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A prospective observational study of the inflammatory profile of Mepolizumab EXacerbations in participants with severe refractory eosinophilic asthma (the MEX study).

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Abstract

Background: Clinical trials with anti-IL5 therapies show a 50% reduction in severe asthma exacerbations. Exacerbations on mepolizumab appear different from placebo, questioning their inflammatory phenotype and physiological characteristics.

Methods: This prospective observational study of 145 mepolizumab-treated participants was undertaken at four UK Severe Asthma Specialist clinics. Participants were aged between 18-80 years old, with eosinophilic asthma (GINA steps 4 and 5) and suitable for access to mepolizumab therapy as per national UK guidelines. All participants received mepolizumab 100mg sc every four weeks, had a scheduled study visit when stable on mepolizumab (≥ 3 months on treatment) and undertook daily peak flow and symptoms diaries throughout the course of the study. Participants attended their study centre for unscheduled exacerbation assessment when their symptoms worsened outside of their normal daily variation and prior to commencing treatment; exacerbation assessment included detailed medical history, examination, FeNO, spirometry, peripheral blood eosinophil count, CRP, and sputum induction, as well as blood and urine samples for biobanking. Where a participant was unable to attend their study centre for exacerbation or had initiated rescue treatment prior to study visit, clinical details of the missed exacerbation were collected by clinical staff. This study was exploratory with the end-point being 100 exacerbations assessed prior to treatment, and all measurements pre-determined to precisely define exacerbation phenotype as per the study aims. Study analysis included description of the cohort and comparisons of subgroups within the cohort, including comparison of the characteristics of those who proceeded to exacerbate on mepolizumab and those who did not, comparison of peak flow and symptoms diaries for assessed and missed exacerbations and comparisons of exacerbation phenotypes defined by sputum cell count. Negative and positive predictive values were used to assess the utility of both FeNO and CRP in determining exacerbation phenotype on mepolizumab treatment. (Clinicaltrials.gov NCT03324230).

Findings: Between 30th November 2017 and 29th May 2019, a total of 172 exacerbations occurred, with 96 assessed prior to commencing treatment. Daily peak flow and symptoms diaries showed no difference in assessed and non-assessed exacerbations. At first assessed exacerbation per participant, 45/69 (65%) produced sputum of whom 47% were sputum eosinophil high $\geq 2\%$ (SE^{HIGH}) and 53% were sputum eosinophil low $< 2\%$ (SE^{LOW}).

SE^{HIGH} exacerbations were FeNO high (57ppb vs 24ppb, median difference 33; 95% CI 8,87; $p < 0.001$), with low FEV₁% predicted (55.6% vs 71.5%, mean difference -15.9; 95% CI -27.0,-4.8; $p = 0.0075$), obstructive spirometry (FEV₁/FVC 57.5% vs 67.8%, mean difference -10.3; 95% CI -17.0,-3.6; $p = 0.0043$)

and higher blood eosinophils (70 vs 30cells/ μ L, median difference 40; 95% CI 20,70; $p<0.001$). In contrast, SE^{LOW} exacerbations were FeNO low, CRP high (15mg/L vs 2.3mg/L, median difference 12.7; 95% CI 3.5,18.5; $p<0.001$), sputum neutrophil high (89.8% vs 37.1%, median difference 52.7; 95% CI 34.5,59.2; $p<0.001$) and 50% received antibiotics (v 19% in SE $\geq$ 2%, $p=0.031$). FeNO ($\leq$ 20 or $\geq$ 50ppb) was the most useful discriminator of inflammatory phenotype at exacerbation. The most common adverse event was hospital admission due to asthma exacerbation (17 of 34 events), none of the adverse events were study procedure related.

Interpretation: Exacerbations on mepolizumab are two distinct entities which can largely be differentiated using FeNO; non-eosinophilic events are driven by infection with a low FeNO and high CRP, while eosinophilic exacerbations are FeNO high. The results of the MEX study challenge the routine use of OCS for the treatment of all asthma exacerbation events on mepolizumab, as well as the switching of biological therapies for treatment failure without profiling the inflammatory phenotype of ongoing asthma exacerbations. The results highlight clinically available tools to enable profiling of these residual exacerbations in mepolizumab treated patients.

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Research in context

Evidence before this study:

The evidence available at the time of study conception was that through selective inhibition of eosinophilic inflammation, mepolizumab reduces exacerbations in those with severe, eosinophilic asthma, and decreases the need for maintenance systemic glucocorticoid therapy. However, the evidence also clearly showed a residual asthma exacerbation rate of approximately 50% in mepolizumab treated patients, for which oral corticosteroids were given.

A PubMed search of evidence available ('asthma', 'mepolizumab', 'exacerbation phenotype', end date 01/01/2017) gave 13 results, 12 of which were review articles outlining potential uses of mepolizumab and one cluster analysis of characteristics predicting response to mepolizumab, with no articles addressing the inflammatory phenotype of residual asthma exacerbations on mepolizumab. Post hoc analysis of mepolizumab phase III clinical trials by our study investigators showed physiological differences between mepolizumab and placebo treated patients, and suggested a differential response to treatment with oral corticosteroids, thus highlighting the need for a prospective study to understand the inflammatory phenotype of these residual asthma exacerbations, an as yet unmet research need.

Added value of this study:

Our study reveals that the exacerbations occurring in mepolizumab-treated patients are heterogeneous in nature with approximately half of the exacerbations due to ongoing T2, eosinophilic inflammation and with the other half being sputum eosinophil-low, sputum neutrophil-high, infection-associated exacerbations with a significantly higher CRP. These events were essentially mutually exclusive and fractional inhaled nitric oxide (FeNO) was a useful discriminator of exacerbation inflammatory phenotype when high (≥ 50 ppb) or low (≤ 20 ppb).

Implications of all available evidence:

Understanding the mechanisms behind residual exacerbations at the individual patient level in those treated with mepolizumab challenges the current clinical approach to management of exacerbations and longer-term treatment of those who continue to have recurrent exacerbations. Our study would suggest that treatment of eosinopenic, neutrophilic exacerbations with oral corticosteroids would be ineffective due to the absence of eosinophilic inflammation and that it could be potentially harmful to switch a patient with recurrent eosinopenic, neutrophilic exacerbations to a more eosinophil-depleting biologic. Conversely, those with breakthrough eosinophilic exacerbations on mepolizumab are likely to respond to treatment with oral corticosteroids at exacerbation and to a more eosinophil-depleting biologic. However, these findings need to be substantiated with further prospective studies

to confirm that differentiating inflammatory phenotype at assessment of exacerbation will help guide better management. Further studies are also required to determine if the absence of airways eosinophils predisposes to airway infection.

Introduction

The treatment of severe eosinophilic asthma has been transformed with the arrival of biological agents targeting the Type 2 cytokine pathway.¹ One of these is mepolizumab, a humanised monoclonal antibody against the eosinophil activating cytokine interleukin 5 (IL5), which has consistently shown a 50% reduction in exacerbation rate when compared to placebo in patients with severe eosinophilic asthma.²⁻⁴

There has been significant interest in the inflammatory phenotype and mechanism of the residual exacerbations in these patients. To date, analyses have compared exacerbations on mepolizumab to those on placebo in randomised controlled trials. Shrimanker *et al* showed that at the time of exacerbation, mepolizumab-treated participants had a lower sputum eosinophil count with fewer symptoms,⁵ and a subsequent post-hoc analysis of three phase III clinical trials^{2,4,6} suggested less impairment of peak expiratory flow (PEF) and fewer symptoms compared to placebo treated participants.⁷ These analyses suggested that exacerbations were less severe and potentially due to different mechanisms in mepolizumab-treated participants. Further study of inflammatory profile and mechanisms of exacerbation in mepolizumab-treated participants is required.

The aim of this study was to explore the inflammatory phenotype and physiological characteristics of exacerbation events from a cohort of severe, eosinophilic asthmatics treated with mepolizumab.

Methods

Participants were aged 18-80 years old with a diagnosis of severe asthma (GINA steps 4 and 5)⁸ and eligible for treatment with mepolizumab in line with UK Clinical Guidelines with an oral corticosteroid (OCS) requirement of either maintenance OCS or 4 exacerbations in the preceding year.^{9,10} Full inclusion and exclusion criteria are in the Online Supplement (online supplement page 1); subjects had to have no asthma exacerbations requiring oral corticosteroids (OCS) in the 4 weeks prior to the first study visit. The Office for Research Ethics Northern Ireland granted ethical approval and local National Health Service Research and Development approval was obtained for individual sites. All participants provided written informed consent prior to study entry. The study was registered on clinicaltrials.gov (NCT03324230) and the study protocol can be found at https://rasp.org.uk/MEX/Exacerbation_mepolizumab_ptcl_V1.0_18AUG17.pdf

Study design

MEX was a multicentre, prospective observational study performed in four UK specialist severe asthma centres and all outcome measures were exploratory. Mepolizumab 100mg was administered by subcutaneous injection every 4 weeks. All patients had a study visit after at least 3 months mepolizumab to define clinical and inflammatory status when clinically stable on treatment. Demographic and clinical details were obtained for all subjects prior to initiation of mepolizumab and in those patients who were initiating mepolizumab at study entry; an additional study visit was performed prior to initiation of drug, Figure E1 (online supplement, page 2). An exacerbation was defined as severe asthma symptoms worsening outside of a patient's normal daily variation and occurring any time after the initial dose of mepolizumab, at which point participants attended their clinical site for assessment prior to commencing rescue treatment. All study visits included detailed medical history, examination, fractional exhaled nitric oxide (FeNO), spirometry, peripheral blood eosinophil count (PBE), C-reactive protein (CRP), and sputum induction with hypertonic saline (up to 5%). Participants completed asthma control and quality of life questionnaires (asthma control questionnaire 5 (ACQ-5), mini-asthma quality of life questionnaire (mini-AQLQ), and St George's respiratory questionnaire (SGRQ), (details in online supplement page 3).¹¹⁻¹³ All participants were provided with an electronic PEF recorder and daily symptoms diary for the duration of the study.

Clinical assessment and management of each exacerbation was in line with standard clinical guidelines¹⁴ and was blind to the assessment of sputum inflammatory characteristics. All unscheduled visits were followed up with a telephone call 2-3 days post-visit. For exacerbation events where the patient was unable to attend for clinical assessment or had initiated rescue treatment prior to study visit (missed exacerbations), patients were advised to manage these according to their self-management plan and clinical staff collected clinical details of the exacerbation. We assumed 0.7 exacerbations per patient per year on mepolizumab (ca 160 events in an 18-month period) and a minimum rate of attendance of 65%. The study endpoint was 100 clinical assessments at exacerbation completed across all sites for participants on mepolizumab prior to initiation of rescue treatment.

Procedures

Twice daily PEF were recorded using Vitalograph Asma1® ePEF meter and daily recording of severity of symptoms, nocturnal waking and daily reliever medication use via a standardized asthma symptoms diary. FeNO measurement was undertaken using NIOX Vero® and spirometry conducted according to the American Thoracic Society/European Respiratory Society consensus statement¹⁵ with FEV₁ and FVC percent-predicted values calculated using global lung function 2012 equations.¹⁶ Sputum was induced and processed in line with the RASP-UK standard operating procedure. Sputum nucleic acid extracts underwent polymerase chain reaction (PCR) using LightCycler® 480 II

instrumentation (Roche Molecular Diagnostics) to indicate presence of a respiratory virus including Influenza A/B, Respiratory syncytial virus A/B, Rhinovirus, Metapneumovirus, Adenovirus, Parainfluenza 1-4, and Coronavirus. DNA was extracted from sputum plugs and bacterial loads measured by quantitative PCR (qPCR), using ThermoFisher Quantstudio 5, based on abundance of 16S ribosomal subunit encoding genes and pathogen specific genes including *Moraxella catarrhalis*, *Haemophilus influenzae*, and *Streptococcus pneumoniae* as previously described.¹⁷ We used a specific bacteria threshold of $\geq 10^6$ genome copies/ml on sputum qPCR as significant because this threshold of detection had a 98% concordance with bacteria detection on routine culture and was associated with an increased percentage sputum neutrophils in COPD exacerbations.¹⁸

Statistical analysis

Descriptive statistics are presented as means (SD), medians [IQR] or counts (%) as appropriate. For normally-distributed variables, we compared groups using the mean difference and independent sample or paired t-test. For non-normally distributed variables, we compared groups using the median difference (with bootstrapped 95% CIs) and Mann-Whitney U or Wilcoxon signed rank tests. Differences for categorical variables were summarised using odd ratios (95% CIs), with p-values derived from chi-squared tests. Spearman's correlation analysis were utilised to describe the relationship between variables. To examine if study assessed exacerbations were different physiological entities from those where the study participant did not attend the clinic, daily PEF and symptoms diaries were compared before and after the index date for the exacerbation. Mean change in PEF and symptom diary scores from 14 days prior to and following the exacerbation nadir were plotted for patient subgroups and compared. To ensure there was no bias introduced from repeated exacerbations in an individual patient, each participant's first exacerbation was used when comparing to the baseline assessment. Inflammatory phenotype at exacerbation was assessed using sputum eosinophils; the first exacerbation with sputum per participant was divided into sputum eosinophil low (SE^{LOW} , sputum eosinophil count <2% at exacerbation) and sputum eosinophil high (SE^{HIGH} , sputum eosinophil count $\geq 2\%$ at exacerbation). A cut-point of 2% sputum eosinophils was used to signify the presence of type 2 inflammation in line with GINA guidelines.¹⁹ As data was collected prospectively during exacerbations, missing data was rare. Therefore, we conducted all analyses under a complete-case framework. Analyses were conducted using STATA 16 (StataCorp, Texas, USA).

Role of the funding

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. All authors had access to all of the data in the study and had final responsibility for the decision to submit for publication.

Results

Between the 30th of November 2017 and 29th of May 2019, 145 participants were recruited, 93 pre-established on mepolizumab (median time on mepolizumab 0.46 years [0.31,0.83]) and 52 new to mepolizumab. Five participants pre-established on mepolizumab had no engagement with any study procedures following their initial study visit and were excluded from further analysis. Demographics, including comorbidities, prior to initiation of mepolizumab are in table E1 (online supplement, page 4) and characteristics when clinically stable on mepolizumab (≥ 3 months treatment) are in Table 1. When clinically stable on mepolizumab, 75% (102/140) of participants were receiving maintenance prednisolone (median dose 10 [5,10] mg/day), participants continued to have considerable symptoms and impaired quality of life (ACQ-5 2.0 (1.0,3.0), mini-AQLQ 4.6 (3.3,6.4), SGRQ 46.1 (21.5,63.3) impaired lung function (FEV₁ 71.8% (20.9), and elevated FeNO (32.5ppb [19,66]). Although PBE were suppressed on mepolizumab compared to the pre-mepolizumab state, 23% (31/135) of participants continued to have PBE of ≥ 100 cells/ μ L detected and 41% (31/75) of sputum producers a sputum eosinophilia of $\geq 2\%$ (Table 1, Figure E2).

Thirty-four serious adverse events were reported, none were directly related to study procedures and half were hospitalisations due to asthma exacerbations, (Table E2, online supplement, page 5). There were 29 study withdrawals (details in Figure 1).

Exacerbations

There were a total of 172 exacerbations in 87/140 (62%) participants; 96 exacerbations were assessed prior to initiation of antibiotic or OCS treatment and of the remaining 76 missed exacerbations, 65 were unable to attend the clinic and 11 were assessed at the clinic but participants had already initiated rescue treatment (OCS n=2, antibiotics n=8 or both n=1, Figure 1). The remaining 53/140 (38%) participants were exacerbation-free.

Comparing those who did and did not exacerbate during the study (Table E3, online supplement page 7), subjects who exacerbated had a higher exacerbation rate and more emergency department (ED) visits in the year prior to commencing mepolizumab, but no other defining clinical characteristics. On

mepolizumab there was no difference in PBE, sputum or FeNO, although those who exacerbated experienced a higher symptom burden.

Missed exacerbations

The symptoms experienced during missed exacerbations are shown in Table E4 (online supplement, page 8). Most participants saw their general practitioner (46%, 35/76) or self-treated as per their personalised asthma action plan (42%, 32/76) with 91% (69/76) commencing high dose OCS and 57% (43/76) antibiotics. There were 12 hospital admissions; four participants commenced rescue treatment but contacted their study teams and were subsequently admitted by the study clinician, the remaining eight were admitted directly through acute medical services. Comparing the PEF of assessed and missed exacerbations, the mean (SD) change in PEF at nadir in the assessed group was -40.5L/min (76.3) and -37.0L/min (93.0) in the missed group, $p=0.84$ and there was no difference in reported symptom burden (Figure 2).

Assessed exacerbations prior to rescue treatment

Details of all 96 exacerbations assessed prior to rescue treatment are illustrated in Table E5 (online supplement, page 9); these occurred in 69 subjects with 21 having a second exacerbation and 6 a third. Mean ACQ-5 was 3.6 (2.6,4.1) with 58 of 91 exacerbations with paired ACQ-5 measurements having an increase of ≥ 0.5 from baseline. Median FeNO was 47ppb [22,81] with 47.3% (43/91) of exacerbations having a FeNO of ≥ 50 ppb and 23% (21/93) of participants a PBE count of ≥ 100 cells/ μ L. Median sputum eosinophil count was 1.3% [0.3,10.4] with 48% (28/59) having an eosinophil count of $\geq 2\%$. Median sputum neutrophil count was 59.5% [33.4,91.9] with 48% (28/59) of exacerbation sputa having a neutrophil count of $\geq 65\%$; there was minimal overlap between the sputum eosinophil high exacerbations and sputum neutrophil high exacerbations (3/59 exacerbations). Treatment at clinic resulted in 64% (61/96) of participants receiving OCS and 27% (26/96) antibiotics; at follow-up telephone call after the unscheduled visit, 13 participants had gone on to commence OCS and three had started antibiotics (Table E5, online supplement page 9). Table E6 illustrates the change in participant characteristics from stable on mepolizumab to first exacerbation per participant ($n=69$) (online supplement, page 10).

To examine the inflammatory phenotype of exacerbation events, the first exacerbation per participant with sputum was divided into SE^{LOW} and SE^{HIGH} . On commencing mepolizumab, there was no difference in the clinical characteristics of the SE^{HIGH} and SE^{LOW} exacerbation subgroups (Table E7, online supplement page 11). When stable on mepolizumab, the SE^{HIGH} exacerbation group had higher sputum

eosinophils, higher FENO but no difference in PBE, CRP, or maintenance prednisolone (Table E8, online supplement page 12). At exacerbation, there was no difference in maintenance prednisolone exposure (Table 2). The SE^{HIGH} group had a higher FeNO (57ppb vs 24ppb, median difference 33; 95% CI 8,87; p<0.001), lower FEV₁ (55.6% vs 71.5%, mean difference -15.88; 95% CI -27.0,-4.8; p=0.0075) and more obstructive spirometry (FEV₁ /FVC 57.5% vs 67.8%, mean difference -10.3; 95% CI -17.0,-3.6; p=0.0043). Pre-defined clinical diagnoses assigned at time of assessment were different between the two inflammatory phenotypes (p=0.04), with the SE^{LOW} group more likely to be diagnosed with an infectious trigger (88% v 40%) and more likely to receive antibiotics as part of their treatment plan (50% vs 19%, p=0.031). There were three hospital admissions in the SE^{LOW} group (1 at the time of assessment and 2 within 3 days of assessment) and none in the SE^{HIGH} group. At exacerbation, the SE^{LOW} subgroup had lower PBE (30cells/μg vs 70 cells/μg, median difference -40; 95% CI -70,-20; p<0.001), higher CRP (15mg/L vs 2.3mg/L, median difference 12.7; 95% CI 3.5,18.5; p<0.001), and higher sputum neutrophils (89.8% vs 37.1%, median difference 52.7; 95% CI 34.5,59.2; p<0.001). Lung function was significantly lower in the SE^{HIGH} group at exacerbation, representing an additive effect of an 8.9% lower FEV₁ in the SE^{HIGH} when stable on mepolizumab alongside a 4.5% greater decrement at exacerbation (Table E8 and Table E9). FeNO did not change at exacerbation in either group, Table E9 (online supplement, page 13).

FeNO correlated significantly with sputum eosinophils at the time of exacerbation (rho 0.56, p<0.001). In total, 57 exacerbations had both sputum differential cell counts and FeNO (Figure 3); 25% (14/57) had a low FeNO (≤20ppb) at exacerbation of which none had a sputum eosinophil count of ≥2% i.e. negative predictive value (NPV) of FeNO≤20ppb for eosinophilic sputum at exacerbation was 100% (95% CI 80,100). Twenty of 26 exacerbations with a high FeNO (≥50ppb) at exacerbation had a sputum eosinophil count of ≥2% (median sputum eosinophil count 7.2% [2.6,30.5]) i.e. positive predictive value (PPV) of FeNO≥50ppb for airway eosinophilia was 77% (95% CI 60,90). The 30% (17/57) of exacerbations with a FeNO between 21-49ppb were more heterogeneous with 53% (9/17) SE^{LOW} and 47% (8/17) SE^{HIGH}.

For all 91 exacerbations with FeNO measurements, there were 22 (24%) FeNO low (≤20ppb), 25 (28%) FeNO intermediate (21-49ppb), and 44 (48%) FeNO high (≥50ppb). Similar to sputum eosinophil analyses, the FeNO subgroups differentiated at exacerbation on PBE (0.30 [20,60], 30 [20,60], 70 [40,120 cells/μL], p=0.0020), CRP (11.5 [4.9,23.0], 5.7 [2.3,13.0], 3.6 [1.4,5.7] mg/L, p=0.009), sputum eosinophils (n=57) 0.3 [0.2,0.5], 1.0 [0.6,4.5], 7.2 [2.5,33.0] %, p<0.001), and respiratory viral PCR positivity (41.7%, 60%, 16.7% p=0.020).

CRP had a significant inverse correlation with sputum eosinophils at the time of exacerbation ($\rho = -0.57$, $p < 0.001$). Of the 57 exacerbations with a paired sputum cell differential and CRP at exacerbation, 31/57 (54.5%) had a CRP of $\leq 5\text{mg/L}$ and of these, 22/31 had a sputum eosinophil count of $\geq 2\%$, therefore CRP $\leq 5\text{mg/L}$ has a 71.0% PPV (95% CI 52.0, 85.8) for predicting a sputum eosinophil count of $\geq 2\%$. The remaining 26/57 exacerbations had a CRP $> 5\text{mg/L}$, of these 21/26 had a sputum eosinophil count of $< 2\%$, therefore CRP $> 5\text{mg/L}$ has an 80.8% (95% CI 60.6, 93.4) NPV for excluding a sputum eosinophil count $\geq 2\%$ at exacerbation. CRP was not of value in providing further differentiation of exacerbation phenotype in the intermediate FeNO group (FeNO 21-49ppb) (data not shown).

To explore the relationship between sputum phenotype when stable and at exacerbation, paired sputum ($n=24$ subjects) were examined; 38% (9/24) were SE^{HIGH} both when stable on mepolizumab and at exacerbation, 33% (8/24) were SE^{LOW} when stable on mepolizumab and at exacerbation (Figure E3, online supplement page 14), in total 71% had a preserved inflammatory phenotype. Similarly for those with paired FeNO measurements when stable on mepolizumab and exacerbation, 68% (13/19) had FeNO $\leq 20\text{ppb}$ when stable and at exacerbation and 60% (26/43) had FeNO $\geq 50\text{ppb}$ when stable and at exacerbation. Of the 75 participants with a sputum sample when clinically stable on mepolizumab, 50% (22/44) of the SE^{LOW} and 53% (18/31) of the SE^{HIGH} went on to have an exacerbation during the study period ($\text{Pr}=0.46$). There was no difference in annualised rate of exacerbations between the SE^{LOW} and SE^{HIGH} groups (1.23 vs 1.23).

When clinically stable on mepolizumab, 57% (39/69) of patients had bacteria detected by PCR, 36% (25/69) had bacteria detected over the 10^6 genome copies/ml threshold and 12% (8/69) had a virus detected on PCR (Table 1). Presence of bacteria or virus on sputum PCR while clinically stable on mepolizumab was not associated with an increase in sputum neutrophils (Table E10, online supplement page 15). When clinically stable on mepolizumab, the study exacerbators were no more likely to have a virus or specific bacteria above the level of detection or above the threshold of 10^6 genome copies/ml, than the non-exacerbators (Table E3, online supplement page 7).

At first exacerbation with sputum, 67% (22/33) of patients had bacteria detected by qPCR, there was no significant difference in sputum neutrophil count for those that had bacteria detected at any threshold on qPCR compared to those that did not (Table E10, online supplement page 15). Thirty-nine percent (13/33) had bacteria $\geq 10^6$ genome copies/ml detected on qPCR with an associated increase in sputum neutrophil count (sputum neutrophils 95% [62.3, 97.7] vs 64% [36.8, 88.4], $p=0.025$). The 36% (12/33) of exacerbations with a virus detected on PCR at exacerbation did not have an associated increase in sputum neutrophils (Table E10).

Applying the SE^{LOW} and SE^{HIGH} phenotypes to the first exacerbation with sputum per participant, SE^{LOW} exacerbations had a two fold increase in the number of viruses detected on PCR (45% vs 23%, p=0.20), a two fold increase in those with a specific bacteria $\geq 10^6$ genome copies/ml (50% vs 23%, p=0.12), and were significantly more likely to have either a virus or specific bacteria $\geq 10^6$ genome copies/ml in sputum at exacerbation (SE^{LOW} 75% vs SE^{HIGH} 38%, mean difference 0.21; 95% CI 0.04,1.2; p=0.036, Table 2).

Discussion

Prospective evaluation of asthma exacerbations on mepolizumab indicates that these are heterogeneous in nature with approximately half driven by eosinophilic inflammation and half being non-eosinophilic events with an increased propensity for pathogen-driven exacerbations. These appear to be broadly mutually exclusive events and FeNO at exacerbation is useful in discriminating between eosinophilic and non-eosinophilic exacerbations.

When stable on mepolizumab, this cohort had continuing asthma symptoms and evidence of ongoing T2 activity. Those who exacerbated on mepolizumab had a higher pre-mepolizumab exacerbation rate and ED attendance, and higher symptom scores. There was no significant difference in clinical or sputum characteristics, in particular no difference in sputum eosinophil count and FeNO, in those who exacerbated and those who did not exacerbate during the study. For all assessed exacerbations, there was no evidence of increased exacerbation towards the end of the 4-week mepolizumab treatment period (Table E5, online supplement page 9), and no difference in time since last mepolizumab administration to exacerbation between SE^{LOW} and SE^{HIGH} subgroups (Table 2), supporting the findings of a recent post-hoc analysis of the DREAM study.²⁰

We anticipated loss of 35% of exacerbation events due to inability to attend (38% during study). Symptom burden and PEF change were similar for assessed and missed exacerbations, suggesting that the assessed exacerbation events were representative of all exacerbations experienced by study participants. For assessed exacerbations, symptom worsening and lung function fall resulted in the majority (64%) receiving rescue OCS at exacerbation assessment; interestingly, 13 of the 35 patients who did not receive OCS treatment at study assessment went on to initiate OCS in the days following this suggesting slow recovery or worsening; in many of these, there was no evidence of eosinophilic inflammation at assessment and the benefit of the OCS is questionable.

There was little difference in the clinical characteristics of the SE^{LOW} and SE^{HIGH} exacerbator groups on initiation of mepolizumab therapy, however, once established on mepolizumab differences become

apparent even when clinically stable. The SE^{HIGH} group had evidence of ongoing T2-inflammation, higher FeNO and higher percentage sputum eosinophils, despite no difference in OCS exposure or PBE suggesting a difference in airways biology.

At exacerbation, the SE^{LOW} subgroup do have a large symptom increase, fall in lung function, and all hospital admissions occurred in this group (confirming these are clinically significant events). The SE^{LOW} events had neutrophilic sputum, higher CRP, were more likely to be clinically diagnosed with a pathogen-driven event, and to have a virus or bacteria ($\geq 10^6$ copies/ml) on PCR at exacerbation. In contrast, SE^{HIGH} exacerbations had higher PBE, lower FEV₁, more obstructed spirometry, and higher FeNO in keeping with T2 cytokine activity and eosinophilic airways inflammation. Importantly, these seem to be dichotomous mutually exclusive events with few patients having evidence of overlapping neutrophilic/eosinophilic sputum, in keeping with previous findings in COPD exacerbations.^{17,21} It is notable that FeNO does not change at acute exacerbation from the stable state, suggesting that substantial upregulation of IL4/IL13 pathways is not an obvious driving factor for eosinophilic exacerbation events, although these inflammatory pathways are still active (accepting FeNO as a surrogate biomarker) and also suggests that non-adherence to background ICS is not a likely driver..²² It has been suggested that *in situ* eosinopoiesis orchestrated by ILC2 cells may occur²³ or other autoimmune mechanisms.²⁴ Understanding the precise mechanism of persistent eosinophilic recruitment despite substantial depletion of the circulating pool and how this translates into a clinical deterioration is an important area for future research.

The fact that non-eosinophilic events are more likely to be associated with pathogen-driven events, alongside the inverse correlation between CRP and sputum eosinophil count at exacerbation, raises the question as to whether suppression of eosinophils in the airway predisposes to airway infection. The role of eosinophils against helminths is well known, but eosinophils may also play a role against viral, bacterial, or fungal organisms and have a variety of pathogen killing mechanisms.^{25,26} Previous studies have suggested the absence of eosinophils in humans does not appear to be associated with significant adverse events,²⁷ however, this topical question is being re-debated in light of the association of eosinopenia with severe COVID-19 infection,^{28,29} and in particular the risks of medication-induced eosinopenia during this global pandemic.³⁰ Furthermore, exacerbations in benralizumab (an eosinophil depleting antibody) treated patients have been described as non-eosinophilic and associated with a significant increase in respiratory infections.³¹ This is currently being further evaluated in the UK Refractory Asthma Stratification Programme.³² The benefit of prophylactic macrolides in reducing exacerbation rate, and importantly the rate of respiratory tract infections, in severe asthmatics is presented in the AMAZES trial.³⁹ Further prospective research into the utility of prophylactic antibiotics as add-on therapy to mepolizumab and other anti-T2 biologics

will be important. Assessment of the presence of airways dilatation/bronchiectasis as part of baseline characteristics will also be an important part of further studies investigating neutrophilic exacerbations in eosinophil-depleting biologics.

PBE count is an established biomarker of T2 biology, exacerbation risk and steroid responsiveness in asthmatic patients,³³⁻³⁸ but the addition of mepolizumab and other anti-IL5 biologics makes this biomarker less useful as a prognostic agent. Sputum differential cell count is not straightforward to measure, has not translated into routine clinical care and is likely to be challenging for routine assessment during an exacerbation. FeNO is an easy to perform, widely available near-patient test, and has useful discriminatory value between SE^{LOW} and SE^{HIGH} events in mepolizumab treated patients at exacerbation. We would suggest that FeNO is measured at exacerbation and when low (FeNO ≤ 20 ppb), careful consideration be given to use of OCS and antibiotics be considered, and when high (FeNO ≥ 50 ppb), OCS are likely to be indicated. Middle-range FeNO values (21-49ppb) are not discriminatory and further consideration of the mechanism of exacerbation and treatment on clinical grounds need to be considered. We acknowledge that this hypothesis, particularly the role of antibiotics in mepolizumab treated participants, needs formal evaluation in a placebo-controlled trial. CRP also has clinical utility in the assessment of exacerbations, particularly where FeNO measurement is not available. The significant inverse correlation between CRP and sputum eosinophils at the time of exacerbation is supportive of sputum-eosinophil low exacerbations being infective in nature. Although a CRP cut point of 5mg/L was useful in discriminating inflammatory phenotype at exacerbation, CRP did not perform as well as FeNO in predictive value of inflammatory phenotype at exacerbation. In addition, CRP did not provide further differentiation of exacerbation phenotype in those with an intermediate FeNO (21-49ppb). The exacerbation rate in SE^{LOW} and SE^{HIGH} subjects when stable on mepolizumab is no different. Inflammatory phenotype assessed using sputum or FeNO is relatively preserved between the stable and exacerbation state, although this is not entirely reliable with approximately a third of subjects changing inflammatory phenotype between baseline and exacerbation. In addition, we would conclude that being on maintenance prednisolone whilst on mepolizumab therapy does not predict clinical response, as there was no difference in maintenance prednisolone dose in those who went on to exacerbate during this study and those who did not. Further, maintenance prednisolone therapy does not exclude breakthrough eosinophilic exacerbations while on mepolizumab or relate to the inflammatory phenotype at exacerbation.

In summary, exacerbation events in mepolizumab-treated severe asthmatics with severe refractory eosinophilic asthma are heterogeneous, but have two mutually exclusive phenotypes; non-eosinophilic pathogen-driven and sputum eosinophil high exacerbations secondary to T2-eosinophilic inflammation. Further studies are needed to evaluate whether differentiating inflammatory

phenotype at assessment of exacerbation will help guide management of increased symptoms triggered by either infectious agents with antibiotics or concomitant macrolide therapy in recurrent exacerbations versus OCS or switching to a more eosinophil-depleting biologic for those with breakthrough T2-driven exacerbations. Studies are also required to assess whether more eosinophil-depleting biological agents, such as anti-IL5-R and anti-TSLP, predispose to infection-related neutrophilic events.

Authorship

LH, IP, RC and CB led the study design process. All authors contributed to the design review. PJM, SD, JY, CB and RS were responsible for data acquisition. LH, PJM and JB were responsible for data analysis. All authors contributed to the interpretation of the data, to the reviews and the edit drafts of the manuscript, approved the final draft of the manuscript for submission and agree to be accountable for all aspects of the work.

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Declaration of interests:

SD, CB, JB, VB, RS and CC have no financial or personal relationships to declare. PJM reports other from Glaxo Smith Kline, outside the submitted work. FY reports personal fees and non-financial support from AstraZeneca, non-financial support from GlaxoSmithKline, outside the submitted work. CEB reports grants and personal fees from GSK, grants and personal fees from AZ, grants and personal fees from Sanofi, grants and personal fees from Novartis, grants and personal fees from Chiesi, grants and personal fees from Genentech, grants and personal fees from Gossamer, grants and personal fees from Mologic, grants and personal fees from 4DPharma for services outside the submitted work. RC reports grants, personal fees and non-financial support from AstraZeneca, personal fees from GSK, personal fees and non-financial support from Teva, personal fees from Novartis, personal fees and non-financial support from Chiesi, non-financial support from Napp Pharmaceuticals, outside the submitted work. IDP reports other from Sanofi, other from Regeneron Pharmaceuticals, Inc., non-financial support from Excerpta Medica, during the conduct of the study; other from Aerocrine AB, other from Almirall, other from AstraZeneca, other from Boehringer

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Data sharing:

Following publication of the primary and secondary analyses, we plan to evaluate how best to share individual de-identified patient data, including data dictionary, in keeping with the data-sharing practices of the RASP-UK consortium. This will allow for maximum utilisation of the data to improve patient care and advance medical knowledge.

References

1. Papadopoulos NG, Barnes P, Canonica GW et al. The Evolving Algorithm of Biological Selection in Severe Asthma. *Allergy*. 2020;**75**, 1555–1563.
2. Ortega HG, Liu MC, Pavord ID, Brusselle GG, FitzGerald JM, Chetta A, et al. Mepolizumab Treatment in Patients with Severe Eosinophilic Asthma. *N Engl J Med*. 2014;**371**(13):1198–1207.
3. Haldar P, Brightling CE, Hargadon B, et al. Mepolizumab and Exacerbations of Refractory Eosinophilic Asthma. *New En*. 2009;**360**(10):973–84.
4. Pavord ID, Korn S, Howarth P et al. Mepolizumab for severe eosinophilic asthma (DREAM): A multicentre, double-blind, placebo-controlled trial. *Lancet*. 2012;**380**(9842):651–9.
5. Shrimanker R, Pavord ID, Yancey S et al. Exacerbations of severe asthma in patients treated with mepolizumab. *Eur Respir J*. 2018;**52**(6):1801127.
6. Chupp GL, Bradford ES, Albers FC et al. Efficacy of mepolizumab add-on therapy on health-related quality of life and markers of asthma control in severe eosinophilic asthma (MUSCA): a randomised, double-blind, placebo-controlled, parallel-group, multicentre, phase 3b trial. *Lancet Respir Med*. 2017;**5**(5):390–400.
7. Shrimanker R, Keene O, Bratton DJ, Yancey S, Heaney L, Pavord ID. Characterisation of Exacerbations of Severe Eosinophilic Asthma on Mepolizumab Compared to Placebo. In: American Thoracic Society International Conference Meetings Abstracts American Thoracic Society International Conference Meetings Abstracts. American Thoracic Society; 2019. p. A2671–A2671.
8. Global Initiative for Asthma. Pocket Guide for Asthma Management and Prevention. *Glob Initiat Asthma*. 2019;1–32. URL: <https://ginasthma.org/reports/> Accessed 03 Mar 2020
9. NICE 2017. 1 Recommendations | Mepolizumab for treating severe refractory eosinophilic asthma | Guidance | NICE. 2017 URL: <https://www.nice.org.uk/guidance/TA431/chapter/1-Recommendations> Accessed 21 Jan 2020
10. Consortium SS medicines. mepolizumab (Nucala) [Internet]. Scottish medicines consortium. 2016. URL: <https://www.scottishmedicines.org.uk/medicines-advice/mepolizumab-nucala-fullsubmission-114916/> Accessed 22 Mar 2020.
11. Juniper EF, O'Byrne PM, Guyatt GH, Ferrie PJ, King DR. Development and validation of a questionnaire to measure asthma control. *Eur Respir J*. 1999;**14**(4):902–7.
12. Juniper EF, Guyatt GH, Cox FM, Ferrie PJ, King DR. Development and validation of the Mini Asthma Quality of Life Questionnaire. *Eur Respir J*. 1999;**14**(1):32–8.
13. Jones PW, Quirk FH, Baveystock CM. The St George's Respiratory Questionnaire. *Respir Med*. 1991;**85**:25–31.
14. SIGN 153 • British guideline on the management of asthma A national clinical guideline 2016. URL: www.sign.ac.uk/pdf/sign50eqia.pdf. Accessed 21 Mar 2020.
15. Miller MR, Hankinson J, Brusasco V et al. Standardisation of spirometry. *Eur Respir J* (2005) **26**, 319–338.
16. Quanjer PH, Stanojevic S, Cole TJ et al. Multi-ethnic reference values for spirometry for the 3–95-yr age range: The global lung function 2012 equations. *Eur Respir J*. 2012;**40**(6):1324–43.
17. Bafadhel M, McKenna S, Terry S et al. Acute exacerbations of chronic obstructive pulmonary disease: Identification of biologic clusters and their biomarkers. *Am J Respir Crit Care Med*. 2011;**184**(6):662–71.
18. Bafadhel M, Haldar K, Barker B et al. Airway bacteria measured by quantitative polymerase chain reaction and culture in patients with stable COPD: Relationship with neutrophilic airway inflammation, exacerbation frequency, and lung function. *Int J COPD*. 2015;**10**:1075–83.
19. GINA. GLOBAL INITIATIVE FOR ASTHMA A GINA Pocket Guide For Health Professionals DIFFICULT-TO-TREAT & SEVERE ASTHMA in adolescent and adult patients Diagnosis and Management A GINA Pocket Guide For Health Professionals DIFFICULT-TO-TREAT & SEVERE ASTHMA in adole. URL: www.ginasthma.org Accessed 13 Mar 2020

20. Pavord ID, Bleeker ER, Buhl R et al. Response to mepolizumab treatment is sustained across 4-weekly dosing periods. *ERJ Open*. 2020 6: 00068-2020; **DOI:** 10.1183/23120541.00068-2020 (In press)
21. Ghebre MA, Pang PH, Diver Set al. Biological exacerbation clusters demonstrate asthma and chronic obstructive pulmonary disease overlap with distinct mediator and microbiome profiles. *J Allergy Clin Immunol*. 2018;**141**(6):2027-2036.
22. Heaney LG, Busby J, Bradding P et al. Remotely monitored therapy and nitric oxide suppression identifies nonadherence in severe asthma. *Am J Respir Crit Care Med*. 2019;**199**(4):454–64.
23. Sehmi R, Smith SG, Kjarsgaard M et al. Role of local eosinophilopoietic processes in the development of airway eosinophilia in prednisone-dependent severe asthma. *Clin Exp Allergy*. 2016;**46**(6):793–802.
24. Mukherjee M, Bulir DC, Radford K et al. Sputum autoantibodies in patients with severe eosinophilic asthma. *J Allergy Clin Immunol*. 2018;**141**(4):1269–79.
25. Ravin KA, Loy M. The eosinophil in infection. *Clin Rev Allergy Immunol*. 2015;**50**(2):214–27.
26. Shamri R, Xenakis JJ, Spencer LA. Eosinophils in innate immunity: An evolving story. *Cell and Tissue Research* 2011;**343**, 57–83.
27. Gleich GJ, Klion AD, Lee JJ, Weller PF. The consequences of not having eosinophils . *Allergy: European Journal of Allergy and Clinical Immunology* 2013;**68**, 829–835.
28. Zhao L, Zhang Y-P, Yang X, Liu X. Eosinopenia is associated with greater severity in patients with coronavirus disease 2019. *Allergy*. 2020;**00**:1–3.
29. Li Q, Ding X, Xia G et al. Eosinopenia and elevated C-reactive protein facilitate triage of COVID-19 patients in fever clinic: A retrospective case-control study. *EClinicalMedicine*. 2020;**23**:100375.
30. Lindsley AW, Schwartz JT, Rothenberg ME. Eosinophil responses during COVID-19 infections and coronavirus vaccination. *J Allergy Clin Immunol*. 2020;**146**, 1–7.
31. Poznanski SM, Mukherjee M, Zhao N et al. Asthma exacerbations on benralizumab are largely non-eosinophilic. *Allergy* 2020;**00**, 1–5.
32. Benralizumab Exacerbation Study - Full Text View - ClinicalTrials.gov. URL: <https://clinicaltrials.gov/ct2/show/NCT04102800>. Accessed 10 07 2020
33. Zeiger RS, Schatz M, Li Q et al. High Blood Eosinophil Count Is a Risk Factor for Future Asthma Exacerbations in Adult Persistent Asthma. *J Allergy Clin Immunol Pract*. 2014;**2**(6):741-750.
34. Price DB, Rigazio A, Campbell JD et al. Blood eosinophil count and prospective annual asthma disease burden: A UK cohort study. *Lancet Respir Med*. 2015;**3**(11):849–58.
35. Pavord ID, Brightling CE, Woltmann G, Wardlaw AJ. Non-eosinophilic corticosteroid inresponsive asthma. *Lancet* 1999; 353:2213-14
36. Woodruff PG, Modrek B, Choy D et al. *Am J Respir Crit Care Med* 2009;180;(5) 388–95
37. McGrath KW, Icitovic N, Boushey H et al. *Am J Resp Crit Care Med* 2012; 185(6)612–9
38. McDowell PJ, Heaney LG. Different phenotypes and Endotypes drive heterogeneity in asthma. *Allergy*. 2019 75;(2)302-310
39. Gibson PG, Yang IA, Upham JW et al. Effect of azithromycin on asthma exacerbations and quality of life in adults with persistent uncontrolled asthma (AMAZES): a randomised, double-blind, placebo-controlled trial. *Lancet*. 2017;390(10095):659-668. doi:10.1016/S0140-6736(17)31281-3

Table 1: Participant characteristics when clinically stable and established on mepolizumab (≥12 weeks mepolizumab treatment).

Participant characteristics (N=140)		
Age (years)		55.47 (11.5)
Male		73/140 (52.1%)
Maintenance prednisolone (mg)		102/140 (74.5%)
Prednisolone Dose (mg) (N=102)		10.0 (5.0,10.0)
ICS dose (BDP µg equivalent)		2000 (2000,2000)
Combined ICS-LABA		140 (100%)
Inhaled anti-muscarinic*		77/140 (55.0%)
Leukotriene receptor antagonist*		74/140 (52.9%)
Oral Theophylline		57/140 (40.7%)
ACQ-5 Total Score		2.0 (1.0,3.0)
ACQ-5 ≤0.75		24/135 (17.8%)
ACQ-5 ≥ 1.5		83/135 (61.9%)
Mini-AQLQ Total Score		4.6 (3.3,6.4)
SGRQ Total Score		46.1 (21.5,63.3)
Total WCC (10 ⁹ /L)		8.5 (7.1,10.2)
Blood Neutrophils (10 ⁹ /L)		5.6 (4.5,8.1)
Blood Eosinophils (cells/µL)		50 (30, 90)
≥100 cells/µL		31/135 (23.1%)
CRP (mg/L)		4.0 (1.8,6.0)
FEV ₁ (% Predicted)		71.8 (20.9)
FVC (% Predicted)		86.9 (18.3)
FENO (ppb)		32.5 (19.0,66.0)
FENO ≤20		35/134 (26.1%)
FENO ≥50		50/134 (37.3%)
Sputum		
Sputum Neutrophils % of cells		43.8 (26.8, 68.7)
≥65%	N=75	20 (26.6%)
Sputum Lymphocyte % of cells	N=75	0.0 (0.0,0.9)
Sputum Eosinophils % of cells		1.3 (0.2,5.7)
≥2%	N=75	31/75 (41.3%)
Sputum Macrophages % of cells	N=75	35.7 (18.4,61.0)
Virus positive on PCR	N=69	8/69 (11.6%)
Total 16s Copies/ml	N=69	9.2x10 ⁸ (2.3x10 ⁸ ,2.2x10 ⁹)
Any qPCR Bacteria Positive above threshold of detection	N=69	39/69 (56.5%)
Any Bacteria Positive (≥10 ⁶ genome copies/ml)	N=69	25/69 (36.2%)
<i>Moraxella catarrhalis</i> (≥10 ⁶ genome copies/ml)	N=69	4/69 (5.8%)
<i>Haemophilus influenzae</i> (≥10 ⁶ genome copies/ml)	N=69	10/69 (14.5%)
<i>Streptococcus pneumoniae</i> (≥10 ⁶ genome copies/ml)	N=69	13/69 (18.8%)
Count of Bacteria Positive (≥10 ⁶ genome copies/ml)	N=69	
0 bacteria specific qPCR >10 ⁶ Copies/ml		44/69 (63.8%)
1 bacteria specific qPCR >10 ⁶ Copies/ml		23/69 (33.3%)
2 bacteria specific qPCR >10 ⁶ Copies/ml		2/69 (2.9%)
Any Virus or Bacteria (≥10 ⁶ genome copies/ml)	N=69	30/69 (43.5%)
Data are presented as mean (SD), median (IQR) and count (%), Virus: PCR for Influenza A, Influenza B, Respiratory Syncytial virus A, Respiratory Syncytial virus B, Rhinovirus, Metapneumovirus, Adenovirus, Parainfluenza 1, Parainfluenza 2, Parainfluenza 3,		

Parainfluenza 4 and Coronavirus. * These add on therapies have not been studied in severe asthma populations.

Table 2. First exacerbation per participant with sputum: low sputum eosinophils (<2%) versus high sputum eosinophils (≥2%).

First assessed exacerbation with sputum		Sputum Eosinophils <2% (n=24)	Sputum Eosinophils ≥2% (n=21)	p
Time between mepolizumab Initiation and Exacerbation (Years)	N=45	1.0 (0.5,1.3)	0.7 (0.5,0.8)	0.20
Days since last mepolizumab administration	N=45	14.5 (6.0,26.5)	14.0 (6.0,24.0)	0.97
Maintenance prednisolone at exacerbation	N=45	16/24 (66.7%)	13/21 (61.9%)	0.74
Maintenance prednisolone dose at exacerbation	N=29	6.9 (4.5,10.0)	5.0 (5.0,10.0)	0.69
Symptoms at exacerbation				
New or Increased Breathlessness	N=45	22/24 (91.7%)	21/21 (100.0%)	0.18
Decrease in Exercise Tolerance	N=44	22/23 (95.7%)	14/21 (66.7%)	0.013
Cough	N=45	24/24 (100.0%)	18/21 (85.7%)	0.055
Change in Sputum Volume	N=45	23/24 (95.8%)	17/21 (81.0%)	0.11
Increased Sputum Purulence	N=45	19/24 (79.2%)	11/21 (52.4%)	0.057
Increased Nocturnal Wakening	N=45	22/24 (91.7%)	17/21 (81.0%)	0.29
Wheeze	N=45	18/24 (75.0%)	17/21 (81.0%)	0.63
Preceding Coryzal Symptoms	N=45	15/24 (62.5%)	9/21 (42.9%)	0.19
Fevers	N=45	12/24 (50.0%)	5/21 (23.8%)	0.071
Haemoptysis	N=45	1/24 (4.2%)	1/21 (4.8%)	0.92
Physiological measurements				
FENO (ppb)	N=44	24.0 (16.0,45.0)	57.0 (30.0,111.0)	<0.001
PEF (L/min)	N=43	359.2 (124.7)	305.1 (88.8)	0.11
FEV ₁ (% Predicted)	N=44	71.5 (21.4)	55.6 (15.3)	0.0075
FEV ₁ /FVC	N=44	67.8 (9.0)	57.5 (13.5)	0.0043
ACQ-5 Total Score	N=45	3.5 (2.1,3.9)	2.8 (2.0,3.6)	0.19
Unscheduled Visit Clinical Diagnosis				0.04
Non-Infective Exacerbation Of Asthma		3/24 (12.5%)	10/20 (50.0%)	
Viral Upper Respiratory Tract Infection		8/24 (33.3%)	5/20 (25.0%)	
Viral Lower Respiratory Tract Infection		5/24 (20.8%)	3/20 (15.0%)	
Bacterial Upper Respiratory Tract Infection		4/24 (16.7%)	0/20 (0.0%)	
Bacterial Lower Respiratory Tract Infection		3/24 (12.5%)	0/20 (0.0%)	
Pneumonia		1/24 (4.2%)	0/20 (0.0%)	
Sinusitis		0/24 (0.0%)	1/20 (5.0%)	
Vocal Cord Dysfunction		0/24 (0.0%)	1/20 (5.0%)	
Unscheduled Visit Treatment				
High Dose OCS Initiated	N=45	14/24 (58.3%)	11 (52.4%)	0.69
Antibiotics Initiated	N=45	12/24 (50.0%)	4 (19.0%)	0.031
Hospital Admission	N=45	1/24 (4.2%)	0 (0.0%)	0.34
Further Medical Assessment Required				0.29
Gp		1/4 (25.0%)	1/3 (33.3%)	
Hospital Admission		2/4 (50.0%)	0/3 (0.0%)	
Further Study Visit		0/4 (0.0%)	1/3 (33.3%)	
Asthma Nurse		1/4 (25.0%)	0/3 (0.0%)	
Haematology				
Blood Neutrophils (10 ⁹ /L)	N=45	6.7 (4.7,8.8)	5.1 (4.2,6.8)	0.13
Blood Eosinophils (cells/μL)	N=45	30 (10,50)	70 (50,90)	<0.001
CRP (mg/L)	N=44	15.0 (5.0,24.0)	2.3 (1.5,4.9)	<0.001
Sputum				
Sputum Neutrophils % of cells	N=45	89.8 (71.6,94.9)	37.1 (28.6,54.2)	<0.001
Sputum Lymphocyte % of cells	N=45	0.0 (0.0,0.3)	1.5 (0.2,2.8)	0.0040
Sputum Eosinophils % of cells	N=45	0.4 (0.2,0.8)	10.4 (4.5,20.6)	<0.001
Sputum Macrophages % of cells	N=45	8.5 (4.1,25.6)	36.0 (21.9,55.6)	<0.001
Virus positive on PCR	N=33	9/20 (45.0%)	3/13 (23.1%)	0.20

Total 16s Copies/ml	N=33	1.0x10 ⁹ (3.9x10 ⁸ ,1.8x10 ⁹)	9.7x10 ⁸ (6.0x10 ⁸ ,1.8x10 ⁹)	0.71
Any qPCR Bacteria Positive above threshold of detection	N=33	14/20 (70.0%)	8/13 (61.5%)	0.61
Any Bacteria Positive (≥10 ⁶ genome copies/ml)	N=33	10/20 (50.0%)	3/13 (23.1%)	0.12
MC (≥10 ⁶ genome copies/ml)	N=33	4/20 (20.0%)	0/13 (0.0%)	0.085
HI (≥10 ⁶ genome copies/ml)	N=33	3/20 (15.0%)	0/13 (0.0%)	0.14
SP (≥10 ⁶ genome copies/ml)	N=33	4/20 (20.0%)	3/13 (23.1%)	0.83
Count of Bacteria Positive >10 ⁶ Copies/ml 0 bacteria specific qPCR >10 ⁶ Copies/ml 1 bacteria specific qPCR >10 ⁶ Copies/ml 2 bacteria specific qPCR >10 ⁶ Copies/ml	N=33	10/20 (50.0%) 9/20 (45.0%) 1/20 (5.0%)	10/13 (76.9%) 3/13 (23.1%) 0/13 (0.0%)	0.27
Any Virus or Bacteria (≥10 ⁶ genome copies/ml)	N=33	15/20 (75.0%)	5/13 (38.5%)	0.036
Data are presented as mean (SD), median (IQR) and count (%). Independent sample T-tests, Mann-Whitney U tests and chi-square tests were used to compare groups.MC: <i>Moraxella catarrhalis</i> . HI: <i>Haemophilus influenzae</i> . SP: <i>Streptococcus pneumonia</i> .				

Figure Legends:

Figure 1. Subject recruitment and exacerbations.

~ Unable to attend study visits for a variety of reasons: work commitments, childcare requirements, distance from home to study site, frequent travel. *Recruited across 4 UK sites

**Excluded from study: Participant enrolled into study but no further engagement with study procedures (no daily PEF, daily symptoms diary and no attendance at exacerbation for assessment). ^ 1 sudden cardiac death in patient awaiting ICD. 1 patient with multi-organ failure secondary to sepsis.^^Four other: wished to conceive, relocated away from clinical site, became too frail to attend unscheduled visits and one patient developed mental health comorbidity.

Figure 2: Comparison of change in peak expiratory flow and daily symptoms diary the fourteen days before and after exacerbation for missed and assessed exacerbations.

Figure 2.1 Mean change with 95% CI in daily peak expiratory flow for the 14 days before and 14 days after exacerbation for assessed and missed exacerbations.

Figure 2.2 Mean change with 95% CI in daily asthma symptom severity score for the 14 days before and 14 days after exacerbation for assessed) and missed exacerbations. Asthma symptoms score from 0 (no symptoms) to 6 (severe symptoms).

Figure 2.3 Mean change with 95% CI in number of night awakenings requiring rescue medication for the 14 days before and 14 days after exacerbation for assessed and missed exacerbations.

Figure 2.4 Mean change with 95% CI in rescue medication requirements for the 14 days before and 14 days after exacerbation for assessed and missed exacerbations.

Figure 3. FeNO as a predictor of sputum eosinophilia: a FeNO 20ppb or less was associated with low sputum eosinophils in sputum (14/57 exacerbations had FeNO $\leq$ 20ppb, all had a sputum eosinophil count of $<2\%$, a positive predictive value of 100%). FeNO of 50ppb or greater was associated with high sputum eosinophils (20/26 exacerbations with FeNO of $\geq$ 50ppb had an eosinophil count $\geq 2\%$, a positive predictive value of 77%).

FeNO and Sputum eosinophils at exacerbation.

Inflammatory profile of Mepolizumab EXacerbations in participants with severe refractory eosinophilic asthma (the MEX study).

PJ McDowell¹, S Diver², F Yang³, K Borg⁴, J Busby⁵, V Brown¹, Shrimanker R⁴, C Cox¹, CE Brightling³, R Chaudhuri², ID Pavord⁴, LG Heaney¹ on behalf of the Medical Research Council: Refractory Asthma Stratification Programme (RASP-UK Consortium).

Online supplement:

NICE and SMC guidance on eligibility to receive mepolizumab treatment:

A) National Institute for Health and Care Excellence (UK):¹

Mepolizumab treatment can be considered in severe, refractory eosinophilic asthmatics in addition to optimised therapy when:

- Blood eosinophil count is 300 cells/microliter or more in the previous 12 months and
- The person has agreed to and followed the optimised standard treatment plan and
- Has had 4 or more asthma exacerbations requiring systemic corticosteroids in the previous 12 months or
- Has had continuous oral corticosteroids of at least the equivalent of prednisolone 5mg/day over the previous 6 months.

B) Scottish medicines consortium (UK):²

Indication for mepolizumab treatment as an add-on treatment for severe refractory eosinophilic asthma in adult patients.

- Patients who have eosinophils of at least 150 cells per microlitre ($0.15 \times 10^9/L$) at initiation of treatment
- Patients who have had at least four asthma exacerbations in the preceding year or
- Patients who are receiving maintenance treatment with oral corticosteroids.

Inclusion and exclusion criteria for the inflammatory profile of mepolizumab exacerbations in participants with severe refractory eosinophilic asthma study:

Inclusion:

Participants must meet the following criteria at visit 1 for study entry (participants can be rescreened for study entry up to 3 times):

1. Age ≥ 18 and ≤ 80 years at Visit 1
2. Able and willing to provide written informed consent and to comply with the study protocol including being able to contact the clinical centre and to attend for assessment during a symptomatic deterioration
3. Severe asthma requiring GINA steps 4 or 5 treatment, confirmed after assessment by an asthma specialist.
4. Suitable for mepolizumab as per NICE/SMC Clinical Guidelines [new and existing participants].

Exclusion:

Participants who meet any of the following criteria will be excluded from study entry:

1. Acute exacerbation requiring oral corticosteroids in previous 4 weeks before the first visit
2. Other clinically significant medical disease or uncontrolled concomitant disease despite treatment that is likely, in the opinion of the investigator, to require a change in therapy or affect the ability to participate in the study
3. History of current alcohol, drug, or chemical abuse or past abuse that would impair or risk the participant's full participation in the study, in the opinion of the investigator
4. Treatment with an investigational agent within 30 days of Visit 1 (or five half-lives of the investigational agent, whichever is longer)

5. Female participants who are pregnant or lactating (excluded as candidates for mepolizumab in clinic and prior to study).

Patient reported outcomes questionnaires

ACQ5

Asthma control was assessed by the five item asthma control questionnaire.³ An ACQ5 score of 0.75 reflects adequate asthma control, whereas a score of 1.5 or over signals inadequate asthma control. The MCID is a reduction in ACQ-5 score of 0.5 or greater.⁴

Mini-AQLQ

The mini-AQLQ consists of 15 questions scored on a scale of between 0 and 7, a lower score reflects greater impairment with MCID being met when the mini-AQLQ improves by 0.5 or more.⁵

St George's respiratory questionnaire

The SGRQ assesses the impact of disease over the preceding 12 months, the higher the score the greater impact disease has on quality of life. The MCID is a score reduction of 4 units.⁶

Study withdrawals

In the period following initial study visit, 29 participants withdrew from the study; 15 had mepolizumab treatment stopped on the basis of non-response, four stopped due to side effects (headache and rash), four participants withdrew consent and 2 died (one of multi-organ failure secondary to sepsis, the other, presumed cardiac arrhythmia in the context of a known cardiomyopathy). Of the remaining four participants who withdrew, one patient wished to conceive, one relocated, one became too frail to attend unscheduled visits and the last had unrelated health concerns.

Figure E1. MEX study visits A) For those initiating mepolizumab on entering the study B) For those entering the study already established on mepolizumab

A. New to mepolizumab on entering study:

B. Established on mepolizumab:

*(≥week 12 on drug)

Table E1. Participant demographics and comorbidities at initiation of mepolizumab therapy (n=140):

Demographics at initiation of mepolizumab (n=140)	
Male	73 (52.1%)
Caucasian	134 (95.7%)
Non-Caucasian	6 (4.3%)
Never Smoker	94 (67.1%)
Ex-Smoker	46 (32.9%)
Pack years	14 (8.26)
Age at Diagnosis Asthma	26.8 (19.2)
Age Commencing Mepolizumab	54.6 (12.0)
BMI	30.4 (8.8)
FENO (ppb)	34.0 (22.0,59.0)
PEF (L/min)	298.4 (205.7)
FEV ₁ (% Predicted)	70.4 (20.0)
FVC (% Predicted)	86.3 (16.1)
ACQ5 Total Score	2.8 (1.8,3.6)
Mini-AQLQ Total Score	3.8 (2.6,4.9)
SGRQ Total Score	54.9 (39.9,73.0)
Highest Blood Eosinophils last 12 months (cells/ μ L)	650 (430, 890)
Sputum at initiation of mepolizumab (n=34*)	
Sputum neutrophils (%)	57.5 (20.1,70.3)
Sputum Lymphocytes (%)	0.0 (0.0,0.7)
Sputum Eosinophils (%)	2.9 (0.5,12.7)
Sputum macrophages (%)	25.4 (16.6,47.4)
Cohort comorbidities (n=140)	
Seasonal Allergic Rhinitis	48 (34.3%)
Perennial Rhinitis	62 (44.3%)
Nasal Polyps	60 (42.9%)
Allergic Dermatitis	35 (25.0%)
Depression or Anxiety	47 (33.6%)
Hypercholesterolaemia	24 (17.1%)
Myocardial Infarction	5 (3.6%)
Osteoporosis or Osteopenia	44 (31.4%)
Cataracts	25 (17.9%)
Diabetes Mellitus	21 (15.0%)
Hypertension	44 (31.4%)
Obstructive Sleep Apnoea	8 (5.7%)
Peptic Ulcer	7 (5.0%)
Chronic Kidney Disease	3 (2.1%)
Glaucoma	2 (1.4%)
Ischaemic Heart Disease	6 (4.3%)
Osteoarthritis	27 (19.3%)
Stroke	4 (2.9%)
Data are presented as mean (SD), median (IQR) and count (%).	
*Sputum samples from taken at the initiation of mepolizumab are available from those commencing mepolizumab on entering the MEX study.	

Figure E2. Flow diagram of sputum differential cell counts used in each analysis.

Each centre in this study has labs experienced in sputum processing, with each sputum count performed by two technicians to ensure accuracy at individual centres with standardized lab parameters for sputum acceptability being a squamous cell count of less than 30% and non-squamous cell viability of over 40%. As part of our rigorous review of sputum analysis and consistency across centres, 50% of all sputum slides from this study were sent to the central lab for further quality control measures where the slides were recounted and viability and proportion of squamous cells assessed. Our quality control process at the central lab revealed that all samples had a squamous cell count of less than 30% and the mean non-squamous cell viability for all samples was 73.0% (SD 15.3%).

Table E2. Serious adverse events (n=34):

SAE	Hospital admission	HDU/ITU
Hospitalisation viral exacerbation of asthma.	YES	NO
Hospitalisation with exacerbation asthma	YES	NO
Death: Found collapsed, unsuccessful resuscitation in community. Discussed with coroner, presumed cardiac arrhythmia as known cardiomyopathy awaiting ICD. On MEPO since 2013 (clinical trial pre-NICE approvals).	NO	NO
Non ST elevation MI: stents to left anterior descending artery and left circumflex.	YES	NO
Hospitalisation for urosepsis and acute kidney injury.	YES	NO
Widespread maculopapular rash: Mepolizumab stopped. No hospitalisation.	NO	NO
Idiopathic pancreatitis (known T2DM)	YES	NO
Infective exacerbation of asthma	YES	NO
Intra-abdominal sepsis due to gastroenteritis with secondary shock requiring ITU admission and vasopressor support.	YES	YES
Death: admitted with sepsis of unknown origin and multi-organ failure. Decision for palliation and died 4-5 days later.	YES	NO
Hospitalisation for asthma exacerbation	YES	NO
Hospitalisation for asthma exacerbation	YES	NO
Hospitalisation for pneumonia	YES	NO
Hospitalisation for asthma exacerbation	YES	NO
Fall and resolving pneumonia	YES	NO
Hospitalisation for asthma exacerbation	YES	NO
Hospitalisation for asthma exacerbation	YES	NO
Hospitalisation for asthma exacerbation	YES	NO
Hospitalisation for asthma exacerbation	YES	NO
Exacerbation of asthma: withdrawn from study as over 3 exacerbations, not responsive to MEPO.	YES	NO
Hospitalisation: Pulmonary embolism treated with Apixaban	YES	NO
Hospitalisation: Influenza A	YES	YES
Hospitalisation: Road traffic accident	YES	NO
Hospitalisation with LLL Streptococcal pneumonia with secondary erythema multiforme	YES	NO
Hospitalised with exacerbation Asthma	YES	NO
Hospitalised with infective exacerbation Asthma	YES	NO
Hospitalised with exacerbation Asthma	YES	NO
Cardiac event: witnessed p wave systole, emergency pace maker inserted. Possible clinical history of similar events previously.	YES	NO
Hospitalised for pneumonia with exacerbation of asthma	YES	NO
Hospitalisation for abdominal pain, query pancreatitis. Previous history of pancreatitis.	YES	NO
Hospitalisation for abdominal pain: cholecystitis.	YES	NO
Loss of consciousness, query syncope. Normal ECG, EF 45-55%, moderate aortic stenosis. For cardiology follow-up.	YES	NO
Hospitalisation for asthma exacerbation	YES	NO
Hospitalisation exacerbation asthma.	YES	NO

Serious adverse events:

A total of 34 serious adverse events reported of which none were study related; 32 of the 34 (94%) adverse events included hospitalisation of which 3 (9%) included an HDU/ITU admission. Two deaths were reported, one a presumed cardiac arrhythmia in a patient with known cardiomyopathy awaiting an ICD, the other sepsis with multi-organ failure and decision to palliate.

Approximately half (17/32) of all reported hospitalisations due to asthma exacerbations. Of the remaining, there were 4 (12%) pneumonias, 3 (9%) cardiac events including an MI and 2 arrhythmias, 3 (9%) intra-abdominal events and 5 (16%) 'other' events include investigation for syncope, a road traffic accident, pulmonary embolism, influenza A and a fall.

Table E3. Comparison of participants with one or more exacerbations (missed or assessed) versus participants who remained exacerbation free for the duration of the study.

	No Exacerbations	≥1 Exacerbation	p
Number of Patients N=140	53 (38%)	87 (62%)	
Time in study (Years)	0.8 (0.4)	0.9 (0.3)	0.01
Exacerbation count			<0.001
0	53 (100.0%)	0 (0.0%)	
1	0 (0.0%)	41 (47.1%)	
2	0 (0.0%)	21 (24.1%)	
3	0 (0.0%)	14 (16.1%)	
4	0 (0.0%)	8 (9.2%)	
5	0 (0.0%)	3 (3.4%)	
Maintenance Prednisolone on study entry	41 (77.4%)	62 (71.3%)	0.43
Maintenance Prednisolone dose (mg) on study entry (n=103)	10.0 (10.0,10.0)	10.0 (6.3,15.0)	0.92
Maintenance Prednisolone on study exit	35 (68.6%)	44 (51.8%)	0.054
Maintenance Prednisolone dose (mg) on study exit (n=79)	5.0 (4.0,5.0)	5.0 (4.5,10.0)	0.32
HPA Axis Suppression (n=93)	17 (45.9%)	18 (32.1%)	0.18
Pre-initiation of mepolizumab N=140	No Exacerbations (n=53)	>1 Exacerbation (n=87)	p
Male	30 (56.6%)	43 (49.4%)	0.41
Age Commencing Mepolizumab	55.5 (11.5)	54.0 (12.3)	0.50
Occupational Worsening of Asthma	0 (0.0%)	6 (6.9%)	0.051
Smoking Status			0.60
Never Smoker	37 (69.8%)	57 (65.5%)	
Ex-Smoker	16 (30.2%)	30 (34.5%)	
Age at Diagnosis Asthma	27.4 (19.8)	26.4 (18.9)	0.77
Seasonal Allergic Rhinitis	14 (26.4%)	34 (39.1%)	0.13
Perennial Rhinitis	29 (54.7%)	33 (37.9%)	0.052
Nasal Polyps	29 (54.7%)	31 (35.6%)	0.027
Allergic Dermatitis	11 (20.8%)	24 (27.6%)	0.37
Number of Exacerbations Prior 12 Months	3.0 (2.0,5.0)	5.0 (3.0,7.0)	0.002
Number of ED Visits Prior 12 Months	0.0 (0.0,0.0)	0.0 (0.0,1.0)	0.01
Number Hospital Admissions Prior 12 Months	0.0 (0.0,0.0)	0.0 (0.0,1.0)	0.067
Number ITU/HDU Admissions Ever	0.0 (0.0,0.0)	0.0 (0.0,0.0)	0.59
Established on mepolizumab and clinically stable N=138	No Exacerbations (n=52)	>1 Exacerbation (n=86)	p
BMI	27.2 (11.6)	28.4 (11.2)	0.64
FENO (ppb)	34.0 (20.0,64.0)	32.0 (18.0,69.0)	0.91
FEV ₁ (% Predicted)	72.1 (20.1)	71.6 (21.5)	0.91
FEV ₁ /FVC	66.0 (10.9)	64.0 (11.7)	0.31
ACQ5 Total Score	1.4 (0.6,2.6)	2.2 (1.2,3.2)	0.002
Mini-AQLQ Total Score	(5.4 (4.3,6.6)	4.1 (3.0,5.9)	0.001
SGRQ Total Score	39.5 (12.8,57.2)	49.9 (26.7,67.0)	0.02
Blood Eosinophils (cells/μL)	70 (40,110)	50 (30,80)	0.073
Fall in PBE from highest PBE in the 12 months prior to mepolizumab to PBE when stable on mepolizumab	580 (360,860)	590 (400,820)	0.98
CRP (mg/L) N=80	4.9 (2.0,6.0)	3.5 (1.5,6.0)	0.46
Sputum Neutrophils % of cells N=75	43.8 (22.1,71.5)	47.0 (28.1,65.5)	0.64
Sputum Eosinophils % of cells N=75	1.3 (0.2,5.8)	1.2 (0.2,5.1)	0.86
Sputum PCR (N=69):			
Virus	5 (16.7%)	3 (7.7%)	0.25
Total 16s Copies/ml	7.5x10 ⁸ (1.6x10 ⁸ ,2.2x10 ⁹)	9.6x10 ⁸ (2.8x10 ⁸ ,3.0x10 ⁹)	0.39
Any qPCR Bacteria Positive above threshold of detection	15 (50.0%)	24 (61.5%)	0.34
Any Bacteria Positive (>10 ⁶)	12 (40.0%)	13 (33.3%)	0.57
MC (≥10 ⁶ genome copies/ml)	2 (6.7%)	2 (5.1%)	0.79
HI (≥10 ⁶ genome copies/ml)	4 (13.3%)	6 (15.4%)	0.81
SP (≥10 ⁶ genome copies/ml)	7 (23.3%)	6 (15.4%)	0.40
Any Virus or Bacteria (>10 ⁶)	16 (53.3%)	14 (35.9%)	0.15
Data are presented as mean (SD), median (IQR) and count (%). Independent sample T-tests, Mann-Whitney U tests and chi-square tests were used to compare groups. *PBE: peripheral blood eosinophils. Specific bacteria: PCR for specific bacteria includes <i>Moraxella catarrhalis</i> (MC), <i>Haemophilus influenzae</i> (HI), <i>Streptococcus pneumoniae</i> (SP). Virus: PCR for Influenza A, Influenza B, Respiratory Syncytial virus A, Respiratory Syncytial virus B, Rhinovirus, Metapneumovirus, Adenovirus, Parainfluenza 1, Parainfluenza 2, Parainfluenza 3, Parainfluenza 4 and Coronavirus.			

Table E4. Details of missed exacerbations

Symptoms of missed exacerbation		
New or Increased Breathlessness	N=76	70 (92.1%)
Cough	N=76	64 (84.2%)
Wheeze	N=76	63 (82.9%)
Change in Sputum Volume	N=76	57 (75.0%)
Increased Nocturnal Wakening	N=76	55 (72.4%)
Increased Sputum Purulence	N=76	47 (61.8%)
Preceding Coryzal Symptoms	N=76	30 (39.5%)
Fevers	N=76	21 (27.6%)
Haemoptysis	N=76	8 (10.5%)
Action at exacerbation		
General Practitioner	N=76	35 (46.1%)
Asthma Action Plan	N=76	32 (42.1%)
Hospital Asthma Nurse	N=76	7 (9.2%)
ED Attendance	N=76	6 (7.9%)
Primary Care Asthma Nurse	N=76	1 (1.3%)
Hospital Admission	N=76	12 (15.8%)
ITU/HDU Admission	N=65	1 (1.5%)
Treatment of missed exacerbation		
Commenced/increased Oral Steroids	N=76	69 (90.8%)
Commenced Antibiotics	N=76	43 (56.6%)
Increased ICS inhaler	N=76	6 (7.9%)
Increased Nebulisers	N=76	27 (35.5%)
Increase in Medication has helped	N=76	53 (69.7%)
Data are presented as counts (%).		

Table E5. All assessed exacerbations on mepolizumab prior to any treatment (n=96).

Symptoms at exacerbation (n=96)	
Time from mepolizumab initiation to exacerbation (years)	0.8 [0.5, 1.2]
Days since last mepolizumab administration	13 (6.24)
New or Increased Breathlessness	93 (96.9%)
Breathless at Rest	48 (52.2%)
Breathless when Walking	36 (39.1%)
Breathless on moderate Exertion (Just Below Usual Level)	8 (8.7%)
Increased Nocturnal Wakening	82 (85.4%)
Cough	82 (85.4%)
Decrease in Exercise Tolerance	78 (83.0%)
Wheeze	78 (81.3%)
Change in Sputum Volume	69 (71.9%)
Increased Sputum Purulence	48 (50.0%)
Preceding Coryzal Symptoms	47 (49.0%)
Fevers	33 (34.4%)
Haemoptysis	6 (6.3%)
Unscheduled Visit: Clinical Diagnosis (n=92)	
Non-Infective Exacerbation Of Asthma	41 (44.6%)
Viral Upper Respiratory Tract Infection	24 (26.1%)
Viral Lower Respiratory Tract Infection	12 (13.0%)
Bacterial Upper Respiratory Tract Infection	5 (5.4%)
Bacterial Lower Respiratory Tract Infection	3 (3.3%)
Pneumonia	1 (1.1%)
Sinusitis	2 (2.2%)
Vocal Cord Dysfunction	1 (1.1%)
Stress	1 (1.1%)
Secondary To Prednisolone Reduction	1 (1.1%)
Other	1 (1.1%)
Unscheduled visit : Treatment (n=96)	
High Dose OCS Initiated	61 (63.5%)
Antibiotics Initiated	26 (27.1%)
Nebulisers Initiated	10 (10.4%)
Hospital Admission	5 (5.2%)
Unscheduled visit: Review Phone Call (n=92)	
Improved	63 (68.5%)
Much The Same	26 (28.3%)
Worsened	3 (3.3%)
Recovery Longer Than Pre-Mepolizumab	13 (13.5%)
Initiated OCS after assessed visit	13 (13.5%)
Initiated antibiotics after assessed visit	3 (3.1%)
Physiological and biochemical measurements at exacerbation	
Respiratory Rate (Bpm)	18.0 (16.0,22.0)
Respiratory Rate ≥ 20 Bpm	42 (44.2%)
SpO2 Room Air	96.0 (95.0,97.0)
SpO2 $\leq 94\%$ Room Air	16 (16.7%)
Heart Rate (Bpm)	86.3 (14.7)
Heart Rate ≥ 100 Bpm	17 (17.7%)
Temperature (°C)	36.2 (3.4)
Temperature $\geq 37.5^\circ\text{C}$	5 (5.2%)
Temperature $\leq 35^\circ\text{C}$	0 (0%)
Systolic Blood Pressure (mmHg)	132.6 (15.7)
FENO (ppb)	47.0 (22.0,81.0)
<20	22 (24.2%)
≥ 50	43 (47.3%)
PEF (L/min)	307.6 (115.9)
FEV ₁ (% Predicted)	61.3 (20.0)
FVC (% Predicted)	84.0 (68.2)
FEV ₁ /FVC (%)	63.3 (12.9)
<70%	59 (63.4%)
ACQ-5 Total Score	3.6 (2.6,4.1)
Blood Neutrophils ($10^9/\text{L}$)	5.5 (4.2,7.5)
Blood Eosinophils (cells/ μL)	50 (20, 90)
≥ 100 cells/ μL	21 (22.6%)
CRP (mg/L)	5.0 (2.0,14.0)
Sputum at exacerbation (N=59)	
Sputum Neutrophils % of cells	59.5 (33.4,91.9)
$\geq 65\%$	28 (47.5%)
Sputum Eosinophils % of cells	1.3 (0.3,10.4)
$\geq 2\%$	28 (47.5%)
Sputum neutrophils $\geq 65\%$ and eosinophils $\geq 2\%$	3 (5.1%)
Sputum Lymphocyte % of cells	0.2 (0.0,1.5)
Sputum Macrophages% of cells	16.6 (5.4,42.7)
Data are presented as mean (SD), median (IQR) and count (%).	

Table E6. Participant characteristics when stable on mepolizumab compared to first exacerbation per exacebator (n=69).

	Number paired data	Stable on mepolizumab	First Exacerbation	p
Time mepo Initiation to Exacerbation (years)	N=69		0.7 (0.4,1.0)	
Temperature (°C)	N=65	36.5 (0.5)	36.1 (4.1)	0.45
Heart Rate (BPM)	N=66	82.7 (13.7)	86.9 (15.7)	0.018
Respiratory Rate (BPM)	N=63	16.0 (14.0,16.0)	18.0 (16.0,22.0)	<0.001
SpO2 Room Air	N=66	96.5 (95.0,98.0)	96.0 (95.0,97.0)	0.078
FeNO (ppb)	N=62	33.5 (19.0,69.0)	42.5 (23.0,78.0)	0.53
PEF (L/min)	N=62	396.2 (141.4)	312.8 (120.8)	<0.001
FEV ₁ (% Predicted)	N=64	73.4 (21.2)	61.4 (20.0)	<0.001
FEV ₁ /FVC	N=64	65.7 (10.6)	63.8 (12.0)	0.066
ACQ-5 Total Score	N=66	2.4 (1.2,3.2)	3.6 (2.6,4.0)	<0.001
Total WCC (10 ⁹ /L)	N=64	8.3 (7.0,10.0)	8.4 (6.4,10.5)	0.52
Blood Neutrophils (10 ⁹ /L)	N=64	5.5 (4.4,7.5)	5.6 (4.2,7.7)	0.41
Blood Lymphocytes (10 ⁹ /L)	N=64	1.7 (1.3,2.4)	1.7 (1.2,2.2)	0.26
Blood Eosinophils (cells/μL)	N=64	50 (30,70)	50 (20,90)	0.96
Blood Monocytes (10 ⁹ /L)	N=64	0.5 (0.4,0.7)	0.6 (0.5,0.7)	0.026
CRP (mg/L)	N=41	3.5 (1.5,6.0)	5.0 (1.5,8.0)	0.028
Data are presented as mean (SD), median (IQR) and count (%). Independent sample T-tests and Mann-Whitney U tests were used to compare groups				

Table E7. Baseline characteristics at initiation of mepolizumab: sputum eosinophil high and sputum eosinophil low first exacerbation per participant with sputum.

	Sputum eosinophils <2% (n=24)	Sputum eosinophils ≥2% (n=21)	P
Male	12 (50.0%)	11 (52.4%)	0.87
Age Commencing Mepolizumab	56.3 (9.2)	54.0 (10.5)	0.44
Age at Diagnosis Asthma	28.2 (20.0)	25.8 (21.6)	0.70
Seasonal Allergic Rhinitis	11 (45.8%)	7 (33.3%)	0.39
Perennial Rhinitis	9 (37.5%)	4 (19.0%)	0.17
Nasal Polyps	6 (25.0%)	7 (33.3%)	0.54
Allergic Dermatitis	7 (29.2%)	5 (23.8%)	0.69
Number of Exacerbations Prior 12 Months	6.0 (3.0,7.0)	5.0 (4.0,9.0)	0.45
Number of ED Visits Prior 12 Months	0.0 (0.0,0.0)	0.0 (0.0,3.0)	0.06
Number Hospital Admissions Prior 12 Months	0.0 (0.0,1.0)	0.0 (0.0,1.0)	0.62
Number ITU/HDU Admissions Ever	0.0 (0.0,0.0)	0.0 (0.0,1.0)	0.09
BMI	33.0 (8.3)	28.5 (11.1)	0.12
FENO (ppb)	33.0 (20.0,42.0)	54.0 (22.0,76.0)	0.17
FEV ₁ (% Predicted)	74.1 (18.7)	67.2 (16.4)	0.20
ACQ5 Total Score	2.7 (2.3,3.6)	3.0 (2.0,4.0)	0.72
Mini-AQLQ Total Score	3.7 (2.9,4.7)	3.5 (1.7,5.2)	0.74
SGRQ Total Score	45.3 (37.2,71.8)	54.2 (45.9,83.8)	0.26
Highest Eosinophils Year Before Mepo (cells/μL)	630 (360, 890)	710 (470, 890)	0.28
Data are presented as mean (SD), median (IQR) and count (%). Independent sample T-tests, Mann-Whitney U tests and chi-square tests were used to compare groups.			

Table E8. Participant characteristics when clinically stable on mepolizumab (≥ 3 months treatment) for those with sputum eosinophils $< 2\%$ and those with sputum eosinophils $\geq 2\%$ at first exacerbation per participant with sputum.

	Eos $< 2\%$ (n=24)	Eos $\geq 2\%$ (n=21)	Effect Size (95% CI)	p
Maintenance prednisolone stable on mepolizumab ^a	18 (75.0%)	16 (76.2%)	1.1 (0.2,5.3)	0.93
Prednisolone Dose (mg) stable on mepolizumab (n=34) ^b	8.8 (5.0,10.0)	10.0 (7.5,20.0)	1.3 (-2.5,12.5)	0.34
FENO (ppb) ^b	22.0 (15.0,37.0)	68.0 (29.5,116.0)	46.0 (11.0,88.0)	< 0.001
FEV ₁ (% Predicted) ^b	79.3 (23.4)	70.4 (15.1)	-8.9 (-20.9,3.2)	0.16
ACQ5 Total Score ^b	1.8 (1.0,3.4)	2.1 (0.9,2.6)	0.3 (-1.2,1.2)	0.72
Mini-AQLQ	4.6 (3.5,6.5)	4.6 (3.1,6.0)	-0.05 (-2.1,1.2)	0.52
SGRQ	47.6 (18.6,66.3)	46.3 (26.0,70.3)	-1.3 (-22.1,33.8)	0.80
Blood Eosinophils (cells/ μ L) ^b	40 (30,70)	50 (20,110)	5 (-20,50)	0.58
CRP (mg/L) ^b	4.9 (2.9,7.0)	2.2 (1.2,6.0)	-2.7 (-4.3,1.5)	0.18
Total cell count $\times 10^6$ /g (n=24) ^b	2.3 (0.5,6.8)	1.7 (1.0,3.0)	-0.6 (-5.1,1.6)	0.71
Sputum Neutrophils % of cells(n=24) ^b	59.5 (40.3,80.5)	30.7 (29.4,40.3)	-28.8 (-42.5,-3.0)	0.06
Sputum Lymphocyte % of cells(n=24) ^b	0.0 (0.0,0.8)	0.0 (0.0,0.3)	0.0 (-0.6,0.4)	0.59
Sputum Eosinophils % of cells(n=24) ^b	0.8 (0.0,2.5)	3.4 (1.3,31.8)	2.7 (0.4,31.5)	0.01
Sputum Macrophages% of cells(n=24) ^b	32.5 (17.3,41.0)	34.9 (15.7,64.7)	2.4 (-18.4,32.7)	0.82
Virus positive on PCR (n=22)	0 (0.0%)	1 (9.1%)		0.31
Total 16s Copies/ml (n=22)	9.8 $\times 10^8$ (2.1 $\times 10^8$,3.2 $\times 10^9$)	1.3 $\times 10^9$ (4.1 $\times 10^8$,3.0 $\times 10^9$)	3.3 $\times 10^8$ (- 1.8 $\times 10^9$,1.8 $\times 10^9$)	0.53
Any Bacteria Positive ($\geq 10^6$) (n=22)	5 (45.5%)	3 (27.3%)	0.45 (0.05,3.59)	0.38
<i>Moraxella catarrhalis</i> ($\geq 10^6$ genome copies/ml) (n=22)	0 (0.0%)	1 (9.1%)		0.31
<i>Haemophilus influenzae</i> ($\geq 10^6$ genome copies/ml) (n=22)	3 (27.3%)	0 (0.0%)	0.00 (0.00,1.12)	0.06
<i>Streptococcus pneumoniae</i> ($\geq 10^6$ genome copies/ml) (n=22)	3 (27.3%)	2 (18.2%)	0.59 (0.04,6.80)	0.61
Count of Bacteria Positive ($\geq 10^6$ genome copies/ml) (N=22)				0.49
0 bacteria specific qPCR $> 10^6$ Copies/ml	6 (54.5%)	8 (72.7%)		
1 bacteria specific qPCR $> 10^6$ Copies/ml	4 (36.4%)	3 (27.3%)		
2 bacteria specific qPCR $> 10^6$ Copies/ml	1 (9.1%)	0 (0.0%)		
Any Virus or Bacteria ($\geq 10^6$ genome copies/ml) (N=22)	5 (45.5%)	3 (27.3%)	0.45 (0.05,3.59)	0.38

^a Data shown as count (%). Effect size shown as odds ratio with 95% CI

^b Data shown as median (IQR). Effect size shown as median difference with bootstrapped 95% CI

Table E9. Change in characteristics from stable on mepolizumab to exacerbation in the sputum eosinophil low (<2%) and sputum eosinophil high (≥2%) subgroups

Δ Change stable on mepolizumab to exacerbation	Eos<2% (n=24)	Eos≥2% (n=21)	Median difference (95% CI)	p
Time between Mepo Initiation and Exacerbation (Years)	1·0 (0·5,1·3)	0·7 (0·5,0·8)	-0·31 (-0·7,0·1)	0·20
Temperature (C)	0·3 (-0·7,0·9)	-0·1 (-0·4,0·2)	-0·40 (-0·9,0·6)	0·26
Systolic Blood Pressure (mmHg)	0·0 (-9·0,18·0)	-0·5 (-9·5,10·5)	-0·5 (-18·0,12·0)	0·68
Heart Rate (BPM)	7·5 (-5·0,15·0)	5·0 (-2·0,12·5)	-2·50 (-9·5,10·0)	0·89
Respiratory Rate (BPM)	4·0 (2·0,6·0)	2·0 (1·0,6·0)	-2·0 (-3·0,2·0)	0·36
SpO2 Room Air	0·0 (-1·0,1·0)	0·0 (-2·0,1·0)	0·0 (-2·0,1·0)	0·59
FENO (ppb)	0·0 (-7·0,11·0)	-0·5 (-14·0,26·5)	-0·5 (-14·5,24·5)	0·95
FEV ₁ (% Predicted)	-10·6 (-16·2,-4·9)	-15·1 (-22·9,-4·7)	-4·5 (-12·9,5·0)	0·31
FEV ₁ /FVC	-3·0 (-6·0,0·0)	-5·0 (-8·5,-1·9)	-2·0 (-5·3,-2·0)	0·11
ACQ5 Total Score	0·8 (0·4,2·4)	1·0 (0·2,1·4)	0·2 (-1·2,0·6)	0·47
Total cell count x10 ⁶ /g (n=24)	2·5 (1·5,14·4)	-0·3 (-0·9,0·9)	-2·7 (-14·5,-1·0)	0·007
Sputum Neutrophils % of cells(n=24)	28·3 (14·7,45·8)	7·1 (-21·8,20·5)	-21·1 (-51·1,-3·8)	0·007
Sputum Neutrophils Absolute Cell Count (cells/g)(n=24)	2·1 (1·4,10·4)	0·2 (-0·7,0·7)	-1·9 (-10·2,-1·0)	0·014
Sputum Eosinophils % of cells(n=24)	-0·2 (-1·5,0·3)	3·3 (-0·7,5·3)	3·5 (-2·2,6·1)	0·068
Sputum Eosinophils Absolute Cell Count (cells/g)(n=24)	0·0 (-0·0,0·0)	0·1 (0·0,0·2)	0·07 (0·03,0·2)	0·005
Sputum Lymphocyte % of cells(n=24)	0·0 (-0·5,0·0)	1·3 (0·0,2·8)	1·3 (0·0,2·8)	0·005
Sputum Macrophages% of cells(n=24)	-24·4 (-31·3,-15·0)	-6·6 (-12·6,9·8)	17·7 (6·7,38·8)	0·006
Data are presented as mean (SD), median (IQR) and count (%). Independent sample T-tests and Mann-Whitney U tests tests were used to compare groups				

Table E10: Detection of pathogens on qPCR and associated sputum neutrophil (%) count, including detection of viruses, bacteria detected at any level on qPCR and bacteria detected above the threshold of 10^6 genome copies/ml. A) Clinically stable and established on mepolizumab with paired sputum differential cell count and qPCR (n=60). B) First exacerbation with sputum per participant with paired sputum differential cell count and qPCR (n=33). C) All exacerbations with paired sputum differential cell count and qPCR (n=45).

	Number positive (%)	Positive: Sputum neutrophils (%)	Negative: Sputum neutrophils (%)	p
A) Clinically stable (n=60)				
Virus	7 (11.6)	49.6 (43.6, 62.2)	43.2 (26.8, 71.5)	0.41
Bacteria at any level of detection	32 (53.3)	58.1 (30.5, 78.1)	39.1 (20.3, 65.0)	0.08
Bacteria detected $>10^6$ genome copies/ml	22 (36.7)	59.3 (29.5, 92.0)	41.2 (29.4, 64.7)	0.13
Virus or Bacteria at any level of detection (genome copies/ml)	35 (58.3)	55.9 (30.7, 75.8)	38.0 (18.5, 65.3)	0.11
Virus or bacteria detected $>10^6$ copies/ml	27 (45.0)	57.2 (30.7, 83.0)	40.3 (23.6, 65.3)	0.15
B) First exacerbation with sputum per participant (n=33)				
Virus	12 (36.3)	87.2 (60.8, 92.9)	70.8 (37.1, 94.7)	0.56
Bacteria at any level of detection	22 (66.7)	83.7 (53.5, 94.8)	70.8 (36.5, 88.5)	0.35
Bacteria detected $>10^6$ genome copies/ml	13 (39.4)	94.7 (62.3, 97.7)	64.1 (36.8, 88.4)	0.025
Virus or Bacteria at any level of detection (genome copies/ml)	27 (81.8)	86.0 (53.5, 94.8)	53.9 (36.5, 72.5)	0.19
Virus or bacteria detected $>10^6$ copies/ml	20 (60.6)	89.8 (65.5, 95.5)	50.5 (36.5, 70.8)	0.002
C) All exacerbations with sputum (n=45)				
Virus	16 (35.6)	87.2 (56.5, 94.7)	70.8 (36.2, 91.9)	0.39
Bacteria at any level of detection	27 (60.0)	85.5 (50.5, 94.8)	69.8 (36.2, 88.5)	0.20
Bacteria detected $>10^6$ genome copies/ml	15 (33.3)	87.1 (59.4, 97.7)	64.1 (36.2, 90.7)	0.07
Virus or Bacteria at any level of detection (genome copies/ml)	33 (73.3)	85.5 (50.5, 94.8)	53.9 (35.8, 85.6)	0.18
Virus or bacteria detected $>10^6$ copies/ml	26 (57.8)	88.4 (59.4, 95.1)	50.5 (35.5, 80.5)	0.011
Data are presented as mean (SD), median (IQR) and count (%). Independent sample T-tests, Mann-Whitney U tests and chi-square tests were used to compare groups				

Figure E3. Paired sputum when stable and at first exacerbation with sputum per participant (n=24).

Analysis of paired sputum when stable on mepolizumab and first exacerbation with sputum per exacerbator: Nine of the paired samples (38%) had elevated sputum eosinophils ($\geq 2\%$) both when stable on mepolizumab and at exacerbation. For these 9 patients, median sputum eosinophils was 14% (3.3,36.5) when stable on mepolizumab and 20.5% [5.3, 39.0] at exacerbation. At exacerbation, median FENO was 57 (40,138), FEV₁ was 59% (13). Eight of the paired samples had a low sputum eosinophil count ($< 2\%$) when stable on mepolizumab and at exacerbation, with a median sputum eosinophil count of 0.12% (0.0,0.8) when stable on mepolizumab and 0.2% (0.04,0.7) at exacerbation. At exacerbation, median FENO was 24 ppb (16,72), FEV₁ 64% (17). Four patients had a sputum eosinophil count of less than two percent (range 0% to 1.25%) when stable on mepolizumab and two percent or greater (range of 2.9% to 5.5%) at exacerbation. Three patients had an eosinophil count of two percent or greater when stable on mepolizumab (range 2.5 to 3%) and less than two percent at exacerbation (range 0.75% to 1%).

Online supplement references:

1. NICE 2017. 1 Recommendations | Mepolizumab for treating severe refractory eosinophilic asthma | Guidance | NICE. 2017 [cited 2020 Jan 21]; URL:<https://www.nice.org.uk/guidance/TA431/chapter/1-Recommendations> Accessed 10 Mar 2020
2. Consortium SS medicines. mepolizumab (Nucala) [Internet]. Scottish medicines consortium. 2016 [cited 2020 Mar 22]. URL: <https://www.scottishmedicines.org.uk/medicines-advice/mepolizumab-nucala-fullsubmission-114916/> Accessed 10 Mar 2020
3. Juniper EF, O'Byrne PM, Guyatt GH, Ferrie PJ, King DR. Development and validation of a questionnaire to measure asthma control. *Eur Respir J*. 1999;**14**(4):902–7.
4. Juniper EF, Bousquet J, Abetz L, Bateman ED. Identifying “well-controlled” and “not well-controlled” asthma using the Asthma Control Questionnaire. *Respir Med*. 2006;**100**(4):616–21.
5. Juniper EF, Guyatt GH, Cox FM, Ferrie PJ, King DR. Development and validation of the Mini Asthma Quality of Life Questionnaire. *Eur Respir J*. 1999;**14**(1):32–8.
6. Jones PW. St. George's respiratory questionnaire: MCID. In: *COPD: Journal of Chronic Obstructive Pulmonary Disease*. 2005;**2**:75–9.