

## Early View

Original article

# **Lung function improvements following inhaled indacaterol/glycopyrronium/mometasone furoate are independent of dosing time in asthma patients: a randomised trial**

Jutta Beier, Henrik Watz, Zuzana Diamant, Jens M. Hohlfeld, Dave Singh, Pascale Pinot, Ieuan Jones, Hanns-Christian Tillmann

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**Lung function improvements following inhaled indacaterol/glycopyrronium/mometasone furoate are independent of dosing time in asthma patients: a randomized trial**

Authors: Jutta Beier<sup>1</sup>, Henrik Watz<sup>2</sup>, Zuzana Diamant<sup>3,4,5</sup>, Jens M. Hohlfeld<sup>6,7,8</sup>, Dave Singh<sup>9</sup>, Pascale Pinot<sup>10</sup>, Ieuan Jones<sup>11</sup>, Hanns-Christian Tillmann<sup>10</sup>

Author Affiliation:

<sup>1</sup>Insaf Respiratory Research Institute, Biebricher Allee 34, 65187 Wiesbaden, Germany (Tel: +49 611 9854410; Fax: +49 611 9854348; E-mail: [j.beier@insaf-wi.de](mailto:j.beier@insaf-wi.de))

<sup>2</sup>Henrik Watz: Pulmonary Research Institute at LungenClinic Grosshansdorf, Airway Research Center North (ARCN), German Center for Lung Research (DZL), Grosshansdorf, Germany; ([h.watz@pulmoresearch.de](mailto:h.watz@pulmoresearch.de))

<sup>3</sup>Department of Respiratory Medicine and Allergology, Institute for Clinical Science, Skåne University Hospital, Lund, Sweden; ([z.diamant@gems.demon.nl](mailto:z.diamant@gems.demon.nl))

<sup>4</sup>Department of Microbiology Immunology & Transplantation, KU Leuven, Catholic University of Leuven, Leuven, Belgium

<sup>5</sup> Department of Clinical Pharmacy & Pharmacology, University of Groningen, University Medical Centre and QPS-NL, Groningen, Netherlands

<sup>6</sup>German Center for Lung Research (BREATH), Hannover, Germany

<sup>7</sup>Department of Respiratory Medicine, Hannover Medical School, Hannover, Germany

<sup>8</sup>Fraunhofer Institute of Toxicology and Experimental Medicine, Hannover, Germany ([jens.hohlfeld@item.fraunhofer.de](mailto:jens.hohlfeld@item.fraunhofer.de))

<sup>9</sup>University of Manchester, Medicines Evaluation Unit, Manchester University NHS Foundation Trust, Manchester, United Kingdom ([dsingh@meu.org.uk](mailto:dsingh@meu.org.uk))

<sup>10</sup>Novartis Institutes for Biomedical Research, Basel, Switzerland ([hanns-christian.tillmann@novartis.com](mailto:hanns-christian.tillmann@novartis.com) and [pascale.pinot@novartis.com](mailto:pascale.pinot@novartis.com))

<sup>11</sup>Novartis Pharma AG, Basel Switzerland ([ieuan.jones@novartis.com](mailto:ieuan.jones@novartis.com))

**Corresponding author:** Jutta Beier, Insaf Respiratory Research Institute, Biebricher Allee 34, 65187 Wiesbaden, Germany (Tel: +49 611 9854410; Fax: +49 611 9854348; E-mail: [j.beier@insaf-wi.de](mailto:j.beier@insaf-wi.de))

**Summary: (229/256 char)**

This randomized study found single inhaler indacaterol/glycopyrronium/mometasone furoate improved respiratory parameters FEV<sub>1</sub> and PEF in asthma patients and showed similar efficacy when taken once daily in the morning or evening.

**Abstract (248/250 words)**

Once-daily asthma treatment should prevent night-time deterioration, irrespective of the time of dosing. IND/GLY/MF, a fixed-dose combination of inhaled indacaterol acetate (IND, long-acting  $\beta_2$ -agonist), glycopyrronium bromide (GLY, long-acting muscarinic antagonist), and mometasone furoate (MF, inhaled corticosteroid [ICS]) delivered by Breezhaler®, is indicated in adult asthma patients inadequately controlled on LABA/ICS.

A randomized, double-blind, placebo-controlled, three-period, crossover, phase II study was performed to investigate the bronchodilator effect of IND/GLY/MF (150/50/80 $\mu$ g) dosed am and pm versus placebo in patients with mild-moderate asthma. The primary endpoint was weighted mean forced expiratory volume in one second (FEV<sub>1</sub>) over 24h following 14 days of IND/GLY/MF dosed am and pm versus placebo. Secondary endpoints included the effect of dosing time on peak expiratory flow (PEF) and safety/tolerability.

Of 37 randomized patients (age 18–72 years, 21 male, 16 female) 34 completed all three treatment periods. At screening, median (range) pre-bronchodilator FEV<sub>1</sub> was 75.8% (60–96). Patients were using stable low- (83.8%) or medium-dose (16.2%) ICS. Morning and evening dosing of IND/GLY/MF improved FEV<sub>1</sub> (AUC<sub>0–24h</sub>) by 610 mL (90% CI: 538, 681) and 615 mL (90% CI: 544, 687), respectively, versus placebo. Mean PEF over 14 days increased by 70.7 L/min (90% CI: 60.5, 80.9) following am dosing, and by 59.7 L/min (90% CI: 49.5, 69.9) following pm dosing of IND/GLY/MF versus placebo. IND/GLY/MF demonstrated a safety profile comparable with placebo.

Once-daily inhaled IND/GLY/MF was well tolerated and provided sustained lung function improvements over 24 hours, irrespective of am or pm dosing, in patients with mild-moderate asthma.

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## Introduction

Despite the availability of combination inhaled corticosteroid (ICS) and long-acting  $\beta_2$ -agonist (LABA) therapies, many patients worldwide are impacted by uncontrolled asthma with clinically relevant symptoms, bronchoconstriction, and exacerbations. Inadequately controlled asthma is associated with a poorer quality of life, daily activity limitations, higher risk of exacerbations, and a disproportionately high use of healthcare resources [1, 2].

The Global Initiative for Asthma (GINA) suggests the addition of a long-acting muscarinic antagonist (LAMA) (tiotropium) for patients with asthma who remain uncontrolled despite treatment with a combination of an ICS with a LABA [3]. There is mounting evidence that add-on LAMA therapy to LABA/ICS can provide additional benefit in terms of fewer exacerbations and improved lung function compared with patients receiving LABA/ICS therapy alone [4, 5]. Therefore, single-inhaler LABA/LAMA/ICS fixed-dose combinations are being developed for patients with asthma.

Increased circadian variation is a hallmark of uncontrolled asthma, with symptoms, airway hyperresponsiveness and airway obstruction worsening at night [6]. Airway eosinophils were shown to correlate significantly with circadian rhythm in induced sputum from patients with mild/moderate asthma, with a peak influx at 04:00, which also coincided with peak sputum eotaxin concentrations.[7] There is also circadian rhythmic variability in a proportion of exhaled volatile organic compounds over 24h.[8] Drugs with a 24h action should prevent night-time lung function deterioration, irrespective of the time of administration. However, studies have shown that for some inhaled therapies, time of administration can affect drug efficacy over a 24h period [9, 10].

IND/GLY/MF is an inhaled combination which is approved, in the EU and other countries worldwide, for once-daily treatment of asthma, inadequately controlled by LABA/ICS. This therapy combines sustained bronchodilation by indacaterol acetate (IND, a LABA) and glycopyrronium bromide (GLY, a LAMA) with anti-inflammatory properties of mometasone furoate (MF), an ICS. The fixed-dose combination is delivered with the Breezhaler® inhalation device. The Breezhaler® device provides feedback on correct delivery [11] and is currently used to deliver a range of medicines in asthma (e.g. budesonide) and COPD, including IND, GLY, and IND/GLY.

The aim of this phase II study was to investigate the bronchodilator effect of once-daily inhaled IND/GLY/MF (150 µg of indacaterol acetate, 50 µg of glycopyrronium bromide and 80 µg of mometasone furoate [medium dose]) when administered in the morning or in the evening compared with placebo in patients with uncontrolled asthma. The individual components of IND/GLY/MF have demonstrated a sustained 24h duration of action as mono- or combination therapies [12, 13]. Therefore, we hypothesized that IND/GLY/MF would demonstrate sustained lung function benefits irrespective of the time of dosing. In addition, the safety and tolerability of the fixed-dose combination versus placebo was evaluated.

## Methods

### *Participants and study design*

Study participants were men and women with asthma aged  $\geq 18$  years who were receiving a stable daily regimen of low- or medium-dose ICS (as defined by GINA [14]) for  $\geq 4$  weeks prior to screening. Eligible patients had a pre-bronchodilator forced expiratory volume ( $FEV_1$ )  $\geq 60\%$ – $<100\%$  of the predicted normal value and demonstrated an  $FEV_1$  increase of  $\geq 12\%$  and  $\geq 200$  mL after administration of 400  $\mu$ g salbutamol/360  $\mu$ g albuterol (or equivalent dose) at screening. Patients who had an asthma exacerbation requiring systemic corticosteroids, hospitalization, or emergency room visit within 1 year prior to the study were excluded. Current smokers and patients who had smoked or inhaled tobacco products within the 6-month period prior to screening, or who had a smoking history of  $\geq 10$  pack years, were also excluded. Details of inclusion and exclusion criteria are available in the Online Supplement.

The study had a randomized, double-blind, placebo-controlled, six-sequence, three-period crossover design and was conducted in 6 European centers (<https://clinicaltrials.gov/NCT03108027>) between 26 June 2017 and 24 February 2018. Prior to study enrollment, there was a 14-day screening period, followed by a 14-day unblinded run-in period where patients were instructed to discontinue their previous asthma medications and were provided with short-acting  $\beta_2$ -agonist (SABA, [100  $\mu$ g salbutamol/90  $\mu$ g albuterol] or another SABA at matching dose strength) as rescue medication. Eligible patients were subsequently randomized to one of the six treatment sequences all consisting of three treatment periods with a minimum duration of 14 days each (maximum 18 days), separated by a 14–21-day washout period (Figure 1A). The final treatment period was followed by a study completion evaluation visit after 1–7 days. The total duration of the study was 13–19 weeks for each patient. The study was ended after the last patient completed follow-up.

For the overall relatively short study duration in patients with mild to moderate asthma it was considered acceptable to have a placebo period/control without ICS background. The run-in period made sure that subjects were able to tolerate not taking ICS for a short, defined period of time. Patients who could not tolerate ICS withdrawal were not randomized.

At the end of the run-in period, patients were randomized in a ratio of 1:1:1:1:1:1 (Williams design) to one of six treatment sequences (Table 1); each sequence consisted of three double-blind treatment periods of 14–18 days (in different orders) as follows:

- A. IND/GLY/MF evening dose:** placebo (am) and IND/GLY/MF (pm)
- B. IND/GLY/MF morning dose:** IND/GLY/MF (am) and placebo (pm)
- C. Placebo:** placebo (am) and placebo (pm)

The dose strength of IND/GLY/MF administered in this trial was 150 µg of indacaterol acetate, 50 µg of glycopyrronium bromide and 80 µg of mometasone furoate which is the medium dose of MF delivered with the Breezhaler® device (corresponding to MF 400 µg delivered with the Twisthaler® inhalation device). More information on the study design including treatment sequences and methods is available in the Online Supplement.

The patient randomization list was produced by the Interactive Response Technology (IRT) provider using a validated system that automated the random assignment of patient numbers to randomization numbers. All patients gave written informed consent. This study was conducted in accordance with the Declaration of Helsinki and was approved by the Independent Ethics Committees of the participating sites.

### *Study objectives*

The primary objective of this study was to investigate the effect of dosing time (morning or evening) on the bronchodilator effect of once-daily inhaled IND/GLY/MF (150/50/80 µg indacaterol acetate/glycopyrronium bromide/mometasone furoate; medium-dose strength of MF) compared with placebo. This was assessed using weighted mean FEV<sub>1</sub> over 24 hours (AUC<sub>0–24h</sub>) following 14 days of treatment with IND/GLY/MF dosed in the morning, IND/GLY/MF dosed in the evening, and placebo, as the primary endpoint.

Secondary objectives were to evaluate the effect of IND/GLY/MF dosing time on peak expiratory flow (PEF) rate from Day 2 to Day 14 during the three treatment periods and with serial measurement during the 24 h spirometry profiling assessment on Day 14 of each treatment period. In addition, the safety and tolerability of IND/GLY/MF were also assessed.



### *Study treatment and assessments*

On Day 1 of each treatment period, patients attended the site for an outpatient visit in the afternoon (approximately 1 to 2 h before the first scheduled pm dose) and Day 1 pre-dose assessments were performed. Patients were evaluated for randomization eligibility (only at treatment period 1) and trained /re-trained on the use of the Breezhaler® inhalation device. Patients were instructed to take IND/GLY/MF or placebo in the morning (at approximately 07:00 h) and in the evening (at approximately 19:00 h) and to record their pre-treatment morning and evening PEF throughout the study in a patient e-diary.

Spirometry measurements followed the American Thoracic Society/European Respiratory Society guidelines [15]. Patients were provided with a combined electronic PEF-meter and e-diary for recording trough (pre-dose) PEF measurements each morning and evening throughout the entire study. FEV<sub>1</sub> measurements were taken 5 min before the evening dose on Day 14 then, +3h, +6h, +9h, +12h, +15h, +18h, +21h and +23h55min (timing from the Day 14 post-evening dose). Spirometry was performed on Day 14 prior to and 3 hours after study drug administration and on Day 15, 6-12 hours after the last dose of IND/GLY/MF. All patients were provided with rescue medication - SABA (100 µg salbutamol/90 µg albuterol or equivalent dose) at day 1 of the run-in period and were instructed to use it on an 'as needed basis' only. SABA use was recorded twice daily throughout the entire study. Adherence to the intake of study drug and the need of rescue medication at home was monitored closely by reviewing the patient e-diary in which all patients were instructed to record the details pertaining to administration each day in the morning and in the evening. Compliance was also assessed by the investigator and/or study personnel at each visit using capsule counts and information provided by the patient.

### *Statistical analysis*

The safety analysis set included all patients who received at least one dose of IND/GLY/MF and descriptive safety statistics are presented. The pharmacodynamic (PD) analysis set included all patients with any available PD data, who received any dose of study drug and experienced no protocol deviations with relevant impact on PD data. The primary and secondary analyses included all patients in the PD analysis set.

The primary variable was the weighted mean forced expiratory volume in 1 second (FEV<sub>1</sub>) over 24 h (AUC<sub>0-24 h</sub>) following 14 days of treatment with QVM149 dosed in the morning,

QVM149 dosed in the evening and placebo. The weighted mean (least square [LS] mean) was calculated as the  $AUC_{0-24\text{ h}}$  divided by the  $t_f - t_l$  h time interval for each patient ( $t_f$ =time of the first observation,  $t_l$ = time of the last observation). The primary variable was determined for each patient on Day 14 of each treatment using the linear trapezoidal rule. Data for the primary and secondary endpoints were analyzed using a linear mixed model. The model included period, treatment (IND/GLY/MF morning, IND/GLY/MF evening, placebo), and sequence as fixed effect factors. The patient effect was assumed to be random. The Kenward-Roger approximation was used to estimate denominator degrees of freedom. From these analyses, point estimates, and their associated 90% confidence intervals (CI) were constructed for each treatment. The difference between adjusted LS means and the corresponding two-sided 90% CI for am dose versus placebo, pm dose versus placebo, and am versus pm dosing were also evaluated within the linear mixed model. The sample size calculation is described in the Online Supplement. Statistical analyses were performed by Novartis Institutes for Biomedical Research, Switzerland and Novartis Healthcare Pvt. Ltd., India using SAS software version 9.4.

## Results

### *Participant characteristics*

Of 129 patients screened for inclusion, 37 eligible patients were randomized to one of the six treatment sequences. Of the 37 eligible patients randomized in this study, 35 patients completed treatment period 1 (Figure 1B). Two patients receiving placebo discontinued the study (subject/guardian decision) during Treatment Period 1 on Day 1 and Day 14 respectively. All 35 patients who entered Treatment Period 2 completed the treatment period as planned. Of the 35 patients who completed Treatment Period 2, 34 patients entered and completed Treatment Period 3. One patient receiving placebo discontinued the study prior to entering Treatment Period 3 due to an asthma exacerbation (Figure 1B).

Patient demographics and baseline characteristics are summarized in Table 2.

At baseline, all patients were receiving maintenance daily treatment with low- (83.8%) or medium-dose (16.2%) ICS (Table 2). Patients had a mean (SD) pre-dose  $FEV_1$  75.8% (9.04) of predicted normal (Table 2). Mean reversibility to SABA was 18.9% (range 12–52) (Table 2).

### *Efficacy outcomes*

#### Effect of IND/GLY/MF dosing time on FEV<sub>1</sub> (AUC<sub>0-24h</sub>)

Fourteen days of IND/GLY/MF treatment significantly improved weighted mean FEV<sub>1</sub> (AUC<sub>0-24h</sub>) compared with placebo, irrespective of time of dosing. LS weighted mean FEV<sub>1</sub> (AUC<sub>0-24h</sub>) after 14 days of IND/GLY/MF am dosing was 3.43 L (90% CI: 3.172, 3.689), and 3.44 L (90% CI: 3.178, 3.694) after 14 days of IND/GLY/MF pm dosing. Patients receiving placebo for 14 days had a substantially lower weighted mean FEV<sub>1</sub> (AUC<sub>0-24h</sub>) of 2.82 L (90% CI: 2.562, 3.080). This was consistent with a LS means difference of 610 mL (90% CI: 538, 681) with am dosing of IND/GLY/MF and 615 mL (90% CI: 544, 687) with pm dosing of IND/GLY/MF versus placebo (Figure 2).

A negligible difference in weighted mean FEV<sub>1</sub> (AUC<sub>0-24h</sub>) was observed between IND/GLY/MF morning and evening dose (-6 mL [90% CI: -76, 65], <1%).

Post-hoc analyses directly compared 24 h post-dose FEV<sub>1</sub> (trough FEV<sub>1</sub>) between IND/GLY/MF am and pm dosing after 14 days of treatment. No difference was observed in mean 24 h post-dose FEV<sub>1</sub> between IND/GLY/MF morning and evening dosing (8.7 mL [90% CI: -60.4, 77.8]).

#### Effect of dosing time of IND/GLY/MF on PEF

Mean morning PEF (measured pre-dose on the morning of Day 15 of each treatment period) was significantly improved by IND/GLY/MF dosed in the morning and the evening (LS means difference 72.1 L/min [90% CI: 61.3, 82.9] and 86.9 L/min [90% CI: 76.1, 97.8], respectively versus placebo) (Table 3).

Similarly, mean evening PEF (measured on the evening of Day 15 of each treatment period) was significantly improved by IND/GLY/MF morning dose (LS means difference 73.1 L/min [90% CI: 61.9, 84.2]) and evening dose (58.7 L/min [90% CI: 47.5, 69.9]) versus placebo (Table 3).

There were negligible differences in overall PEF values between morning and evening dosing (versus placebo) (Figure 3). With IND/GLY/MF dosed in the morning, the next morning pre-dose PEF was lower compared with dosing in the evening (−14.8 L/min [90% CI: −25.6, −4.1]). Analogously, pre-dose PEF was higher in the evening with morning dosing than with evening dosing (+14.4 L/min [90% CI: 3.3, 25.5]).

### *Safety and tolerability*

Safety and tolerability were assessed in the safety analysis set (n=37). No serious adverse events (SAEs), deaths, or new safety findings for IND/GLY/MF were reported during this study. The incidence of treatment-emergent adverse events (AEs) affecting >5% of patients by treatment group is available in Table 4. The safety and tolerability profiles of IND/GLY/MF were comparable between am and pm dosing and were similar to placebo (Table 4). Two patients discontinued in the placebo period due to subject/guardian decision and one patient discontinued during a washout-period due to an asthma exacerbation of moderate severity.

Two patients experienced an AE graded as severe (one bacterial food poisoning [Day 15 during the IND/GLY/MF pm dosing period] and one case of influenza [Day 9 during IND/GLY/MF pm dosing period]). These were deemed unrelated to the study treatment by the investigator and did not lead to discontinuation of the patients from the study.

Rescue medication use details are available for 35 patients. The number of patients who did not take rescue medication (SABA) in the last 7 days in the respective periods were 24 (71%) with IND/GLY/MF morning dosing, 24 (71%) with IND/GLY/MF evening dosing, and 10 (29%) with placebo. The odds ratio of being rescue medication free with morning dosing versus placebo was 11.5 (95% CI 2.6, 50.3;  $p = 0.0015$ ). Evening dosing results were almost identical.

## Discussion and Conclusions

The results of this study show that once-daily IND/GLY/MF at 150 µg of indacaterol acetate, 50 µg of glycopyrronium bromide and 80 µg of mometasone furoate (medium ICS dose category) delivered with the Breezhaler® device elicits substantial and sustained bronchodilation in patients with asthma receiving ICS at baseline after 14–18 days of treatment, irrespective of the time of dosing (am or pm). In addition, the fixed-combination was overall well-tolerated. These data support the once-daily use of IND/GLY/MF as treatment in patients with asthma.

Evidence generated from randomized controlled trials is supportive of the use of LABA/LAMA/ICS in the treatment of patients with poorly controlled asthma despite the use of ICS and LABA [16]. Tiotropium was tested as an add-on to LABA/ICS treatment in two replicate studies, showing that addition of tiotropium 5 µg (via the Respimat (R) inhalation device) resulted in significant changes in peak FEV<sub>1</sub> from baseline compared with placebo (increases of 86±34 mL and 154±32 mL) in patients with a mean baseline predicted FEV<sub>1</sub> of 62%. [17] In the 52-week TRIMARAN and TRIGGER studies, twice daily inhalations of formoterol fumarate/glycopyrronium/beclometasone dipropionate (FF/G/BDP) from a single device showed significant improvements of 57 mL and 73 mL in pre-dose FEV<sub>1</sub>, respectively, versus FF/BDP.[18] The individual lung function contribution of the LABA indacaterol and the LAMA glycopyrronium were not investigated in this clinical trial. To put results into perspective it is helpful to consider that in large phase 3 trials that investigated IND/MF versus MF alone and IND/GLY/MF versus IND/MF adding IND to MF increased trough FEV<sub>1</sub> by 132 to 211 mL after 26 weeks of treatment [19] and adding GLY to IND/MF by an additional 65 to 76 mL.[20]

Adherence to treatment is a key element of sustained asthma control. While once-daily dosing has been suggested to improve adherence,[21] a patient may prefer a (flexible) time of the day to take his or her medication. Flexibility of dosing irrespective of the time of day may hence further support adherence. Based on the present data, IND/GLY/MF allows this flexibility. In this study, the 2-week run-in period allowed for an assessment of likely study adherence since patients used short-acting rescue medication only, and ensured that those who could not tolerate withdrawal from ICS for a short period of time were not randomized. Since the increases in FEV<sub>1</sub> as the primary outcome with morning and evening dosing of

IND/GLY/MF versus placebo were substantial and comparable, it is reasonable to assume that adherence was high. The formulation of fixed-dose LABA/LAMA/ICS combinations in a single device may have contributed to a good level of adherence.[16]

To benchmark the presented increases in FEV<sub>1</sub> (AUC<sub>0-24h</sub>) of >600 mL with IND/GLY/MF over placebo, previous reports for LABA/ICS fixed-dose combination effects can be considered. For vilanterol/fluticasone furoate administered using a dry powder inhaler, increases of 377 mL and 422 mL in FEV<sub>1</sub> (AUC<sub>0-24h</sub>) following morning and evening dose over 14 days, respectively, versus placebo were observed in a similar population of asthmatic patients aged 18–70 with predicted FEV<sub>1</sub> of ≥60% [22]. Furthermore, when these increases are added to those observed in adult asthmatics with baseline predicted FEV<sub>1</sub> of 60–85%, the LAMA umeclidinium dosed at 5.6, 31.25, 62.5, 125, or 250 µg once daily, or 15.6 or 31.25 µg twice daily, administered for 14 days (range 68–121 mL for increase in FEV<sub>1</sub> (AUC<sub>0-24h</sub>) over placebo) [23], improvements appeared to be less than those achieved with IND/GLY/MF in the present study. Since comparing observations across studies carries limitations (e.g. differences in drugs and doses used, treatment duration, patient populations), the authors caution against the over-interpretation of these cross-study observations. PEF is measured twice daily every day and is therefore a reliable, consistent, and accurate measure of lung function variation with some correlation to symptoms, even for patients who cannot perform full spirometry manoeuvres. The PEF improvements versus placebo observed with IND/GLY/MF in this study are well above the range (15–20 L/min) suggested to be clinically relevant and perceptible by the patient [24, 25]. The consistency in PEF over the treatment period suggests good and stable lung function control with IND/GLY/MF in patients with asthma.

The safety and tolerability profile of IND/GLY/MF was similar to placebo. The two severe adverse events of influenza and food poisoning that occurred during this study were deemed unrelated to the study drug by the investigator, and there were no serious adverse events, deaths or new safety findings reported. One patient discontinued the study during the placebo period due to an asthma exacerbation which was treated with prednisolone.

Potential limitations to this study include the relatively short duration of treatment, participants being limited to those with mild to moderate asthma which differs from the labeled indication of IND/GLY/MF, and the rate of adherence to study medication which was

self-reported via an electronic diary. While 14 days of treatment cannot give a reliable estimate of asthma control sustained benefits were demonstrated over each IND/GLY/MF treatment period as evidenced by substantial, consistent, and constant PEF improvements compared to placebo (Figure 3). Although lack of data on asthma control status can be perceived as a limitation, it should be noted that this is a comparatively small study embedded in the overall development program of IND/GLY/MF, the results of which have been previously published.[19, 20, 26]

Overall, these results demonstrate that IND/GLY/MF is effective irrespective of time of dosing, and therefore, that this fixed-dose combination can be administered effectively and safely either in the morning or the evening.

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## **Data availability statement**

Novartis is committed to sharing with qualified external researchers, access to patient-level data and supporting clinical documents from eligible studies. These requests are reviewed and approved by an independent review panel on the basis of scientific merit. All data provided is anonymized to respect the privacy of patients who have participated in the trial in line with applicable laws and regulations.

This trial data availability is according to the criteria and process described on [www.clinicalstudydatarequest.com](http://www.clinicalstudydatarequest.com).

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## Tables

**Table 1. Definition of treatment sequences**

Treatment sequence	Treatment period 1	Treatment period 2	Treatment period 3
<b>1</b>	A	B	C
<b>2</b>	B	A	C
<b>3</b>	C	B	A
<b>4</b>	C	A	B
<b>5</b>	A	C	B
<b>6</b>	B	C	A

**A. IND/GLY/MF evening dose:** placebo (am) and IND/GLY/MF (pm); **B. IND/GLY/MF morning dose:** IND/GLY/MF (am) and placebo (pm); **C. Placebo:** placebo (am) and placebo (pm)

**Table 2. Baseline participant demographics and characteristics (PD analysis set)**

Parameter	All (n=37)
Median age, years (range)	46.0 (18–72)
Male, n (%)	21 (56.8)
Body mass index, kg/m <sup>2</sup>	26.2 (4.67)
Blood Eosinophils, 10 <sup>9</sup> /L	0.242 (0.1588)
Race, n (%)	
White	35 (94.6)
Other	2 (5.4)
Screening ICS category, n (%)	
Low-dose	31 (83.8)
Medium-dose	6 (16.2)
Pre-bronchodilator FEV <sub>1</sub> , L	2.9 (0.72)
Post-bronchodilator FEV <sub>1</sub> , L	3.4 (0.81)
Mean predicted FEV <sub>1</sub> pre-dose, % (range)	75.8 (60–96)
Reversibility, L	0.5 (0.21)
Mean reversibility, % (range)	18.9 (12–52)
Baseline morning <sup>†</sup> PEF, L/min	422.4 (107.42)
Baseline evening <sup>†</sup> PEF, L/min	454.9 (107.51)

All values shown are mean (SD) unless otherwise stated. FEV<sub>1</sub>: forced expiratory volume in one second; ICS: inhaled corticosteroids; n: number of patients contributing to the analysis; PEF: peak expiratory flow. <sup>†</sup>Morning and evening PEF are defined as the first adequate results prior to dosing for each time point.

**Table 3. Comparison of PEF measured in the morning and evening after 14 days of treatment (PD analysis set)**

LS means (90% CI)		LS means difference (90% CI)
<b>Morning mean PEF (L/min)</b>		
IND/GLY/MF am (n=35) 489.6 (456.2, 523.1)	vs. Placebo (n=36) 417.5 (384.1, 450.9)	72.1 (61.3, 82.9)
IND/GLY/MF pm (n=35) 504.4 (471.0, 537.9)	vs. Placebo (n=36) 417.5 (384.1, 450.9)	86.9 (76.1, 97.8)
IND/GLY/MF am (n=35) 489.6 (456.2, 523.1)	vs. IND/GLY/MF pm (n=35) 504.4 (471.0, 537.9)	-14.8 (-25.6, -4.1)
<b>Evening mean PEF (L/min)</b>		
IND/GLY/MF am (n=35) 522.0 (488.7, 555.4)	vs. Placebo (n=36) 449.0 (415.7, 482.3)	73.1 (61.9, 84.2)
IND/GLY/MF pm (n=35) 507.7 (474.3, 541.0)	vs. Placebo (n=36) 449.0 (415.7, 482.3)	58.7 (47.5, 69.9)
IND/GLY/MF am (n=35) 522.0 (488.7, 555.4)	vs. IND/GLY/MF pm (n=35) 507.7 (474.3, 541.0)	14.4 (3.3, 25.5)

LS: least squares; CI: confidence interval; n: number of patients contributing to the analysis; PEF: peak expiratory flow. Morning PEF assessments were performed 24 h after the last morning dose and 12 h after the last evening dose. Analogously, the evening PEF assessments were performed 24 h after the last evening dose and 12 h after the last morning dose.

**Table 4 Incidence of treatment-emergent AEs by preferred MedDRA term affecting >5% of patients (safety analysis set)**

<b>Preferred term</b>	<b>IND/GLY/MF am (N=35) n (%)</b>	<b>IND/GLY/MF pm (N=35) n (%)</b>	<b>Placebo (N=36) n (%)</b>	<b>Total (N=37) n (%)</b>
<b>Number of patients with <math>\geq 1</math> AE</b>	18 (51.4)	23 (65.7)	18 (50.0)	32 (86.5)
<b>Headache</b>	5 (14.3)	3 (8.6)	7 (19.4)	10 (27.0)
<b>Nasopharyngitis</b>	2 (5.7)	2 (5.7)	5 (13.9)	8 (21.6)
<b>Oropharyngeal pain</b>	3 (8.6)	4 (11.4)	2 (5.6)	7 (18.9)
<b>Cough</b>	1 (2.9)	2 (5.7)	1 (2.8)	4 (10.8)
<b>Dysphonia</b>	2 (5.7)	3 (8.6)	1 (2.8)	4 (10.8)
<b>Asthma</b>	1 (2.9)	1 (2.9)	1 (2.8)	3 (8.1)
<b>Throat clearing</b>	1 (2.9)	1 (2.9)	0 (0.0)	2 (5.4)

AE: adverse event; N: number of patients studied; n: number of patients with at least one adverse event in the category. Values shown are n (%).

## Figures

**Figure 1. Study design and participant inclusion**

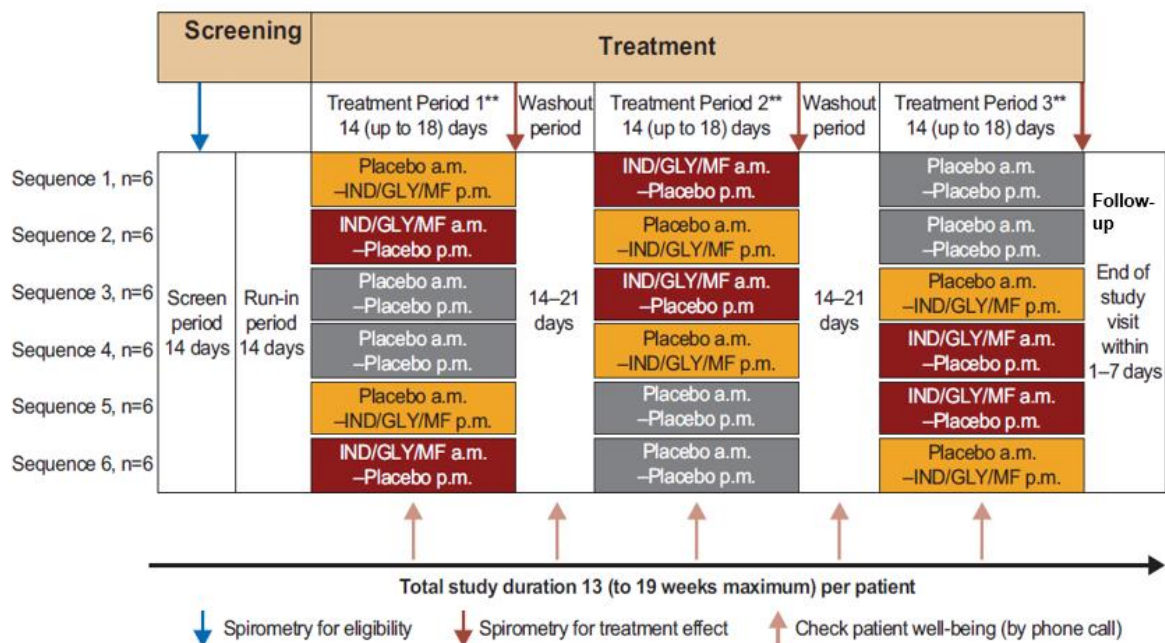
A. Study design. This was a randomized, placebo-controlled, double-blind, six-sequence, three-period, crossover study.

\*\*Primary endpoint – mean FEV<sub>1</sub> (L) (AUC<sub>0–24h</sub>)

B. Study disposition. Of 129 screened for enrolment in this study, 37 patients were randomized to one of 6 treatment sequences (each consisting of 3 treatment periods) in an allocation ratio of 1:1:1:1:1:1. Thirty-four patients completed all three treatment periods.

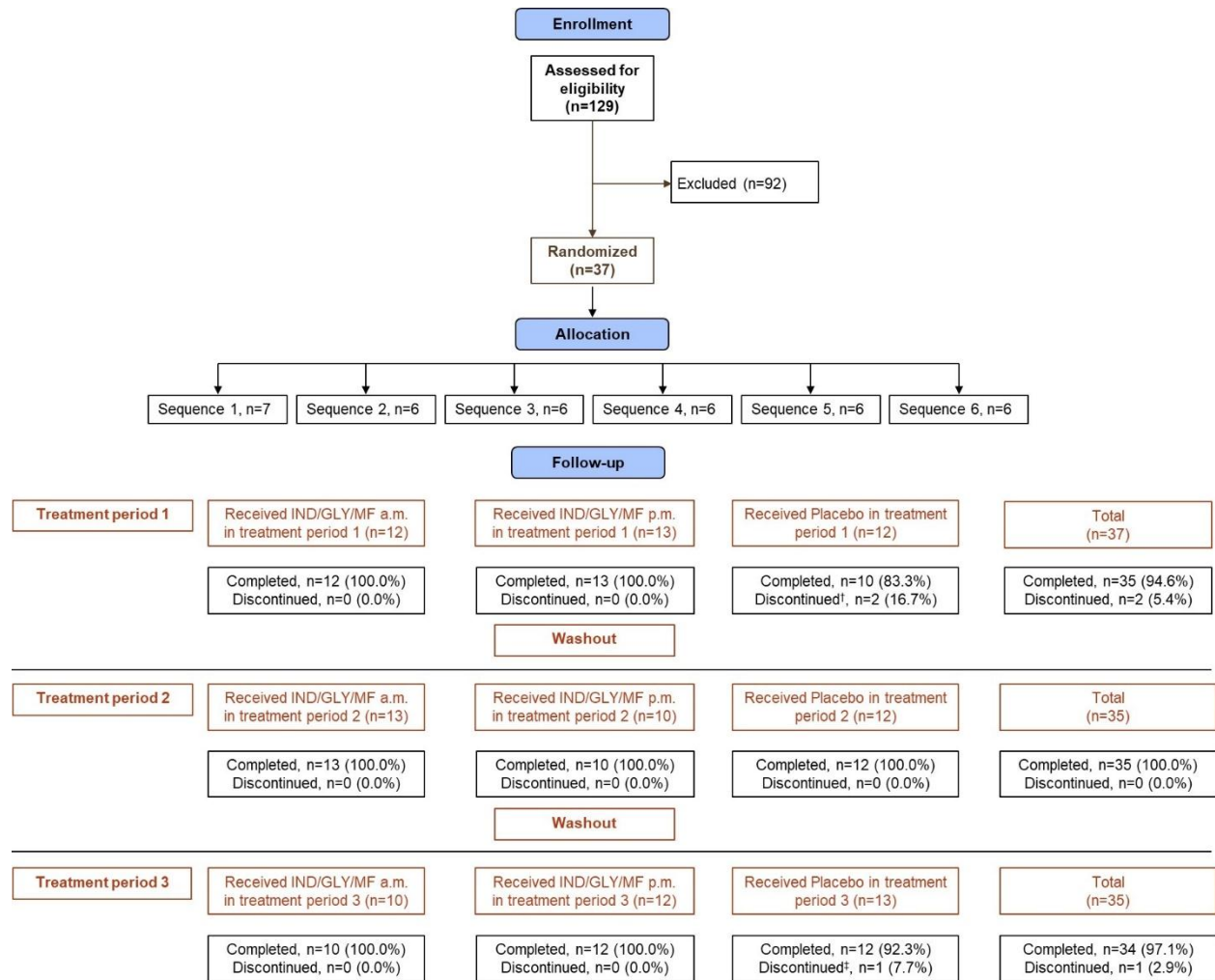
<sup>†</sup>Two patients discontinued from the study during treatment period 1 due to subject/guardian decision. <sup>‡</sup>One patient experienced an asthma exacerbation and discontinued from the study on the first day of treatment period 3.

A.



\*\*Primary endpoint – mean FEV<sub>1</sub> (AUC<sub>0–24h</sub>)

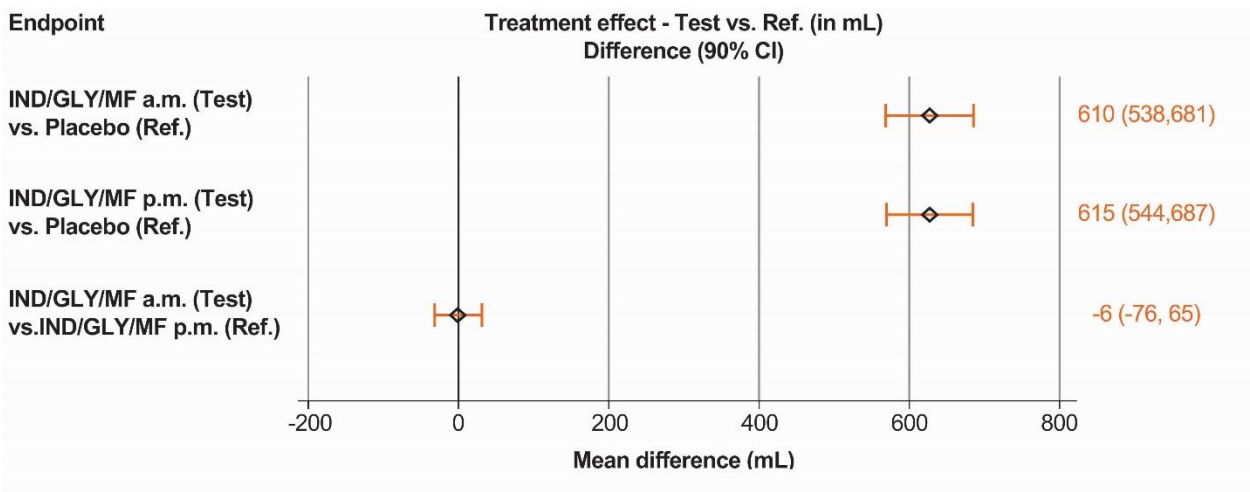
B.



**Figure 2: Effect of morning or evening dosing of IND/GLY/MF on weighted mean FEV<sub>1</sub> (AUC<sub>0-24h</sub>) versus placebo (PD analysis set)**

Comparison of morning or evening dosing of IND/GLY/MF on weighted mean FEV<sub>1</sub> (mL) (AUC<sub>0-24h</sub>) with placebo in the PD analysis set. Parameters were analyzed using a mixed model adjusting for period, treatment, and sequence as fixed effect factors, and patient as a random effect. Data are presented as LS means treatment difference (90% CI) compared with placebo.

CI: confidence interval; FEV<sub>1</sub>: forced expiratory volume in one second; LS: least squares; PD: pharmacodynamics; Ref: Reference



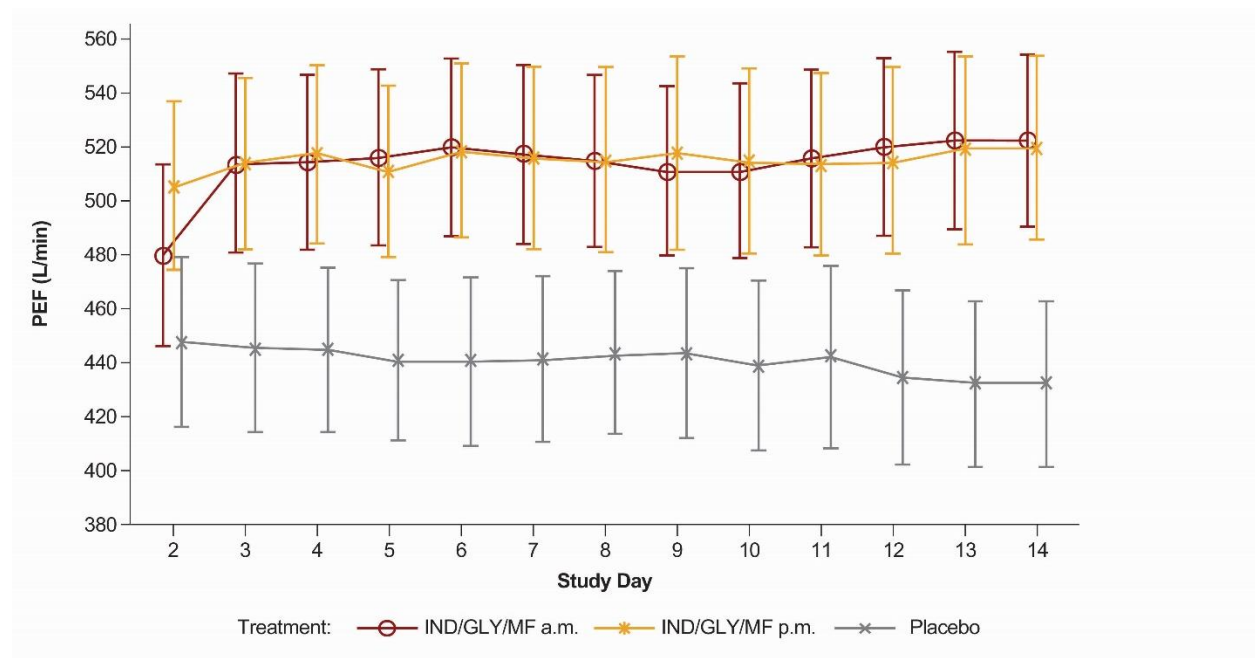


**Figure 3: Effect of IND/GLY/MF on overall mean peak expiratory flow (L/min) (90% CI) over days 2–14 by treatment (PD analysis set)**

Parameters were calculated using a mixed effects model including period, treatment (IND/GLY/MF morning, IND/GLY/MF evening, placebo), and sequence as fixed effects.

Note: if a treatment period for an individual patient exceeded 14 days, the patient's PEF values contributed only up to Day 14 for the respective period.

CI: confidence interval; FEV<sub>1</sub>: forced expiratory volume in one second; LS: least squares; n: number of patients contributing to the analysis; PD: pharmacodynamics; PEF: peak expiratory flow



## **Online Supplement**

### **Lung function improvements following inhaled indacaterol/glycopyrronium/mometasone furoate are independent of dosing time in asthma patients: a randomized trial**

Jutta Beier, Henrik Watz, Zuzana Diamant, Jens M. Hohlfeld, Dave Singh, Pascale Pinot, Ieuan Jones, Hanns-Christian Tillmann

## **Full inclusion and exclusion criteria**

### **Inclusion criteria**

- Patients with a documented physician diagnosis of asthma and who additionally met the following criteria:
  - patients receiving daily treatment with an inhaled corticosteroid at a low or medium daily dose
  - on a stable regimen for at least 4 weeks prior to screening
- Pre-bronchodilator forced expiratory volume in one second (FEV<sub>1</sub>)  $\geq 60\%$  and  $< 100\%$  of the predicted normal value for the patient during screening.
- Patients who demonstrated an increase in FEV<sub>1</sub> of  $\geq 12\%$  and  $\geq 200$  mL after administration of 400 µg salbutamol/360 µg albuterol (or equivalent dose) at screening. All patients were required to perform a reversibility test at screening.
- At screening, and baseline (day 1 pre-dose time) of the first treatment period, vital signs (systolic and diastolic blood pressure and pulse rate) were assessed in the sitting position and again in the standing position. Sitting and standing vital signs had to be within the following ranges:
  - oral body temperature 35.0–37.5 °C
  - systolic blood pressure 90–159 mmHg
  - diastolic blood pressure 50–99 mmHg
  - pulse rate 40–90 beats per minute
- Hypertensive patients must have been on stable antihypertensive therapy for at least 4 weeks prior to screening to be included in the trial.

### **Exclusion criteria**

- Contra-indicated for treatment with, or having a history of reactions/hypersensitivity to any drugs of a similar class.
- Patients who had an asthma attack/exacerbation requiring systemic steroids, hospitalization, or emergency room visit within 1 year of screening.
- Patients who were previously intubated for a severe asthma attack/exacerbation.

- Patients with a history of clinically relevant bronchoconstriction upon repeated forced expiratory manoeuvres.
- History of paradoxical bronchospasm in response to inhaled medicines.
- Patients who during the run-in period prior to randomization required the use of  $\geq 12$  puffs/24 h of rescue medication for 48 h (over two consecutive days) or who had a decline in peak expiratory flow (PEF) from the reference PEF of  $\geq 30\%$  for 6 consecutive scheduled PEF readings.
- Patients who did not maintain regular day/night, waking/sleeping cycles (e.g. night shift workers).
- Current smokers and patients who had smoked or inhaled tobacco products within the 6-month period prior to screening, or who had a smoking history of greater than 10 pack years (Note: one pack is equivalent to 20 cigarettes. 10 pack years = 1 pack/day x 10 years or pack/day x 20 years).

### **Patient numbering, treatment assignment, and randomization**

#### *Patient numbering*

Each patient was uniquely identified by a 7-digit patient number, which was comprised of the site number assigned by Novartis and a sequential number assigned by the investigator at screening. Once assigned to the patient, the patient number was not re-used. This number was the definitive, unique identifier for the patient and was used to identify the patient throughout the study for all data collected, sample labels, etc. Upon signing the informed consent form, the patient was assigned the next sequential number by the investigator. The investigator or his/her staff contacted the Interactive Response Technology (IRT) and provided the requested identifying information of the patient to register them into the IRT.

#### *Treatment assignment, and randomization*

Randomized treatment was assigned to individual patients by way of a randomization number, which was in the range of 5101–5137. After randomizing 35 patients, two patients met eligibility criteria for randomization on the same day. Consequently, after confirming with the clinical trial team that the randomization system is able to accommodate an additional patient than planned, both the patients were randomized. This resulted in randomizing 37 patients instead of the planned 36 patients.

The randomization number was only used to identify which treatment sequence the patients were randomized to receive. The patient number assigned to the patient at screening remained the unique identifier for the patient throughout the study. The investigator entered the screening number that was kept throughout the study in the electronic case report form (eCRF). The randomization numbers were generated using the following procedure to ensure that treatment assignment remained unbiased and concealed from patients and investigator staff: a patient randomization list was produced by the IRT provider using a validated system that automated the random assignment of patient numbers to randomization numbers. These randomization numbers were linked to the different treatment arms, which in turn were linked to the medication numbers. The randomization scheme for patients was reviewed and approved by a member of the Randomization Office.

#### *Treatment blinding*

Patients, investigators and the sponsor remained blinded to study treatment throughout the study. The identity of the treatments was concealed by the use of study drugs that were all identical in packaging, labeling, schedule of administration, appearance, and odor. No emergency unblinding occurred during the study.

### **Study design**

#### *Treatment period Day 1*

On Day 1 of each treatment period, patients attended the site for an outpatient visit in the afternoon (approximately 1 to 2 h before the first scheduled evening dose) and Day 1 pre-dose assessments were performed. Patients were evaluated for randomization eligibility (only at treatment period 1), and trained/retrained on the use of the Breezhaler<sup>®</sup> inhalation device. Only the evening dose was inhaled onsite (at approximately 19:00 h) on Day 1 under the guidance and supervision of site personnel.

#### *Treatment period Day 2 to Day 13*

The first morning dose of each treatment period was administered on Day 2 of each treatment period. Patients self-administered the study drug from Day 2 to Day 13 of each treatment period at home. Patients were instructed to take the study drug in the morning (at approximately 07:00 h) and in the evening (at approximately 19:00 h) and to record their pre-treatment morning and

evening PEF throughout the study in a patient e-diary. Site staff were recommended to call the patient once per week during the treatment and washout periods to verify the patient's well-being and to ensure that none of the study discontinuation criteria were met.

#### *Treatment period Day 14 and Day 15*

Patients visited the site on the morning of Day 14 of each treatment period to inhale the morning dose of study drug or placebo under the guidance and supervision of site staff. Site personnel also reviewed PEF measurements and rescue medication intake as well as study drug compliance by checking the patient's e-diary. Patients were retrained on the use of the Breezhaler<sup>®</sup> inhalation device under the guidance of site personnel. Patients were then admitted in the afternoon of Day 14 of treatment period 1 and treatment period 2 (approximately 4 h prior to Day 14 assessments) to ensure that the patients did not take any rescue medication within 4 h prior to the start of spirometry assessments. The last evening dose of study drug or placebo on Day 14 (at approximately 19:00 h) and morning dose on Day 15 (at approximately 07:00 h) was inhaled under the guidance and supervision of site personnel. Spirometry was performed from Day 14 starting before the evening dose (at approximately -5 min in relation to planned evening dosing time, i.e. at approximately 19:00 h) and then every 3 h after administration of the evening dose until 24 h after the Day 14 evening dose, i.e. until approximately 19:00 h on Day 15. After completion of the spirometry assessments and the last Day 15 visit assessment, the patients were discharged and started the washout period of 14 to 21 days' duration.

#### *Washout period*

The treatment periods were separated by washout periods of 14 to 21 days duration. Washout periods started from the evening of Day 15 of treatment periods 1 and 2. During the washout period, patients were instructed to continue measuring PEF, and to register PEF measurements and rescue medication intake in the patient e-diary. Site personnel continued to call the patient weekly to check the patient's well-being. Procedures in treatment periods 2 and 3 were identical to the procedures of treatment period 1 apart from:

- Eligibility check, which occurred only prior to randomization on Day 1 of treatment period 1.
- At the end of treatment period 3, the patients completed the end of study visit assessments instead of entering into a washout period.

### *Study completion*

At the end of the last treatment period (treatment period 3), patients were discharged from the site after completing study completion evaluations (1–7 days following the last dose). All patients had a safety follow-up call 30 days after their last visit.

### **Concomitant and rescue medication**

At Day 1 of the run-in period, all patients were instructed to discontinue their previous asthma medications and were provided with a short-acting  $\beta_2$ -agonist (100  $\mu$ g salbutamol/90  $\mu$ g albuterol, or equivalent dose) as rescue medication on an “as-needed” basis. Rescue medication was withheld for at least 4 hours prior to spirometry assessments. Prohibited asthma medications during the treatment period included long-acting anticholinergic agents (other than the study drug); short-acting anticholinergics; fixed combinations of long-acting  $\beta_2$ -agonists and inhaled corticosteroids; long-acting  $\beta_2$ -agonists (other than the study drug); short-acting  $\beta_2$ -agonists (other than those prescribed as rescue medication in the study); theophylline and other xanthines; and inhaled (other than the study drug), parenteral, or oral corticosteroids.

### **Statistical analysis**

Sample size calculation: to ensure that at least 30 patients would complete the study, 36 patients were planned to be enrolled assuming a dropout rate of up to 20% and assuming equal assignment to the six sequences. With a sample size of 30 completers, ie - 5 patients per sequence, a two-sided 90% confidence interval for the difference (between IND/GLY/MF morning dosing, IND/GLY/MF evening dosing and placebo in weighted mean FEV<sub>1</sub> over 24 h) after 2 weeks of treatment had an interval that extended no more than 88 mL from the observed difference in means. This calculation assumed a within-patient standard deviation of 200 mL in weighted mean FEV<sub>1</sub> over 24 h (AUC<sub>0–24h</sub>).