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Mepolizumab for urban children with exacerbation-prone eosinophilic asthma in the USA (MUPPITS-2): a randomised, double-blind, placebo-controlled, parallel-group trial

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Mepolizumab for urban children with exacerbation-prone eosinophilic asthma in the USA (MUPPITS-2): a randomised, double-blind, placebo-controlled, parallel-group trial

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Summary

Background Black and Hispanic children living in urban environments in the USA have an excess burden of morbidity and mortality from asthma. Therapies directed at the eosinophilic phenotype reduce asthma exacerbations in adults, but few data are available in children and diverse populations. Furthermore, the molecular mechanisms that underlie exacerbations either being prevented by, or persisting despite, immune-based therapies are not well understood. We aimed to determine whether mepolizumab, added to guidelines-based care, reduced the number of asthma exacerbations during a 52-week period compared with guidelines-based care alone.

Methods This is a randomised, double-blind, placebo-controlled, parallel-group trial done at nine urban medical centres in the USA. Children and adolescents aged 6–17 years, who lived in socioeconomically disadvantaged neighbourhoods and had exacerbation-prone asthma (defined as ≥two exacerbations in the previous year) and blood eosinophils of at least 150 cells per µL were randomly assigned 1:1 to mepolizumab (6–11 years: 40 mg; 12–17 years: 100 mg) or placebo injections once every 4 weeks, plus guideline-based care, for 52 weeks. Randomisation was done using a validated automated system. Participants, investigators, and the research staff who collected outcome measures remained masked to group assignments. The primary outcome was the number of asthma exacerbations that were treated with systemic corticosteroids during 52 weeks in the intention-to-treat population. The mechanisms of treatment response were assessed by study investigators using nasal transcriptomic modular analysis. Safety was assessed in the intention-to-treat population. This trial is registered with ClinicalTrials.gov, NCT03292588.

Findings Between Nov 1, 2017, and Mar 12, 2020, we recruited 585 children and adolescents. We screened 390 individuals, of whom 335 met the inclusion criteria and were enrolled. 290 met the randomisation criteria, were randomly assigned to mepolizumab (n=146) or placebo (n=144), and were included in the intention-to-treat analysis. 248 completed the study. The mean number of asthma exacerbations within the 52-week study period was 0·96 (95% CI 0·78–1·17) with mepolizumab and 1·30 (1·08–1·57) with placebo (rate ratio 0·73; 0·56–0·96; p=0·027). Treatment-emergent adverse events occurred in 42 (29%) of 146 participants in the mepolizumab group versus 16 (11%) of 144 participants in the placebo group. No deaths were attributed to mepolizumab.

Interpretation Phenotype-directed therapy with mepolizumab in urban children with exacerbation-prone eosinophilic asthma reduced the number of exacerbations.

Funding US National Institute of Allergy and Infectious Diseases and GlaxoSmithKline.

Introduction

Asthma exacerbations are common events, particularly in school-age children living in socioeconomically disadvantaged urban neighbourhoods,[1] and have important short-term and long-term sequelae.[2–4] Although conventional therapies, such as daily inhaled corticosteroids with or without long-acting β agonists, often improve asthma control, a substantial proportion of children and adolescents have asthma that remains inadequately controlled and at serious risk of exacerbations.[5,6] The identification of strategies to prevent asthma exacerbations remains an important unmet need, particularly for underserved urban Black and Hispanic children with exacerbation-prone disease.[7]

The emergence of biologic therapies for asthma provided opportunities for phenotype-directed approaches to asthma management. Elevated concentrations of eosinophils in the blood[8,9] and airway[10] have been identified as biomarkers for increased risk of asthma exacerbation. Targeted therapy with mepolizumab, a humanised monoclonal antibody directed at interleukin (IL)-5, significantly reduces exacerbations in adults with severe,

Research in context

Evidence before this study

Black and Hispanic children living in urban environments in the USA have an excess burden of morbidity and mortality from asthma. Phenotype-directed biologic therapies for eosinophilic asthma have revolutionised treatment for adults with severe disease, but data are scarce in children and diverse populations. Furthermore, precision biomarkers for responsiveness to these therapies are needed.

Added value of this study

Therapy targeting eosinophils in urban children and adolescents living in disadvantaged neighbourhoods significantly reduced the number of asthma exacerbations, albeit to a lesser extent than observed previously in adults, but did not affect other asthma outcomes. Uniquely, we used airway transcriptomic analyses to identify inflammatory pathways underlying differential responses to mepolizumab therapy in these children. We identified multiple inflammatory pathways associated with eosinophils and the epithelium as drivers of differential exacerbation risk in placebo and mepolizumab treated participants.

Implications of all the available evidence

These findings highlight the importance of well powered paediatric clinical trials to evaluate treatment responses for biologics and other interventions that were evaluated initially in adults. Furthermore, it is essential to evaluate treatment responses in Black and Hispanic individuals, who are at the greatest risk of morbidity and mortality from asthma and are often under-represented in clinical trials. Finally, airway transcriptomic profiling provides detailed molecular insights into treatment response and non-response, thereby identifying essential novel biomarkers and disease mechanisms.

exacerbation-prone asthma and blood eosinophils of 150 or more cells per µL.[11,12] However, data in children, adolescents, and racially and ethnically diverse populations are scarce.[7,13,14]

Despite the use of targeted biologic therapies, exacerbations continue to occur, albeit at reduced rates, and the mechanisms underlying these variable treatment responses have not been established. Although mepolizumab markedly depletes blood eosinophils, the relative change in blood eosinophils is not associated with treatment efficacy and more precise biomarkers that predict response have not been determined. Patterns of inflammation in the airway could explain asthma mechanisms with greater detail and serve as increasingly precise biomarkers for treatment responsiveness. Transcriptome profiling allows for an unbiased assessment of these inflammatory patterns. We previously identified[15] upper airway transcriptome modules, which are networks of co-expressed and functionally related genes, associated with asthma exacerbations in urban children, and hypothesised that mepolizumab would reduce exacerbations through suppression of eosinophil-associated airway transcriptome modules. We further hypothesised that airway transcriptome profiling would identify alternative mechanisms associated with reduced responses to therapy.

To address these gaps in asthma care and to gain insight into mepolizumab's clinical and mechanistic effects,[16] we did this Mechanisms Underlying Asthma Exacerbations Prevented and Persistent with Immune Based Therapy: a Systems Approach Phase 2 (MUPPITS-2) randomised controlled trial to assess the efficacy, safety, and mechanisms of phenotype-directed therapy with mepolizumab added to guidelines-based care for urban children with exacerbation-prone eosinophilic asthma.

Methods

Study design

The MUPPITS-2 study is a randomised, double-blind, placebo-controlled, parallel-group trial done at nine urban medical centres in the USA. The study was approved by the Western Institutional Review Board. The protocol is in the appendix (pp 20–97).

Participants

To identify potential participants, research staff used methods approved by the independent review board, including hospital admission records, clinicians' specialty-clinic records, and local advertising campaigns. Research staff screened potential participants using a standardised recruitment questionnaire. Inclusion criteria included children and adolescents (aged 6–17 years) with exacerbation-prone asthma and blood eosinophils of 150 or more cells per µL who lived in prespecified low-income US census tracts, had been diagnosed by a medical doctor as having had asthma for at least 1 year, had two or more exacerbations treated with systemic corticosteroids in the previous year, required a minimum inhaled corticosteroids regimen of twice-daily treatment with fluticasone propionate 250 µg or equivalent (ages 6–11 years) or fluticasone 250 µg plus salmeterol 50 µg or equivalent (ages 12–17 years), had been vaccinated for chickenpox, and had documentation of current medical insurance with prescription coverage at the time of randomisation. Exclusion criteria included pregnancy or lactation, smoking, a life-threatening asthma exacerbation in the past 2 years that required intubation, mechanical ventilation, or resulted in a hypoxic seizure, and specified medical conditions and treatments (appendix pp 43–44). Parents provided written informed consent and children aged 12–17 years provided written assent and children aged 7–11 years provided verbal assent.

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See **Online** for appendix

Randomisation and masking

Randomisation was done using a validated automated system at the US National Institute of Allergy and Infectious Diseases (NIAID), Division of Allergy, Immunology and Transplantation Statistical and Clinical Coordinating Center (Rho, Durham, NC, USA). Participants were randomly assigned to mepolizumab or placebo, by a covariate adaptive randomisation algorithm developed by Pocock and Simon[17] to maintain a balance between treatment arms with respect to study site, number of exacerbations in the previous year, peripheral blood eosinophils, BMI, total serum IgE, and treatment dose. This method also helped to balance the distribution of covariates that are known to be highly associated with our primary endpoint (eg, number of previous exacerbations and peripheral blood eosinophils) and covariates that determine if a participant fits the dosing tables for omalizumab therapy approved by the US Food and Drug Administration (FDA; eg, BMI and total serum IgE; appendix pp 94–95). The criteria for exclusion from randomisation included: participant could not perform acceptable spirometry; participant did not have documentation of medical insurance; and clinically significant laboratory abnormality. Participants, investigators, research staff collecting outcome measures, and data analysts remained masked to group assignments. Unmasked pharmacists prepared the mepolizumab and placebo syringes, which were identical in appearance.

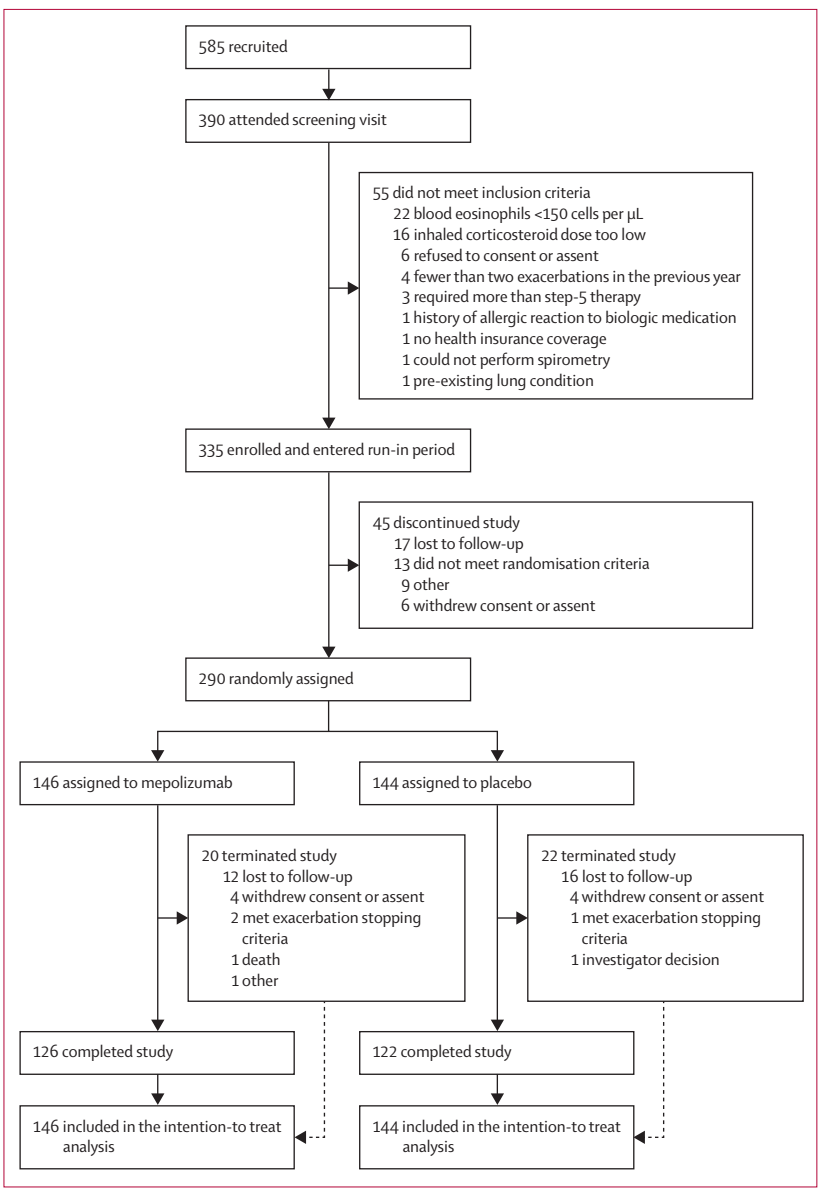

Figure 1: **Study design**

Procedures

After screening, eligible participants were enrolled in a 4-week run-in period and study teams assumed the management of participants' asthma care according to a guideline-based treatment algorithm.[18] After the run-in period, participants that met the randomisation criteria were randomly assigned to adjunctive therapy with either mepolizumab (6–11 years: 40 mg; 12–17 years: 100 mg) or matching placebo, by subcutaneous injection once every 4 weeks for 52 weeks. Study outcomes and adverse events were assessed at each study visit. Spirometry, impulse oscillometry, and the measurement of fractional exhaled nitric oxide (FeNO) were done according to American Thoracic Society and European Respiratory Society guidelines.[19] Peripheral blood eosinophil counts were determined by a central laboratory (Marshfield Hospital, Marshfield, WI, USA), with the study team masked to the results. Total serum IgE and specific IgE to aeroallergens were quantified by a commercial laboratory (Viracor, Lee's Summit, MO, USA). Nasal lavage samples were collected and processed for RNA sequencing using a previously published protocol[15] and analysed by study investigators using a validated module analysis framework (see appendix pp 2–6). The exacerbation criterion for stopping study participation was initially more than six exacerbations, modified to more than three exacerbations on Jan 21, 2021 as an additional safety measure.

Outcome measures

The primary outcome was the number of severe asthma exacerbations treated with systemic corticosteroids during the masked study treatment period. Systemic corticosteroids were started after consultation with a study-site clinician according to previously published criteria (ie, albuterol needed for more than six individual treatments in 24 h; moderate–severe wheeze, cough, shortness of breath, or chest tightness or pain for at least 5 of the preceding 7 days; wheeze, cough, shortness of breath, or chest tightness or pain severe enough to place a participant in their red zone on their asthma action plan that did not substantially improve after three doses of albuterol; unscheduled visit for acute asthma care

requiring repeated doses of albuterol; or hospitalisation was needed for asthma).[20] Secondary outcome measures, not adjusted for multiplicity, included time to first asthma exacerbation, changes in Composite Asthma Severity Index (CASI) score,[21] physician–patient global assessment of response to therapy since starting the study, and lung function (forced expiratory volume [FEV_1] % predicted). Adverse events were identified by research staff via observation, participant interview, or were self-reported by the participant, and were collated by the research staff from the time of participant consent to the end of their participation in the study. Exploratory outcomes included blood eosinophil counts, nasal lavage cell counts, transcriptomics in relationship to the number of exacerbations, and effects of treatment to modulate immune inflammatory pathways.

Statistical analysis

The target sample size of 290 children (reduced from 320 because of lower drop-out than anticipated) provided power of at least 0·9, with a two-sided type 1 error rate of 0·05 if the relative exacerbation rate ratio (RR) of the mepolizumab versus placebo treatment was less than 0·6. This calculation assumed an exacerbation rate of 1·2 events per year for the inferior treatment and allowed for a 15% drop-out. The estimated RRs and corresponding 95% CIs for the primary and secondary analyses of exacerbation rates were calculated using a negative binomial model with an offset term that accounted for per-participant follow-up time in the intention-to-treat population. The model adjusted for randomisation variables: study site, number of exacerbations in the year before the study (2 *vs* ≥3), peripheral blood eosinophils (<400 cells per µL *vs* ≥400 cells per µL), BMI (<95th percentile *vs* ≥95th percentile), total serum IgE (<540 kUA/L *vs* ≥540 kUA/L) and treatment dose (40 mg *vs* 100 mg). An interaction term was included when testing the difference in RRs between participants who fitted the omalizumab dosing table approved by the FDA, and those who did not. To assess whether mepolizumab affected exacerbation RR in relation to the omalizumab dosing table approved by the FDA, we used the recommended statistical test for interaction adjusted for the randomisation variables described earlier.[22] Secondary outcome measures were not adjusted for multiplicity.[23] We generated time to exacerbation survival function estimates using the Kaplan–Meier method, and compared survival curves by treatment group using a Cox regression model adjusted for these covariates. Continuous secondary and exploratory endpoints were estimated using a mixed model for repeated measures (and a generalised additive mixed model for exacerbation seasonality), which included the treatment arm as a fixed effect, and adjusted for randomisation variables, with weeks specified as an unstructured covariance matrix for the repeated effects. With a mixed model, no imputation of missing data was required, as the estimates were unbiased according to the missing-at-random assumption.[24] The interaction terms of weeks and treatment were used to estimate the least squares mean for each treatment group at each week, and the difference in the least squares means was used to

	All (n=290)	Mepolizumab (n=146)	Placebo (n=144)
Demographic characteristics			
Site			
Boston	21 (7%)	11 (8%)	10 (7%)
Chicago	26 (9%)	13 (9%)	13 (9%)
Cincinnati	31 (11%)	17 (12%)	14 (10%)
Dallas	36 (12%)	18 (12%)	18 (13%)
Denver	26 (9%)	13 (9%)	13 (9%)
Detroit	39 (13%)	19 (13%)	20 (14%)
New York	39 (13%)	19 (13%)	20 (14%)
St Louis	29 (10%)	14 (10%)	15 (10%)
Washington, DC	43 (15%)	22 (15%)	21 (15%)
Age, years	10·0 (9·0–13·0)	10·0 (9·0–13·0)	10·5 (9·0–13·0)
Female	126 (43%)	71 (49%)	55 (38%)
Male	164 (57%)	75 (51%)	89 (62%)
Race			
Black or African American	203 (70%)	106 (73%)	97 (67%)
White	31 (11%)	15 (10%)	16 (11%)
More than one race	26 (9%)	12 (8%)	14 (10%)
Unknown or not reported	27 (9%)	12 (8%)	15 (10%)
Other	3 (1%)	1 (1%)	2 (1%)
Ethnicity			
Hispanic or Latino	72 (25%)	35 (24%)	37 (26%)
Not Hispanic or Latino	218 (75%)	111 (76%)	107 (74%)
Total monthly household income			
<US$1250 per month	112 (39%)	54 (37%)	58 (40%)
US$1251–2500 per month	100 (34%)	47 (32%)	53 (37%)
>US$2501 per month	73 (25%)	42 (29%)	31 (22%)
Unknown	5 (2%)	3 (2%)	2 (1%)
Caretaker completed high school education	248 (86%)	123 (85%)	125 (88%)
One or more smokers in the household	82 (28%)	36 (25%)	46 (32%)
Clinical characteristics			
Weight, kg	47 (35–66)	46 (32–67)	48 (36–65)
BMI percentile	88 (63–98)	87 (64–98)	88 (61–98)
BMI greater than 95th percentile	106 (37%)	52 (36%)	54 (38%)
Fractional exhaled nitric oxide, parts per million	34 (18–64)	29 (16–62)	35 (20–66)
FEV_1% predicted	93 (17)	94 (16)	92 (18)
FEV_1:FVC ratio	0·76 (0·09)	0·77 (0·09)	0·74 (0·10)
FEV_1 % reversibility	14 (13)	13 (11)	15 (15)
Any skin test positive			
No	13 (4%)	4 (3%)	9 (6%)
Yes	263 (91%)	135 (92%)	128 (89%)
Missing data	14 (5%)	7 (5%)	7 (5%)
Any allergen with IgE ≥0·35 kU/L	277 (96%)	137 (94%)	140 (97%)
Total number allergens with IgE ≥0·35 kU/L	7 (4–11)	7 (4–11)	7 (4–11)
Eosinophils, cells per µL	430 (300–690)	400 (300–655)	455 (300–700)
Total serum IgE, kUA/L	404 (172–1008)	390 (170–914)	416 (174–1112)

(Table 1 continues on next page)

	All (n=290)	Mepolizumab (n=146)	Placebo (n=144)
(Continued from previous page)			
Asthma characteristics			
Composite Asthma Severity Index score	6·9 (2·7)	6·9 (2·8)	7·0 (2·6)
Treatment step			
Step 3: fluticasone 250 µg twice a day	35 (12%)	18 (12%)	17 (12%)
Step 4: fluticasone 250 µg twice a day plus long-acting β agonists	86 (30%)	38 (26%)	48 (33%)
Step 5: fluticasone 500 µg twice a day plus long-acting β-agonists	169 (58%)	90 (62%)	79 (55%)
Number of exacerbations in previous year			
2	168 (58%)	86 (59%)	82 (57%)
3 or more	122 (42%)	60 (41%)	62 (43%)
Number of oral steroids burst in the previous year	2·8 (1·5)	2·7 (1·4)	2·8 (1·5)
Number of hospitalisations in the previous year			
0	156 (54%)	74 (51%)	82 (57%)
1	80 (28%)	39 (27%)	41 (28%)
2	41 (14%)	26 (18%)	15 (10%)
3 or more	13 (4%)	7 (5%)	6 (4%)

Data are n (%), median (IQR), or mean (SD). FEV_1=forced expiratory volume in 1 second. FVC=forced vital capacity.

Table 1: **Baseline characteristics**

compare mepolizumab and placebo. All analyses presented are for the intention-to-treat population, clinical analyses were done using SAS (version 9.4) and mechanistic analyses using R (version 4.1.0). All figures were constructed using R package ggplot2 (version 3.3.6). Non-normally distributed data were log-transformed before statistical analysis. Description of the methods used in the nasal transcriptome analyses are provided in the appendix (pp 2–6). The protocol was approved by the NIAID Inner-City Asthma Consortium steering committee, protocol review committee, data safety monitoring board, and single institutional review board. The statistical analysis plan is in the appendix (pp 98–138). This trial is registered with ClinicalTrials.gov, NCT03292588.

Role of the funding source

NIAID project scientists participated collaboratively in the study design, data analysis and interpretation, and the writing of the report. Study medication (mepolizumab) was donated by GlaxoSmithKline, which had the opportunity to comment on the study design but had no role in data collection, analysis, or interpretation.

Results

This study was done between Nov 7, 2017, and April 20, 2021. Between Nov 1, 2017, and Mar 12, 2020, 585 children and adolescents were recruited, of whom 390 (67%) attended a screening visit (figure 1). 55 people did not meet the inclusion criteria and 335 did and were enrolled. 45 of these participants discontinued the study before being randomly assigned, including 13 who did not meet the randomisation criteria. 290 participants were randomly assigned to mepolizumab (n=146) or placebo (n=144) groups. 42 participants terminated the trial early after randomisation (mepolizumab n=20; placebo n=22). Baseline characteristics were similar between treatment groups; 43% were female, 70% were Black, 25% were Hispanic, and 37% had a BMI in the 95th or higher percentile (table 1). Asthma and type 2 inflammatory characteristics included mean FEV_1% predicted 93%, FEV_1:FVC ratio 0·76, FEV_1% reversibility 14%, median blood eosinophils 430 cells per µL, FeNO 34 parts per billion, and total serum IgE 404 kUA/L. Baseline asthma therapy was fluticasone 500 µg plus salmeterol 50 µg twice daily or equivalent for 58% of participants, and the mean number of oral corticosteroid courses in the year before study entry was 2·8.

The primary outcome of the number of asthma exacerbations that were treated with systemic corticosteroids within the 52-week study period was 0·96 (95% CI 0·78–1·17) with mepolizumab *vs* 1·30 (1·08–1·57) with placebo (rate ratio 0·73 [0·56–0·96]; p=0·027; figure 2A).

The time to first asthma exacerbation was not significantly different between treatment groups (hazard ratio [HR] 0·86 [95% CI 0·63–1·18]; p=0·36; figure 2B). There were no between-group differences in FEV_1% predicted, FEV_1:FVC, or measures of impulse oscillometry during the study (appendix p 7). The CASI improved from baseline in both the mepolizumab and placebo groups during study follow up; however, there was no significant difference between treatment groups (appendix p 8). Similarly, 109 (84%) of 129 patients in the mepolizumab group and 110 (89%) of 124 in the placebo group self-reported clinically moderate or significant improvements in their asthma via the patient global assessment[12] at the end of study (odds ratio [OR] 0·72 [95% CI 0·42–1·24]; p=0·24; appendix p 13). Study clinicians completed the same assessment and reported clinically moderate or significant improvement in 85 (66%) of 129 participants in the mepolizumab group and 87 (71%) of 122 in the placebo group (OR 1·01 [95% CI 0·62–1·64]; p=0·97; appendix p 13). When the MUPPITS-2 study was designed, omalizumab was the only approved biologic in children, so we included a secondary analysis based upon whether participants met FDA-approved omalizumab dosing criteria; the effect of mepolizumab on exacerbation risk did not differ based on whether participants fitted the omalizumab FDA-approved dosing table or not ($p_{interaction}$ 0·41). At the end of the study, blood eosinophil counts were significantly reduced in the mepolizumab group, with a difference from baseline of –299 (95% CI –363 to –235; p<0·0001), but remained unchanged in the placebo group (appendix p 9). Nasal eosinophil percentage was also significantly decreased in the mepolizumab group (–13·4 [95% CI –20·5 to –6·3]; p=0·0003; appendix p 9),

although to a lesser extent than for blood eosinophils, and unchanged in the placebo group. FeNO was not significantly changed by therapy (appendix p 10).

As observed in previous studies in children,[20,25,26] a post-hoc analysis identified a strong seasonal exacerbation pattern in the placebo group. This seasonal pattern was significantly altered by mepolizumab (p=0·0006; figure 2C), particularly with a blunting of the autumn (fall) exacerbation peak (OR 0·64 [95% CI 0·42–0·98]; p=0·041).

Most (79·1%) of the study follow-up occurred before the COVID-19 pandemic (appendix p 14). The overall exacerbation rate was 58% lower during the COVID-19 pandemic; however, the relative reduction in the exacerbation rate observed with mepolizumab was not different before versus during the pandemic.

Mepolizumab treatment was generally well tolerated; there were modest between-group differences in the adverse events reported by participants, except for higher rates of injection-site reactions associated with mepolizumab than with placebo (table 2). Five episodes of anaphylaxis occurred (mepolizumab n=3, placebo n=2), none of which was related to study therapy. One death occurred in the mepolizumab group during the trial owing to an acute, severe asthma exacerbation leading to cardiopulmonary arrest.

In prespecified exploratory analyses, nasal lavage RNA-sequencing at baseline (week 0) and end of study (week 52) was used to identify molecular patterns associated with exacerbation risk and treatment responses. Whole genome transcriptome data were summarised using a previously defined and validated repertoire of 52 cell-associated nasal gene networks (modules), a subset of which were previously linked to risk for asthma exacerbations in urban children (appendix pp 15–16).[15] Multivariate partial least squares regression identified 12 (23%) of 52 modules for which expression values at baseline were related to exacerbation numbers during the course of the study in the placebo, or mepolizumab, or both, treatment groups (figure 3). Univariate negative binomial modelling of exacerbation rates as a function of baseline module expression yielded similar results (table 3; appendix p 11).

Baseline expression values of three eosinophil-associated modules functionally representative of type 2 inflammation, eicosanoid metabolism, and cytoplasmic proteins were associated with increased exacerbation risk in participants treated with placebo, but not mepolizumab. Rate ratios (RRs), representing the estimated exacerbation RR for a doubling of module expression ranged from 1·25–1·74 in the placebo group (p=0·0012–0·015; table 3). Furthermore, these three modules were significantly downregulated by mepolizumab from week 0 to week 52 (figure 3; appendix p 17; fold changes 0·77–0·90; p=0·00017–0·011), whereas these modules remained unchanged in the placebo group (p>0·05).

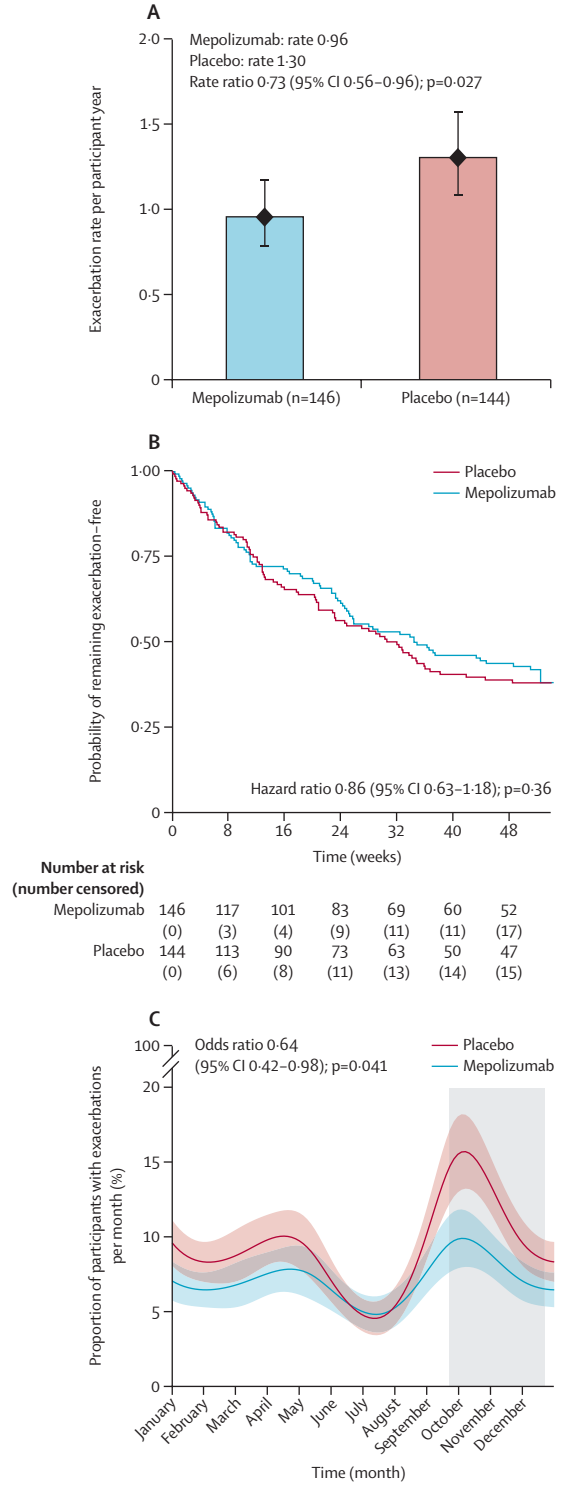

Figure 2: **Effect of mepolizumab on asthma exacerbations**
(A) Asthma exacerbations with error bars showing 95% CIs. (B)T ime to first asthma exacerbation. (C) Post-hoc analysis of the seasonal pattern of asthma exacerbation, with 95% CIs shown by red or blue shading and autumn months shown by grey shading.

By contrast, baseline expression of five epithelial-associated modules was significantly related to exacerbation risk in the mepolizumab group, but not in the placebo group (RR 1·24–1·41; p=0·0035–0·030); these modules represent multiple functional and inflammatory pathways of the epithelium including keratinisation and tight junctions, epidermal growth factor receptor (EGFR) signalling and cell–cell adhesion, IL-33 response and cilia function, extracellular matrix production, and induction of tissue kallikreins and IL-23 and IL-17 pathway signalling. A sixth epithelial module identified by partial least squares regression, labelled transforming growth factor β (TGF-β)/Smad3 associated cell differentiation, showed a similar trend (RR 1·36; p=0·077). Five of these six modules were significantly upregulated by mepolizumab therapy (fold changes 1·25–1·37, p=0·0002–0·0089); all were unchanged by placebo.

Uniquely, one module related to eosinophil activation and mucus hypersecretion (linked to both eosinophils and the epithelium) was positively associated with exacerbation risk in both treatment groups (RR 1·20–1·29; p=0·014–0·039) and was unchanged by therapy. Two modules related to type 1 interferon regulation and neutrophil chemotaxis were inversely associated with exacerbations in both groups (RR 0·70–0·76; p=0·0099–0·062) and were unchanged by therapy.

In addition to providing important mechanistic information, the nasal module expression values more accurately defined exacerbation risk and distinguished treatment effects than previously studied biomarkers including nasal cell percentages, blood eosinophils,

	Mepolizumab (n=146)	Placebo (n=144)
Any treatment-emergent adverse event	42/146 (29%)	16/144 (11%)
Injection site reactions	19/146 (13%)	7/144 (5%)
Skin and subcutaneous tissue disorders	10/146 (7%)	1/144 (<1%)
Gastrointestinal disorders	7/146 (5%)	3/144 (2%)
Changes in laboratory values or vital signs	5/146 (3%)	3/144 (2%)
Nervous system disorders (eg, headache, dizziness, and syncope)	7/146 (5%)	0/144 (0%)

Data are n/N (%). The table shows adverse events with an overall incidence of more than 3%.

Table 2: **Adverse events related to study treatment**

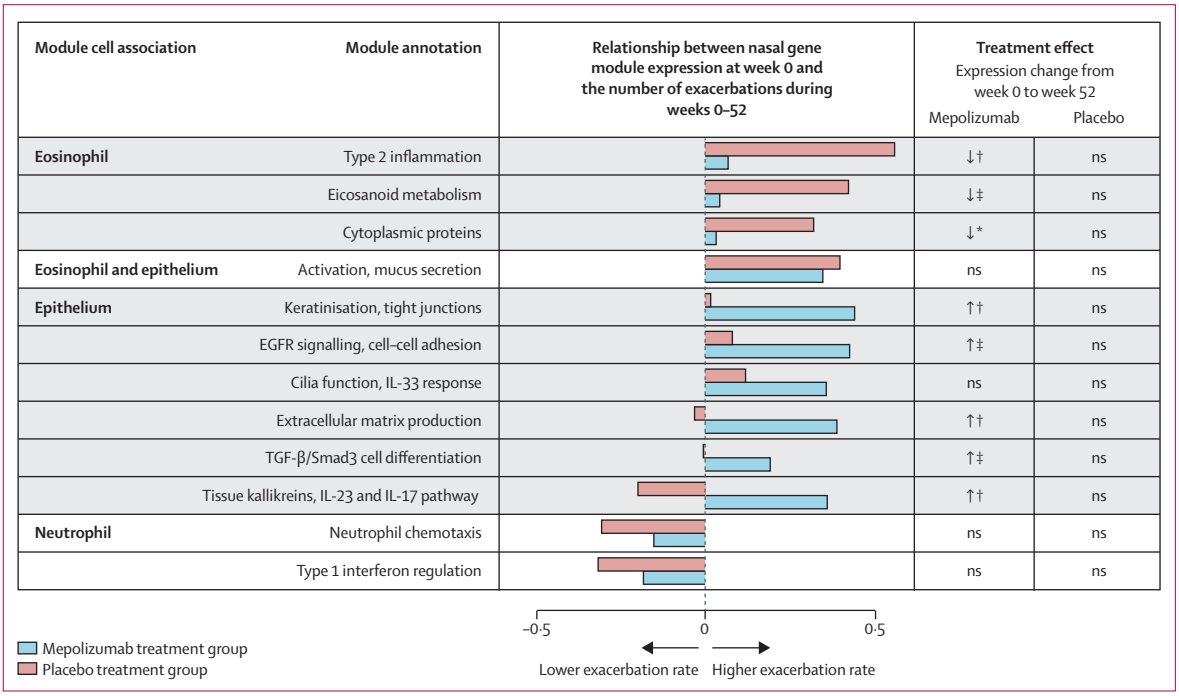

Figure 3: **Nasal gene module expression and exacerbation rates and treatment**

12 gene-expression modules, measured from nasal lavage at the randomisation visit (week 0), showed significant relationships to the primary clinical outcome of the number of exacerbations during the study in either the placebo group, or the mepolizumab group, or both, by multivariate partial least squares regression. The figure shows the weights for each of the 12 modules and for each treatment group, with the weight value shown for the placebo group and the mepolizumab group. A positive weight (with the bar to the right of the centre) indicates a positive association with the number of exacerbations, whereas a negative weight (with the bar to the left of the centre) indicates an inverse association. Weight values can range from 0 to 1 and partly reflect the relative importance of each module in the modelling exacerbation number. Eight of the 12 modules showed differential expression from week 0 to week 52 in the mepolizumab group, whereas no modules were differentially expressed from week 0 to week 52 in the placebo group. Downward arrows indicate a decrease in expression, whereas upward arrows indicate an increase. For further details about each module see the appendix (p- 15–16). EGFR=epidermal growth factor receptor. ns=no significant change in expression. IL=interleukin. TGF-β=transforming growth factor β. *p<0·05. †p<0·01. ‡p<0·001.

pulmonary functions, and FeNO (appendix p 18). Although nasal eosinophils were numerically related to an increased exacerbation risk in the placebo group, and nasal epithelial cells were related to an increased exacerbation risk in the mepolizumab group, neither relationship was statistically significant. Furthermore, the eosinophil-associated eicosanoid metabolism and cytoplasmic protein modules remained significantly associated with exacerbation numbers in the placebo group after adjustment for cell percentage ($p<0·05$); only the type 2 inflammation module did not retain significance independent of cell percentage ($p=0·26$). Similarly, the epithelial-associated keratinisation and tight junctions, EGFR signalling and cell–cell adhesion, IL-33 and cilia production, and extracellular matrix production modules remained significantly associated with the exacerbation number in the mepolizumab group after adjustment for cell percentage ($p<0·05$); the tissue kallikreins IL-23 and IL-17 pathway module did not retain significance adjusted for cell percentages ($p=0·12$). Blood eosinophils (appendix p 12), FEV_1% predicted, and FeNO did not show significant relationships with exacerbations rates, although lower FEV_1:FVC percent predicted related to higher exacerbation rates in both groups (appendix p 18).

	Module annotation	Mepolizumab (n=125)		Placebo (n=124)	
		RR	p value	RR	p value
Eosinophil	Type 2 inflammation	1·10 (0·89–1·35)	0·36	1·25 (1·05–1·5)*	0·015
Eosinophil	Eicosanoid metabolism	1·15 (0·84–1·57)	0·37	1·62 (1·21–2·2)*	0·0012
Eosinophil	Cytoplasmic proteins	1·19 (0·77–1·82)	0·42	1·74 (1·21–2·52)*	0·0027
Eosinophil and epithelium	Activation, mucus secretion	1·29 (1·06–1·58)*	0·014	1·20 (1·00–1·44)*	0·039
Epithelium	Keratinisation, tight junctions	1·32 (1·09–1·61)*	0·0053	1·11 (0·90–1·39)	0·30
Epithelium	EGFR signalling cell–cell adhesion	1·34 (1·10–1·64)*	0·0049	1·10 (0·90–1·36)	0·31
Epithelium	Cilia function, IL-33 response	1·25 (1·04–1·51)*	0·020	1·11 (0·92–1·33)	0·24
Epithelium	Extracellular matrix production	1·41 (1·12–1·77)*	0·0035	1·06 (0·86–1·32)	0·58
Epithelium	TGF-β/Smad3 cell differentiation	1·36 (0·98–1·89)	0·077	1·04 (0·74–1·45)	0·83
Epithelium	Tissue kallikreins, IL-23 and IL-17 pathway	1·24 (1·02–1·51)*	0·030	1·03 (0·81–1·31)	0·82
Neutrophil	Neutrophil chemotaxis	0·72 (0·51–1·02)	0·062	0·70 (0·51–0·94)*	0·011
Neutrophil	Type 1 interferon regulation	0·76 (0·58–1·00)*	0·050	0·74 (0·58–0·95)*	0·0099

Data are RR (95% CI). Results of negative binomial regression of exacerbation number compared with baseline nasal lavage module expression on a log2 scale for each module depicted in figure 2. RRs represent the estimated rate ratio for a one-unit increase in log2 module expression (ie, a doubling of expression). Modules are listed by their cell association and annotation. For further details about each module see the appendix (p 10). RR=rate ratio. EGFR=epidermal growth factor receptor. IL=interleukin. TGF-β=transforming growth factor β. *p≤0·05.

Table 3: **Baseline module expression and exacerbation rates**

Discussion

Phenotype-directed therapy with mepolizumab added to guideline-based care reduced the number of recurrent exacerbations in urban children aged 6–17 years with eosinophilic exacerbation-prone asthma. We did not observe significant between-treatment differences in lung function as assessed by spirometry or impulse oscillometry, CASI scores, or physician–patient global assessments. There were improvements in asthma control both in participants treated with mepolizumab and those treated with placebo, highlighting the effectiveness of interventions associated with clinical trials that promote adherence to guideline-based care in children at high-risk.

Our incorporation of longitudinal nasal transcriptomic analyses to more precisely characterise mepolizumab treatment responsiveness than in the past provided an advancement in the understanding of mepolizumab's effects on asthma exacerbations. These analyses provided crucial insights into the mechanisms underlying the response to mepolizumab therapy and identified those inflammatory pathways that contribute to exacerbations despite reducing eosinophil-related inflammation. As anticipated, higher baseline expression of inflammatory pathways involving the eosinophil, inclusive of canonical type 2 inflammation and eicosanoid metabolism, and eosinophil cytoplasmic proteins, identified risk for more exacerbations in the placebo group compared with the mepolizumab group. However, components of eosinophil activation that were associated with airway mucus hypersecretion persisted, despite mepolizumab therapy, and were associated with a continued exacerbation risk. These observations suggest that despite mepolizumab's overall reduction in eosinophil numbers and type 2 inflammation, refractory mechanisms of eosinophils and epithelium-regulating mucins contribute to exacerbation risk and incomplete responses to mepolizumab. Indeed, our previous work identified this pathway as key to asthma exacerbation progression and lower lung function.[15,27,28] Similarly, mucus overproduction has been established as having a key role in severe asthma in adults[29,30] and is only partially related to airway eosinophil numbers.

A novel observation of our study was that elevated baseline expression of multiple non-type 2 inflammatory pathways from the epithelium identified risk for exacerbations in the mepolizumab group, and expression of many of these pathways increased while on mepolizumab therapy. These epithelial pathways have been identified previously[15,31] as underlying aspects of both viral and pollution triggered exacerbations in urban children and can occur independent of type 2 inflammation. These observations suggest that when type 2 inflammation is dampened with mepolizumab, epithelial inflammatory pathways can promote disease activity and might even show reciprocal elevation. Furthermore, adverse environmental exposures in urban

children driving these epithelial inflammatory pathways might partially account for the small therapeutic response observed in this population.

Additionally, these findings highlight the complexity of treating heterogeneous inflammatory pathways that exist in asthma, even among individuals meeting accepted criteria for an eosinophilic phenotype, and might help explain the persistent risk for exacerbations, albeit at lower rates, observed across studies of biologics that target type 2 inflammation. Perhaps most importantly, our results highlight key molecular pathways that can be considered for future interventional strategies, such as mucus hypersecretion, the kallikrein-kinin system, extracellular matrix overproduction, EGFR signalling, and TGF-β/Smad3 signalling. Our data also highlight the need to tailor therapies towards each individual's airway inflammatory profile, including the potential importance of targeting multiple inflammatory pathways, or their proximal drivers, or both.

Post-hoc analyses identified significant seasonal variation in exacerbations with the most prominent mepolizumab treatment effect in the fall. The overall effects of mepolizumab therapy on exacerbations were more modest than expected despite similar substantial reductions in blood eosinophils to those in previous mepolizumab trials in adults and children,[11,32] and also significant reductions in nasal airway eosinophils (although to a lesser extent than in blood). In contrast to our study, previous trials done in adults had low numbers of Black and Hispanic participants. Importantly, the social and environmental determinants of health facing disadvantaged urban Black and Hispanic children are unique relative to populations studied in previous mepolizumab trials. Furthermore, the pathways driving type 2-high, eosinophilic asthma might differ between children and adults. For example, adult participants with childhood-onset versus adult-onset eosinophilic asthma have been less responsive to benralizumab, another biologic therapy targeting IL-5.[16,33] Our findings also raise the possibility that exacerbations in adults are more commonly driven by eosinophils, whereas in children, other inflammatory processes are instrumental.

The strengths of our study include a diverse range of participants who were at greatest risk of asthma morbidity, predominantly Black and Hispanic children living in disadvantaged urban communities, an understudied population. Another study strength was the high retention and adherence to injections despite a portion of the trial occurring during the COVID-19 pandemic. Furthermore, integration of airway transcriptomic network analyses into this multisite paediatric clinical trial enabled the identification of novel mechanisms of response and non-response to mepolizumab therapy.

A study limitation is the use of nasal airway samples as a proxy for lower airway disease; however, this issue is mitigated by the direct relevance of these pathways to asthma outcomes.[15,34,35] The COVID-19 pandemic affected the frequency of asthma exacerbations; however, this did not significantly affect the observed treatment effects of mepolizumab.

In conclusion, in urban children and adolescents with exacerbation-prone eosinophilic asthma, adjunctive therapy with mepolizumab reduced asthma exacerbations, but did not affect other asthma outcomes. Airway transcriptomic analyses identified primarily eosinophil-associated and epithelial-associated molecular pathways underlying differential clinical responses to mepolizumab and identified potential future targets to precisely and effectively diminish disease burden from exacerbations in these youth at high risk. Our findings also highlight the importance of evaluating treatment responses for biologics, and other interventions, in urban Black and Hispanic children, populations often underrepresented in clinical trials and at greatest risk for morbidity and mortality from asthma.

Contributors

DJJ, PJG, CAS, WWB, and MCA participated in the study design. DJJ, PJG, SW, LG, and PMB did the project administration. LBB, LG, MAG, JS, AHL, RSG, RTC, MM, GKKH, GTO, JAP, MGS, KR-S, EMZ, SJT, MK, CMD, HK, CL, WJS, and JEG did the data collection. DJJ, RMS, KAD-M, AC, CMV, and MCA oversaw or did the data analysis. AC and MCA directly accessed and verified the underlying data. DJJ and MCA wrote the first draft of the manuscript. LBB, PJG, AC, JS, CMV, PMB, CAS, and WWB provided initial manuscript review and revisions. All authors critically reviewed the manuscript and approved submission. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

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Data sharing

All study design information and data used in the analyses can be accessed through ImmPort (https://www.immport.org/) at accession number SDY1903. The raw RNA-sequencing FASTQ format data and minimum information about a high-throughput nucleotide sequencing experiment have been deposited with the US National Center for Biotechnology Information Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/) at accession number GSE192861.

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