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

1. Global Initiative for Chronic Obstructive Lung Disease (GOLD). Global strategy for the diagnosis, management and prevention of chronic obstructive lung disease. https://goldcopd.org/wp-content/uploads/2019/12/GOLD-2020-FINAL-ver1.2-03Dec19_WMV.pdf. Date last updated: 2020. Date last accessed: 10 Jul 2021.

2. McGuinness K, Holt K, Dockry R, et al. Validation of the VitaloJAK™ 24 hour ambulatory cough monitor. Thorax 2012; 67(Suppl 2):A131.

3. Sumner H, Woodcock A, Kolsum U, et al. Predictors of objective cough frequency in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2013; 187: 943–949.