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The novel bronchodilator navafenterol: a phase 2a, multi-centre, randomised, double-blind, placebo-controlled crossover trial in COPD

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Take home message: Navafenterol, a novel dual-pharmacology bronchodilator for COPD, improved lung function, reduced COPD symptoms and decreased objective cough counts, to a similar extent to umeclidinium/vilanterol.

ABSTRACT

Background: Navafenterol (AZD8871) belongs to a new class of bronchodilator, the single-molecule muscarinic antagonist and beta agonist (MABA), being developed for the treatment of chronic obstructive pulmonary disease (COPD). This study aimed to evaluate the efficacy, pharmacokinetics and safety of navafenterol *versus* placebo and an active comparator treatment for moderate-to-severe COPD.

Methods: This phase 2a, randomised, multicentre (Germany and UK), double-blind, double-dummy, three-way complete crossover study (ClinicalTrials.gov identifier: NCT03645434) compared 2 weeks' treatment of once-daily navafenterol 600 µg via inhalation with placebo and a fixed-dose combination bronchodilator (umeclidinium/vilanterol [UMEC/VI]; 62.5 µg/25 µg) in participants with moderate-to-severe COPD. The primary outcome was change from baseline in trough FEV_1 on day 15. Secondary endpoints included: change from baseline in peak FEV_1; change from baseline in breathlessness, cough and sputum scale (BCSS); change from baseline in COPD assessment tool (CAT); adverse events; and pharmacokinetics.

Results: Seventy-three participants were randomised. After 14 days, trough FEV_1 was significantly improved with navafenterol compared with placebo (least-squares [LS] mean difference 0.202 L; $p<0.0001$). There was no significant difference in FEV_1 between navafenterol and UMEC/VI (LS mean difference −0.046 L; $p=0.075$). COPD symptoms (CAT and BCSS) showed significantly greater improvements with both active treatments *versus* placebo (all $p<0.005$). Novel objective monitoring (VitaloJAK) showed that cough was reduced with both active treatments compared with placebo. Safety profiles were similar across the treatment groups and no serious adverse events were reported in the navafenterol treatment period.

Conclusion: Once-daily navafenterol was well tolerated, improved lung function and reduced COPD-related symptoms, similar to an established once-daily fixed-dose combination bronchodilator.
(255/250 words)

Keywords: MABA; drug therapy, combination; forced expiratory volume; cough

Introduction

Chronic obstructive pulmonary disease (COPD) is a common condition that is a significant cause of morbidity and mortality worldwide [1, 2]. The major clinical symptoms of COPD are chronic and progressive dyspnoea, cough and sputum production [1, 3]. Cough and sputum production are often reported as troubling symptoms, with increased incidence in the morning, which negatively affect health-related quality of life [4, 5].

Regular pharmacological treatment with inhaled long-acting bronchodilators can alleviate and reduce COPD symptoms [1]. Long-acting muscarinic antagonists (LAMAs) and long-acting β_2-agonists (LABAs) are mainstays of treatment for COPD, and are preferred over short-acting treatments [1]. LAMA/LABA combination therapy, such as umeclidinium/vilanterol (UMEC/VI), has greater efficacy than monotherapy for improving lung function, symptoms and health-related quality of life in patients with COPD [6, 7]. LAMAs and LABAs induce smooth muscle relaxation and bronchodilation via different mechanisms of action, which leads to additive effects in clinical practice [8].

Dual-pharmacology bronchodilators, a novel class of compounds that combine muscarinic antagonist and β_2-adrenoceptor agonist functions in a single molecule, termed muscarinic antagonists and beta agonists (MABAs), may offer advantages over combination therapy that uses two separate drug entities [9]. Because MABAs are single molecules, both pharmacologies are delivered as a fixed ratio, have a single pharmacokinetic profile and have a simplified clinical development programme relative to LAMA/LABA combination therapies [9, 10].

In vitro studies have demonstrated that navafenterol (AZD8871), an inhaled long-acting MABA, has potent M_3 antimuscarinic and β_2-adrenoceptor agonist activities [11]. In a phase 1, randomised, double-blind crossover study in patients with COPD that compared single doses of navafenterol 400 µg or 1800 µg with placebo, indacaterol and tiotropium, navafenterol delivered sustained bronchodilation over 36 h; both doses of navafenterol were superior to placebo and the higher dose was superior to both indacaterol and tiotropium, with no emerging safety concerns [12]. A phase 2a, randomised, double-blind crossover study of navafenterol 100 µg, navafenterol 600 µg and placebo once daily for 14 days in patients with COPD demonstrated dose-dependent clinically meaningful improvements in bronchodilation over 24 h at day 15, compared with placebo [13]. It is anticipated that navafenterol, as a single-molecule MABA, can provide a novel approach to the treatment of patients with COPD, with greater efficacy than long-acting bronchodilator monotherapy, at least equivalent efficacy to LAMA/LABA dual therapy, a similar safety profile and also potentially provide a platform for future combination with inhaled anti-inflammatory agents.

There are limited data on the effect of COPD treatments on the reduction of cough and little is known about what affects cough frequency in patients with COPD. It is hoped that objective cough monitoring will provide important information on the impact of treatment of cough in COPD [14].

The present study (ClinicalTrials.gov identifier: NCT03645434) was the first to compare navafenterol with a LAMA/LABA combination treatment (UMEC/VI) used in clinical practice. The primary aim of the study was to compare the effects of navafenterol *versus* UMEC/VI on

lung function. Secondary endpoints included COPD symptoms and safety assessment, while an objective reduction in cough count was an exploratory endpoint.

Methods

Study design

This phase 2a, randomised, double-blind, double-dummy, three-way complete crossover study compared navafenterol 600 µg with placebo and an active comparator LAMA/LABA bronchodilator (UMEC/VI) 62.5 µg/25 µg, administered once daily by dry powder inhaler devices (Genuair®/Pressair® device [SD3FL] for navafenterol and the Ellipta® device for UMEC/VI) in participants with moderate-to-severe COPD. The study was conducted between October 10, 2018 and August 7, 2019 at three sites in Germany and two sites in the UK.

Over the three 14-day treatment periods, participants received all three treatments in differing sequences, with a 42–49-day washout period (figure 1a). Patients were maintained on daily ipratropium (20 µg × 2 puffs 4 times per day) during washout periods. Participants were randomised using interactive web and interactive voice response systems. Full methodological details of the study are provided in the supplementary methods.

This study was performed in accordance with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with International Council for Harmonisation/Good Clinical Practice applicable regulatory requirements, as well as the AstraZeneca policy on bioethics. The study protocol was approved by independent ethics

committees according to local requirements. All patients provided written informed consent. This manuscript has been written in accordance with Consolidated Standards of Reporting Trials (CONSORT) guidelines [15].

Patients

Men and women aged 40–85 years with moderate-to-severe COPD were included. Patients were current or former smokers, with a post-bronchodilator forced expiratory volume in 1 s (FEV_1)/forced vital capacity ratio of <70% after inhalation of salbutamol 400 µg and a post-bronchodilator FEV_1 ≥40% and ≤80% of the predicted normal value at the second visit.

Patients were excluded if they had significant comorbidities (*e.g.* significant cardiovascular disease such as myocardial infarction within the 6 months before the screening visit, severe hepatic impairment), alpha-1 antitrypsin deficiency, other active pulmonary disease (predominant asthma, active tuberculosis, lung cancer, bronchiectasis, sarcoidosis, idiopathic interstitial pulmonary fibrosis, primary pulmonary hypertension, or uncontrolled sleep apnoea), two or more moderate-to-severe COPD exacerbations in the year before screening, acute worsening of COPD requiring antibiotic or corticosteroid treatment in the 3 months before screening, or had been hospitalised owing to poorly controlled COPD in the 3 months before screening.

Eligible patients were switched from their regular maintenance COPD medication to ipratropium (20 µg × 2 puffs 4 times per day) at enrolment. LABA, LAMA, LABA/LAMA and inhaled corticosteroid/LABA therapies were withdrawn at the start of the study. Patients

receiving an inhaled corticosteroid component were allowed to continue taking it as a monotherapy as a stable dose throughout.

A reversibility test was conducted upon washout of prior COPD medication where a reversible status was defined as increased post-bronchodilator FEV_1 of ≥12% (percentage reversibility) and ≥200 mL (absolute reversibility) compared with the pre-bronchodilator test. Reversibility status was not part of the eligibility criteria.

Outcomes

The primary objective was to assess the efficacy of navafenterol 600 µg in patients with moderate-to-severe COPD. The primary endpoint was the change from baseline in trough FEV_1 at day 15.

Secondary endpoints included: FEV_1 area under the curve (AUC); change from baseline in trough FEV_1 measured on day 2 and day 8; change from baseline in peak FEV_1; change from baseline in total score of the breathlessness, cough and sputum scale (BCSS) questionnaire [16, 17]; change from baseline in the COPD assessment tool (CAT) score [18]; use of rescue medication (salbutamol 100 µg); treatment-emergent adverse events; tolerability; and pharmacokinetics of navafenterol and its primary metabolite, LAS191861 (supplementary figure S1).

Objective cough counts were captured as an exploratory outcome using the VitaloJAK cough monitor on day 1 and day 14 (Vitalograph; Buckingham, UK) [19]. The cough monitor records a patient's cough frequency over a 24-hour period via wearable microphones. A

condensed recording is produced and analysed to assess the number of coughs per hour [20, 21]. Perceived cough severity was assessed using a visual analogue scale [20].

Statistical analysis

All participants were included in the full analysis set (FAS), which was used for the analysis of efficacy variables. The FAS was defined as all participants randomised and receiving study treatment, irrespective of their protocol adherence and continued participation in the study. Change from baseline in trough FEV_1 and change from baseline in peak FEV_1 were analysed using a mixed model with fixed effects for treatment, sequence and period. The participant was fitted as a random effect and the pre-dose FEV_1 of each period was included as a covariate. SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) was used for the data analysis. The study was powered to demonstrate superiority of navafenterol compared with UMEC/VI for the primary efficacy endpoint (see supplementary methods). All randomised patients who received at least one dose of study treatment were included in the safety population. Patients who specifically consented were included in the pharmacokinetic analysis subset. Details of the pharmacokinetic analysis are included in the supplementary methods.

Results

Patients

In total, 116 patients were enrolled, 73 of whom were randomised into the study. All randomised patients received at least one dose of study treatment, 71 patients completed at least one study period and 66 patients completed all three treatment periods (figure 1b).

Two patients discontinued treatment during a study period: one owing to a serious adverse event of acute coronary syndrome while receiving UMEC/VI and one owing to moderate COPD exacerbation while receiving placebo. The mean duration of participation was 186 days. The pharmacokinetic analysis subset included 41 participants.

Patient demographics and baseline data are summarised in table 1. The mean age of participants was 66.0 years and the majority were male (68.5%). The mean post-bronchodilator value for predicted FEV_1 at screening was 58.7%; 46 patients (63.0%) had a reversible status at screening. Most patients (76.7%) had not had a COPD exacerbation in the past year and 28 patients (38.4%) were maintained on an inhaled corticosteroid. The mean (standard deviation) CAT score at baseline was 15.4 (6.1).

Lung function

At day 15, trough FEV_1 was significantly improved by treatment with either navafenterol or UMEC/VI compared with placebo (navafenterol least-squares [LS] mean difference 0.202 L; 95% confidence interval [CI] 0.151 to 0.253 L; p<0.0001; UMEC/VI LS mean difference 0.248 L; 95% CI 0.197 to 0.300 L; p<0.0001) (figure 2a). The effect of UMEC/VI was numerically greater compared with navafenterol, but the difference was not statistically significant (LS mean difference −0.046 L; 95% CI −0.097 to 0.005 L; p=0.075).

At day 14, there was a significant difference in change from baseline in peak FEV_1 for navafenterol and for UMEC/VI compared with placebo (navafenterol LS mean difference 0.388 L; 95% CI 0.329 to 0.447 L; p<0.0001; UMEC/VI LS mean difference 0.326 L; 95% CI 0.226 to 0.385 L; p<0.0001; figure 2b). Navafenterol showed a fast onset of action, with the

effect of navafenterol on change from baseline in peak FEV_1 significantly greater than with UMEC/VI (LS mean difference 0.062 L; 95% CI 0.003 to 0.121 L; p=0.0385). Navafenterol also showed a greater effect on peak FEV_1 than UMEC/VI on day 1 ($p<0.05$). On day 2, trough FEV_1 was higher for UMEC/VI than for navafenterol ($p<0.05$); however, this difference reduced during the treatment period (figure 3).

Navafenterol demonstrated significantly greater improvements in FEV_1 AUC than placebo on day 14 at all time points measured (0–4, 0–8, 0–12 and 0–24 h post-dose). Navafenterol showed significantly greater improvements in FEV_1 AUC than UMEC/VI from 0 to 4 h post-dose at day 14 (LS mean difference 0.062 L; 95% CI 0.006 to 0.117 L; p=0.031; table 2 and supplementary table S1).

Post hoc analysis of subgroups defined by reversibility status suggested that, for both navafenterol and UMEC/VI, the increase in peak FEV_1 from baseline was numerically smaller in patients with a non-reversible status at screening compared with patients who had a reversible status (supplementary figure S2a). The pattern of a greater peak response to navafenterol versus UMEC/VI was observed in both subgroups, although not statistically significant (p=0.12 for both subgroups), while UMEC/VI showed a significant increase in trough FEV_1 compared with navafenterol at day 15 in the reversible subgroup only (p=0.008); there was no treatment difference in the non-reversible subgroup (p=0.65) (supplementary figure S2b).

Subgroups defined by eosinophil counts ≥ or $<150 \times 10^6$/L at baseline, inhaled corticosteroid use at baseline and current smokers *versus* former smokers were analysed *post hoc*. All

analyses showed navafenterol and UMEC/VI caused significant improvements in trough and peak FEV_1 compared with placebo that were not dependent on eosinophil counts, inhaled corticosteroid use or current smoking status (supplementary figures S3–S5).

Symptom reduction

The CAT and BCSS questionnaires showed that, compared with baseline assessments, navafenterol and UMEC/VI each significantly improved symptoms of COPD relative to placebo during both the first (days 1–8) and second week (days 9–14) of treatment (all comparisons *versus* placebo, $p<0.005$; figure 4). Between the first and second week of treatment, the LS mean changes from baseline in CAT scores were similar (days 1–8, navafenterol −2.10; UMEC/VI −2.74; placebo −0.53; days 9–14, navafenterol −2.85; UMEC/VI −3.22; placebo −0.47). For BCSS, the LS mean changes from baseline score between the first and second weeks were also similar (day 1–8, navafenterol −0.39; UMEC/VI −0.63; placebo 0.16; days 9–14, navafenterol −0.36; UMEC/VI −0.65; placebo 0.51). There were no significant differences between navafenterol and UMEC/VI in CAT or total BCSS (all navafenterol *versus* UMEC/VI comparisons $p>0.05$; figure 4). The proportion of CAT responders (defined as a 2.0-point improvement) was higher for both navafenterol and UMEC/VI *versus* placebo, but there were no differences between UMEC/VI and navafenterol (supplementary table S2). The use of rescue medication was significantly lower with both active treatments than with placebo (all comparisons *versus* placebo $p<0.0001$) and was similar between the navafenterol and UMEC/VI treatment groups (all navafenterol *versus* UMEC/VI comparisons $p>0.05$; supplementary figure S6).

In an exploratory analysis, VitaloJAK objective cough monitoring measured cough frequency over 24 hours at baseline and on day 14. Current smokers reported a higher frequency of cough at baseline than ex-smokers (supplementary table S3). At day 14, cough frequency was numerically lower over the 0–24-h period with navafenterol and UMEC/VI treatment compared with placebo (navafenterol *versus* placebo p=0.108; UMEC/VI *versus* placebo p=0.018; figure 5). Additionally, over the time period corresponding to maximum peak effect from spirometry (0–4 h), navafenterol and UMEC/VI showed significantly greater improvements in cough frequency compared with placebo (p=0.038 and p=0.027, respectively). This effect was sustained throughout the daytime period (0–12 h; p=0.004 and p=0.001, respectively). At day 14, improvements in cough with navafenterol and UMEC/VI compared with placebo were also seen using a visual analogue scale (supplementary table S4).

Safety

A total of 61 participants (83.6%) experienced an adverse event during the study period. The proportions of patients who reported treatment-emergent adverse events were similar among the treatment groups: 39 participants (55.7%) during the navafenterol treatment period, 38 participants (55.1%) during the UMEC/VI treatment period and 35 participants (51.5%) during the placebo treatment period (table 3 and described in detail in the supplementary results).

No serious adverse events were reported during the navafenterol treatment period. Four participants reported serious adverse events: vestibular neuronitis (1 [1.5%]) and humerus fracture (1 [1.5%]) were reported during the placebo treatment period, and tooth abscess

(1 [1.4%]) and acute coronary syndrome (1 [1.4%]) were reported during the UMEC/VI treatment period. None of the serious adverse events were considered by the investigators as related to the study treatment. The participant who reported acute coronary syndrome discontinued UMEC/VI and was withdrawn from the study. No clinically relevant differences in vital signs, laboratory findings or echocardiogram results were identified among the treatment groups. No deaths were reported during the study.

Pharmacokinetics

Navafenterol was rapidly absorbed after single (day 1) and multiple (day 14) doses (supplementary table S5; supplementary figure S7). The range for median time to maximum plasma concentration (t_{max}) was 0.45–2.05 h after dose administration. Evidence of accumulation was seen after repeated dosing, with accumulation ratios of 1.72 for maximum plasma concentration (C_{max}) and 2.41 for AUC. The metabolite LAS191861 was rapidly formed (median t_{max} was ~2 h after dosing) with AUC approximately twofold lower than for navafenterol. Additional pharmacokinetic parameters are shown in supplementary table S5. In 15 out of 25 samples, navafenterol at concentrations of 3.63–15.85 pg/mL was detected in pre-dose samples for the treatment period immediately following the navafenterol treatment period. LAS191861 was also detected in 20 out of 25 samples at concentrations of 2.02–17.71 pg/mL. This is likely due to the long terminal half-life of both navafenterol and LAS191861. Sensitivity analysis of change in trough FEV_1 from baseline to day 15 suggests that the influence of the carryover on the estimated treatment effect observed for navafenterol or the active comparator was likely to be small (data not shown).

Discussion

This randomised controlled trial showed that navafenterol was superior to placebo in improving lung function outcomes in patients with moderate-to-severe COPD. The primary endpoint analysis on day 15 showed no statistically significant difference between navafenterol and UMEC/VI on trough FEV_1. For both treatments, there were similar improvements in COPD symptoms as measured by CAT, BCSS and objective cough monitoring.

The lung function profiles of navafenterol and UMEC/VI showed different patterns, when assessed over 24 h on day 1 and day 15. On day 1, navafenterol showed a greater peak effect than UMEC/VI. However, navafenterol exhibited a numerically lower trough FEV_1 than UMEC/VI on day 2. After 14 days of treatment, navafenterol retained a greater peak FEV_1 than UMEC/VI, but with a more gradual decline in FEV_1 after the peak and therefore both treatments had similar values from 12 to 24 h post-dose. It is possible that the greater peak effect of navafenterol could result in a lower burden of symptoms in the mornings, although this was not studied here and needs to be further evaluated. The effect of navafenterol on trough FEV_1 increased from day 1 to day 15; this effect was less evident in patients treated with UMEC/VI. Pharmacokinetic analysis showed that the absorption of navafenterol and appearance of its metabolite LAS191861 was rapid. Importantly, there was substantial accumulation after repeated dosing. This accumulation of navafenterol from day 1 to day 14 was consistent with the pattern of change in FEV_1 over the same time period.

The minimal clinically important difference (MCID) has been estimated at 1–3 points for CAT and >0.3 points for BCSS [17, 22]. For BCSS, a 1-point change represents substantial symptomatic improvement, a 0.6-point change is considered moderate, and a 0.3-point changes is considered small [22]. In this study, improvements greater than MCIDs were reported for navafenterol versus placebo for both CAT and BCSS at the end of the 14-day treatment period. Objective cough monitoring and the visual analogue scale were evaluated as exploratory endpoints. The cough visual analogue scale is a practical tool used to evaluate cough severity, but is a subjective assessment that reflects the patient's perception of their own symptoms [20, 23]. The VitaloJAK is an objective method of measuring cough frequency over time and may be more sensitive than the subjective visual analogue score [24]. Several clinical studies in patients with COPD and asthma have used the VitaloJAK for the assessment of cough and its relationship with other disease parameters [21, 25-27]. Although the MCID for reduction in cough counts has not been determined for COPD, data from refractory chronic cough suggests that a 20% reduction would be considered clinically meaningful [28]. The present study provides the first evidence that long-acting bronchodilators can decrease cough frequency in patients with COPD, with significant and sustained reductions during the daytime period compared with placebo. Because cough can reduce the quality of life of patients, sensitive objective cough monitoring techniques may have considerable value in COPD clinical trials [4].

In this study, we found that a higher proportion of patients met bronchodilator reversibility criteria than was observed in phase 3 trials of long-acting bronchodilators and a previous real-world study of patients with COPD [29-32]. In published studies, 11%–34% of patients

were classified as having a reversible status, whereas in the present study 63% of patients were classified as having a short-acting bronchodilator reversible status.

A number of other MABAs have been in development for the treatment of COPD including batefenterol, AZD8999, AZD2115, CHF6366 and THRX200495 [33]. The majority of these are no longer in active clinical development. Batefenterol completed phase 2b clinical trials but has not progressed to phase 3. In terms of β_2-adrenoceptor agonist/muscarinic antagonist activity ratios, batefenterol has a stronger β_2-adrenoceptor agonist function whereas navafenterol has a stronger M_3 muscarinic antagonist activity [33]. In a phase 2 trial, batefenterol 300 µg in combination with fluticasone furoate 100 µg showed improvements in change from baseline in FEV_1 compared with placebo over 42 days of treatment [34]. Differences in trial design make it challenging to compare across studies. Although synergy between LABA and LAMA therapies has been demonstrated *in vitro*, this remains unproven in clinical practice. A potential benefit of MABAs is the future possibility of co-formulation with an anti-inflammatory compound(s) which could offer an opportunity for a novel triple (or quadruple) pharmacology fixed-dose combination products that would be technically less demanding to develop than a product containing LAMA and LABA as separate molecular entities.

This study had a crossover design that used double-dummy and double-blinding. The crossover design minimised interparticipant variability and optimised sample size. To reduce the possibility of carryover effects as a consequence of the long terminal half-life of navafenterol and LAS191861, a long washout period was implemented. Although there were measurable concentrations of navafenterol after washout, sensitivity analyses

including carryover variables were performed and did not indicate the presence of significant carryover in the FEV_1 efficacy results. A limitation of the study is that the therapeutic dose of navafenterol is unknown, because the full dose response has not yet been explored. Although this may limit comparisons with known therapeutic doses of established bronchodilators, efficacy comparisons with placebo are unaffected. Given that navafenterol is well tolerated so far, higher doses of navafenterol may be explored to examine the potential positive effects on efficacy. An additional limitation of the study was that there was no robust control of type I errors for any of the secondary endpoints, although this is standard for phase 2 studies. This study may not be fully representative of the broader COPD patient population owing to the high proportion of patients with a short-acting bronchodilator reversible status.

Conclusions

Treatment with navafenterol 600 µg once daily was well tolerated and provided improvements in overall lung function and COPD symptoms reduction, to a similar extent to UMEC/VI, an established LAMA/LABA combination bronchodilator. The results from this study support further investigation of navafenterol in larger and longer clinical trials in patients with COPD.

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Tables

TABLE 1. Patient demographics and baseline characteristics

Demographics	**All patients (N=73)**
Mean age, years (SD)	66.0 (7.6)
Sex, n (%)	
Female	23 (31.5)
Male	50 (68.5)
Race, n (%)	
White	73 (100.0)
Smoking status, n (%)	
Ex-smoker	45 (61.6)
Current smoker	28 (38.4)
Time since COPD diagnosis, years (SD)	12.1 (7.4)
Number of exacerbations in previous 12 months, mean (SD) [min, max]	0.2 (0.4) [0, 1]
Number of patients with 0 exacerbations in the previous 12 months, n (%)	56 (76.7)
Number of patients with 1 exacerbation in the previous 12 months, n (%)	17 (23.3)
Time since last exacerbation to randomisation, mean month (SD)	22.6 (23.8)
Inhaled corticosteroid use, n (%)[#]	28 (38.4)
CAT score at baseline, mean (SD)	15.4 (6.1)
Eosinophil count, mean 10^9/L (SD)	0.3 (0.2)
Lung function at screening (post bronchodilator)	
FEV_1, % predicted (SD)	58.7 (10.4)
FEV_1/FVC (SD)	50.1 (9.1)
Severity of airflow limitation, n (%)	
Moderate (≥50%, <80%)	56 (76.7)
Severe (≥30%, <50%)	17 (23.3)
FEV_1 reversibility, % predicted (SD)	20.8 (12.8)
Reversibility status, n (%)	
Reversible	46 (63.0)
Non-reversible	27 (37.0)
Relevant baseline medical history, n (%)	
Cardiac disorders	8 (11)
Myocardial infarction	2 (2.7)
Myocardial ischaemia	2 (2.7)
Atrioventricular block second degree	1 (1.4)
Cardiac aneurysm	1 (1.4)
Coronary artery disease	1 (1.4)

Extrasystoles	1 (1.4)
Left ventricular dysfunction	1 (1.4)
Palpitations	1 (1.4)
Ventricular hypokinesia	1 (1.4)
Asthma	4 (5.5)
Immune system disorders	13 (17.8)
Seasonal allergy	5 (6.8)
Drug hypersensitivity	4 (5.5)
Allergy to metals	2 (2.7)
Allergy to animal	1 (1.4)
Allergy to arthropod bite	1 (1.4)
Iodine allergy	1 (1.4)

CAT: COPD assessment tool; COPD: chronic obstructive pulmonary disease; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; SD: standard deviation. #: These participants were maintained on stable inhaled corticosteroid treatment throughout the study.

TABLE 2 AUC FEV_1 at day 14

Time period	Navafenterol *versus* placebo		Navafenterol *versus* UMEC/VI	
	FEV_1 AUC LS mean difference, L (95% CI)	**p-value**	**FEV_1 AUC LS mean difference, L (95% CI)**	**p-value**
0–4 h	0.376 (0.320 to 0.432)	<0.0001	0.062 (0.006 to 0.117)	0.0308
0–8 h	0.328 (0.271 to 0.385)	<0.0001	0.020 (−0.037 to 0.077)	0.4948
0–12 h	0.296 (0.242 to 0.349)	<0.0001	0.001 (−0.053 to 0.054)	0.9827
0–24 h	0.244 (0.194 to 0.294)	<0.0001	−0.030 (−0.079 to 0.020)	0.2426

AUC: area under the curve; CI: confidence interval; FEV_1: forced expiratory volume in 1 s; LS: least-squares; UMEC/VI: umeclidinium/vilanterol.

TABLE 3 Summary of treatment-emergent adverse events

Adverse events, n (%)	**Navafenterol (n=70)**	**UMEC/VI (n=69)**	**Placebo (n=68)**	**All participants (N=73)**
Any AE	39 (55.7)	38 (55.1)	35 (51.5)	61 (83.6)
Any SAE[#]	0 (0.0)	2 (2.9)	2 (2.9)	4 (5.5)
Any AE leading to discontinuation	0 (0.0)	1 (1.4)	0 (0.0)	1 (1.4)
Most frequently reported AEs[¶]				
Headache	14 (20.0)	13 (18.8)	14 (20.6)	23 (31.5)
Nasopharyngitis	6 (8.6)	8 (11.6)	3 (4.4)	16 (21.9)
Rhinitis	2 (2.9)	3 (4.3)	3 (4.4)	8 (11.0)
Cough	3 (4.3)	3 (4.3)	0 (0.0)	6 (8.2)

AE: adverse event; MedDRA: Medical Dictionary for Regulatory Activities; SAE: serious adverse event; UMEC/VI: umeclidinium/vilanterol.

[#]: Participants with multiple SAEs were counted once for each system organ class/preferred term. The summary includes SAEs starting on or after the first administration of study drug, up to and including 42 days after the final dose of study treatment. Washout period was considered part of the prior treatment; [¶]: AEs reported in more than five patients are included using the MedDRA (version 21.0) preferred term.

Figure legends

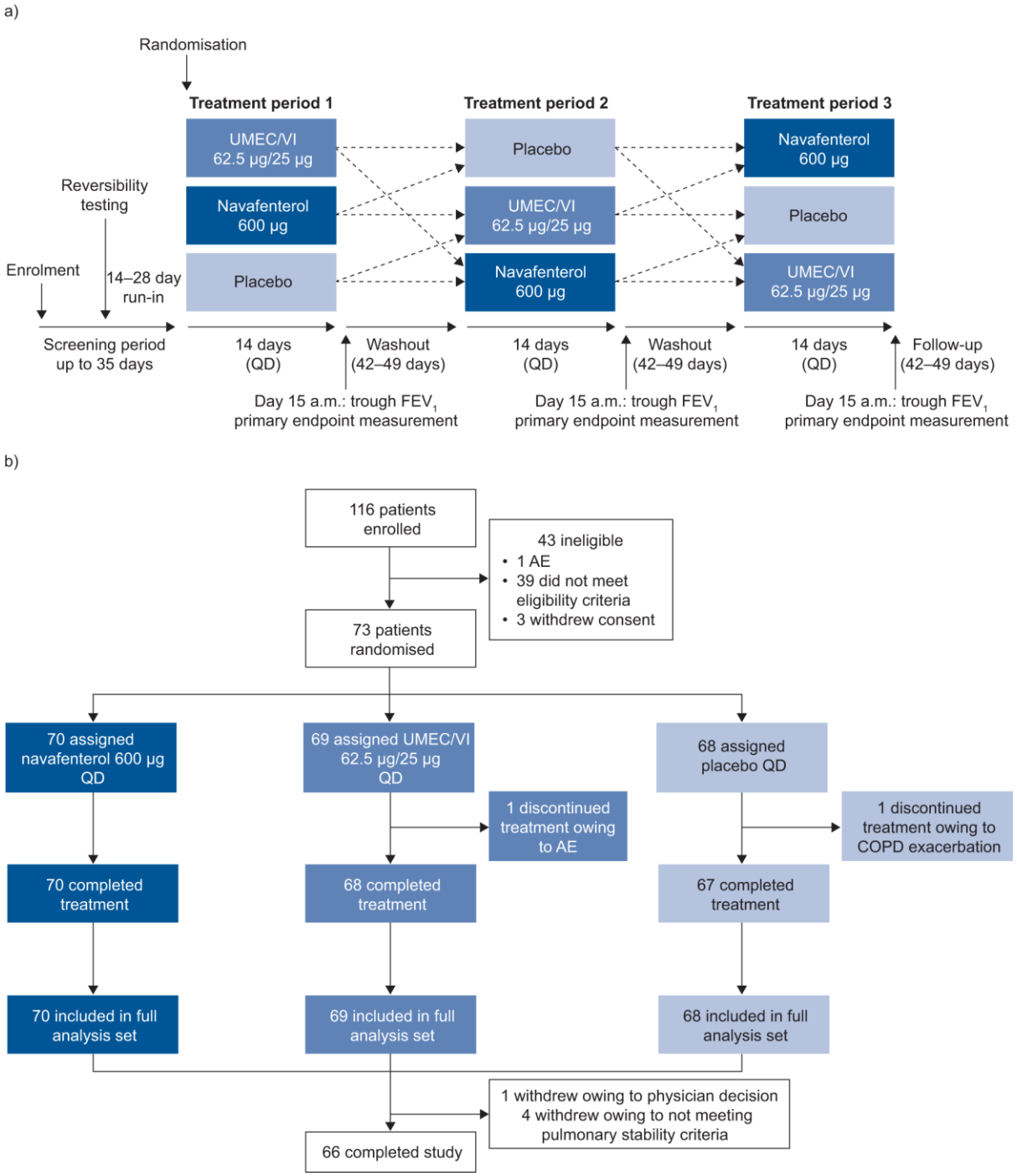

FIGURE 1 a) Study design and b) patient disposition. AE: adverse event; COPD: chronic obstructive pulmonary disease; FEV_1: forced expiratory volume in 1 s; QD: once daily; UMEC/VI: umeclidinium/vilanterol

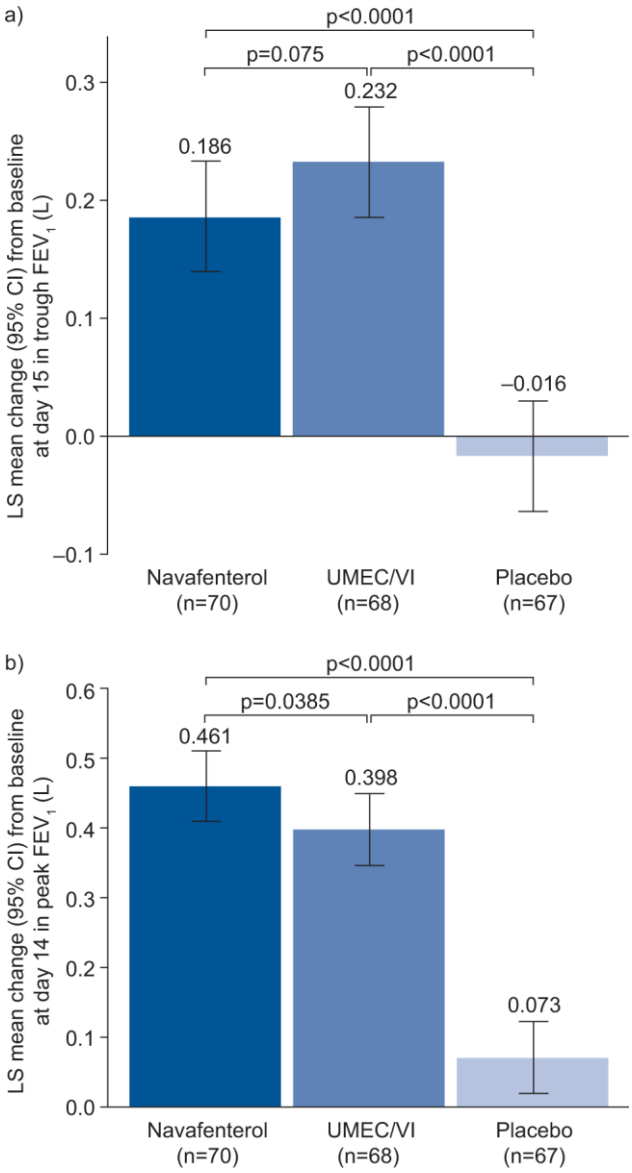

FIGURE 2 a) LS mean change from baseline in trough FEV_1 at day 15. b) LS mean change from baseline in peak FEV_1 at day 14. CI: confidence interval; FEV_1: forced expiratory volume in 1 s; LS: least-squares; UMEC/VI: umeclidinium/vilanterol.

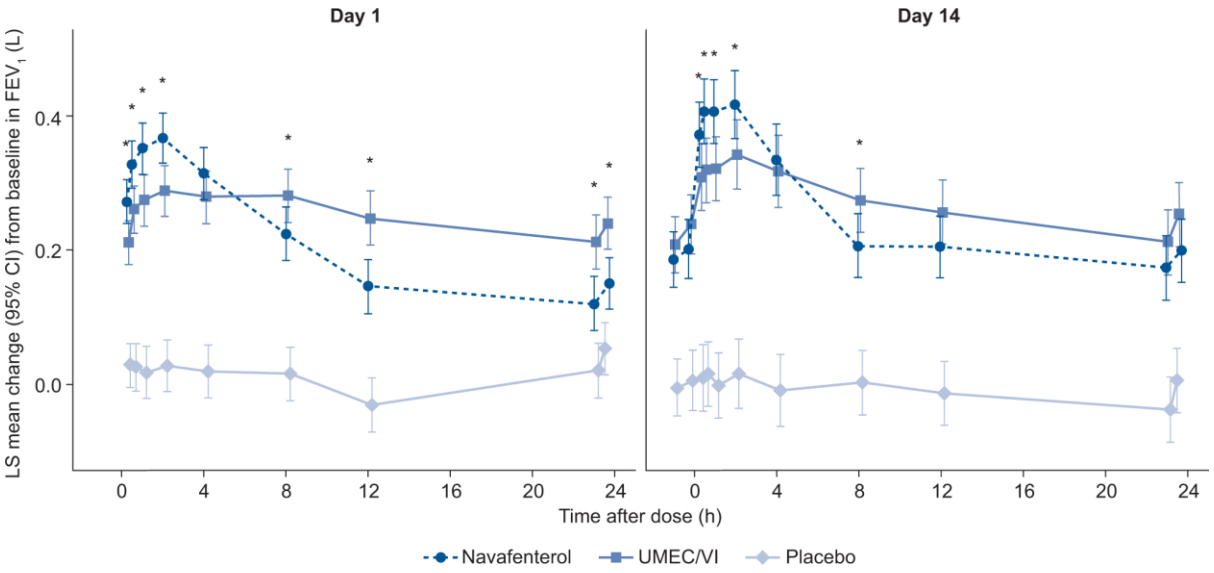

FIGURE 3 FEV_1 over 24 h on day 1 and day 14. CI: confidence interval; FEV_1: forced expiratory volume in 1 s; LS: least-squares; UMEC/VI: umeclidinium/vilanterol. *: $p<0.05$, significant difference between navafenterol and UMEC/VI.

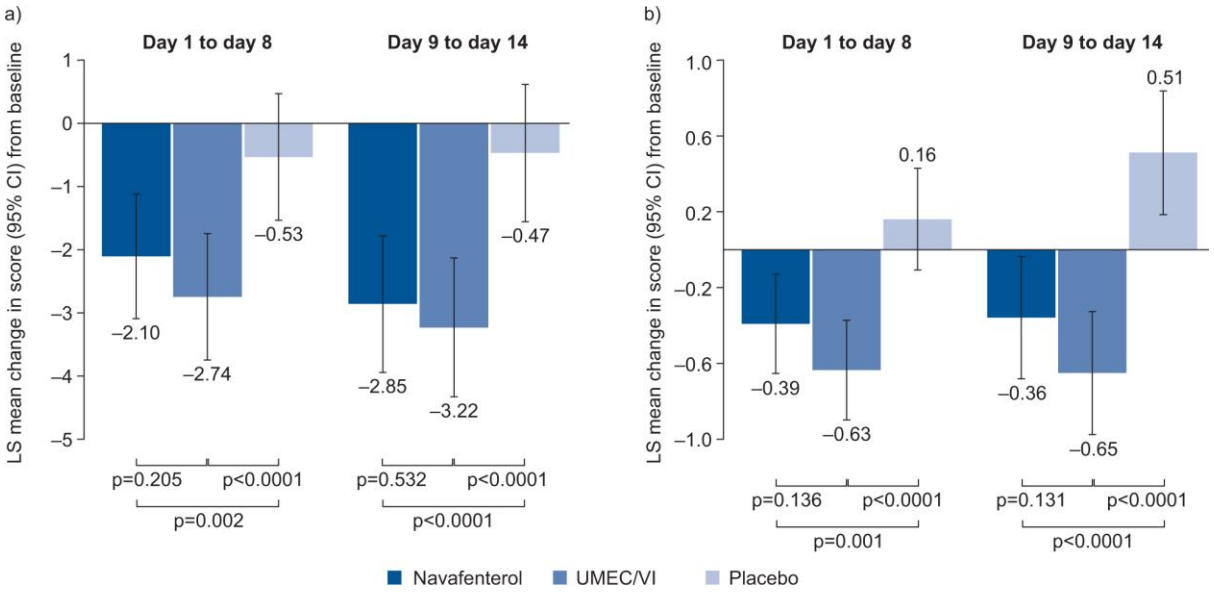

FIGURE 4 Effect of treatment on the symptoms of COPD, as measured by a) the COPD assessment tool (CAT) and b) the breathlessness, cough and sputum scale (BCSS). CI: confidence interval; COPD: chronic obstructive pulmonary disease; LS: least-squares; UMEC/VI: umeclidinium/vilanterol.

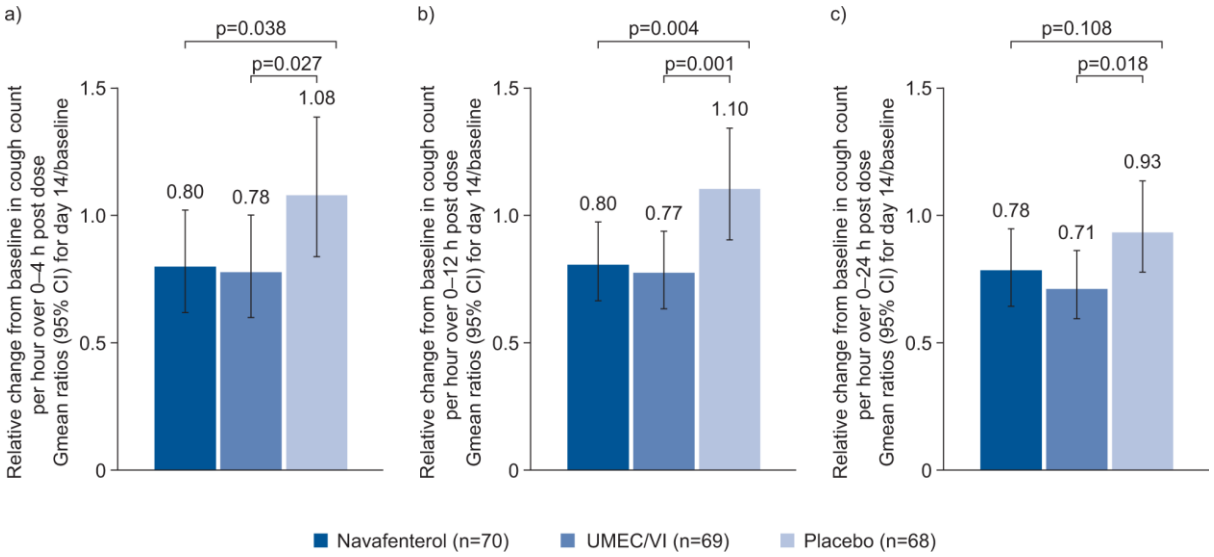

FIGURE 5 Relative change from baseline (Gmean ratios) in objective cough count for day 14 over a) 0–4 h post-dose, b) 0–12 h post-dose and c) 0–24 h post-dose. CI: confidence interval; Gmean: geometric mean; UMEC/VI: umeclidinium/vilanterol.

Supplementary tables

TABLE S1 AUC FEV_1 at day 14 for UMEC/VI *versus* placebo

Time period	UMEC/VI *versus* placebo	
	FEV_1 AUC LS mean difference, L (95% CI)	**p-value**
0–4 h	0.314 (0.258 to 0.371)	<0.0001
0–8 h	0.309 (0.251 to 0.366)	<0.0001
0–12 h	0.295 (0.242 to 0.348)	<0.0001
0–24 h	0.274 (0.224 to 0.324)	<0.0001

AUC: area under the curve; CI: confidence interval; FEV_1: forced expiratory volume in 1 s;

LS: least-squares; UMEC/VI: umeclidinium/vilanterol.

TABLE S2 CAT responder analysis for day 1 to day 14

Navafenterol *versus* placebo		**UMEC/VI *versus* placebo**		**Navafenterol *versus* UMEC/VI**	
Proportion of responders by treatment	**p-value**	**Proportion of responders by treatment**	**p-value**	**Proportion of responders by treatment**	**p-value**
Navafenterol 0.62 Placebo 0.45	0.022	UMEC/VI 0.67 Placebo 0.44	0.008	Navafenterol 0.61 UMEC/VI 0.66	0.63

CAT: COPD assessment tool; UMEC/VI: umeclidinium/vilanterol.

TABLE S3 Mean frequency of baseline cough (coughs/h) stratified by smoking status

Time period	**Current smoker**			**Ex-smoker**		
	N	**Mean**	**SD**	**N**	**Mean**	**SD**
0–24 h	26	13.8	9.9	41	6.7	7.4
0–12 h	26	12.7	10.6	41	7.0	7.9
0–4 h	27	14.0	12.0	41	8.6	10.9

SD: standard deviation.

TABLE S4 Cough severity using the visual analogue scale at baseline and day 14

Treatment	Day[#]	Cough severity			Mean ratios day 14/baseline			Comparison with placebo[¶]		
		n	Geometric mean	Geometric CV%	n	Geometric mean	Geometric CV%	Estimated ratio	95% CI	p-value
Navafenterol (N=70)	Baseline	67	2.10	1.40						
	Day 14	67	1.42	2.04	67	0.71	1.10	0.83	0.67 to 1.03	0.087
UMEC/VI (N=69)	Baseline	69	1.90	1.31						
	Day 14	68	1.25	1.58	68	0.66	1.14	0.76	0.61 to 0.94	0.013
Placebo (N=68)	Baseline	65	1.86	1.17						
	Day 14	64	1.55	1.77	64	0.89	0.75	–	–	–

CI: confidence interval; CV: coefficient of variation; UMEC/VI: umeclidinium/vilanterol. [#]: Baseline was measured at day −1; [¶]: estimated ratio and 95% CI are transformed back to original scale.

TABLE S5 Pharmacokinetic parameters for navafenterol and LAS191861

Parameter		**Navafenterol**		**LAS191861**	
		Day 1	**Day 14**	**Day 1**	**Day 14**
C_{max}	n	40	41	41	41
	Geometric mean, pg/mL	310.4	532.9	26.64	63.29
	Geometric CV%	61.30	46.58	53.33	52.12
t_{max}	n	40	41	41	41
	Median, h	0.99	0.98	2.00	2.00
	Range, h	0.38–2.02	0.45–2.05	0.98–4.00	0.98–4.03
$AUC_{0–24}$	n	41	41	39	41
	Geometric mean, h × pg/mL	1661	3996	289.5	941.7
	Geometric CV%	83.60	55.66	57.83	63.11
$R_{ac(Cmax)}$	n	NA	40	NA	41
	Geometric mean	NA	1.725	NA	2.377
	Geometric CV%	NA	44.77	NA	40.02
$R_{ac(AUC0–24)}$	n	NA	41	NA	39
	Geometric mean	NA	2.406	NA	3.443
	Geometric CV%	NA	50.37	NA	47.15
$MR_{AUC0–24}$	n	NA	NA	41	41
	Geometric mean	NA	NA	0.1522	0.2356
	Geometric CV%	NA	NA	41.66	30.33

$AUC_{(0–24)}$: area under the plasma concentration–time curve from time 0 h to 24 h post-dose; C_{max}: maximum plasma concentration; CV: coefficient of variation; $MR_{AUC0–24}$: metabolic ratio of area under the plasma concentration–time curve from time 0 h to 24 h post-dose; NA: not applicable; $R_{ac(AUC0–24)}$: accumulation ratio calculated from $AUC_{0–24}$; $R_{ac(Cmax)}$: accumulation ratio calculated from C_{max}; t_{max}: time to C_{max}.

Supplementary figure legends

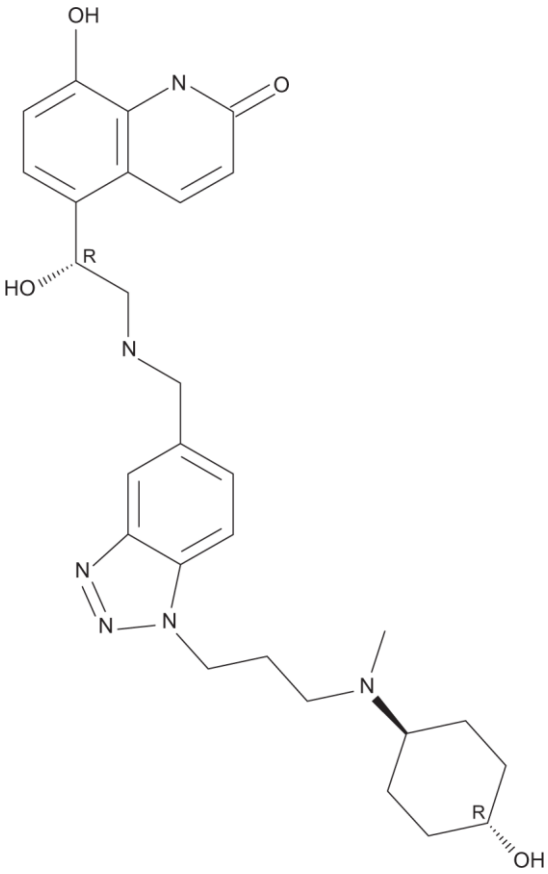

FIGURE S1 Chemical structure of LAS191861, the primary metabolite of navafenterol (AZD8871).

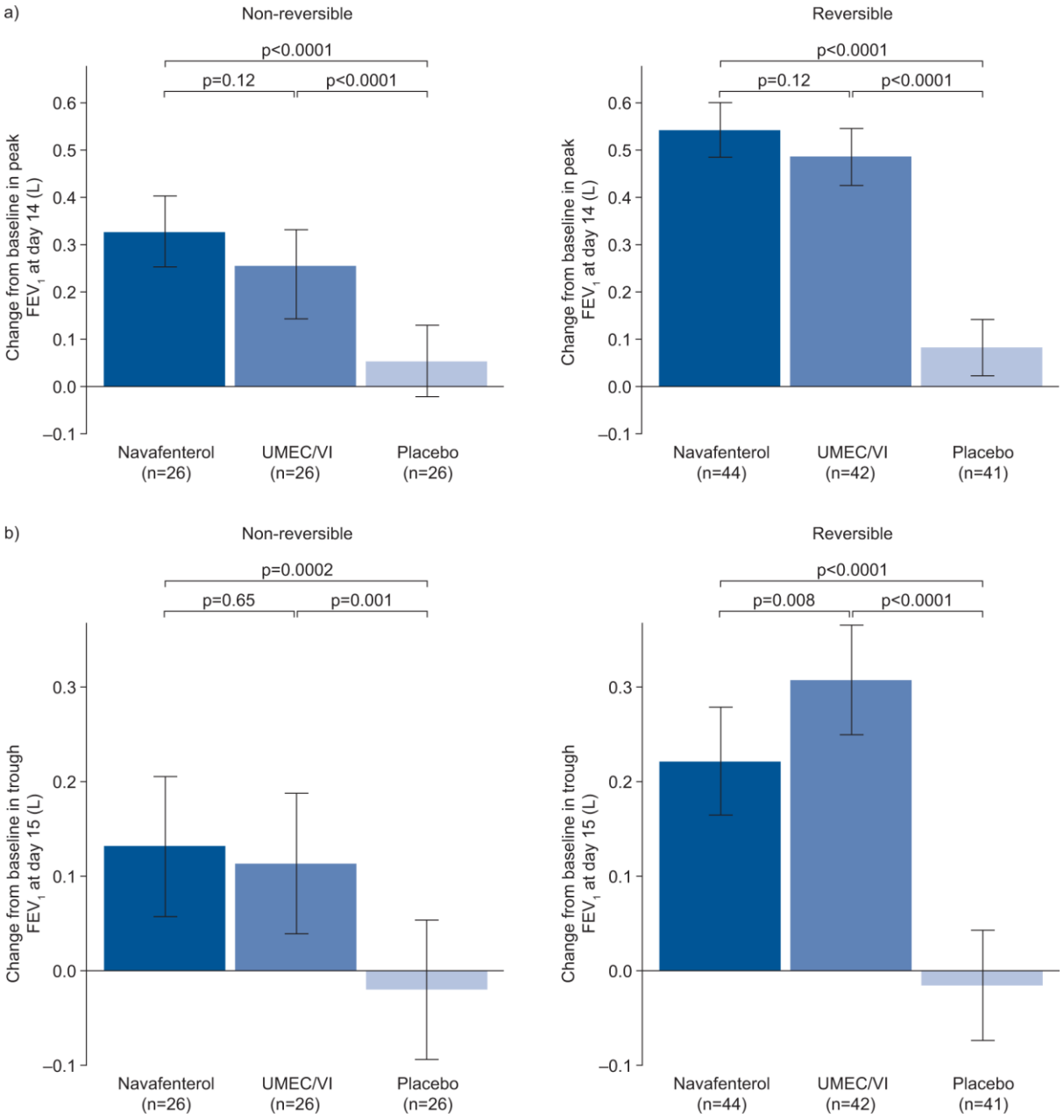

FIGURE S2 FEV_1 in patients with reversible and non-reversible status at screening. a) LS mean change from baseline in peak FEV_1 at day 14. b) LS mean change from baseline in trough FEV_1 at day 15. FEV_1: forced expiratory volume in 1 s; LS: least-squares; UMEC/VI: umeclidinium/vilanterol.

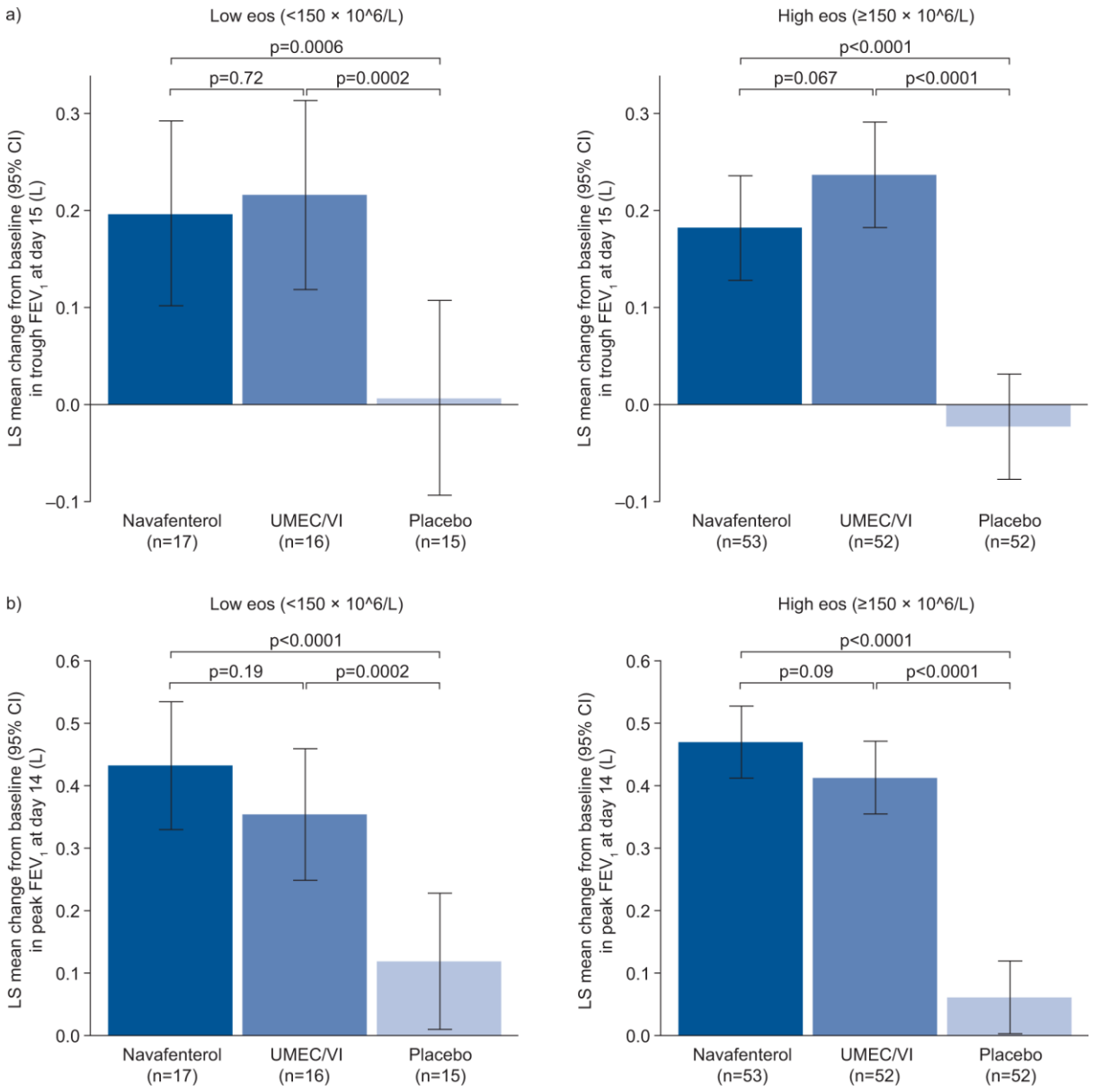

FIGURE S3 Subgroup analysis of low (<150 × 10^6/L) and high (≥150 × 10^6/L) eosinophil count. a) LS mean change from baseline in trough FEV_1 at day 15. b) LS mean change from baseline in peak FEV_1 at day 14. CI: confidence interval; eos: eosinophil count; FEV_1: forced expiratory volume in 1 s; LS: least-squares; UMEC/VI: umeclidinium/vilanterol.

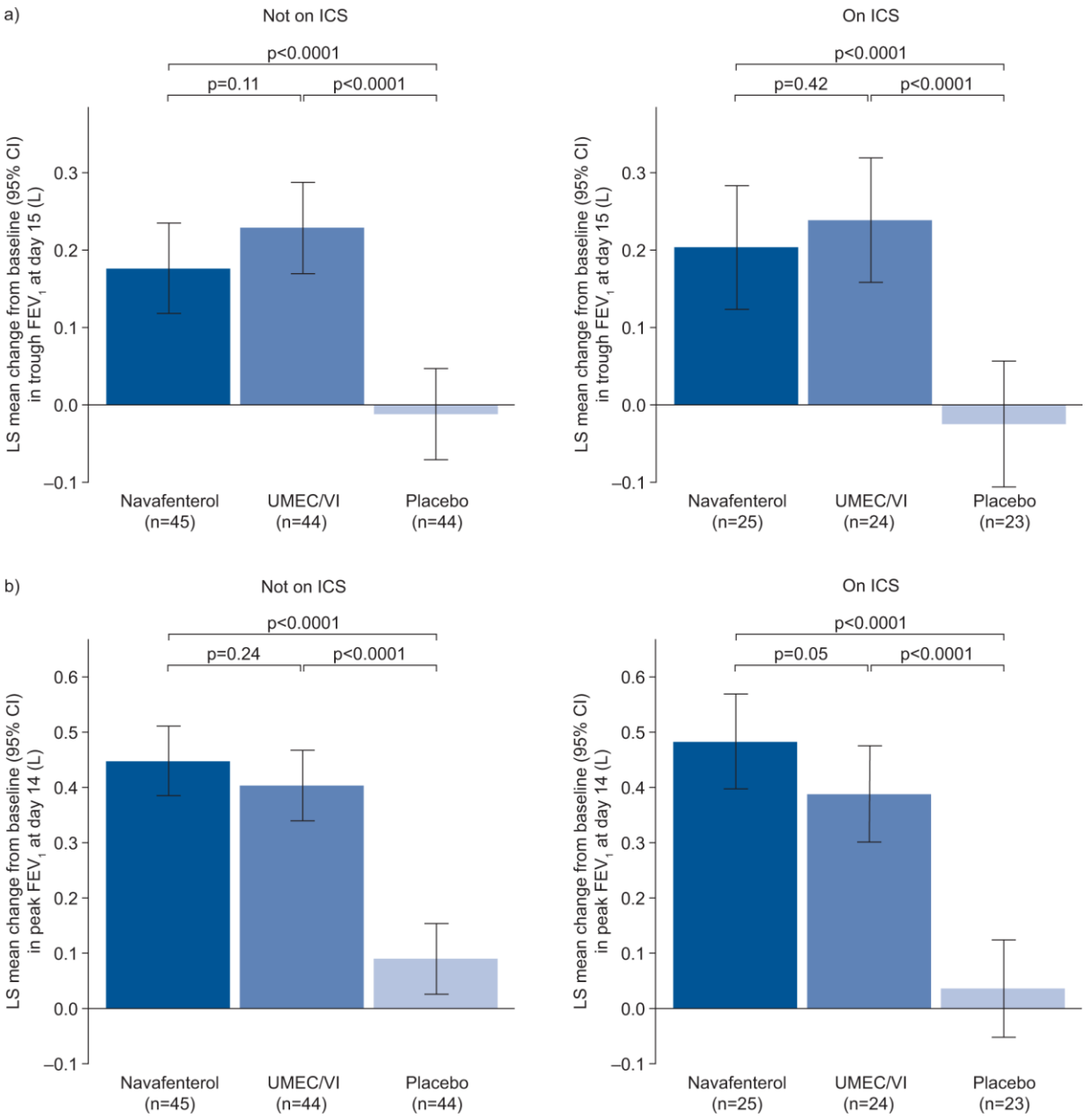

FIGURE S4 Subgroup analysis of patients receiving and not receiving ICS. a) LS mean change from baseline in trough FEV_1 at day 15. b) LS mean change from baseline in peak FEV_1 at day 14. CI: confidence interval; FEV_1: forced expiratory volume in 1 s; ICS: inhaled corticosteroid; LS: least-squares; UMEC/VI: umeclidinium/vilanterol.

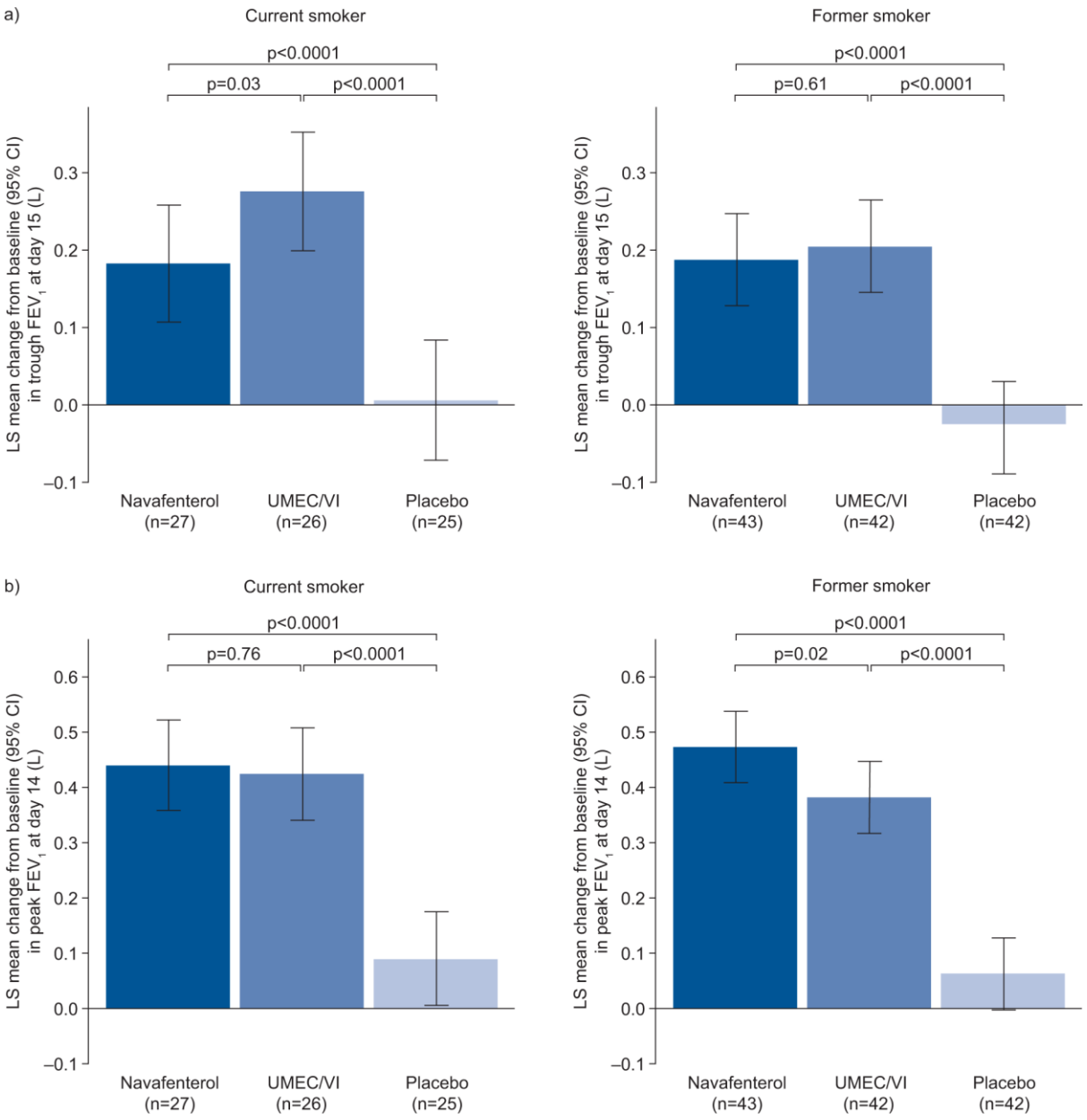

FIGURE S5 Subgroup analysis of current and former smokers. a) LS mean change from baseline in trough FEV_1 at day 15. b) LS mean change from baseline in peak FEV_1 at day 14. CI: confidence interval; FEV_1: forced expiratory volume in 1 s; LS: least-squares; UMEC/VI: umeclidinium/vilanterol.

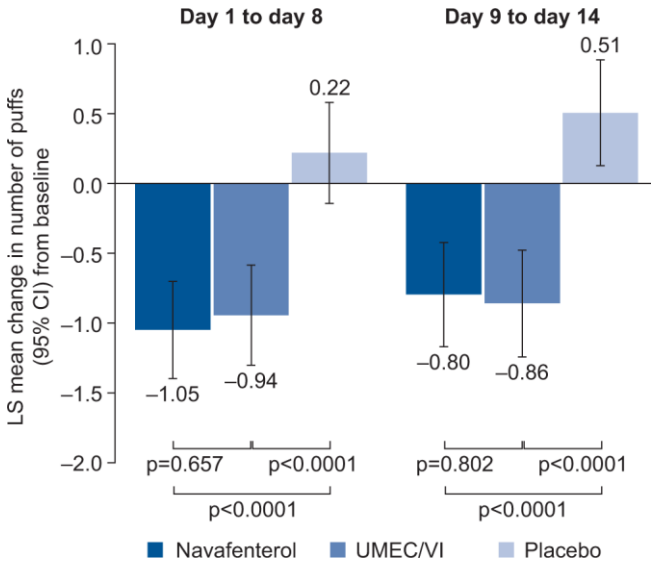

FIGURE S6 LS mean change from baseline in number of puffs of rescue medication per day from days 1–8 and 9–14. CI: confidence interval; LS: least-squares; UMEC/VI: umeclidinium/vilanterol.

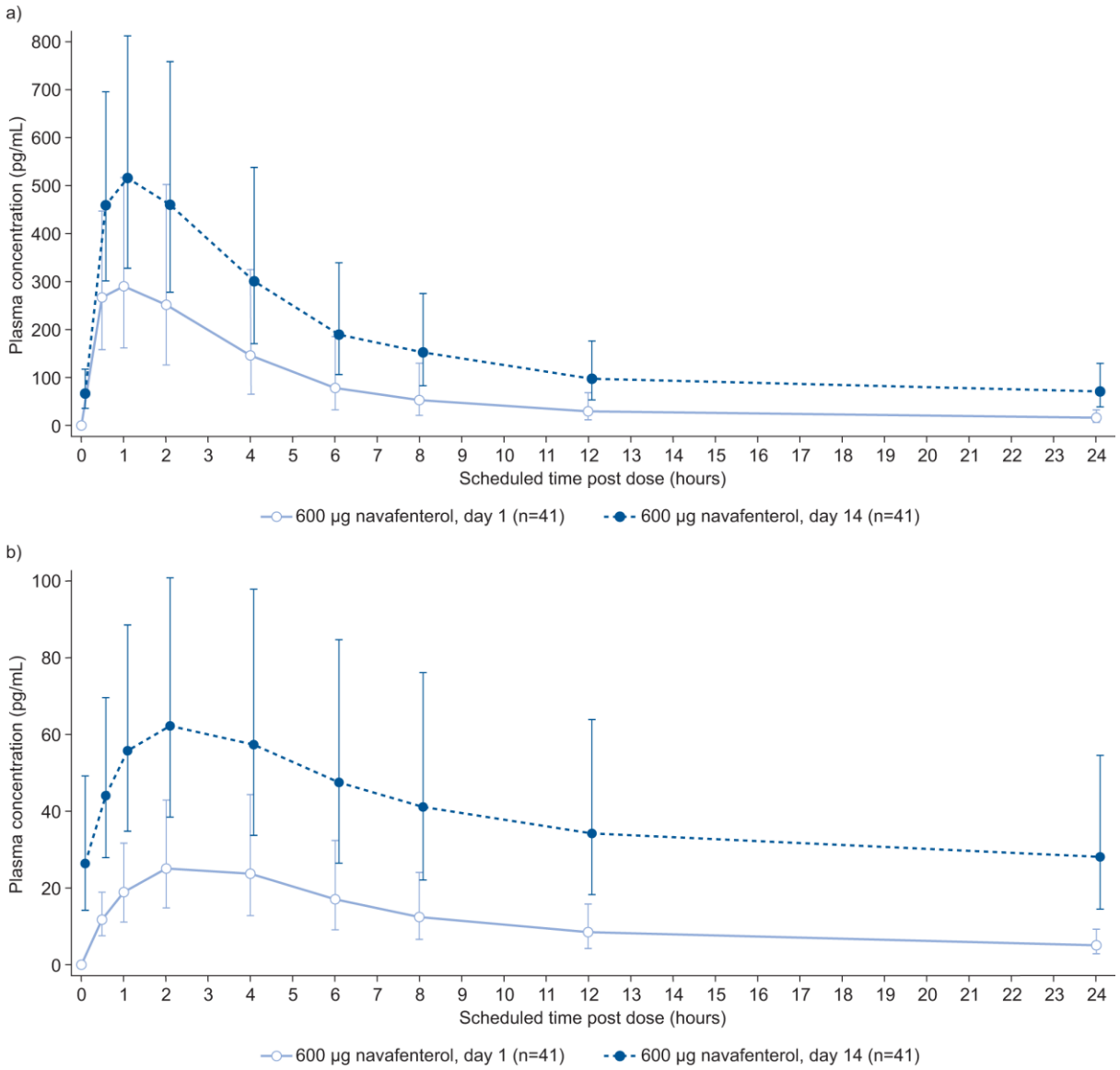

FIGURE S7 Geometric mean plasma concentration–time profiles for a) navafenterol and b) LAS191861. Error bars represent geometric standard deviation.

Supplementary materials

Supplementary methods

Study design

This study was a randomised, phase 2a, double-blind, double-dummy, three-way complete crossover Williams' design study (ClinicalTrials.gov identifier: NCT03645434). It compared the active treatment navafenterol and a long-acting muscarinic antagonist/long-acting β_2-agonist combination bronchodilator, umeclidinium/vilanterol (UMEC/VI), with placebo, administered once daily by dry powder inhaler devices to participants with moderate-to-severe chronic obstructive pulmonary disease (COPD). The study was conducted between October 10, 2018 and August 7, 2019 at three sites in Germany and two sites in the UK. It consisted of 12 visits, starting with a 14–28-day screening period (visits 1 and 2), followed by three 14-day treatment periods (visits 3–11). Following the first and second treatment periods, there was a 42–49-day washout period. After completion of the third treatment period, there was a 42–49-day follow-up period before the final site visit (visit 12) (figure 1a).

The treatments were assigned according to a Williams' design with three periods and six sequences, using a balanced randomisation ratio (1:1:1:1:1:1) per treatment sequence using an interactive voice/web response system (figure 1a). Throughout the run-in, washout and follow-up periods, patients received open-label ipratropium, two inhalations of 20 µg, four times daily; salbutamol 100 µg was provided open-label as a rescue medication. Both ipratropium and salbutamol were discontinued 8 h and 6 h, respectively, before any

pulmonary function test. At visit 1, participants ceased their usual COPD medication and, if required, were maintained on a stable dose of mono-component inhaled corticosteroid throughout the study. Reversibility, defined as increased post-bronchodilator forced expiratory volume in 1 s (FEV_1) of ≥12% (percentage reversibility) and ≥200 mL (absolute reversibility) compared with the pre-bronchodilator test, was measured at visit 2.

Patients

Moderate-to-severe COPD was defined as per the Global Initiative for Chronic Obstructive Lung Disease guidelines [1]. Patients were either current or former smokers.

In both countries, before study initiation the study protocol was approved at each site by the independent ethics committee or institutional review board (Germany: the Ethics Committee at the State Medical Association of Hesse, Frankfurt; the Ethics Committee of the State of Berlin, Berlin; and the Ethics Committee of the Schleswig-Holstein Medical Association, Bad Segeberg. UK: the South Central – Berkshire Research Ethics Committee, Bristol). All patients provided written informed consent before study enrolment.

Outcomes

The primary objective was to assess the efficacy of navafenterol 600 µg. The primary endpoint was the change from baseline in trough FEV_1 at day 15.

Secondary endpoints included: FEV_1 area under the curve $(AUC)_{[0–4]/4\ h}$ at day 1, day 8 and day 14; FEV_1 $AUC_{(0–8)/8\ h}$, $AUC_{(0–12)/12\ h}$ and $AUC_{(0–24)/24\ h}$ at day 1 and day 14; change from

baseline in trough FEV_1 on day 2, day 8 and over the treatment duration; change from baseline in peak FEV_1 on day 1, day 8, day 14 and over the treatment duration; change from baseline in total score of the breathlessness, cough and sputum scale (BCSS) questionnaire from day 1–8, day 9–14 and over the treatment duration; change from baseline in the COPD assessment tool (CAT) from day 1–8, day 9–14 and over the treatment duration; use of rescue medication from day 1–8 and day 9–14; treatment-emergent adverse events; tolerability; and pharmacokinetics of navafenterol and its primary metabolite, LAS191861.

Objective cough counts were also captured as an exploratory outcome using the VitaloJAK cough monitor (Vitalograph; Buckingham, UK) and perceived cough severity assessed using a visual analogue scale [2, 3]. Change from baseline in number of coughs, as measured by cough monitoring, was assessed on day 14, and change from baseline in cough visual analogue scale was assessed on day 8 and day 15. Cough monitoring was conducted for 24 h, starting 24 h before dosing on day 1 and starting pre-dose on day 14.

Statistical analysis

All participants were included in the full analysis set, which was used for the analysis of efficacy variables. Sensitivity analyses were performed on the full analysis set for the change from baseline in trough FEV_1 at day 15 to assess potential carryover effects between treatment periods. An additional sensitivity analysis was performed for the change from baseline in trough FEV_1 at day 15 on the per protocol population.

The study was powered to demonstrate superiority of navafenterol compared with UMEC/VI for the primary efficacy endpoint. With a total of 54 patients, the study would

have 90% power to detect a 100 mL difference between navafenterol and UMEC/VI treatment for the change from baseline in trough FEV_1 at day 15, assuming a standard deviation of 220 mL, a two-sided 5% significance level and a normal distribution. Assuming a dropout rate of ~25%, a sample size of 72 randomised patients would be required. Due to the exploratory nature of the study, no adjustments for multiple testing were made.

FEV_1 AUC at day 14 was analysed by means of a linear mixed-effect model: the fixed effects were for treatment, sequence and period, with a random effect for patient (nested within the sequence) and baseline as a continuous covariate.

The change from baseline for cough visual analogue scale, BCSS, CAT and use of rescue medication were each analysed using a mixed model: the fixed effects were for treatment, sequence and period, a random effect for patient (nested within the sequence) and baseline was included as a covariate. For the cough visual analogue scale, a log-transformation was applied to the score, which was then transformed back to the linear scale. The change from baseline in number of coughs, measured by objective cough monitoring, was analysed using a similar model after data transformation (a log-transformation was applied to the counts, which was then transformed back to the linear scale). A summary of raw counts per hour was also produced.

Blood samples for pharmacokinetic evaluation were drawn on day 1 and 14, pre-dose and 1, 2, 4, 6, 8, 12 and 24 h post-dose on days 1 and 14 of treatment and pre-dose and 1 h post-dose on day 8 of treatment. Determination of the plasma concentrations of navafenterol and LAS191861 were performed using liquid chromatography with tandem mass

spectrometry (LC-MS/MS). Pharmacokinetic parameters were derived using non-compartmental methods within Phoenix WinNonlin Version 8.1.

Supplementary results

Safety

Treatment-emergent adverse events were reported by similar proportions of participants across the treatment groups. Headache was the most common treatment-emergent adverse event, reported by 23 participants (31.5%) in the total safety population: 14 participants (20.0%) receiving navafenterol, 13 participants (18.8%) receiving UMEC/VI and 14 participants (20.6%) receiving placebo. Nasopharyngitis, rhinitis and cough were the next most common adverse events, reported in 16 (21.9%), 8 (11.0%) and 6 (8.2%), respectively, of participants in the total safety population, with similar incidences among the study drug treatments. Three participants (4.1%) experienced a cardiac adverse event. Acute coronary syndrome (n=1 [1.4%]) and tachycardia (n=1 [1.4%]) were reported in the UMEC/VI treatment period, and palpitations (n=1 [1.4%]) were reported in the navafenterol treatment period.

References

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