

ORIGINAL ARTICLE

Clinical Allergy

A phase 2 study to evaluate the safety, efficacy and pharmacokinetics of DP2 antagonist GB001 and to explore biomarkers of airway inflammation in mild-to-moderate asthma

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Funding information

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Abstract

Background: GB001 is an oral antagonist of the prostaglandin D2 receptor that may inhibit recruitment and activation of airway eosinophils, reducing airway inflammation.

Objective: To assess GB001 safety, efficacy and pharmacokinetics from a Phase 2 study and explore the association between type 2 biomarkers (fractional exhaled nitric oxide and blood eosinophils) and asthma control markers following GB001 administration.

Methods: A randomized, placebo-controlled, double-blind study evaluating 36 patients with mild-to-moderate atopic asthma. Patients receiving fluticasone propionate ≤ 500 mcg/day or equivalent were randomized (2:1) to GB001 (30 mg) or placebo once daily for 28 days. Safety, pharmacokinetics, forced expiratory volume in 1 second, asthma control questionnaire and rescue medication use were assessed. Clinical outcomes were analysed post hoc by baseline fractional exhaled nitric oxide (<35 and ≥ 35 ppb) and blood eosinophil (<250 and ≥ 250 cells/ μ L) subgroups.

Results: GB001 was well tolerated and rapidly absorbed with a 14.5-hour terminal half-life. Overall, GB001 demonstrated greater improvement relative to placebo in forced expiratory volume in 1 second at Day 28 (102 mL [95% CI: -110, 314]). Greater effects on forced expiratory volume in 1 second were observed in the high baseline fractional exhaled nitric oxide and blood eosinophil subgroups (207 mL [95% CI: -283, 698]; 133 mL [95% CI: -422, 687], respectively). These effects were observed as early as Day 2 (229 mL [95% CI: -170, 628]; 163 mL [95% CI: -223, 550] for the high baseline fractional exhaled nitric oxide and blood eosinophil subgroups, respectively) and were sustained through treatment completion.

Conclusion and clinical relevance: GB001 was well tolerated, with the estimated half-life supporting once-daily (QD) dosing. GB001 may have a rapid and sustained

ClinicalTrials.gov Identifier: NCT03683576

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effect on lung function, particularly in patients with type 2 phenotype. Further studies are needed to confirm these findings.

KEYWORDS

atopic asthma, CRTH2, DP2 antagonist, eosinophilic asthma, prostaglandin D2

1 | INTRODUCTION

Asthma is a heterogeneous condition characterized by different phenotypes and endotypes related to specific biomarkers that may predict therapeutic response in selected patient populations.¹⁻³ Blood eosinophils and fractional exhaled nitric oxide (FeNO) measurement can facilitate identification of patients exhibiting type 2-mediated airway inflammation.³⁻⁶ FeNO has been investigated as a surrogate marker of airway inflammation, which is closely associated with eosinophilic inflammation.⁵⁻⁷ FeNO and blood eosinophils could each independently serve as prognostic markers of airway inflammation and severity, and also as predictive biomarkers of treatment effect.^{4,8,9}

DP2 is a G protein-coupled receptor selectively expressed by type 2 T lymphocytes (Th2), type 2 innate lymphoid cells (ILC2), basophils and eosinophils. The main source of PGD2 in the airway tissue is the mast cell. DP2 signalling promotes the recruitment and activation of basophils and eosinophils and stimulates Th2 and ILC2 cells to release type 2 cytokines [IL-4 and IL-13 (primarily produced by basophils), and IL-5 (primarily produced by eosinophils)], leading to the development, amplification and persistence of type 2 inflammation.^{10,11} Hence, it is hypothesized that DP2 antagonists will inhibit recruitment and activation of Th2 and ILC2 cells, basophils and eosinophils, with a consequent reduction in airway inflammation and improvement in lung function.

A study involving healthy controls, mild (steroid naïve), moderate [inhaled corticosteroid (ICS) use] and severe asthma patients identified the highest CRTH2 (DP2) levels in bronchoalveolar lavage (BAL) cells in patients with severe asthma.¹² These results support the association of the PGD2 pathway activation with type 2 inflammatory markers, despite the use of corticosteroids.

GB001 is a potent and highly selective oral DP2 antagonist being developed as a once-daily oral treatment in patients with asthma. In this randomized, placebo-controlled, double-blind, parallel-group study in mild-to-moderate atopic asthma patients on concurrent ICS, the effects of treatment with GB001 or placebo once daily for 28 days were assessed. The primary objective of the study was to evaluate the safety of GB001, while characterization of pharmacokinetics (PK) was a secondary objective and efficacy, including forced expiratory volume in 1 second (FEV₁), asthma control questionnaire (ACQ) and rescue medication use, was an exploratory objective. In addition, a post hoc analysis of biomarkers of type 2 inflammation was performed to identify baseline markers of treatment response and to investigate biomarker changes associated with treatment over time.

2 | MATERIALS AND METHODS

2.1 | Study population

The analysis of this study was comprised of two parts: (a) a prospective plan analysis (primary study) and (b) a post hoc analysis (biomarker characterization). The study was a phase 2 randomized, placebo-controlled, double-blind, parallel-group study assessing, as the primary objective, the safety and tolerability of GB001 (formulation uncorrected for salt form) in patients with mild-to-moderate asthma. The secondary objective was to characterize the PK profile; we also conducted exploratory analyses on markers of asthma control and characterized FeNO, blood eosinophils and eosinophil shape change (ESC) profiles. Patients were required to have a physician's diagnosis of asthma at least 12 months prior to randomization and a positive skin prick test within the last 5 years. Thirty-six male or female patients, 18-55 years of age, with partially controlled asthma receiving a total daily dose of fluticasone propionate ≤ 500 mcg or equivalent were planned to be randomized in a 2:1 ratio to GB001 30 mg (N = 24) or placebo (N = 12) once daily for 28 days. Patients were permitted to use their usual inhaled short-acting β_2 agonist rescue medication on an as-needed basis throughout the study. Patients were partially controlled at screening according to asthma guidelines.¹³ Patients had a pre-bronchodilator FEV₁ $\geq 50\%$ predicted. Current smokers were excluded. Oral or injectable steroids, antihistamine preparations (prescribed or OTC), LTRAs, methylxanthines, chromones and biologics for asthma were not permitted. All patients provided written informed consent. The protocol was approved by local or study research ethics committees and conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki (NCT01448954).

2.2 | Treatment period

Up to 10 days after the screening visit (Visit 2), patients were randomized. Patients were treated for 28 days starting at Visit 3 (Day 1) and ending at Visit 6 (Day 28). Patients were admitted to the study site for Visit 3 (Day 0) the day before Day 1 and remained in the study site until the morning of Day 2 (24 hours after the first dose). On the morning of Day 1, patients were randomized and PK, safety and ESC (at one site only) were evaluated over a 24-hour period. Patients were discharged from the study site on the morning of Day 2 after taking their second dose of investigational product (IP) and continued with daily administration of IP at home. Patients returned to the study site on Days 7, 14 and 27 (Visits 4-6). At Visits 4 and

5, patients attended the study site for safety assessments and to receive further supplies of IP to take home.

2.3 | Lung function assessment

FEV₁, FVC and PEF were assessed by spirometry. Spirometry was performed according to American Thoracic Society (ATS) guidelines on the Standardization of Spirometry¹⁴ and measured using a calibrated spirometer by qualified staff. A minimum of three manoeuvres was performed at each time-point.

2.4 | Asthma control questionnaire

The ACQ-7 consists of seven questions (5 asthma symptoms, daily rescue bronchodilator use and FEV₁% predicted), each of which was scored from 0 to 6 (0 = no impairment, 6 = maximum impairment).¹⁵ The first six questions (asthma symptoms and daily rescue bronchodilator use) were completed by the patient.

2.5 | Rescue medication use

Patients recorded their daily rescue medication use during the study in a diary later transcribed into the database. The rescue medication use at Visits 3 and 6 was recorded at the site.

2.6 | Exhaled nitric oxide

FeNO was collected at Day 1 (baseline) and Day 28, using an ozone/NO₂ chemiluminescence-based analyser, in accordance with ATS/ERS recommendations,¹⁶ at a target constant flow rate of 0.05 L/s. Patients refrained from eating/drinking for 1 hour before measurement.

2.7 | Blood eosinophils

Peripheral blood eosinophil counts were assessed at Day 1 (pre-treatment) and Day 30. Results were from the differential cell counts performed as part of the routine haematology testing at these visits.

2.8 | Eosinophil shape change

Whole blood samples were taken to assess the effect of GB001 on inhibition of PGD₂-mediated ESC response. This assessment was performed only at one centre (Medicines Evaluation Unit) using methods previously reported.¹⁷ Samples were analysed immediately after collection. Citrated whole blood was pre-treated with GB001 prior to the addition of PGD₂ (30 nmol/L) to stimulate ESC. Cells

were fixed and red blood cells lysed. The eosinophil population was identified based on its granularity (side scatter) and autofluorescence using a FACS cell analyser; change in shape was measured as a change in forward scatter.

2.9 | Pharmacokinetics

Blood samples (approximately 5 mL) for the determination of concentrations of GB001 in plasma were collected at Days 1 and 28 to assess PK. Plasma concentration of GB001 was measured with a validated liquid chromatography with tandem mass spectrometry (LC-MS/MS) assay. The lower limit of quantitation (LLOQ) of LC-MS/MS assay was 5 ng/mL. PK parameters were estimated by non-compartmental methods using software Phoenix[®] WinNonlin (version 6.2, Certara[®]). Actual PK sampling times were used to estimate PK parameters.

2.10 | Safety assessments

All adverse events (AEs) were coded according to the MedDRA version 14.1 and classified as either pre-treatment AEs or treatment-emergent AEs (TEAEs), defined as events that started on or after the first dose of IP, or events that started prior to the first dose of IP whose severity subsequently worsened. Electrocardiograms (ECGs) (12-lead) were performed, and standard ECG intervals including RR, PR, QRS and QT were determined. ECG was measured after resting semi-supine for 5 minutes. The measurements were obtained from a minimum of three intervals. Blood and urine samples were taken for determination of biochemistry, haematology and urinalysis.

2.11 | Statistical methods

The sample size was not based on statistical considerations and was considered sufficient to address the primary and secondary objectives of assessing safety and tolerability, characterizing PK and exploring efficacy. The safety population was defined as all subjects who received at least one dose of study drug and who were used for safety analyses and efficacy analyses, with the exception of ESC analyses. The PK population consisted of all subjects who received at least one dose of GB001 and who had sufficient blood samples taken to obtain the maximum plasma concentration at one or more of the PK assessment days. The ESC substudy population (*n* = 15) consisted of all patients who received at least one dose of IP (GB001 or placebo) at the Medicines Evaluation Unit and had evaluable shift in forward scatter (FSC) at Visit 3, Day 1, pre-dose (baseline). To determine evaluable shifts in forward scatter at baseline, the percentage difference between FSC incubated with 30 nmol/L prostaglandin D₂ (+PGD₂) and FSC control was calculated as follows: $100 \times (1 - (\text{FSC control} / \text{FSC} + \text{PGD}_2))$. If the percentage difference at baseline was $\leq 10\%$, the datum was considered non-evaluable (and was therefore excluded from the ESC population).

Descriptive summary statistics were calculated for continuous variables, and the number and percentage of patients in each category were presented for categorical variables. Descriptive summary statistics generally included the number of patients (n), mean, standard deviation (SD), median, minimum and maximum, with geometric mean and percent coefficient variation also included in PK analyses. Post hoc subgroup analyses of baseline characteristics and outcomes were performed by baseline FeNO and blood eosinophil categories of low and high, defined using cut-offs for FeNO of 35 ppb and for blood eosinophils of 250 cells/ μ L. These cut-offs were selected based on published data and are consistent with an eosinophilic phenotype.^{3,7,18} Differences in mean change from baseline and 95% confidence intervals (CIs) were calculated for outcomes of interest using two-sample t tests and assuming equal variances in both treatment groups. Statistical analyses were performed using SAS software version 9.2 or higher (SAS Institute Inc).

3 | RESULTS

3.1 | Demographic and baseline characteristics

The two treatment groups were generally similar with respect to demographic and baseline characteristics, with an overall mean age of 35 years and BMI of 27 kg/m². Most patients were male, and the majority were Caucasian (92% each). Three-quarters of patients had never smoked. There were 8 (33.3%) former smokers in the GB001 treatment group compared with 1 (8.3%) in the placebo treatment group. Overall, pulmonary function was within normal limits (mean FEV₁% predicted 97%) in the majority of the patients (Table 1).

Baseline characteristics evaluated by biomarker subgroups were generally similar in the low and high baseline FeNO (n = 22 [61%] and 14 [39%], respectively) and in the low and high blood eosinophil (n = 25 [69%] and 11 [31%], respectively) subgroups, except for total IgE and rescue medication. A total of 14 (39%) patients had high baseline FeNO (\geq 35 ppb), and 6 (17%) patients had both high baseline FeNO and blood eosinophils (\geq 250 cells/ μ L). There was a weak correlation between baseline FeNO and blood eosinophils ($r = 0.29$). As expected, FeNO and blood eosinophils were greater in the high baseline biomarker subgroups. While lung function was normal in the overall population, FEV₁ was slightly lower in the high baseline biomarker (FeNO and blood eosinophils) subgroups (Table 1). Most of the patients had a baseline ACQ-7 score representative of partly controlled asthma, with a mean (SD) baseline score of 0.84 (0.46) in the overall population.

3.2 | Change in FEV₁

In the overall population, GB001 demonstrated greater improvement relative to placebo in FEV₁ at Day 28 (102 mL [95% CI: -110, 314]) with larger differences from placebo observed for patients in the high FeNO and high blood eosinophil subgroups (207 mL [95% CI: -283, 698] and 133 mL [95% CI: -422, 687], respectively) (Table 2). Consistent with the absolute changes in FEV₁, the change

in FEV₁% predicted demonstrated greater changes in the high FeNO and high blood eosinophil subgroups (6.5% [95% CI: -5.3, 18.2] and 3.7% [95% CI: -9.6, 16.9], respectively). Notably, the changes in FEV₁ were observed as early as Day 2, with a difference from placebo of 229 mL [95% CI: -170, 628] and 163 mL [95% CI: -223, 550] in the high FeNO and high blood eosinophil subgroups, respectively (Table 2). These changes were sustained through completion of treatment on Day 28.

3.3 | Asthma control

GB001 demonstrated greater improvement relative to placebo in ACQ-7 score in the high FeNO subgroup (-0.13 [95% CI: -0.85, 0.59]). There were no meaningful differences between GB001 and placebo in ACQ-7 score in the overall population and the low FeNO and blood eosinophil subgroups (Table 2).

3.4 | Rescue medication

During screening, the mean (SD) daily rescue medication use (number of puffs per day) was 0.76 (0.92) in the overall population. While an increase in use of rescue medications was observed in the low FeNO and low blood eosinophil subgroups, GB001 demonstrated similar reductions during Days 22-28 relative to placebo in the use of rescue medication in the high FeNO and high blood eosinophil subgroups (-0.08 [95% CI: -0.86, 0.69] and -0.07, [95% CI: -1.22, 1.08], respectively) (Table 2).

3.5 | Change in exhaled nitric oxide

The mean (SD) baseline FeNO was 32.53 (20.34) ppb in the overall population. In the overall study population, GB001 demonstrated a modest reduction relative to placebo in FeNO (-4.47 ppb [95% CI: -13.70, 4.77]) with larger reductions demonstrated for patients in the high FeNO subgroup (-13.42 ppb [95% CI: -29.55, 2.72]) compared to the low FeNO subgroup (-1.26 ppb [95% CI: -9.08, 6.56]) (Table 2 and Figure 1). A larger reduction was observed in the low blood eosinophil subgroup compared to the high blood eosinophil subgroup (-7.05 ppb [95% CI: -16.99, 2.89] and 2.79 ppb [95% CI: -20.79, 26.37]), respectively (Table 2). There was a greater proportion of GB001-treated patients with a decrease from baseline in FeNO > 10 ppb at Day 28 (or >20% decrease if baseline FeNO was >50 ppb) compared to placebo (10 [42%] versus 2 [18%]) in the overall population.

3.6 | Change in blood eosinophils

The mean (SD) baseline blood eosinophil count was 235 (196) cells/ μ L in the overall population. There were no reductions in blood eosinophil counts at the end of the study, although a small increase

TABLE 1 Baseline demographic and clinical characteristics by low and high baseline biomarker subgroups and the overall population

| Characteristics | Low FeNO (<35 ppb) (N = 22) | High FeNO (≥35 ppb) (N = 14) | Low Eosinophils (<250 cells/μL) (N = 25) | High Eosinophils (≥250 cells/μL) (N = 11) | Overall Population (N = 36) |
|---------------------------------|-----------------------------------|------------------------------------|--|---|-----------------------------------|
| Age (years) | 33.5 (8.43) | 36.4 (9.65) | 34.0 (8.51) | 35.8 (10.06) | 34.6 (8.90) |
| Male | 20 (91) | 13 (93) | 23 (92) | 10 (91) | 33 (92) |
| BMI (kg/m ²) | 28.02 (4.62) | 26.42 (3.94) | 27.76 (4.29) | 26.58 (4.68) | 27.40 (4.38) |
| Former smoker | 6 (27) | 3 (21) | 4 (16) | 5 (46) | 9 (25) |
| Total IgE (kIU/L) | 355 (341) | 772 (1295) | 343 (388) | 912 (1401) | 517 (857) |
| Blood eosinophils (cells/μL) | 175 (84) | 330 (276) | 137 (54) | 459 (220) | 235 (196) |
| FeNO (ppb) | 18.60 (7.34) | 54.44 (13.48) | 28.74 (16.87) | 41.16 (25.44) | 32.53 (20.34) |
| ACQ-7 score | 0.82 (0.51) | 0.86 (0.41) | 0.84 (0.48) | 0.83 (0.43) | 0.84 (0.46) |
| Rescue medication (puffs) | 1.06 (1.05) | 0.29 (0.32) | 0.89 (1.00) | 0.47 (0.68) | 0.76 (0.92) |
| FEV ₁ % predicted | 100 (19) | 91 (18) | 100 (20) | 90 (15) | 97 (19) |
| FEV ₁ (mL) | 3951 (943) | 3534 (852) | 3904 (889) | 3527 (978) | 3789 (920) |

Note: Values are mean (SD) for continuous parameters and n (%) for categorical parameters.

ACQ-7, Asthma Control Questionnaire, 7-item; BMI, body mass index; FeNO, fractional exhaled nitric oxide; FEV₁, forced expiratory volume in one second; SD, standard deviation.

from baseline to Day 30 was observed in the overall population (55 cells/μL [95% CI: -26, 136]). There were small increases across all biomarker subgroups, with the largest change seen in the high blood eosinophil subgroup (Table 2).

3.7 | Eosinophil shape change

Mean (SD) % inhibition of ESC in placebo (n = 4) was -21.48% (65.52) at 24 hours post-dose on Day 1 and 35.05% (6.77), 16% (60.24) and -13.63% (20.85) on Day 28 pre-dose, 24 and 48 hours post-dose, respectively. In contrast, near-maximal inhibition of ESC was observed following dosing with GB001 (n = 11). The mean (SD) inhibition of ESC was 97.28% (4.40) at 24 hours post-dose on Day 1 and 96.78% (3.46), 97.03% (2.82) and 96.57% (4.44) on Day 28 pre-dose, 24 and 48 hours post-dose, respectively. Inhibition of ESC was >85% for all patients in the GB001 treatment group (Figure 2). No patient in the placebo treatment group had >85% inhibition of ESC at any time-point, except for one patient for whom 100% inhibition of ESC was recorded at Day 28, 24 hours post-dose.

3.8 | Pharmacokinetics

GB001 was rapidly absorbed following oral dosing, with a median T_{max} value of 2.5 hours (range approximately 1-5 hours) at Days 1 and 28. There was minimal accumulation of GB001 following multiple doses for 28 days (AUC accumulation index (R_{ac}) of 1.24). The mean t_{1/2} value of GB001 was 14.5 hours (Table 3), supporting a QD dosing regimen.

3.9 | Safety

There were no serious TEAEs, severe TEAEs or TEAEs leading to study drug discontinuation. The overall incidence of TEAEs was 75% (n = 18) and 83% (n = 10) for GB001 and placebo, respectively. The most common TEAE was headache (47%, n = 17) with no differences between GB001 (46%, n = 11) and placebo (50%, n = 6). Nine patients had a respiratory tract infection, three patients in the GB001 treatment group (12.5%) and six patients in the placebo treatment group (50%). There were no clinically significant findings for laboratory values, vital signs and ECGs.

4 | DISCUSSION

This study demonstrated that GB001 was well tolerated and rapidly absorbed following oral administration, with a median T_{max} of 2.5 hours and a half-life of 14.5 hours, supporting once-daily dosing. Although efficacy was an exploratory objective, numeric differences in FEV₁ with GB001 versus placebo were seen at the first evaluation at Day 2 and maintained throughout the 28-day intervention period. Specifically, in the overall population, GB001 demonstrated greater improvement relative to placebo in FEV₁ (102 mL) at Day 28. Notably, larger increases in FEV₁ were demonstrated for patients in the high FeNO and high blood eosinophil subgroups (207 and 133 mL), respectively.

Over the past decade, several studies have been conducted to evaluate the efficacy and safety of DP2 antagonists in asthma, with mixed results. Busse and colleagues evaluated the effect of the DP2 antagonist (AMG-853) for 12 weeks (n = 396) in patients on background ICS. The investigators reported no changes in FeNO,

TABLE 2 Difference (95% CI) in mean change from baseline for GB001 vs placebo in clinical outcomes and biomarkers by low and high baseline biomarker subgroups and the overall population

| Outcome/Biomarker* | Low FeNO (<35 ppb) (N = 22) | High FeNO (≥35 ppb) (N = 14) | Low Eosinophils (<250 cells/μL) (N = 25) | High Eosinophils (≥250 cells/μL) (N = 11) | Overall Population (N = 36) |
|---|--------------------------------|---------------------------------|--|--|--------------------------------|
| FEV ₁ (mL) (Day 2) | 187 (-42, 416) | 229 (-170, 628) | 190 (-64, 443) | 163 (-223, 550) | 184 (-13, 382) |
| FEV ₁ (mL) (Day 28) | 34 (-184, 253) | 207 (-283, 698) | 104 (-113, 322) | 133 (-422, 687) | 102 (-110, 314) |
| FEV ₁ % predicted | 0.8 (-4.8, 6.4) | 6.5 (-5.3, 18.2) | 3.2 (-2.3, 8.6) | 3.7 (-9.6, 16.9) | 3.0 (-2.2, 8.3) |
| ACQ-7 score | 0.02 (-0.42, 0.46) | -0.13 (-0.85, 0.59) | -0.09 (-0.57, 0.40) | 0.03 (-0.50, 0.57) | -0.05 (-0.40, 0.31) |
| Rescue medication (puffs) during Days 22-28 | 0.34 (-0.33, 1.01) | -0.08 (-0.86, 0.69) | 0.17 (-0.45, 0.79) | -0.07 (-1.22, 1.08) | 0.10 (-0.41, 0.61) |
| FeNO (ppb) | -1.26 (-9.08, 6.56) | -13.42 (-29.55, 2.72) | -7.05 (-16.99, 2.89) | 2.79 (-20.79, 26.37) | -4.47 (-13.70, 4.77) |
| Blood eosinophils (cells/μL) | 24 (-44, 92) | 82 (-116, 280) | 7 (-42, 55) | 169 (-20, 357) | 55 (-26, 136) |

Note: Differences in mean change from baseline and 95% CI are calculated using two-sample t tests and assuming equal variances in both treatment groups.

ACQ-7, Asthma Control Questionnaire, 7-item; CI, confidence interval; FeNO, fractional exhaled nitric oxide; FEV₁, forced expiratory volume in one second.

*All outcomes were assessed at end of treatment on Day 28, except for blood eosinophils, which was assessed on Day 30, unless otherwise noted.

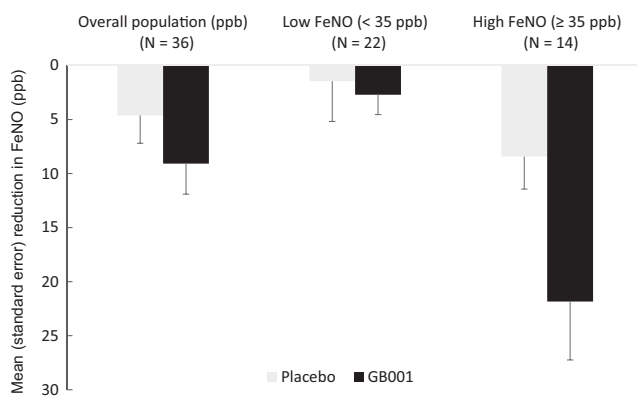


FIGURE 1 Mean reduction in fractional exhaled nitric oxide levels at Day 28 compared to baseline in all subjects (N = 36) and low FeNO (n = 22) and high FeNO (n = 14) subgroups. Bars represent mean ± standard error. Grey bars = placebo; black bars = GB001-treated subjects. Abbreviations: FeNO, fractional exhaled nitric oxide; ppb, parts per billion

IgE, ACQ, AQLQ, FEV₁, symptoms and exacerbations.¹⁹ In a smaller study, Singh and colleagues studied timapiprant for 16 days (n = 16) in an allergy challenge model. Mild atopic asthmatics had a reduction in the late but not early asthmatic response. There was a reduction in sputum eosinophils, but no changes in FeNO or FEV₁ were observed.²⁰ Similarly, Diamant and colleagues studied another DP2 antagonist (setipiprant) for 12 weeks (n = 18) in atopic patients to house dust mite. The investigators reported a reduction in the late but not early asthmatic response following allergen challenge, but no changes in FeNO and blood eosinophils were observed.²¹ Recently, a meta-analysis²² including a total of 14 trials with 4671 participants evaluated the effect of DP2 antagonists in asthma. The results show that DP2 antagonists used as monotherapy improve pre-bronchodilator FEV₁ and FEV₁% predicted. In addition, there

were improvements in other markers of asthma control including reduction in asthma exacerbations, improvement in asthma quality of life (AQLQ) and lower rescue medication use. However, data regarding the use of DP2 antagonists as add-on treatment to an ICS were inconsistent. Some of these inconsistencies might be related to baseline asthma severity and phenotypes that differ across studies, which could influence the results of clinical outcomes. Furthermore, DP2 antagonists have unique pharmacological characteristics related to selectivity, specificity, affinity and potency that may further limit the detection of differences in clinical outcomes across different studies. More data are needed to determine whether some of these in vitro characteristics will be enough to differentiate these compounds in vivo. The current study, although limited by a small sample size, demonstrated changes in FEV₁ in patients receiving add-on treatment with an ICS in both the overall population and the high baseline biomarker subgroups in a post hoc analysis. Only 6 (17%) patients had both high baseline FeNO (≥35 ppb) and blood eosinophils (≥250 cells/μL), limiting our ability to adequately characterize this subgroup.

A clinical phenotyping approach has been advocated to identify patient subgroups with clinical characteristics that are associated with treatment response or prognosis.¹⁻⁴ Endotyping has gained prominence as the identification of a patient subgroup defined by the presence of a biological mechanism.² Recently, biological treatments for asthma have been developed using biomarkers to identify patients with specific mechanisms (ie endotypes).^{9,18,23} The use of both clinical phenotype information and biomarkers to select patients for novel anti-inflammatory treatments aligns with the precision medicine strategy that takes an individualized approach to optimize treatment. Until recently, only the allergen-dependent immune pathway was an important target for asthma treatment. However, it is now clear that both the non-allergen- and

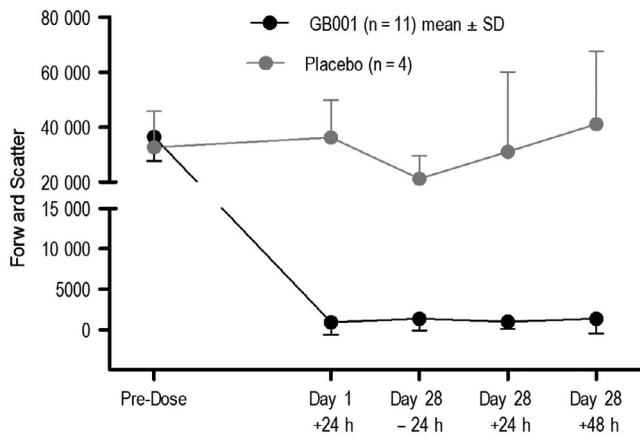


FIGURE 2 Effect of treatment with GB001 (black line) or placebo (grey line) on eosinophil shape change up to 48 hours post-dose on Day 28. Bars represent mean \pm standard deviation. Grey line = placebo; black line = GB001

allergen-dependent immune pathways are involved in the pathophysiological and immunological responses in asthma.²⁴ The type 2 inflammatory endotype is likely to respond to DP2 antagonism based on the activity of the PGD2-DP2 pathway in these patients. However, most clinical trials of DP2 antagonists have selected patients based on disease severity (eg ICS use or low FEV₁)²⁵ rather than phenotype or endotype (eg blood eosinophil counts or FeNO). Type 2 cytokines (IL-5, IL-4 and IL-13) are particularly prominent during exacerbations in these patients, as demonstrated by the marked reduction in exacerbations using targeted monoclonal antibody therapies using different thresholds for blood eosinophil counts.^{9,18} While there are different blood eosinophil thresholds that correlate with response to treatment, in the current study we used a cut-off of 250 cells/uL, which falls within the range to define the eosinophilic phenotype. PGD2 has also been demonstrated to increase during asthma exacerbations, with levels correlating with increases in IL-5 and measures of exacerbation severity.²⁶ Thus, it is biologically plausible that DP2 antagonists could be effective in reducing inflammation and consequently attenuating asthma exacerbations. The current study, while limited by its small sample size and the post hoc nature of the subgroup analyses, has generated hypotheses to be further evaluated prospectively using a biomarker-endotype-driven approach in larger studies.

DP2 is expressed by Th2 cells, ILC2, epithelial cells, basophils and eosinophils.¹⁰ Antagonism of DP2 inhibits activation of

eosinophils as measured by CD11b expression and inhibits ESC induced by PGD2 ex vivo. The analysis of ESC and mediator secretion has been used as a tool to help understand how eosinophils respond to stimuli and chemotactic factors. Eosinophils undergo shape changes, along with secretion of the granule-derived enzyme eosinophil peroxidase (EPX) in response to chemotactic stimuli, including PAF and CCL11 (eotaxin-1).²⁷ It has been proposed that this morphologic change could be associated with activation of the intracellular motile apparatus, as well as exocytosis, such as in the case of PAF-induced ESC.²⁸ In a recent study reported by Hilvering and colleagues, recombinant human IL-5 induced ESC in a dose-dependent manner, which was inhibited by anti-IL-5 neutralizing antibody. This change in eosinophil shape indicates changes in cell activation and highlights the key role of IL-5 and other eosinophil-active factors in the type 2 inflammatory response.²⁹ In the current study, ESC inhibition was consistent with other reports about DP2 antagonists.^{20,30} Receptor expression is an important component to determine activity in this pathway. These data combined with data from Fajit and colleagues¹² provide further evidence of the role of PGD2 in moderate-to-severe asthma.

Two previous studies with fevipiprant,^{31,32} a DP2 antagonist, demonstrated improvements in FEV₁ in patients with different levels of treatment regimen and disease severity. In one of these studies reported by Gonem and colleagues,³² sputum eosinophils were measured in patients with moderate-to-severe asthma. The mean sputum eosinophil percentage was significantly reduced in the fevipiprant group in contrast to the placebo group. Notably, there were no changes reported in FeNO in the two studies. Further analyses are needed to better understand if these observations are reproducible. More recently, Saunders and colleagues³³ showed a reduced airway smooth muscle mass in bronchial biopsies from patients who had participated in the study by Gonem and colleagues.³² We hypothesize that reduction of type 2 biomarkers such as eosinophils and FeNO can lead to decrease in inflammation of the airway smooth muscle.

Matsunaga and colleagues⁸ reported, in a 3-year prospective study, on the use of FeNO as a biomarker to monitor patients at risk of a progressive loss of lung function, an indirect marker of airway remodelling. FeNO > 40 ppb was independently associated with an accelerated decline in FEV₁. These data, combined with the relevant implications on airway smooth muscle inflammation work by

TABLE 3 Pharmacokinetic parameter estimates of GB001 following single and multiple doses

| Day | N | PK Parameter | | | | |
|--------|----|-------------------------|--------------------------|---------------------------------|-------------------------|-----------------|
| | | T _{max} (hour) | C _{max} (ng/ml) | AUC _(0-t) (ng-hr/ml) | t _{1/2} (hour) | R _{ac} |
| Day 1 | 24 | 2.5 (1-5.03) | 606 (27.4%) | 5195 (29.9%) | NA | NA |
| Day 28 | 24 | 2.5 (0.98-4.98) | 740 (34.1%) | 6473 (32.1%) | 14.5 (22.3%) | 1.24 (21.5%) |

Note: Data are presented as geometric mean (CV%), except for T_{max} which is presented as median (range).

AUC_(0-t), area under the concentration-time curve during a dosing interval; C_{max}, maximum plasma concentration; NA, not available; R_{ac}, accumulation index of AUC; t_{1/2}, terminal elimination half-life; T_{max}, time to reach maximum plasma concentration.

Saunders,³³ suggest that FeNO is potentially a valuable prognostic marker for identifying patients who are at risk of progressive loss of lung function. While blood eosinophils have been effectively used as a biomarker of type 2 pathway-driven disease and offer an easy way for evaluating eosinophils in clinical practice, the use of an additional biomarker such as FeNO may aid in selecting patients linked to the type 2 pathway-driven disease and therefore may be particularly well suited to DP2 antagonism.

In conclusion, GB001 was well tolerated and rapidly absorbed following oral dosing with minimal accumulation in patients with mild-to-moderate atopic asthma. The terminal half-life of GB001 supports its once-daily dosing regimen. In addition, greater improvements in FEV₁ relative to placebo at Day 28 were seen in patients with a type 2 biomarker signature of high FeNO or high blood eosinophils, suggesting the importance of endotyping patients to identify those who may be most likely to respond to treatment. There was a marked difference in the magnitude of FeNO reduction with GB001 treatment relative to placebo on Day 28 for patients with high (≥ 35 ppb) versus low (< 35 ppb) baseline FeNO. FeNO, in addition to blood eosinophils, may be a useful predictive and prognostic marker of GB001 treatment response. These data provide a foundation to further explore type 2 pathway blockade, in the context of airway inflammation and to continue to develop GB001 as a treatment for moderate-to-severe type 2 high asthma.

ACKNOWLEDGEMENT

DS is supported by the National Institute for Health Research Manchester Biomedical Research Centre.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author, Hector Ortega. The data are not publicly available due to their containing information that could compromise the privacy of research participant.

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How to cite this article: Ortega H, Fitzgerald M, Raghupathi K, et al. A phase 2 study to evaluate the safety, efficacy and pharmacokinetics of DP2 antagonist GB001 and to explore biomarkers of airway inflammation in mild-to-moderate asthma. *Clin Exp Allergy.* 2020;50:189-197. <https://doi.org/10.1111/cea.13524>