated surrogate airway inflammatory markers. In the study of Peters *et al.* where exhaled breath nitric oxide was performed [26], values at baseline were already suppressed by ICS during run-in, such that any further reductions conferred by tiotropium were also of small magnitude and unlikely to be clinically relevant. A small but significant fall in nitric oxide was reported by Fardon *et al.* [30] when tiotropium was added to ICS/LABA. One possibility might be to look at the corticosteroid sparing activity of adding LAMA vs. placebo during tapered ICS step down, using mannitol challenge, nitric oxide and other non-invasive inflammatory surrogates [46]. This type of design might be able to demonstrate *in vivo* anti-inflammatory activity conferred by LAMA, in keeping with *in vitro* observations [25]. Such *in vivo* anti-inflammatory activity conferred by LAMA might help explain the apparent disconnect between the small improvements and FEV₁ and the reduction in exacerbations with tiotropium, when used as triple therapy [42]. In this regard it has been shown that repeated inhalations of methacholine, a muscarinic receptor agonist, may result in biopsy changes of airway remodelling without producing eosinophilic inflammation in asthmatic patients [47]. This in turn suggests the possibility that long term prevention of bronchoconstriction by LAMA may prevent airway remodelling, in keeping with the observed inhibitory effect of tiotropium in the murine and guinea pig models of antigen induced chronic asthma [48–50].

Another pharmacological mechanism might invoke a protective effect of tiotropium due to blockade of augmented cholinergic transmission due to down regulation and uncoupling of pre-junctional β_2 ADR from prolonged LABA stimulation, which normally acts as a cholinergic brake. Moreover as acetylcholine acts via M3 receptors to promote β_2 ADR uncoupling via protein kinase C phosphorylation, LAMA might also prevent against LABA induced subsensitivity [51]. These mechanisms might infer potential synergism between LAMA and LABA, perhaps also when given with ICS as triple therapy. Hence, it is possible that concomitant administration of LAMA might confer relative protection against potential adverse effects due to LABA exposure. As triple combination inhalers (i.e. ICS/LABA/LAMA) are currently in development for both asthma and COPD, further studies may provide interesting insights into the role of acetylcholine in the pathophysiol-

ogy of persistent asthma. In the context of asthma, unlike COPD, there will be no place for dual LAMA/LABA combination inhalers unless given in conjunction with a separate ICS inhaler.

Competing Interests

All authors have completed the Unified Competing Interest form (available on request from the corresponding author) and declare: BJL has had support from Boehringer to attend the British Thoracic Society and European Society Respiratory meetings as well as being paid to attend advisory board meetings, BJL received consulting fees from Gurnos, Hexal, Sandoz, Chiesi, Nycomed and 3 M, grant support from Teva Inc and Chiesi, a speaker bureau fee from Teva Inc and travel/accommodation/meeting expenses from Chiesi (ERS Meeting), Nycomed (ERS Meeting) and Pharmaxis (AAAAI Meeting) in the previous 3 years and no other relationships or activities that could appear to have influenced the submitted work.

REFERENCES

- 1 Bateman ED, Hurd SS, Barnes PJ, Bousquet J, Drazen JM, FitzGerald M, Gibson P, Ohta K, O'Byrne P, Pedersen SE, Pizzichini E, Sullivan SD, Wenzel SE, Zar HJ. Global strategy for asthma management and prevention: GINA executive summary. *Eur Respir J* 2008; 31: 143–78.
- 2 Westby MJ, Benson MK, Gibson PG. Anticholinergic agents for chronic asthma in adults. *Cochrane Database Syst Rev* 2004; (3): CD003269.
- 3 Vestbo J, Hurd SS, Agustí AG, Jones PW, Vogelmeier C, Anzueto A, Barnes PJ, Fabbri LM, Martinez FJ, Nishimura M, Stockley RA, Sin DD, Rodriguez-Roisin R. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. *Am J Respir Crit Care Med* 2013; 187: 347–65.
- 4 Bateman ED, Boushey HA, Bousquet J, Busse WW, Clark TJ, Pauwels RA, Pedersen SE. Can guideline-defined asthma control be achieved? The Gaining Optimal Asthma Control study. *Am J Respir Crit Care Med* 2004; 170: 836–44.
- 5 Lipworth BJ. Long-acting beta2-adrenoceptor agonists: a smart choice for asthma? *Trends Pharmacol Sci* 2007; 28: 257–62.
- 6 Lipworth BJ, Aziz I. A high dose of albuterol does not overcome bronchoprotective subsensitivity in asthmatic subjects receiving regular salmeterol or formoterol. *J Allergy Clin Immunol* 1999; 103: 88–92.
- 7 Lee DK, Jackson CM, Currie GP, Cockburn WJ, Lipworth BJ. Comparison of combination inhalers vs. inhaled corticosteroids alone in moderate persistent asthma. *Br J Clin Pharmacol* 2003; 56: 494–500.

- 8 Green RH, Brightling CE, McKenna S, Hargadon B, Neale N, Parker D, Ruse C, Hall IP, Pavord ID. Comparison of asthma treatment given in addition to inhaled corticosteroids on airway inflammation and responsiveness. *Eur Respir J* 2006; 27: 1144–51.
- 9 Thomas M, von Ziegenweidt J, Lee AJ, Price D. High-dose inhaled corticosteroids versus add-on long-acting beta-agonists in asthma: an observational study. *J Allergy Clin Immunol* 2009; 123: 116–21.
- 10 Gibson PG, Powell H, Ducharme FM. Differential effects of maintenance long-acting beta-agonist and inhaled corticosteroid on asthma control and asthma exacerbations. *J Allergy Clin Immunol* 2007; 119: 344–50.
- 11 Salpeter SR, Wall AJ, Buckley NS. Long-acting beta-agonists with and without inhaled corticosteroids and catastrophic asthma events. *Am J Med* 2010; 123: 322–8. e2.
- 12 Lee DK, Currie GP, Hall IP, Lima JJ, Lipworth BJ. The arginine-16 β_2 -adrenoceptor polymorphism predisposes to bronchoprotective subsensitivity in patients treated with formoterol and salmeterol. *Br J Clin Pharmacol* 2004; 57: 68–75.
- 13 Wechsler ME, Kunselman SJ, Chinchilli VM, Bleecker E, Boushey HA, Calhoun WJ, Ameredes BT, Castro M, Craig TJ, Denlinger L, Fahy JV, Jarjour N, Kazani S, Kim S, Kraft M, Lazarus SC, Lemanske RF Jr, Markezich A, Martin RJ, Permaul P, Peters SP, Ramsdell J, Sorkness CA, Sutherland ER, Szeffler SJ, Walter MJ, Wasserman SI, Israel E. Effect of beta2-adrenergic receptor polymorphism on response to longacting beta2 agonist in asthma (LARGE trial): a genotype-stratified, randomised, placebo-controlled, crossover trial. *Lancet* 2009; 374: 1754–64.
- 14 Basu K, Palmer CN, Tavendale R, Lipworth BJ, Mukhopadhyay S. Adrenergic beta(2)-receptor genotype predisposes to exacerbations in steroid-treated asthmatic patients taking frequent albuterol or salmeterol. *J Allergy Clin Immunol* 2009; 124: 1188–94. e3.
- 15 Palmer CN, Lipworth BJ, Lee S, Ismail T, Macgregor DF, Mukhopadhyay S. Arginine-16 beta2 adrenoceptor genotype predisposes to exacerbations in young asthmatics taking regular salmeterol. *Thorax* 2006; 61: 940–4.
- 16 Lipworth BJ, Basu K, Donald HP, Tavendale R, Macgregor DF, Ogston SA, Palmer CNA, Mukhopadhyay S. Tailored second-line therapy in asthmatic children with the Arg16 genotype. *Clin Sci (Lond)* 2013; 124: 521–8.
- 17 Bleecker ER, Postma DS, Lawrance RM, Meyers DA, Ambrose HJ, Goldman M. Effect of ADRB2 polymorphisms on response to longacting beta2-agonist therapy: a pharmacogenetic analysis of two randomised studies. *Lancet* 2007; 370: 2118–25.
- 18 Bleecker ER, Nelson HS, Kraft M, Corren J, Meyers DA, Yancey SW, Anderson WH, Emmett AH, Ortega HG. Beta2-receptor polymorphisms in patients receiving salmeterol with or without fluticasone propionate. *Am J Respir Crit Care Med* 2010; 181: 676–87.
- 19 Kramer JM. Balancing the benefits and risks of inhaled long-acting beta-agonists - the influence of values. *N Engl J Med* 2009; 360: 1592–5.
- 20 Chowdhury BA, Dal Pan G. The FDA and safe use of long-acting beta-agonists in the treatment of asthma. *N Engl J Med* 2010; 362: 1169–71.
- 21 Moulton BC, Fryer AD. Muscarinic receptor antagonists, from folklore to pharmacology; finding drugs that actually work in asthma and COPD. *Br J Pharmacol* 2011; 163: 44–52.
- 22 Lipworth BJ, Williamson PA. Think the impossible: beta-blockers for treating asthma. *Clin Sci (Lond)* 2010; 118: 115–20.
- 23 Ind PW, Dixon CM, Fuller RW, Barnes PJ. Anticholinergic blockade of beta-blocker-induced bronchoconstriction. *Am Rev Respir Dis* 1989; 139: 1390–4.
- 24 Short P, Williamson P, Anderson W, Lipworth B. Randomised placebo controlled trial to evaluate chronic dosing effects of propranolol in steroid treated persistent asthmatics. *Thorax* 2012; 67: A68.
- 25 Kistemaker LE, Oenema TA, Meurs H, Gosens R. Regulation of airway inflammation and remodeling by muscarinic receptors: perspectives on anticholinergic therapy in asthma and COPD. *Life Sci* 2012; 91: 1126–33.
- 26 Peters SP, Kunselman SJ, Icitovic N, Moore WC, Pascual R, Ameredes BT, Boushey HA, Calhoun WJ, Castro M, Cherniack RM, Craig T, Denlinger L, Engle LL, DiMango EA, Fahy JV, Israel E, Jarjour N, Kazani SD, Kraft M, Lazarus SC, Lemanske RF Jr, Lugogo N, Martin RJ, Meyers DA, Ramsdell J, Sorkness CA, Sutherland ER, Szeffler SJ, Wasserman SI, Walter MJ, Wechsler ME, Chinchilli VM, Bleecker ER. Tiotropium bromide step-up therapy for adults with uncontrolled asthma. *N Engl J Med* 2010; 363: 1715–26.
- 27 Bateman ED, Kornmann O, Schmidt P, Pivovarov A, Engel M, Fabbri LM. Tiotropium is noninferior to salmeterol in maintaining improved lung function in B16-Arg/Arg patients with asthma. *J Allergy Clin Immunol* 2011; 128: 315–22.
- 28 Britton J, Hanley SP, Garrett HV, Hadfield JW, Tattersfield AE. Dose related effects of salbutamol and ipratropium bromide on airway calibre and reactivity in subjects with asthma. *Thorax* 1988; 43: 300–5.
- 29 Raemdonck K, de Alba J, Birrell MA, Grace M, Maher SA, Irvin CG, Fozard JR, O'Byrne PM, Belvisi MG. A role for sensory nerves in the late asthmatic response. *Thorax* 2012; 67: 19–25.
- 30 Fardon T, Haggart K, Lee DKC, Lipworth BJ. 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* 2007; 101: 1218–28.
- 31 Santanello NC, Zhang J, Seidenberg B, Reiss TF, Barber BL. What are minimal important changes for asthma measures in a clinical trial? *Eur Respir J* 1999; 14: 23–7.
- 32 Kerstjens HA, Disse B, Schroder-Babo W, Bantje TA, Gahlemann M, Sigmund R, Engel M, van Noord JA. Tiotropium improves lung function in patients with severe uncontrolled asthma: a randomized controlled trial. *J Allergy Clin Immunol* 2011; 128: 308–14.
- 33 Kerstjens HA, Engel M, Dahl R, Paggiaro P, Beck E, Vandewalker M, Sigmund R, Seibold W, Moroni-Zentgraf P,

- Bateman ED. Tiotropium in asthma poorly controlled with standard combination therapy. *N Engl J Med* 2012; 367: 1198–207.
- 34** Pedersen SE, Bateman ED, Bousquet J, Busse WW, Yoxall S, Clark TJ. Determinants of response to fluticasone propionate and salmeterol/fluticasone propionate combination in the Gaining Optimal Asthma control study. *J Allergy Clin Immunol* 2007; 120: 1036–42.
- 35** Clearie KL, McKinlay L, Williamson PA, Lipworth BJ. Fluticasone/Salmeterol combination confers benefits in people with asthma who smoke. *Chest* 2012; 141: 330–8.
- 36** Beasley R, Singh S, Loke YK, Enright P, Furberg CD. Call for worldwide withdrawal of tiotropium Respimat mist inhaler. *Br Med J* 2012; 345: e7390.
- 37** Tiotropium Respimat Pharmacokinetic Study in COPD. 2013. Available at <http://www.clinicaltrials.gov/ct2/show/NCT01222533?term=205.458&rank=11> (last accessed 11 April 2013).
- 38** Short PM, Williamson PA, Elder DH, Lipworth SI, Schembri S, Lipworth BJ. The impact of tiotropium on mortality and exacerbations when added to inhaled corticosteroids and long-acting beta-agonist therapy in COPD. *Chest* 2012; 141: 81–6.
- 39** Gavalda A, Miralpeix M, Ramos I, Otal R, Carreno C, Vinals M, Domenech T, Carcasona C, Reyes B, Vilella D, Gras J, Cortijo J, Morcillo E, Llenas J, Ryder H, Beleta J. Characterization of aclidinium bromide, a novel inhaled muscarinic antagonist, with long duration of action and a favorable pharmacological profile. *J Pharmacol Exp Ther* 2009; 331: 740–51.
- 40** Juniper EF, Guyatt GH, Willan A, Griffith LE. Determining a minimal important change in a disease specific Quality of Life Questionnaire. *J Clin Epidemiol* 1994; 47: 81–7.
- 41** Juniper EF, Svensson K, Mork AC, Stahl E. Measurement properties and interpretation of three shortened versions of the asthma control questionnaire. *Respir Med* 2005; 99: 553–8.
- 42** Lipworth B, Manoharan A, Short P. Tiotropium in asthma. *N Engl J Med* 2012; 367: 2552. author reply 3.
- 43** Lipworth B, Tan S, Devlin M, Aiken T, Baker R, Hendrick D. Effects of treatment with formoterol on bronchoprotection against methacholine. *Am J Med* 1998; 104: 431–8.
- 44** Aziz I, Tan KS, Hall IP, Devlin MM, Lipworth BJ. Subsensitivity to bronchoprotection against adenosine monophosphate challenge following regular once-daily formoterol. *Eur Respir J* 1998; 12: 580–4.
- 45** Fardon TC, Fardon EJ, Hodge MR, Lipworth BJ. Comparative cut off points for adenosine monophosphate and methacholine challenge testing. *Ann Allergy Asthma Immunol* 2004; 93: 365–72.
- 46** Lipworth BJ, Short PM, Williamson PA, Clearie KL, Fardon TC, Jackson CM. A randomized primary care trial of steroid titration against mannitol in persistent asthma: STAMINA trial. *Chest* 2012; 141: 607–15.
- 47** Grainge CL, Lau LC, Ward JA, Dulay V, Lahiff G, Wilson S, Holgate S, Davies DE, Howarth PH. Effect of bronchoconstriction on airway remodeling in asthma. *N Engl J Med* 2011; 364: 2006–15.
- 48** Gosens R, Bos IS, Zaagsma J, Meurs H. Protective effects of tiotropium bromide in the progression of airway smooth muscle remodeling. *Am J Respir Crit Care Med* 2005; 171: 1096–102.
- 49** Bos IS, Gosens R, Zuidhof AB, Schaafsma D, Halayko AJ, Meurs H, Zaagsma J. Inhibition of allergen-induced airway remodelling by tiotropium and budesonide: a comparison. *Eur Respir J* 2007; 30: 653–61.
- 50** Kang JY, Rhee CK, Kim JS, Park CK, Kim SJ, Lee SH, Yoon HK, Kwon SS, Kim YK, Lee SY. Effect of tiotropium bromide on airway remodeling in a chronic asthma model. *Ann Allergy Asthma Immunol* 2012; 109: 29–35.
- 51** Boterman M, Elzinga CR, Wagemakers D, Eppens PB, Zaagsma J, Meurs H. Potentiation of beta-adrenoceptor function in bovine tracheal smooth muscle by inhibition of protein kinase C. *Eur J Pharmacol* 2005; 516: 85–92.