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A Multicentre, Randomized, Double-Blind, Placebo-Controlled, Crossover Study To Investigate the Efficacy, Safety, Tolerability, and Pharmacokinetics of Repeat Doses of Inhaled Nemiralisib in Adults with Persistent, Uncontrolled Asthma[S]

Sanjeev Khindri, Anthony Cahn, Malcolm Begg, Mickael Montembault, Claudia Leemereise, Yi Cui, Annabel Hogg, Hannah Wajdner, Shuying Yang, Jon Robertson, J. Nicole Hamblin, Andrea Ludwig-Sengpiel, Oliver Kornmann, and Edith M. Hessel

Respiratory Therapy Area Unit (S.K.), Clinical Pharmacology Science and Study Operations (M.M.), Global Clinical Safety and Pharmacovigilance (Y.C.), and Clinical Pharmacology Modelling and Simulation, Quantitative Sciences, RD Projects Clinical Platforms and Sciences (S.Y.), GSK, Stockley Park, Uxbridge, Middlesex, United Kingdom; Discovery Medicine (A.C.), Refractory Respiratory Inflammation Discovery Performance Unit (M.B., H.W., J.N.H., E.M.H.), Clinical Pharmacology Science and Study Operations (A.H.), and Clinical Statistics (J.R.), GSK, Stevenage, Hertfordshire, United Kingdom; Clinical Operations Department, GSK, Zeist, The Netherlands (C.L.); KLB Gesundheitsforschung Lübeck, Lübeck, Germany (A.L.-S.); and IKF Pneumologie Frankfurt, Clinical Research Centre Respiratory Diseases, Frankfurt, Germany (O.K.)

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ABSTRACT

Phosphoinositide 3-kinase δ (PI3Kδ) is a lipid kinase involved in leukocyte recruitment and activation. Activation of PI3Kδ has been linked to airway inflammation and asthma pathogenesis. This randomized, double-blind, placebo-controlled, crossover study investigated the efficacy, safety, tolerability, and pharmacokinetics of a PI3Kδ inhibitor, nemiralisib (GSK2269557), in patients with persistent, uncontrolled asthma. Patients (n = 50) received once-daily inhaled nemiralisib (1000 μg) or placebo for 28 days, with a crossover to the alternative treatment following a 4-week washout period. Spirometry demonstrated no discernible difference in trough forced expiratory volume in 1 second (FEV_1) from baseline (adjusted posterior median 7 ml; 95% credible interval −83, 102 ml) between nemiralisib and placebo treatment at day 28 (primary endpoint). These results were supported by most secondary endpoints, including weighted mean FEV_1 (0–4 hours) and change in trough forced vital capacity at day 28. Nemiralisib was generally well-tolerated, with few side effects except for post-inhalation cough (nemiralisib: 35%; placebo: 9%). At day 14, sputum interleukin (IL)-5, IL-13, IL-6, and IL-8 levels were reduced by a median of 17%, 7%, 15%, and 8%, respectively, when comparing nemiralisib with placebo [n = 15 (IL-5, IL-8) or 16 (IL-6, IL-13); posterior probability of a true ratio >0%: 78%, 64%, 76%, and 63%, respectively]. These results suggest that nemiralisib inhibited PI3Kδ locally; however, this did not translate into meaningful clinical improvement. Further studies will investigate the potential efficacy of nemiralisib in patients with asthma with other specific more severe phenotypes, including those who are colonized with bacteria and frequently exacerbate.

Introduction

Asthma, a chronic respiratory disease, affects approximately 358 million people worldwide, and the prevalence is increasing, rising by 12.6% globally between 1990 and 2015 (Soriano et al., 2017). Asthma is ranked 23rd among the global burden of disease causes, contributing 1.1% of global disability-adjusted life years (Soriano et al., 2017). Common symptoms of asthma include shortness of breath, chest tightness, wheeze, and cough, and patients may experience periodic flare-ups (exacerbations) (www.ginasthma.org).

This work has not been previously presented.

S.K., A.C., M.B., M.M., Y.C., A.H., H.W., S.Y., J.R., and E.M.H. are employees of, and hold stocks/share options in, GSK. C.L. is an employee of GSK. J.N.H. was an employee of GSK at the time this study was conducted and is a stockholder in GSK. J.N.H. and E.M.H. are listed as coinventors on patents relating to nemiralisib. A.L.-S. and O.K.'s institutions each received honoraria for conducting the clinical trial described in this paper.

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ABBREVIATIONS: ACT, asthma control test; AE, adverse event; COPD, chronic obstructive pulmonary disease; CrI, credible interval; ECG, electrocardiogram; FeNO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; ICS, inhaled corticosteroids; IL, interleukin; ITT, intent-to-treat; LABA, long-acting β_2-agonist; LLQ, lower limit of quantification; PD, pharmacodynamics; PEF, peak expiratory flow; PI3K, phosphoinositide 3-kinase; PK, pharmacokinetics; PP, per-protocol; QC, quality control; SHIP1, Src homology 2–containing inositol-5′-phosphatase 1; TNF, tumor necrosis factor.

Current guidelines recommend inhaled corticosteroids (ICS) for the initial treatment of asthma (www.ginasthma.org); however, a subpopulation of patients with asthma remains uncontrolled despite taking ICS, and long-term ICS usage may be associated with side effects (Lipworth, 1999; Reddy and Little, 2013). Alternative therapies include leukotriene receptor antagonists and, in patients experiencing persistent symptoms or exacerbations, long-acting β_2-agonist (LABA) in free- or fixed-dose combination with ICS (www.ginasthma.org). Patients with severe asthma or persistent symptoms despite these treatments may be prescribed biologics, such as anti-IgE or anti-interleukin (IL)-5 agents (www.ginasthma.org). However, whereas biologics may be beneficial in reducing exacerbations, they are costly and they may not be cost effective for many patients (Wu et al., 2007; Menzella et al., 2016). Thus, there remains a need for novel therapies to achieve disease control in patients with asthma.

Asthma is characterized by chronic airway inflammation and hyper-responsiveness to inhaled allergens and indirect stimuli (www.ginasthma.org). In atopic individuals, T cells produce inflammatory mediators upon antigen exposure, such as IL-4, IL-5, and IL-13, which recruit and activate mast cells, eosinophils, and IgE-producing B cells, mediating local allergic inflammation and contributing to the pathogenesis of asthma (Robinson et al., 1992, 1993; Matangkasombut et al., 2009; Deo et al., 2010). Correspondingly, an increase in inflammatory cytokines, such as IL-6 and IL-8, is observed in the lungs of patients with asthma experiencing an exacerbation (Yokoyama et al., 1995; Norzila et al., 2000; Maneechotesuwan et al., 2007).

Phosphoinositide 3-kinases (PI3Ks) are a large family of lipid-signaling kinases (Park et al., 2008). The class I family of PI3Ks phosphorylates the membrane phospholipid phosphatidylinositol 4,5-biphosphate into phosphatidylinositol 3,4,5-trisphosphate, which acts in various signaling pathways (Vanhaesebroeck et al., 2010). The class IA PI3Kδ isoform is expressed predominantly in leukocytes (Chantry et al., 1997) and has roles in mediating antigen receptor and cytokine signaling in T cells (including Th2 cells), mast cells, and B cells, as well as in eosinophil migration (Clayton et al., 2002; Okkenhaug et al., 2002, 2007; Ali et al., 2004; Patton et al., 2006; Nashed et al., 2007; Stark et al., 2015; Way et al., 2016). In asthma mouse models, PI3Kδ inhibition has been shown to attenuate recruitment of inflammatory cells, including lymphocytes and eosinophils, and reduce the release of proinflammatory T cell cytokines (Lee et al., 2006). Additionally, PI3Kδ inhibition may prevent IgE release from B cells and reduce degranulation of mast cells (Ali et al., 2004; Lee et al., 2006).

Src homology 2–containing inositol-5′-phosphatase 1 (SHIP1) is predominantly expressed in hematopoietic cells and dephosphorylates phosphatidylinositol 3,4,5-trisphosphate to phosphatidylinositol 4,5-biphosphate, thereby inducing selective downregulation of the PI3Kδ pathway (Leaker et al., 2014). In patients with mild-to-moderate asthma, activation of SHIP1 with oral AQX-1125 was shown to significantly attenuate allergen-induced responses, accompanied by a trend toward reduced recruitment of eosinophils, neutrophils, and macrophages (Leaker et al., 2014).

Nemiralisib (GSK2269557) is a potent and highly selective inhaled PI3Kδ inhibitor being developed as a potential therapeutic for the treatment of inflammatory airway diseases (Down et al., 2015; Cahn et al., 2017). The effects of nemiralisib (1000 μg) in patients with stable chronic obstructive pulmonary disease (COPD) were recently investigated in a randomized, double-blind, placebo-controlled study, which found a reduction in sputum IL-6 (29%) and IL-8 (32%) levels following inhalation of nemiralisib, suggesting nemiralisib may reduce airway inflammation (Cahn et al., 2017), consistent with reduced disease severity (Franciosi et al., 2006; Hacievliyagil et al., 2006). The present proof-of-concept study was the first administration of inhaled nemiralisib (1000 μg once daily for 28 days) to patients with persistent, uncontrolled asthma who had not received ICS or LABA treatment of $\geq$12 weeks prior to the first dose of study medication. We aimed to investigate the efficacy, safety, tolerability, and systemic pharmacokinetics (PK) of nemiralisib in this patient group.

Materials and Methods

Study Design. This was a multicentre, randomized, double-blind, placebo-controlled, two-period crossover study in patients with persistent, uncontrolled asthma, currently not treated with an ICS or a LABA. The study was conducted at 12 centers in Germany, of which three closed without activity and one center experienced a screening failure. Data were collected from eight centers between October 7, 2015 and September 28, 2016 (ClinicalTrials.gov identifier: NCT02567708).

Patients were screened for eligibility up to 28 days before randomization. Those patients meeting the eligibility criteria entered a run-in period of approximately 2 weeks, during which baseline asthma status was measured and safety evaluations were performed. At the end of the run-in period, eligible patients were randomized to one of two sequences. Patients randomized to sequence one received matched placebo once daily for 28 $\pm$ 2 days in an initial treatment period (treatment period 1) and nemiralisib 1000 μg once daily for 28 $\pm$ 2 days in a second treatment period (treatment period 2). Patients randomized to sequence two received nemiralisib 1000 μg once daily for 28 $\pm$ 2 days in treatment period 1 and matched placebo once daily for 28 $\pm$ 2 days in treatment period 2. There was a washout period of $\geq$4 weeks between treatment periods. Patients visited the clinic on the first day of treatment (day 1), and at days 7, 14, and 28 of each treatment period, and attended a follow-up visit 1 to 2 weeks after their last dose.

Nemiralisib and placebo were administered using the DISKUS dry powder inhaler (owned by or licensed to the GSK group of companies). The dose of nemiralisib was selected based on previous safety, tolerability, and biomarker data in patients with COPD; the same nemiralisib formulation and device were also used (Cahn et al., 2017). Use of this dose is also supported by extensive preclinical research (Down et al., 2015).

To maintain blinding, the devices used to administer nemiralisib and placebo were identical in appearance. An interactive web response system was used to assign patients to a treatment sequence, and participating sites were provided with only the number of the device to use, not the treatment sequence.

The study was conducted in accordance with the International Conference on Harmonization Good Clinical Practice and the Declaration of Helsinki. All patients provided written informed consent prior to participation in the study.

Patients. Patients were eligible for inclusion if they were aged 18–70 years with persistent, uncontrolled bronchial asthma treated only with an intermittent short-acting β_2-agonist or other noncorticosteroid controllers, and had not received ICS or LABA treatment of $\geq$12 weeks prior to the first dose of study medication. In addition, patients were required to have a best prebronchodilator forced expiratory volume in 1 second (FEV_1) $\geq$60% of the predicted normal value at screening, and a FEV_1 increase by $\geq$12% and $\geq$200 ml over the baseline value within

10–40 minutes of inhalation of 400 μg salbutamol via a metered dose inhaler.

Key exclusion criteria included a history of life-threatening asthma, severe asthma exacerbation, or respiratory infection; and current smokers or patients with a history of smoking within 6 months of screening. Full exclusion and withdrawal criteria are listed in Supplemental Materials.

All patients were allowed to use salbutamol on an as-needed basis during the entire study, except for the 4 hours prior to each FEV_1 and fractional exhaled nitric oxide (FeNO) test conducted at the clinic, if possible.

Study Objectives and Endpoints. The primary study objective was to investigate the efficacy of inhaled nemiralisib administered once daily for 28 days in patients with persistent, uncontrolled asthma, compared with placebo. The primary endpoint was defined as the change from baseline in trough FEV_1 at day 28. This primary endpoint chosen as FEV_1 is a recognized efficacy measure in this population and enables direct comparison with established therapeutics.

The secondary study objectives were to characterize the clinical response, safety, and tolerability, and plasma PK of inhaled nemiralisib administered once daily for 28 days in patients with persistent, uncontrolled asthma. Secondary efficacy endpoints were as follows: change from baseline in trough FEV_1 at days 7 and 14; percent change from baseline in trough FEV_1 at days 7, 14, and 28; weighted mean (0–4 hours) FEV_1 at day 28; change from baseline in forced vital capacity (FVC) at days 7, 14, and 28; change from baseline in FEV_1/FVC at days 7, 14, and 28; change from baseline in daily FEV_1 (morning) and peak expiratory flow (PEF) (morning and evening) averaged over the treatment period; change from baseline in asthma control test (ACT) score at day 28; change from baseline in trough FeNO at days 7, 14, and 28; and the mean number of inhalations per day of rescue medication. Safety endpoints included the incidence of adverse events (AEs), monitoring of vital signs, 12-lead electrocardiogram (ECG), and clinical laboratory tests (hematology and clinical chemistry). PK assessments to determine the trough plasma concentration after 7, 14, and 28 days of treatment and day 28 plasma exposure up to 3.5 hours postdose were also conducted. Exploratory endpoints included pharmacodynamic (PD) analyses, which investigated the levels of proinflammatory cytokines in induced sputum prior to and after 14 days of treatment.

Efficacy Assessments. FEV_1 and FVC were measured in the clinic using a spirometer in accordance with the American Thoracic Society/European Respiratory Society standards (Miller et al., 2005). Single time point spirometry was performed predose on days 1, 7, and 14, and serial spirometry was performed on day 28: predose, and 1–4 hour postdose of each treatment period. Daily FEV_1 and PEF were measured morning and evening by patients using a handheld device before any rescue salbutamol use. FeNO was measured at days 1, 7, 14, and 28 of each treatment period, using a handheld electronic device in accordance with the American Thoracic Society/European Respiratory Society Recommendations (American Thoracic Society and European Respiratory Society, 2005), and interpreted as per the American Thoracic Society Clinical Practice Guideline (Dweik et al., 2011). Patients self-completed the five-item ACT on days 1 and 28 before any other assessment at a clinic visit.

Safety Assessments. AE monitoring was conducted throughout the study. In each treatment period, 12-lead ECG was performed at screening and predose on days 1, 7, and 28. Vital signs were measured at screening; predose on days 1, 7, 14, and 28; and at the follow-up visit. Routine laboratory assessments were carried out at screening and predose on days 1, 14, and 28.

PK Assessments. Blood samples were collected via an indwelling cannula or by direct venipuncture predose on days 7, 14, and 28. In addition, on day 28, samples were taken between 5 and 10 minutes postdose and also between 2.5 and 3.5 hours postdose. Plasma samples were analyzed for nemiralisib using an internally validated analytical method based on protein precipitation, followed by high performance liquid chromatography/tandem mass spectrometry analysis. The lower limit of quantification (LLQ) was 20 pg/ml using a 50 μl aliquot of ethylenediaminetetraacetic acid plasma. The higher limit of quantification was 10,000 pg/ml.

Calibration standards and quality control (QC) samples prepared at three different analyte concentrations were analyzed with each batch of samples. For the analysis to be acceptable, no more than one-third of the QC results were to deviate from the nominal concentration by more than 15%, and at least 50% of the results from each QC concentration needed to be within 15% of nominal concentration. The applicable analytical runs met all predefined run acceptance criteria.

Cytokine Levels in Induced Sputum. Hypertonic saline-induced sputum samples were collected during screening (for confirmation of eligibility) and predose on days 1 and 14 in a subset of patients. Time points were selected to match those used previously in the study of nemiralisib in patients with COPD to enable comparison (Cahn et al., 2017). Cytokines [IL-4, IL-5, IL-6, IL-8, IL-13, and tumor necrosis factor (TNF)-α] in sputum supernatant were analyzed using custom multiplex assay (V-plex) platform from Meso Scale Discovery (Gaithersburg, MD), according to the manufacturer's instructions. Imputed values (half of the LLQ) were used for cases in which the data obtained from a sample were below the LLQ.

Statistical Methods. A crossover design was selected to provide tighter precision for the treatment comparisons, compared with a parallel group design using the same number of patients. The sample size was determined by feasibility; however, this was deemed to provide sufficient precision around the estimation for the comparisons of interest to enable progression given positive data. Assuming the within-patient S.D. was similar to that observed in previous studies, it was estimated that the lower and upper bounds of the 95% credible intervals for the difference between nemiralisib and placebo for the primary endpoint with the proposed sample size would be within $\pm$94 ml of the observed treatment difference. Approximately 50 patients were planned to be randomized to the study to ensure completion of both treatment periods by approximately 40 patients, assuming a 20% dropout rate. Randomization was stratified by patients who were able and willing to provide induced sputum samples (able to produce >100 mg induced sputum at screening or during the run-in period) and those who were not. Approximately 16 patients were planned to be randomized into the induced sputum stratum. Randomization to treatment sequence was in a 1:1 ratio into each stratum.

The primary efficacy analyses were based on the intent-to-treat (ITT) population (in which patients were analyzed according to the treatment they received), and a sensitivity analysis was performed in the per-protocol (PP) population, which included all patients with no major protocol violation. The secondary efficacy, safety, and PK analyses were conducted in the ITT, safety, and PK populations, respectively. Exploratory PD endpoints were assessed in the PD population. The safety population comprised all randomized patients, and the PK and PD populations comprised all patients in the ITT population who had a blood sample for PK analysis, or who participated in the sputum substudy, respectively.

Adjusted posterior medians and corresponding 95% credible intervals (CrI) for the change from baseline in trough FEV_1 were produced for both study groups, as well as the difference between nemiralisib and placebo. Posterior probabilities that the true difference was greater than various thresholds (0, 0.05, and 0.1 l) were also produced. The change from baseline in FVC was analyzed in the same way.

The weighted mean (0–4 hours) FEV_1 was calculated for each treatment period based on clinic FEV_1 data obtained at predose and 1–4 hours postdose on day 28. The weighted mean was derived by calculating the area under the curve, and then dividing by the actual relevant time interval.

Multiple readings ($\leq$8) were taken for each FeNO assessment, which were log-transformed and analyzed using the same technique as for the FEV_1 analyses. Results were back-transformed to provide estimated geometric medians per treatment.

The ACT questionnaire had five possible response options for each question, with a score of 1 (poor control) to 5 (good control), and the scores from each question were summed to give a total score.

Each efficacy endpoint was analyzed used a Bayesian model using a noninformative prior for all model parameters, and adjusting for the following covariates: patient-level baseline, adjusted period-specific baseline, period, and treatment. For each FEV_1 outcome, including weighted mean (0–4 hours) FEV_1, the baseline was the trough FEV_1.

The plasma nemiralisib PK concentration–time data were summarized by study day and sampling time. The trough value of plasma nemiralisib concentration was determined as the concentration measured at predose on each study visit.

Results

Patient Population. Of 108 patients screened, 50 patients were eligible to enter the study, all of whom were randomized (24 patients received placebo, and 26 patients received nemiralisib during treatment period 1) (Fig. 1). The study was completed by 42 (84%) patients. All patients were included in the safety population. Forty-four patients were included in the ITT population; six patients had an inclusion criteria deviation and were randomized when screening spirometry overread was not acceptable. Because they entered the study without spirometric evidence of asthma, they were excluded from the ITT population. Forty-two patients were included in the PK population and 41 in the PP population. Sixteen patients (eight assigned to each treatment sequence) participated in the sputum substudy (PD population).

All patients were white, 22–67 years of age, and 56% were female (Table 1). Prestudy spirometry, FeNO (predose day 1, treatment period 1), and blood eosinophil counts for the ITT population are shown in Table 2. The baseline characteristics of the patients who participated in the sputum substudy are provided in Supplemental Table 1.

Spirometry Assessments. Overall, posterior median trough FEV_1 at day 28 increased from baseline in both nemiralisib and placebo treatment groups (Fig. 2). The adjusted median increase from baseline in FEV_1 at day 28 (primary endpoint) was 27 ml (95% CrI: −66, 117 ml) in the placebo group and 35 ml (95% CrI: −61, 124 ml) in the nemiralisib treatment group. The posterior median treatment difference (nemiralisib vs. placebo) at day 28 was 7 ml (95% CrI: −83, 102 ml). The posterior probability that the true difference between treatments is greater than zero at day 28 was 0.57. This result indicates that patients in the nemiralisib group received no discernible benefit of treatment with respect to trough FEV_1 at day 28.

Similar results were observed in trough FEV_1 at days 7 and 14; the posterior median trough FEV_1 levels increased from baseline at days 7 and 14 in both nemiralisib and placebo

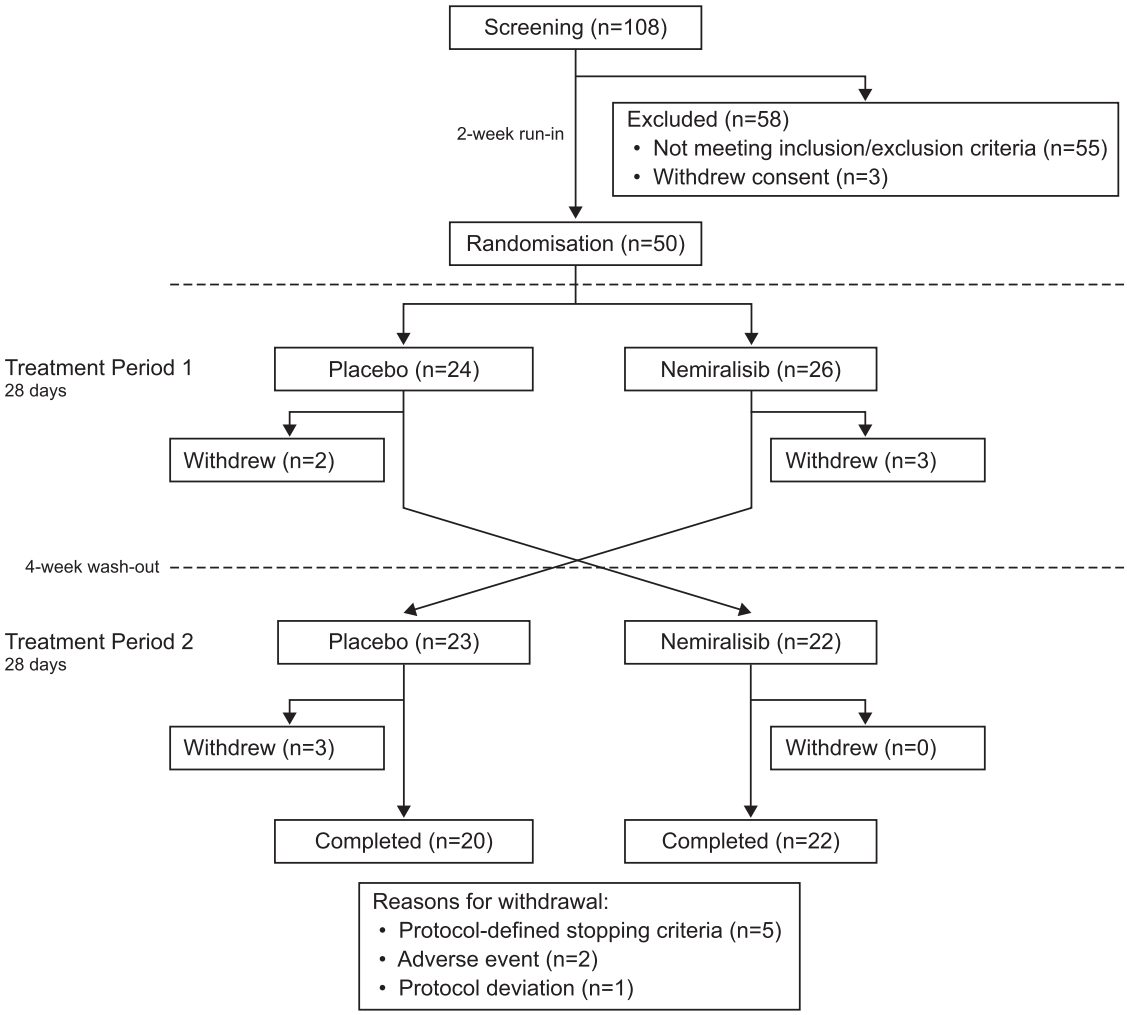

Fig. 1. CONSORT flow diagram of study participants.

TABLE 1
Patient demographics (safety population)

Characteristic	Total (N = 50)
Age, years, mean (S.D.)	44.5 (13.86)
Sex	
Female, n (%)	28 (56)
Male, n (%)	22 (44)
BMI, kg/m^2, mean (S.D.)	26.94 (3.66)

BMI, body mass index.

treatment groups (Fig. 2). The posterior median treatment difference (nemiralisib vs. placebo) was −9 ml (95% CrI: −151, 131 ml) at day 7, and 24 ml (95% CrI: −87, 137 ml) at day 14. The posterior probability that the true difference is greater than zero was 0.44 and 0.66 at days 7 and 14, respectively. Sensitivity analyses in the PP population showed similar results to the ITT population.

Similarly, the adjusted median percent change from baseline in trough FEV_1 showed a small increase in both groups at days 7, 14, and 28, with the exception of day 7 in the nemiralisib group, in which a small percentage decrease was observed (Table 3). Overall, however, these data supported the observation that no discernible benefit was detected in patients treated with nemiralisib when compared with placebo-treated patients.

Values for the adjusted median of the weighted mean (0–4 hours) FEV_1 at day 28, median trough FVC, and the adjusted median change from baseline in trough FEV_1/FVC for days 7, 14, and 28 are reported in Table 3 for both placebo and nemiralisib groups. Whereas small differences were observed, overall the data indicate that there was no benefit of nemiralisib treatment when compared with placebo. An increase from baseline was observed in daily evening FEV_1 and PEF compared with daily morning FEV_1 and PEF, respectively, in both placebo and nemiralisib groups (Supplemental Table 2).

ACT. The total ACT score at day 28 increased from baseline by a posterior median of 1.4 points (95% CrI: 0.5, 2.2) in the placebo group and 1.9 points (95% CrI: 1.0, 2.7) in the nemiralisib group (Table 3). The posterior median treatment difference at day 28 was 0.5 points (95% CrI: −0.6, 1.7). The posterior probability that the true difference is greater than zero at day 28 was 0.81.

FeNO. Posterior median change from baseline in trough FeNO is shown in Table 3. In the placebo group, trough FeNO levels increased from baseline by 3% at days 7 and 28, and marginally changed at day 14 (−1%). In the nemiralisib group, trough FeNO decreased from baseline by 9% at day 7 and 5% at day 14, and increased at day 28 by 3%. The posterior median ratios (nemiralisib:placebo) at days 7 and 14 were 0.89 (95% CrI: 0.78, 1.00) and 0.95 (95% CrI: 0.83, 1.09), respectively, favoring treatment with nemiralisib compared with placebo. The posterior median ratio at day 28 was 1.00 (95% CrI: 0.84, 1.18). The posterior probability that the true treatment ratio is less than one was 0.97, 0.76, and 0.51 at days 7, 14, and 28, respectively.

TABLE 2
Patient baseline characteristics measured at screening (ITT population)
These data were obtained by post hoc analyses.

Characteristic	Total (N = 44)
Predicted normal FEV_1, %, mean (range)	73.87 (60.3, 96.5)
FEV_1/FVC, %, mean (range)	67.98 (45.9, 87.2)
FEV_1 reversibility, %, mean (range)	21.17 (11.9, 50.8)
FeNO, ppb, geo mean[a]	28.64
Blood eosinophil count, 10^9/l, geo mean	0.266

geo, geometric; ppb, parts per billion.
[a]Measurements taken at pre-dose day 1 of treatment period 1.

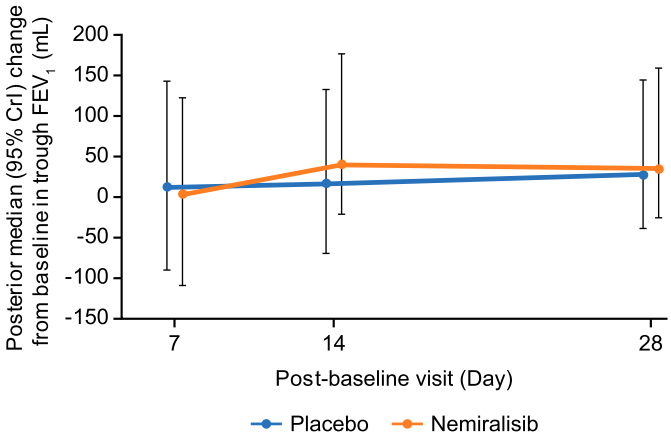

Fig. 2. Adjusted change from baseline in trough FEV_1 (L) by visit (ITT population).

Use of Rescue Medication. The number of inhalations per day of rescue medication [mean (S.D.)] was lower in the nemiralisib group [2.7 (1.53)] than the placebo group [3.0 (1.86)]. The percentage of rescue-free days [mean (S.D.)] was marginally higher in the placebo group [49.5 (35.04)] compared with the nemiralisib group [47.3 (34.79)].

PK. With the exception of three patients, all nemiralisib-treated patients had a quantifiable plasma concentration of nemiralisib 5–10 minutes following inhalation, suggesting quick absorption of the drug. The plasma concentration of nemiralisib was similar, 5–10 minutes and 2.5–3.5 hours after dosing (Supplemental Table 3), suggesting a slow decline in plasma concentration. For the majority of patients, trough plasma concentration was stable after day 7. Mean trough levels of nemiralisib (predose values) were 556, 521, and 649 pg/ml on days 7, 14, and 28, respectively (Supplemental Table 3). The overlapping confidence intervals suggest there was no significant change in trough concentration between these time points.

Safety. A total of 31/50 (62%) patients experienced AEs, including 25/48 (52%), who reported AEs while receiving nemiralisib and 15/47 (32%) while receiving placebo (Table 4). AEs considered by the investigators to be drug-related occurred at a higher frequency in the nemiralisib group (35%) compared with the placebo group (4%). Two (4%) patients in the nemiralisib group discontinued treatment due to AEs: one due to acute bronchitis and another to exertional dyspnoea; neither was considered by the investigator to be treatment-related.

The most commonly reported AE was post-inhalation cough, with more patients reporting cough in the nemiralisib group (17/48, 35%) compared with placebo (4/47, 9%); 14 patients in the nemiralisib group and two patients in the placebo group experienced a cough considered by the investigator to be treatment-related. Cough commonly occurred immediately after inhalation of nemiralisib and was generally mild or moderate in intensity and short of duration, with no patient discontinuing treatment due to cough.

TABLE 3
Summary of statistical analysis of secondary endpoint data (ITT population)

		Placebo (N = 42)			Nemiralisib 1000 μg (N = 42)		
		n	Posterior Median (S.D.)	95% CrI	n	Posterior Median (S.D.)	95% CrI
Trough FEV_1 (L) fold change from baseline	Day 7	39	1.24 (1.99)	−2.63, 5.12	40	−0.77 (1.97)	−4.70, 3.04
	Day 14	38	0.50 (1.91)	−3.34, 4.13	41	0.54 (1.89)	−3.26, 4.10
	Day 28	37	0.97 (1.72)	−2.52, 4.30	39	0.31 (1.72)	−3.23, 3.50
Weighted mean (0–4 h) FEV_1 (L)	Day 28	38	2.67 (0.06)	2.56, 2.78	40	2.72 (0.06)	2.61, 2.84
Trough FVC (L) change from baseline	Day 7	39	0.04 (0.06)	−0.08, 0.15	40	0.02 (0.06)	−0.10, 0.13
	Day 14	38	−0.00 (0.06)	−0.11, 0.11	41	0.04 (0.06)	−0.07, 0.15
	Day 28	37	0.02 (0.05)	−0.09, 0.12	39	0.05 (0.05)	−0.05, 0.16
Trough FEV_1/FVC (%) change from baseline	Day 7	39	−0.96 (0.72)	−2.34, 0.50	40	−0.86 (0.71)	−2.29, 0.52
	Day 14	38	−0.46 (0.75)	−1.94, 0.99	41	−0.44 (0.73)	−1.90, 0.99
	Day 28	37	−0.69 (0.69)	−2.05, 0.64	39	−0.79 (0.68)	−2.16, 0.52
ACT score change from baseline	Day 28	38	1.4 (0.45)	0.5, 2.2	40	1.9 (0.43)	1.0, 2.7
Trough FeNO (ppb) fold change from baseline	Day 7	38	1.03 (0.05)	0.93, 1.13	34	0.91 (0.05)	0.82, 1.01
	Day 14	34	0.99 (0.06)	0.89, 1.11	35	0.95 (0.06)	0.85, 1.05
	Day 28	34	1.03 (0.07)	0.91, 1.17	34	1.03 (0.06)	0.91, 1.17

ppb, parts per billion.

There was no increased report of AEs due to infections/infestations in patients receiving nemiralisib compared with placebo (3/48, 6% vs. 8/47, 17%), and the majority of infection/infestation-related AEs were nasopharyngitis. No serious AEs or deaths were reported, and there were no trends or changes of clinical concern in laboratory evaluations, vital signs, or ECG for nemiralisib when compared with placebo.

Cytokine Levels in Induced Sputum. Levels of inflammatory cytokines IL-5, IL-6, IL-8, IL-13, and TNF-α were measured in induced sputum samples, and the adjusted posterior median change from baseline for each cytokine is shown in Table 5. When comparing cytokine levels across treatment groups, a 17% reduction in IL-5 levels in nemiralisib-treated patients was observed (posterior probability that the true ratio is less than one was 0.78). The ratio between nemiralisib and placebo treatment was 0.93 (reduction of 7%) for changes in IL-13 (posterior probability that the true ratio is less than one was 0.64). The ratio between nemiralisib and placebo treatment of IL-6 and IL-8 levels was 0.85 (a reduction of 15%) and 0.92 (a reduction of 8%), respectively (posterior probability that the true ratio is less than one was 0.76 for IL-6, and 0.63 for IL-8). No notable changes were observed in sputum TNF-α levels. Measurement of IL-4 was also attempted, but all values were below the assay LLQ.

TABLE 4
Summary of on-treatment adverse events (safety population)

Adverse Event	Number (%) of Subjects		
	Placebo (N = 47)	Nemiralisib 1000 μg (N = 48)	Total (N = 50)
Total	15 (32)	25 (52)	31 (62)
Cough	4 (9)	17 (35)	19 (38)
Nasopharyngitis	5 (11)	1 (2)	6 (12)
Headache	3 (6)	1 (2)	4 (8)
Throat irritation	0	2 (4)	2 (4)
Oropharyngeal pain	1 (2)	1 (2)	2 (4)
Rhinitis	2 (4)	0	2 (4)
Bone contusion	0	1 (2)	1 (2)
Diarrhea	0	1 (2)	1 (2)
Dysphonia	0	1 (2)	1 (2)
Dyspnoea exertional	0	1 (2)	1 (2)
Fall	0	1 (2)	1 (2)
Hand fracture	0	1 (2)	1 (2)
Hypersensitivity	0	1 (2)	1 (2)
Influenza	0	1 (2)	1 (2)
Migraine	0	1 (2)	1 (2)
Muscle strain	0	1 (2)	1 (2)
Oral herpes	0	1 (2)	1 (2)
Rash	0	1 (2)	1 (2)
Sciatica	0	1 (2)	1 (2)
Sputum increased	0	1 (2)	1 (2)
Thermal burn	0	1 (2)	1 (2)
Arthralgia	1 (2)	0	1 (2)
Back pain	1 (2)	0	1 (2)
Dizziness	1 (2)	0	1 (2)
Dyspnoea	1 (2)	0	1 (2)
Epicondylitis	1 (2)	0	1 (2)
Migraine with aura	1 (2)	0	1 (2)
Respiratory tract infection	1 (2)	0	1 (2)

TABLE 5
Summary of statistical analysis of inflammatory cytokine biomarkers (PD population)

Cytokine (pg/ml)	Placebo (N = 16)		Nemiralisib 1000 μg (N = 16)				
	n	Posterior Median Change from Baseline (95% CrI)	n	Posterior Median Change from Baseline (95% CrI)	Posterior Median Nemiralisib/Placebo (95% CrI)	Nemiralisib/Placebo % Inhibition	Posterior Probability of True Ratio >0%
IL-5	15[a]	1.06 (0.65, 1.76)	15[a]	0.88 (0.54, 1.41)	0.83 (0.48, 1.42)	17%	78%
IL-6	16	0.97 (0.59, 1.57)	16	0.83 (0.49, 1.36)	0.85 (0.51, 1.39)	15%	76%
IL-8	15	1.01 (0.54, 1.91)	16	0.94 (0.51, 1.73)	0.92 (0.54, 1.58)	8%	63%
IL-13	16	1.27 (0.90, 1.82)	16	1.18 (0.82, 1.68)	0.93 (0.61, 1.40)	7%	64%
TNF-α	16	0.95 (0.57, 1.58)	16	0.99 (0.60, 1.67)	1.04 (0.62, 1.75)	−4%	44%

[a]Seven of 15 values for IL-5 were imputed as half the lower limit of quantification.

Collectively, these data suggest that treatment with nemiralisib may reduce proinflammatory cytokine levels in the lungs of these patients.

Discussion

There remains an unmet need for novel therapies for patients with uncontrolled asthma. Nemiralisib, a potent and highly selective PI3Kδ inhibitor, has previously been studied in patients with stable COPD and in patients experiencing a COPD exacerbation (Cahn et al., 2017; https://www.clinicaltrials.gov/ct2/show/NCT02294734). The present study was the first time that nemiralisib was administered via the inhaled route to patients with persistent, uncontrolled asthma, treated only with an intermittent short-acting β_2-agonist or other noncorticosteroid controllers. The study was undertaken using the same dose shown to be effective, and with an acceptable safety profile, in patients with COPD. Our aim was to investigate the efficacy, safety, tolerability, and PK of nemiralisib in patients with asthma. The results demonstrated no discernible difference in trough FEV_1 from baseline between nemiralisib and placebo treatment at day 28 (primary endpoint). These observations were also supported by most secondary endpoints. At day 14, sputum IL-5, IL-13, IL-6, and IL-8 levels were reduced by a median of 17%, 7%, 15%, and 8%, respectively, when comparing nemiralisib with placebo. Although the effects on proinflammatory cytokines suggest that nemiralisib was able to inhibit PI3Kδ in the lung, this did not translate into a meaningful clinical improvement in this group of patients.

When the primary endpoint of a study is not achieved, clarity on whether drug plasma levels were sufficient is crucial for a correct interpretation of the data. The PK data demonstrated that nemiralisib was quickly absorbed into the systemic circulation and that the steady state level was attained after a week of treatment, which was consistent with the previous COPD study with nemiralisib (Cahn et al., 2017). Notably, similar plasma concentrations of nemiralisib were observed in patients with asthma and patients with stable COPD; mean trough concentrations ranged from 521 to 649 pg/ml in patients with asthma and from 604 to 711 pg/ml in those with stable COPD. Mean peak concentrations of nemiralisib at 5–10 minutes postdose were 1189 and 1109 pg/ml, in patients with asthma and COPD, respectively (Cahn et al., 2017).

The presence of proinflammatory cytokines in induced sputum derived from patients with persistent, uncontrolled asthma aligns with the role of PI3Kδ in promoting cytokine production and airway inflammation (Way et al., 2016). Although the levels of inhibition observed were modest, treatment with nemiralisib reduced the levels of sputum-derived proinflammatory cytokines (including IL-5, IL-13, IL-6, and IL-8), relative to placebo. Reductions in IL-6 and IL-8 (although in some cases nonsignificant) have been observed in broadly similar populations treated with ICS (Inoue et al., 1999; Carpagnano et al., 2005; Zuiker et al., 2015); improvements in lung function parameters were also observed in these studies. Furthermore, reductions in IL-6 and IL-8 sputum levels were observed following 14-day nemiralisib inhalation in patients with stable COPD (reductions of 29% and 32% in IL-6 and IL-8, respectively) (Cahn et al., 2017). More recently, a study using the same dose of nemiralisib (1000 μg) administered to patients with COPD experiencing exacerbations achieved its primary efficacy endpoint (https://www.clinicaltrials.gov/ct2/show/NCT02294734).

Similar to our study, a randomized, double-blind, placebo-controlled, crossover study of another PI3K inhibitor, duvelisib, which inhibits both PI3Kδ and PI3Kγ and is given via the oral route, did not meet the primary efficacy assessment of the maximum decrease in FEV_1, following an allergen challenge in patients with asthma (Schmalbach et al., 2015). Significant improvements were observed in the secondary endpoints of FEV_1 area under the curve and in methacholine challenge, suggesting some activity of this compound in patients with mild asthma (Schmalbach et al., 2015). The difference between the current nemiralisib study and the study with duvelisib may be due to route of administration (inhaled vs. oral) and/or inhibition of one (PI3Kδ) versus two (PI3Kδ and PI3Kγ) PI3K pathways.

Activation of SHIP1 with AQX-1125 via the oral route in patients with mild-to-moderate asthma was shown to significantly attenuate allergen-induced responses, accompanied by a trend toward reduced recruitment of eosinophils, neutrophils, and macrophages (Leaker et al., 2014). Whether the route of administration or whether activation of SHIP1 results in a more effective downregulation of the PI3Kδ pathway remains to be elucidated.

Overall, nemiralisib was well-tolerated. The most commonly reported AE was short-duration post-inhalation cough, which was generally considered to be mild or moderate in intensity and did not lead to patients discontinuing treatment. The presence of a short-duration post-inhalation cough in some patients was also reported in the nemiralisib study in patients with stable COPD (Cahn et al., 2017).

Although there was an improvement in ACT score with nemiralisib, the score remained less than 20 at day 28 in both groups, indicating a lack of good asthma control in both groups. In addition, although the mean daily frequency of

rescue medication was slightly lower in the nemiralisib group compared with placebo, the percentage of rescue-free days was also lower in the nemiralisib group, again indicating a lack of good control with nemiralisib alone.

FeNO can help identify airway inflammation and can be used as a predictor of ICS response (http://www.niox.com/Global/Documents/ATS-recommendations-for-interpreting-FeNO.pdf). In the current study, a small improvement in FeNO was observed following nemiralisib versus placebo at the start of the treatment period (days 7 and 14), but the difference was negligible at day 28, suggesting the effect was not sustained over time.

The absence of a meaningful clinical improvement in this group of patients with asthma treated with nemiralisib may also be explained by low levels of inflammation in these patients. The geometric mean baseline FeNO of 28.64 parts per billion suggests that the patient population had generally mild asthma. Furthermore, whereas the PD findings suggest that nemiralisib is able to inhibit the production of inflammatory cytokines in the lungs, thus potentially reducing inflammation, there was no clinically relevant change in FeNO at the end of the treatment period, suggesting little change to the extent of eosinophilic airway inflammation in these patients following nemiralisib inhalation.

The precision of treatment comparisons in this study was improved by using a crossover design compared with a parallel group design, because the comparisons would be made within an individual rather than between individuals. A washout period of 4 weeks was considered sufficient based on the steady state and PD effects of previous studies with nemiralisib. Although the sample size was small, it was sufficient to detect meaningful changes in FEV_1 (the primary endpoint); however, the study was unable to measure changes in the rate of exacerbations and patient-reported outcomes, both of which would require a larger sample size and/or longer study duration. Hence, larger studies with a longer period of treatment are required to further investigate the effect of nemiralisib on these endpoints in patients with asthma. In addition, an alternative endpoint could have been to explore the effect of nemiralisib on methacholine-evoked bronchial hyper-reactivity; however, this would have been an additional procedure for the patients to undertake, and it is not widely recognized as a proof-of-concept efficacy endpoint.

In conclusion, this study has shown that nemiralisib demonstrates an acceptable safety and tolerability profile in patients with persistent, uncontrolled asthma, but not currently on ICS treatment, with the most common AE a short-duration post-inhalation cough. Inhalation of nemiralisib appears to act upon the PI3Kδ pathway, leading to a reduction in sputum proinflammatory cytokine levels, including IL-5, IL-6, IL-8, and IL-13 when compared with placebo, suggestive of reduced airway inflammation in these patients; however, this effect did not translate to marked clinical improvements in lung function in this population. Based on the collective results of our clinical studies in both patients with COPD and asthma, and the published observations in patients with the activating mutation in PI3Kδ (APDS) who suffer from severe recurrent respiratory infections, we hypothesize that nemiralisib may be most effective in patients who are colonized with bacteria and frequently exacerbate, regardless whether they have asthma or COPD. Further studies are needed to investigate the potential efficacy of nemiralisib in patients with asthma with other specific more severe phenotypes, including those who are colonized with bacteria and frequently exacerbate.

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Authorship Contributions

Participated in research design: Khindri, Begg, Montembault, Cui, Robertson, Hamblin, Hessel.

Contributed new reagents or analytic tools: Hogg, Wajdner, Ludwig-Sengpiel, Kornmann.

Performed data analysis: Khindri, Cahn, Begg, Leemereise, Cui, Wajdner, Yang, Robertson, Hamblin, Hessel.

Wrote or contributed to the writing of the manuscript: Khindri, Cahn, Begg, Montembault, Leemereise, Cui, Hogg, Wajdner, Yang, Robertson, Hamblin, Ludwig-Sengpiel, Kornmann, Hessel.

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Address correspondence to: Dr. Edith M. Hessel, Refractory Respiratory Inflammation Discovery Performance Unit, GSK, Gunnels Wood Road, Stevenage SG1 2NY, United Kingdom. E-mail: edith.m.hessel@gsk.com

A multicentre, randomised, double-blind, placebo-controlled, crossover study to investigate the efficacy, safety, tolerability and pharmacokinetics of repeat doses of inhaled nemiralisib in adults with persistent, uncontrolled asthma

Sanjeev Khindri, Anthony Cahn, Malcolm Begg, Mickael Montembault, Claudia Leemereise, Yi Cui, Annabel Hogg, Hannah Wajdner, Shuying Yang, Jon Robertson, J. Nicole Hamblin, Andrea Ludwig-Sengpiel, Oliver Kornmann, Edith M. Hessel

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Supplementary materials

Methods

Study population

Patients were excluded from the study if they had a history of life-threatening asthma, any severe asthma exacerbation or respiratory infection, concurrent respiratory disease, or were current smokers or had smoked within 6 months of screening. Patients were excluded if levels of alanine aminotransferase and bilirubin were more than 2 and 1.5 times the upper limit of normal, respectively. Patients with a current or chronic history of liver disease or known hepatic/biliary abnormalities (with the exception of Gilbert's syndrome or asymptomatic gallstones), a corrected QT interval >450 msec, and other laboratory abnormalities or concurrent diseases that may have affected their safety or confounded the efficacy results in the event of an exacerbation were also excluded. Other exclusion criteria included the following: history of regular alcohol consumption within 6 months of the study; history of sensitivity to any components of the study medications or a history of drug or other allergy; presence of hepatitis B surface antigen or positive hepatitis C test result at screening or within 3 months prior to the first dose of study medication; participation in a clinical trial and administration of an investigational product within 30 days, 5 half-lives or twice the duration of the biological effect (whichever was longer) prior to beginning treatment period; and exposure to more than 4 investigational medicinal products within 12 months before the first dose of study medication.

Patients were excluded if they had any previous treatment with an anti-immunoglobulin E monoclonal antibody. Prohibited medications during the study (from screening until the end of Treatment Period 2) included leukotriene-modifying agents, theophyllines, slow-release bronchodilators, ketotifen, nedocromil sodium, orally inhaled sodium cromoglycate and β-adrenergic blocking agents. Anticholinergics and regular/chronic treatment with strong inhibitors of cytochrome P450 (CYP) 3A4 or CYP2D6 were prohibited from 1 week and 2 weeks, respectively, of screening until the end of

Treatment Period 2. Systemic or inhaled corticosteroids, oral or inhaled long acting β_2-agonists and immunosuppressive medications were disallowed within 12 weeks of the first dose of study medication until the end of Treatment Period 2. Nicotine replacement or containing products were prohibited within 5 half-lives of the first dose of study medication until the end of Treatment Period 2.

Withdrawal criteria were defined as safety stopping criteria and efficacy stopping and alert criteria. Safety stopping criteria included liver chemistry stopping and increased monitoring criteria, developed in alignment with the US Food and Drug Administration premarketing clinical liver safety guidance, 2009 (http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation /Guidances/UCM174090.pdf); corrected QT of >500 msec, uncorrected QT >600 msec or change from baseline of corrected QT >60 msec; unacceptable adverse events; clinically significant changes in laboratory parameters/electrocardiogram recordings; paradoxical bronchospasm; and pregnancy. Efficacy stopping criteria were defined as clinic forced expiratory volume in one second (FEV_1) below the FEV_1 stability limit calculated at randomisation; at least 3 days in which ≥12 inhalations/day of salbutamol were used within the 7-day period immediately preceding contact; an asthma exacerbation (worsening asthma requiring any treatment [including use of systemic or inhaled corticosteroids, and/or emergency room visit or hospitalisation] other than rescue salbutamol). Patients were instructed to contact the investigator as soon as possible for further evaluation if they experienced 3 days in which the morning or evening peak expiratory flow (PEF) had fallen below the PEF stability limit calculated at randomisation.

Results

Supplementary Table 1. Patient baseline characteristics measured at screening (PD population)

Characteristic	**Total (N=16)**
Predicted normal FEV_1, %, mean (range)	72.53 (67.72, 77.33)
FEV_1/FVC, %, mean (range)	67.10 (61.56, 72.64)
FEV_1 reversibility, %, mean (range)	20.09 (14.79, 25.40)
FeNO, ppb, geo. mean*	26.43

Blood eosinophil count, 10^9/L, geo. mean	0.235

BMI, body mass index; FeNO, fractional exhaled nitric oxide; FEV_1, forced Expiratory Volume in 1 second; FVC, forced vital capacity; geo, geometric; ppb, parts per billion.

*Measurements taken at pre-dose Day 1 of Treatment Period 1.

These data were obtained by post hoc analyses.

Supplementary Table 2. Summary of change from baseline in daily FEV_1 and PEF, by treatment (ITT population).

Change from baseline	Placebo (N=42)			Nemiralisib (N=42)		
	n	Mean (SD)	95% CI	n	Mean (SD)	95% CI
Daily FEV_1 (L)						
Morning	38	0.00 (0.20)	-0.07, 0.07	40	0.07 (0.30)	-0.03, 0.16
Evening	34	0.01 (0.26)	-0.08, 0.11	39	0.12 (0.38)	-0.01, 0.24
Daily PEF (L/min)						
Morning	38	-4.80 (32.44)	-15.40, 5.90	40	14.20 (46.11)	-0.50, 29.00
Evening	34	9.80 (36.05)	-2.80, 22.40	39	29.80 (64.28)	9.00, 50.60

CI, confidence interval; FEV_1, forced expiratory volume in 1 second; ITT, intent-to-treat; PEF, peak expiratory flow; SD, standard deviation.

Mean baseline (mean of the planned pre-dose measurements on Day 1) FEV_1 was 2.547 L and 2.377 L for the placebo and nemiralisib groups, respectively.

Mean baseline PEF was 381.285 L/min and 354.927 L/min for placebo and nemiralisib, respectively.

Supplementary Table 3. Summary of plasma nemiralisib pharmacokinetic concentration-time data (pg/mL) by study day and time (pharmacokinetic population)

Treatment	N	Day	Planned time	n	No. imputed	Geometric mean	95% CI
Nemiralisib 1000 µg	42	7	Pre-dose	40	2	555.7	(446.2, 692.0)
		14	Pre-dose	40	3	520.7	(380.3, 712.9)
		28	Pre-dose	38	3	649.2	(519.9, 810.8)
			5–10 min	40	3	1188.8	(990.5, 1426.8)
			2.5–3.5 h	40	3	1170.8	(976.1, 1404.4)

CI, confidence interval.