Mepolizumab decreases tissue eosinophils while increasing type-2 cytokines in eosinophilic chronic rhinosinusitis

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Abstract

Background: Eosinophilic chronic rhinosinusitis is an often treatment-resistant inflammatory disease mediated by type-2 cytokines, including interleukin (IL)-5. Mepolizumab, a monoclonal antibody drug targeting IL-5, has demonstrated efficacy and safety in inflammatory airway disease, but there is negligible evidence on direct tissue response. The study aim was to determine the local effect of mepolizumab on inflammatory biomarkers in sinonasal tissue of eosinophilic chronic rhinosinusitis patients. *Methods:* Adult patients with eosinophilic chronic rhinosinusitis received 100mg mepolizumab subcutaneously at four-weekly intervals for 24 weeks in this prospective phase 2 clinical trial. Tissue eosinophil counts, eosinophil degranulation (assessed as submucosal eosinophil peroxidase deposition by immunohistochemistry) and cytokine levels (measured in homogenates by immunoassay) were evaluated in ethmoid sinus tissue biopsies collected at baseline and at weeks 4, 8, 16 and 24. *Results*: Twenty patients (47.7±11.7 years, 50% female) were included. Sinonasal tissue eosinophil counts decreased after 24 weeks of treatment with mepolizumab (101.64±93.80 vs 41.74±53.76 cells per 0.1mm ^{2}; p=0.035), eosinophil degranulation remained unchanged (5.79±2.08 vs 6.07±1.20, p=0.662), and type-2 cytokine levels increased in sinonasal tissue for IL-5 (10.84±18.65 vs 63.98±50.66, p=0.001), IL-4 (4.48±3.77 vs 9.38±7.56, p=0.004), IL-13 (4.02±2.57 vs 6.46±3.99, p=0.024) and GM-CSF (1.51±1.74 vs 4.50±2.97, p=0.001). *Conclusions:* Mepolizumab reduced eosinophils in sinonasal tissue, demonstrating that antagonism of IL-5 suppresses eosinophil trafficking. With reduced tissue eosinophils, a local type-2 inflammatory feedback loop may occur. The study exposes mechanistic factors which may explain incomplete treatment response.

Original Article

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Short title: Tissue effect of mepolizumab in chronic rhinosinusitis

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Conflict of Interest

Richard J Harvey is a consultant with Medtronic, Stryker, Novartis, Meda, and NeilMed pharmaceuticals. Research grant funding received from Glaxo-Smith-Kline and Stallergenes. He has been on the speakers' bureau for Glaxo-Smith-Kline, Meda Pharmaceuticals and Seqirus. Janet Rimmer has honoraria with Sanofi Aventis, Novartis, Mundipharma, BioCSL, and Stallergenes. Larry H Kalish is on the speakers' bureau at Mylan, Sequiris and Care Pharmaceuticals. Raymond Sacks is a consultant for Medtronic and is on the speaker's bureau for Meda Pharmaceuticals. All other authors have no financial disclosures or conflicts of interest.

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Methods: Adult patients with eosinophilic chronic rhinosinusitis received 100mg mepolizumab subcutaneously at four-weekly intervals for 24 weeks in this prospective phase 2 clinical trial. Tissue eosinophil counts, eosinophil degranulation (assessed as submucosal eosinophil peroxidase deposition by immunohistochemistry) and cytokine levels (measured in homogenates by immunoassay) were evaluated in ethmoid sinus tissue biopsies collected at baseline and at weeks 4, 8, 16 and 24.

Results : Twenty patients (47.7±11.7 years, 50% female) were included. Sinonasal tissue eosinophil counts decreased after 24 weeks of treatment with mepolizumab (101.64±93.80 vs 41.74±53.76 cells per 0.1mm^2;

p =0.035), eosinophil degranulation remained unchanged (5.79±2.08 vs 6.07±1.20, p=0.662), and type-2 cytokine levels increased in sinonasal tissue for IL-5 (10.84±18.65 vs 63.98±50.66, p =0.001), IL-4 (4.48±3.77 vs 9.38±7.56,p =0.004), IL-13 (4.02±2.57 vs 6.46±3.99, p =0.024) and GM-CSF (1.51±1.74 vs 4.50±2.97, p =0.001).

Conclusions: Mepolizumab reduced eosinophils in sinonasal tissue, demonstrating that antagonism of IL-5 suppresses eosinophil trafficking. With reduced tissue eosinophils, a local type-2 inflammatory feedback loop may occur. The study exposes mechanistic factors which may explain incomplete treatment response.

Key words

Biologics, chronic rhinosinusitis, eosinophils, mepolizumab, type-2 inflammation

Introduction

Chronic rhinosinusitis (CRS) is a heterogeneous inflammatory condition of the upper airways that often has a profound negative impact on sufferers' quality of life and exacts a major societal economic burden.[1] CRS is a complex disease consisting of several disease variants, divided by endotype dominance into type-2 or non type-2. The type-2 CRS endotype generally translates to an eosinophilic CRS (eCRS) phenotype and is diagnosed by marked infiltration of eosinophils in sinonasal mucosa [eosinophil count >10/high power field (HPF 400x) on histopathological analysis].[1] eCRS is associated with high rates of treatment resistance and post-treatment recurrence,[2]accentuating the need to develop targeted therapies for eCRS.

Type-2 inflammation in CRS is driven by an accumulation of type-2 innate lymphoid (ILC2) cells, type-2 T helper (Th2) cells, and cytokines, including interleukin (IL)-5, granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-4, and IL-13, that work in concert to generate eosinophilic infiltration in sinonasal tissue.[3,4, 5]

On the basis of IL-5's actions as an upstream cytokine that stimulates eosinophils,[3] blocking this cytokine is a promising treatment approach in eCRS. Mepolizumab, a humanised IgG1 monoclonal antibody (mAb) drug, binds with and neutralises IL-5.[6, 7] Mepolizumab has shown efficacy in refractory eosinophilic asthma,[6, 8-10] which commonly co-occurs with eCRS in a 'united airway disease'.[11] However, only two asthma trials have studied the effect of mepolizumab in bronchial mucosa,[12, 13] and there are no data regarding the effect of the drug on cytokines.[14] Mepolizumab has been shown to reduce the total eosinophil count in bronchial mucosa in asthma.[12] Mepolizumab reduces nasal polyp size and need for surgery in CRS.[15-17] The effect of mepolizumab in sinonasal tissue and in the eCRS phenotye have not yet been reported. By examining the effects of mepolizumab on local tissue, more knowledge may be gained about the drug's mechanism of action and underlying disease processes. The study aim was to assess tissue outcomes following treatment of severe eCRS with mepolizumab.

Methods

Study design

A prospective open-label single-arm phase II clinical trial of patients undergoing mepolizumab treatment at a tertiary Ear, Nose and Throat practice was performed (trial registration ID ACTRN12618000113257). The study received ethics approval from St Vincent's Hospital Human Research Ethics Committee (REGIS ID 2019/PID04424). Written informed consent was obtained from all patients prior to study participation.

Patient population

Adult patients (>18 years) diagnosed with eCRS who had undergone endoscopic sinus surgery and whose upper airway disease was not controlled with long term continuous oral corticosteroids were eligible for participation. Exclusion criteria included patients previously treated with mepolizumab, mepolizumab hypersensitivity, established immunodeficiency, cystic fibrosis, pregnancy, lactation and hypereosinophilic syndromes.

Patients were recruited to receive 100mg subcutaneous mepolizumab (GlaxoSmithKline, UK) at four-weekly intervals for 24 weeks. Demographic characteristics, including age, gender, asthma status, atopy, smoking status, date of previous surgery, and use of intranasal and systemic corticosteroid medication, were recorded. Asthma status was determined by either a 15 percent change in forced expiratory volume in 1 second on spirometry with challenge testing or β-agonist use, or current use of regular inhaled bronchodilator or corticosteroid therapy. Atopy was determined by an spIgE automated immunoassay (ImmunoCAPVR, Thermo Fisher Scientific, Massachusetts, United States). Concentration of any allergen group >0.35 kUA/L was classed as a positive result. The grass mix consisted of *Lolium perenne* , *Phleum pratense* ,*Poa pratensis* (Kentucky blue), *Sorghum halepense*(Johnson), *Paspalum notatum* (Bahia) and *Cynodon dactylon*(Bermuda). The dust mite mix contained *Dermatophagoides pteronyssinus* , *Dermatophagoides farina* and *Blatella germanica* (German cockroach). The mould mix comprised *Penicillium chrysogenum* , *Cladosporium herbarum* , *Aspergillus fumigatus*and *Alternaria alternata* . The animal epithelium mix included*Felis domesticus* (Cat), *Equus caballus* (Horse), *Bos Taurus* (Cow) and *Canis familiaris* (Dog). Smoking status was defined by having smoked within the last 12 months. Medication name and dosage were recorded at screening and reviewed at each treatment visit. All trial participants were precluded from changing medication in the four weeks prior to commencement of mepolizumab treatment.

Blood eosinophil measurement

Blood was collected via venepuncture. Blood eosinophil count, as an indicator of systemic inflammation, was measured on full blood count (Haematology Analyzer, DxH 800, Beckman Coulter, Brea, United States) and expressed as $x10^9$cells/μL.

Tissue collection

Two ethmoid sinus tissue biopsies were collected by an Ear, Nose and Throat surgeon at baseline and at weeks 4, 8, 16 and 24 following the administration of local anaesthetic to each nostril (300μL, 5% lignocaine hydrochloride/0.5% phenylephrine hydrochloride). One biopsy was fixed in 10% neutral buffered formalin for histopathology and the second snap-frozen in liquid nitrogen, and stored at -170°C until further processing.

Histopathology of general inflammatory markers

Formalin-fixed samples were processed with standard haematoxylin and eosin (H&E) staining and immunohistochemistry (IHC). A structured, manual histopathological assessment congruent with current CRS diagnostic criteria[18] was performed for each H&E-stained section by an anatomical pathologist (PE) in a blinded manner. Overall degree of inflammation (defined by extent of general inflammatory cell infiltrate, oedema and tissue distortion), sub-epithelial oedema, fibrosis, basement membrane thickening, hyperplastic papillary change, mucosal ulceration, squamous metaplasia, and tissue neutrophil infiltrate per HPF (400x) were each scored according to subcategories.

Tissue eosinophil count on whole slide image

As an alternative method for histopathological assessment of samples, glass slides were prepared for whole slide image (WSI) acquisition using previously described methods.[19] The slides were digitalised (Ventana DP 200, Roche, Basel, Switzerland). Component TIF image tiles from each slide were then imported into the open source digital pathology software QuPath version 0.2.3.[20] In order to more precisely quantify tissue eosinophils, two $0.1mm^2$ fields in sub-epithelial layers in each H&E-stained tissue section with high eosinophil density were selected on QuPath. Areas of crush were not selected. Crush areas were defined as abnormally elongated dark thinned nuclei in stroma.[21]Eosinophils were independently counted on H&E-stained tissue sections by the first author (SW) and a senior anatomical pathology registrar (SL) in a blinded manner. Eosinophils in the epithelium and intravascular space were excluded.

The eosinophil cell count for each of the two reviewers was calculated as the average number of eosinophil cells across the two $0.1mm^2$ areas and was expressed as eosinophils per $0.1mm^2$. The final eosinophil cell count was calculated as the mean of the two reviewers' scores.

Immunohistochemistry staining

To assess eosinophil degranulation, the second slide per sinonasal biopsy was immunostained for an eosinophil cytotoxic granule protein, eosinophil peroxidase (EPX), using primary anti-EPX antibodies (rabbit polyclonal anti-human EPX, 1:400 dilution, ab238506, Abcam, Cambridge, United Kingdom) (no retrieval) and the Ventana BenchMark Ultra automated staining system (Ventana Medical Systems, Inc., Tucson, United States). Immunoreactivity of EPX antibodies was enhanced with Ventana OptiView detection kit (Ventana Medical Systems, Inc., Tucson, United States). EPX-stained slides were compared with positive ovarian cancer controls. Slides were counter-stained with haematoxylin, and dehydrated at the end of the procedure. Positive cells and degranulation products stained brown.

Scoring of eosinophil degranulation

Eosinophil degranulation was assessed by a semi-quantitative scoring system, adapted from previously reported scoring systems.[13, 22] EPX was assessed in the sub-epithelial stroma on IHC-stained tissue sections by two independent reviewers (SW, SL) in a blinded manner. A total numerical value (0-10) was assigned to each tissue section. The score was calculated as the sum of three parameters: overall staining intensity (0=minimal, 0.5=moderate, 1=excessive), percent area of staining (0=<1%, 1=1-24%, 2=25-49%, 3=50-74%, 4=75-100%), and location of staining (0=no eosinophils/no EPX release, 1=eosinophils present/no detectable EPX release, 2=EPX release limited to areas surrounding eosinophils, 3=nominal deposition, 4=considerable deposition, 5=dense deposition).

Tissue homogenate preparation

As previously described,[23] 1mL of 0.9% NaCl and EDTA-free protease inhibitor (Roche, Basel, Switzerland) was added per 0.1g of frozen tissue. Tissue was disrupted using a bead mill homogeniser (FastPrep-24 5G, MP Biomedicals, Santa Ana, United States) at 6m/sec, 40 sec, over 4 cycles at 4°C, with 180 sec between runs, in 2mL tubes with a single 3.175mm stainless steel bead (Metal Bead Lysing Matrix 1/8" Matrix S, MP Biomedicals, Santa Ana, United States). Homogenates were centrifuged (10,000xg, 10 min, 4°C) (Heraeus Fresco 17 Centrifuge, Thermo Fisher Scientific, Waltham, United States). [37] Supernatants were aliquoted into microcentrifuge tubes (Eppendorf, Hamburg, Germany), and stored at -80°C until analysis.

Cytokine analysis

Cytokine levels in tissue homogenates were measured using the MILLIPLEX Human High Sensitivity T Cell Magnetic Bead Panel HSTCMAG-28SK (Merck Millipore, Darmstadt, Germany), which contained beads for IL-1β, IL-4, IL-5, IL-10, IL-13, GM-CSF, and TNF-α.

The protein concentration of samples was determined using Bradford Protein Assay (Bio-Rad Laboratories, Hercules, United States), according to the manufacturer's instructions and read by a spectrophotometer (FLUOstar Omega, BMG LabTech, Ortenberg, Germany) at 595nm. The immunoassay was run according to the manufacturer's protocols. Samples were diluted with assay buffer to a standard total protein concentration of 2mg/mL in a total volume of 50µL. Plates containing standards, controls and samples were read using a validated and calibrated Bio-Plex MAGPIX Multiplex Reader (Bio-Rad, Hercules, United States). Median fluorescent intensity data were analysed using a regression equation for the standard curve to calculate the unknown cytokine concentrations in sample and control wells.

Statistical Analysis

The primary endpoint was comparison of tissue outcomes between baseline and 24 weeks (six months) of treatment with mepolizumab. Statistical analyses were performed using SPSS version 26 (SPSS Inc., United States),[24] and graphs were presented using GraphPad Prism 8 version 8.2.1 (GraphPad Software, United States).[25] Descriptive statistics were given for demographics, baseline characteristics, and outcome measures. Mean and standard deviation (SD) were summarised for normally distributed continuous variables. Median and interquartile range (IQR) were summarised for continuous variables that were not normally distributed and for ordinal variables. Frequencies were summarised for nominal variables. Parametric results were

expressed as mean±1 SD. Non-parametric results were expressed as median [IQR]. Paired samples t-test was utilised for continuous data. For ordinal data with less than four levels, data was transformed into binary nominal variables. Pairwise analysis for baseline versus Week 24 was performed using McNemar's test for proportions. For variables scored by more than one reviewer, the average measures Intraclass Correlation Coefficient was calculated. All p -values were 2-tailed and a value of <0.05 was considered statistically significant.

Results

Study population

Twenty participants were included (age 47.72±11.65 years, 50% female), of whom 100% had comorbid asthma and 55% were atopic (Table 1). Time since last endoscopic sinus surgery was 4.1±3.5 years. Intranasal corticosteroids were used by 50% and oral steroids by 10% patients prior to study enrolment.

Blood eosinophil count

At the end of treatment at Week 24, blood eosinophils were depleted (0.48±0.28 vs 0.08±0.07x10^9cells/L, p=<0.001), representing an 83% reduction from baseline (Figure 1).

Tissue eosinophil count

There was a decreased tissue eosinophil count from baseline at Week 24 of treatment (101.64±93.80 vs 41.74±53.76 cells/0.1mm^2, p =0.035) (Figure 1, 2), representing a reduction of 59%. Interobserver scoring of tissue eosinophil count demonstrated excellent agreement (ICC 0.984).

Eosinophil degranulation

When sinonasal biopsies were stained with a high-affinity antibody to detect both intracellular and released EPX, there was no change in staining from baseline after mepolizumab (5.79±2.08 vs 6.07±1.20, p=0.662), despite a 59% reduction in intact eosinophils. Interobserver scoring of EPX staining showed excellent agreement (ICC 0.976).

General tissue inflammatory markers

Mepolizumab reduced the proportion of patients with moderate or severe tissue inflammation (100% vs 68%, p=0.031) and subepithelial oedema (100% vs 63%, p=0.016) from baseline at treatment conclusion (Table 2). Mepolizumab did not change the following histopathological outcomes in sinonasal tissue from baseline at Week 24: neutrophils, inflammatory predominance, basement membrane thickening, hyperplastic papillary change, mucosal ulceration, squamous metaplasia and fibrosis.

Cytokine concentration

Mepolizumab treatment increased type-2 biomarker levels in sinonasal tissue at Week 24 vs baseline for IL-4 (4.48±3.77 vs 9.38±7.56,p =0.004), IL-5 (10.84±18.65 vs 63.98±50.66, p =0.001), IL-13 (4.02±2.57 vs 6.46±3.99, p =0.024) and GM-CSF (1.51±1.74 vs 4.50±2.97, p =0.001), as well as increased the regulatory cytokine IL-10 (5.07±3.98 vs 9.74±6.00, p =0.006) (Figure 3). There was no change in type-1 pro-inflammatory cytokines IL-1β (6.94±19.66 vs 1.41±1.24, p =0.264) and TNF-α (4.92 ± 7.08 vs 5.78 ± 2.98,p =0.625).

Discussion

Previous studies found that mepolizumab decreased symptoms of CRSwNP.[15-17] However, those studies did not investigate mepolizumab-induced changes to eosinophilic inflammation in target sinonasal tissue. There is also negligible data about the response of local tissue to mepolizumab in other eosinophilic diseases.[12, 13]

A key finding in the current study was a reduction in tissue eosinophils with mepolizumab. This study found a reduction in tissue eosinophils of 59% versus a reduction in blood eosinophils of 83%. This finding is consistent with the only other study – an asthma trial – to have assessed the drug's effect on airway

tissue eosinophils.[12] The magnitude of the mepolizumab-mediated decrease in airway eosinophil numbers in the present study (59%) was similar to the reduction (55%) in the asthma study.[12] The paucity of tissue eosinophils at Week 24 may be largely attributable to reduced eosinophil production in bone marrow and reduced trafficking of the cell to tissue, which is demonstrated by reduced blood eosinophil levels. IL-5 promotes eosinophilopoiesis in bone marrow,[26, 27] and introducing an anti-IL-5 agent curtails bone marrow production of the cell.[26] Eosinophils in tissue may live up to several weeks,[28-30] compared to the lifespan of an eosinophil in circulation, which is approximately 24 h.[31] Given the study duration is sufficiently long to account for the difference in half-life between tissue eosinophils and blood eosinophils, factors other than half-life presumably account for fewer circulating eosinophils and a relatively higher number in tissue. Resident tissue eosinophils, with an immunosuppressive and regulatory role in airway tissue, may account for the cells persisting in tissue, despite very low circulating eosinophils.[32] However, it remains unclear whether the remaining eosinophils found in sinonasal tissue of treated patients are inflammatory or resident tissue eosinophils.

The finding of a reduced tissue eosinophil count with mepolizumab is clinically salient because high tissue eosinophil counts are strongly associated with polyp recurrence.[33]

A study component was the investigation of eosinophil degranulation in view of its purported role in eCRS pathophysiology.[34, 35] Activation and degranulation of eosinophils involves the extra-cellular release of pre-formed granules, including cytotoxic proteins (major basic protein, EPX and eosinophil cationic protein), which mediate sinonasal pathology through epithelial damage and fibrosis.[35-37] In contrast to cationic protein and major basic protein, EPX is an eosinophil-specific granule protein.[36, 38, 39] Interestingly, the current study found no change in EPX staining following mepolizumab. In an asthma study, sputum EPX concentration also did not change in responders to mepolizumab, but was elevated if symptoms worsened post-treatment.[40]

This study was the first to investigate mepolizumab's effect on cytokine concentrations in any disease-specific tissue. In line with this study's finding of increased IL-5 in tissue, increased IL-5 after mepolizumab has been found in bronchiolar lavage fluid[12, 13] and plasma of asthma patients.[41-43] Sputum IL-5 was also elevated in asthma patients who showed inadequate response to mepolizumab.[40] IL-5 signalling impacts on key stages of the eosinophil lifecycle, including mobilisation from bone marrow, migration in circulation, and trafficking to and survival in tissue, which generates tissue eosinophilia.[30, 44-47] It is possible that the assay in the current study measures IL-5 bound to mepolizumab. A future direction would involve evaluating the concentration of IL-5 bound to mepolizumab when a commercially anti-mepolizumab antibody becomes available. Elevated levels of IL-5 may also suggest inadequate neutralisation of this key eosinophilic cytokine, and may indicate treatment underdosing, which has been raised as a concern in a previous report.[48] A recent study[40] found elevated IL-5 immunoglobulin-G immune-complex deposition in the sputa of asthma patients who worsened on mepolizumab therapy – the autoimmune-mediated pathology may have sustained the eosinophilic inflammation and prevented symptom resolution.[40]

There are several possible explanations for the increase in IL-5 in sinonasal tissue in the current study. First, the functional absence of IL-5 (bound to mepolizumab) and/or reduction in eosinophils may trigger a feedback loop, stimulating local tissue cells known to release IL-5, such as ILC2 cells, Th2 cells, and mast cells.[49, 50] A similar feedback loop may also occur systemically, given reports of increased plasma IL-5 with mepolizumab in other eosinophilic diseases.[41-43, 51] Second, a decreased rate of elimination of IL-5 from tissue, when bound in a mepolizumab-IL-5 complex compared with free ligand, may be responsible.[52-54]

The study found a significant increase in the tissue concentration of other type-2 cytokines IL-4, IL-13 and GM-CSF with mepolizumab. Similar to the possible mechanism for IL-5 upregulation after mepolizumab, the decrease in airway eosinophils may have stimulated local production of other type-2 cytokines in a feedback pathway. Increased type-2 cytokines after mepolizumab, including IL-4, IL-13 and GM-CSF, may provide another explanation for the lack of complete removal of blood and tissue eosinophils.[12, 13, 55, 56] Cytokines other than IL-5 have an ability, albeit weaker than IL-5, to stimulate bone marrow and local tissue eosinophilopoiesis.[12, 26, 45, 57-59] For the first time, the present study provides evidence that, after mepolizumab administration, increased type-2 cytokines in tissue may be partly responsible for continued

bone marrow eosinophilopoiesis, via distant signalling, and local tissue eosinophilopoiesis. The study also found increased IL-10 in sinonasal tissue. Evidence suggests that IL-10 acts as an anti-inflammatory cytokine through its inhibition of release of cytokines such as GM-CSF and IL-5, thereby reducing eosinophilic inflammation.[60]

The upregulation of type-2 cytokine concentrations, particularly the large increase in IL-5 concentration, raises concerns that ceasing mepolizumab might exacerbate type-2 inflammation. In a clinical trial of another anti-IL-5 drug (reslizumab), there was a rebound increase in eosinophil levels following treatment discontinuation.[61] In patients treated with an anti-IL-4 and anti-IL-13 drug (dupilumab), a transient increase in blood eosinophils was not associated with disease worsening.[62] Therefore, while the reduced level of eosinophils found in the current study may be clinically beneficial, this reduction is not the complete answer to disease control. There has been no rebound effect described for mepolizumab in the 12-month period following its stoppage.[63] Nevertheless, monitoring of patients after mepolizumab cessation in further drug trials and clinical practice is advisable in light of the potential risk of recurrent disease.

There was no change in non-type-2 cytokines IL-1β and TNF-α after mepolizumab therapy. TNF-a and IL-B are proinflammatory cytokines that enhance inflammation.[64] IL-4 has been reported to suppress IL-1B and TNF-a production.[65, 66] Elevated levels of IL-4 and IL-10 may have contributed to the lack of change in IL-1β and TNF-α.

Therefore, it is suggested that in patients with inflammatory airway disease, mepolizumab's antagonism of IL-5-mediated eosinophilopoiesis and eosinophil trafficking to tissue is responsible for reduced sinonasal eosinophils. The novel finding of upregulation of type-2 cytokines, particularly IL-5, in sinonasal tissue indicates that mechanistic factors, such as a possible mepolizumab-induced feedback loop, may explain incomplete response to therapy, highlighting the need for more data to improve current treatments.

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Tables

Table 1: Baseline demographic characteristics

Characteristic	**n=20**
Age (years) (mean±Standard Deviation)	47.7 ± 14.5
Gender (% female)	50
Years since last surgery (years)	4.1 ± 3.5
Asthma (%)	95
Atopy (%)	50
Smoking (%)	15
Intranasal corticosteroids (%)	50
Systemic corticosteroids (%)	10

n = number

Table 2: Effect of mepolizumab on tissue inflammatory markers

	Baseline n=20	24 Weeks n=19	p value
Degree of inflammation (% moderate/severe)	100	68	0.031
Subepithelial oedema (% moderate/severe)	100	63	0.016
Neutrophils (% present)	85	53	0.070
Basement membrane thickening (% >7.5um)	20	26	1.000
Hyperplastic papillary change (% present)	0	26	0.063
Mucosal ulceration (% present)	0	11	0.500
Squamous metaplasia (% present)	5	21	0.375
Fibrosis (% present)	20	32	0.688

n = number

Figure Legends

Figure 1: Effect of mepolizumab on blood and sinonasal tissue eosinophil levels. Blood samples and tissue biopsies were collected during baseline, 4, 8, 16, 24 week visits, prior to Mepolizumab administration. Blood eosinophil levels and tissue eosinophil counts per $0.1mm^2$ were evaluated and represented as mean values±standard deviation of 20 eCRS participants.

Figure 2: Effect of mepolizumab on sinonasal tissue eosinophil levels. Representative microscopic images of medium power view of haematoxylin and eosin (H&E) (A, B) of a single patient at baseline (A) and end of treatment (B).

Figure 3: Cytokine response in sinonasal tissue following treatment with mepolizumab. Type-2 (A-D), type-1 (E, F) and regulatory (G) cytokine levels in homogenised sinonasal tissue biopsies were measured by immunoassay at baseline and end of treatment of 20 patients with eosinophilic chronic rhinosinusitis patients treated with mepolizumab. Data was statistically analysed by paired T tests. Data is presented as mean values +/- SD and analysed by paired T test. ns = P > 0.05; * = P [?] 0.05; ** = P [?] 0.01; *** = P [?] 0.001.

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