Clinical Trial Protocol

Protocol Title: Mepolizumab for the Treatment of Chronic Cough with Eosinophilic Airways Disease.

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The study will be performed in accordance with the ethical principles of the Declaration of Helsinki, and consistent with International Conference on Harmonization (ICH)/Good Clinical Practice (GCP) and applicable regulatory requirements. Approval will be obtained from the local ethics committee, and all patients provided written and informed consent.

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Table of Contents

List of Tables

List of Figures

List of Abbreviations

ACQ – asthma control questionnaire
COPD – Chronic obstructive pulmonary disease
EAR – Early asthmatic response
EB – Eosinophilic bronchitis
GERD – gastro-esophageal reflux disease
ICS – Inhaled corticosteroid
IL5 - Interleukin 5
LAR – Late asthmatic response
NAEB – Non-asthmatic eosinophilic bronchitis
OCS – Oral corticosteroid
RCT – randomized controlled trials
REB – Research Ethics Board
SOP – Standard operating procedure
TRPV1 – Transient receptor potential vanilloid type-1
WOCBP – Women of childbearing potential

1. Introduction

1.1. Study Summary

Cough is the most common presenting symptom to family physicians (1). Chronic Cough affects approximately 10-12% of the general population (2) and is one of the commonest reasons for referral to secondary care (3). Unfortunately, there are no licensed treatments for this debilitating condition, which is associated with a poor quality of life, affecting the social, physical and psychological well-being of patients (4). Asthma and non-asthmatic eosinophilic bronchitis (NAEB) are known causes of chronic cough and 30-50% of patients have evidence of eosinophilic airway diseases (5-7). Inhaled corticosteroids (ICS) are commonly used to treat cough in asthma and EB but there are no randomized controlled studies showing evidence of improvements in objective cough rates. Our group has recently demonstrated increased sputum eosinophils after allergen challenge to be associated with increased neuronal sensitivity and increased objectively measured cough frequency over 24 hours (8). Eosinophils have also been found to be co-localised with airway nerves (9). Increased airway eosinophilia in asthma and non-asthmatic eosinophilic bronchitis (NAEB) may be driving excessive coughing and this can potentially be targeted by anti-interleukin 5 (IL5) therapy.

Therefore, the aim of this single centre proof-of-concept study is, for the first time, to investigate whether mepolizumab reduces objective cough frequency in patients with eosinophilic asthma and NAEB presenting with chronic cough. Secondary outcomes including the effects on quality of life, intensity of irritant sensations, airway hyper-reactivity and inflammatory cells will also be evaluated.

1.2 Background and Rationale

Chronic Cough – An Unmet Need

Cough is the single most common reason for seeking medical attention (1). Many individuals experience acute coughing lasting up to three weeks after a viral respiratory tract infection. However, approximately 10% suffer from chronic cough (lasting more than eight weeks) with detrimental effects on quality of life (10-12). The prevalence increases with age, but peaks in the 50's and 60's and is twice as common in females than in males (13). The typical

history is described as a non-productive cough often occurring in distressing bouts, which the patient cannot control, usually preceded by an irresistible urge to cough associated with an irritating sensation in the throat. Cough can be evoked by innocuous triggers such as changes in temperature, strong smells from perfumes or aerosols, dust and passive smoking (14, 15). Chronic cough has major psychological, social and physical consequences (16). Individuals with chronic cough are embarrassed socially and in the workplace. They become physically exhausted by the frequent prolonged coughing bouts, and women may experience stress incontinence. The treatment options for chronic cough are limited. In the US, over $8 billion is spent each year on over-the-counter cough and cold medications, most of which contain dextromethorphan which has very limited if any clinical effectiveness (17). Unfortunately, there are no current licensed treatments for chronic cough, and this represents a major clinical unmet need.

Aetiology of Chronic Cough

Chronic cough can be associated with many respiratory conditions such as asthma, NAEB, bronchitis in smokers and chronic obstructive pulmonary disease (COPD), where the cough is typically related to the pathophysiology of the underlying disease, e.g. smoking in COPD or excess airway mucus. Other causes include eosinophilic bronchitis, interstitial lung diseases, bronchiectasis or use of angiotensin-converting enzyme inhibitors. Extra-pulmonary diseases such as gastro-esophageal reflux disease (GERD) and post-nasal drip secondary to rhinosinusitis can also be identified as potential triggers.

Observational studies detailing the aetiology of chronic cough in patients presenting to specialist cough clinics show large variations in the prevalence of asthma (6-36%), GERD (0-41%), and rhinitis (8-56%) (18). Furthermore, up to a quarter of patients may have multiple aetiologies combined (19). Non-asthmatic eosinophilic bronchitis was first described in 1989 at McMaster University as a potential cause of chronic cough (20). However, as access to measuring sputum eosinophilia is limited, the prevalence of NAEB is uncertain. Studies in specialist clinics where assessments of airway inflammation in chronic cough patients have been made, report between 10-30% as having evidence of eosinophilic airways (7, 21, 22).

Treatments for cough in asthma and non-asthmatic eosinophilic bronchitis

Both asthma and NAEB are associated with airway eosinophilia, but in the latter, there is no evidence of increased airway hyper-reactivity or variable airflow limitation, and cough is the predominant symptom. This has led to guidelines recommending a trial of treatment with ICS. However, the strength of the recommendation is weak due to a lack of evidence showing reduction in objective coughs rates in placebo-controlled studies (23). The use of ICS is well established in asthma from the large phase 3 randomized controlled trials (RCT), however, cough is not independently assessed in most studies, and current asthma questionnaires such as the asthma control questionnaire (ACQ) do not capture cough as a symptom. In patients with asthma or NAEB, use of inhaled corticosteroids can also make coughing worse, occasionally leading to use of low-dose oral corticosteroids. This can result in long term side effects which are intolerable to patients.

Airway Nerves, Eosinophils and Cough

Cough is the archetypal airway defensive reflex mediated by sensory afferents of the parasympathetic vagus nerves and its receptor profile on the nerve terminals. Activation of two main sub-types of sensory vagal afferent nerves is responsible for evoking cough (24):

I. C fibres: networks of un-myelinated nerves found throughout the airways, sensitive to chemical stimuli including inflammatory mediators (e.g. bradykinin, prostaglandins) and environmental stimuli (e.g. pollutants, temperature). These nerves can be activated by capsaicin, a specific agonist of transient receptor potential vanilloid type-1 (TRPV1)

II. A-delta fibres: proximal airways are also innervated by sub-epithelial myelinated nerves also known as 'cough receptors', as they evoke cough. They respond to punctate mechanical stimuli, low osmolarity and acidity.

To directly assess the role of airway eosinophils in asthma, coughing and neuronal sensitivity, we previously performed whole lung allergen challenge in patients with mild steroid naïve atopic asthma who demonstrated both an early and late asthmatic response (EAR/LAR) to inhaled allergen (8). We showed that capsaicin evoked coughs increased during the early asthmatic response but also remained heightened 24 hours after allergen when

bronchoconstriction had resolved. This heighted cough response was associated with an increase in sputum eosinophils from 3% to 15% and also increased objective hourly cough frequency. This suggests that in individual patients, an increase in airway eosinophilia, increases neuronal sensitivity which enhances the cough reflexes, resulting in an increase in spontaneous coughs. How eosinophils actually sensitise airway nerves is still unknown, however recent wholemount imaging studies in moderate to severe asthmatics has shown greater co-localisation of eosinophils and airway nerves in patients with moderate-severe asthma compared with healthy controls and patients with mild asthma (9). Furthermore, in an animal model, eosinophils mediated an increase in nerve density (9).

Mepolizumab as a treatment for chronic cough in eosinophilic airways diseases

Mepolizumab is a humanised monoclonal antibody against IL-5, which is currently licensed for treatment in patients with severe uncontrolled exacerbations. Interleuklin-5 is a key cytokine controlling eosinophil growth, differentiation, recruitment, activation and survival (25-27). Treatment with mepolizumab results in a reduction in serum eosinophils and sputum eosinophils (28, 29). The main clinical benefits are in the reduction in exacerbations requiring treatment bursts of oral corticosteroids (OCS) and/or hospitalisations by approximately 50-60% (29, 30). There have been no studies which have recruited patients with chronic cough or assessed objective cough frequency as a study outcome with anti-IL5 therapy.

Therefore, the aim of this study is to evaluate the effectiveness of mepolizumab for the treatment of chronic cough in patients with eosinophilic airways disease, including both asthma and NAEB.

2. Hypothesis

We hypothesise that in patients with asthma and NAEB, eosinophils are involved in sensitising airway nerves and thereby increasing spontaneous objective coughs. We therefore predict that treatment with mepolizumab will reduce airway eosinophilia in patients with chronic cough due to eosinophilic asthma and NAEB, thereby causing a reduction in objective cough frequency.

3. Study Aim

This is a proof-of-concept study to investigate if anti-IL-5 treatment aimed at reducing airway eosinophilia causes a reduction in objective cough frequency.

4. Study Objectives

Primary Objectives:

In patients with refractory chronic cough with airway eosinophilia to measure the effects of mepolizumab on 24-hour objective cough frequency.

Secondary Objectives:

1. To determine the effects of mepolizumab on:

   i. Awake /Night-time cough frequency
   ii. Intensity of irritant sensations
   iii. Quality of life
   iv. Airway hyper-responsiveness
   v. Inflammatory cells

All the above secondary objectives will also be analysed based on disease group, i.e., asthma and NAEB.

2. The relationship between change in cough frequency and change in eosinophils

5. Study Design

5.1. Design

This is a 9-visit randomized, double-blind, placebo controlled, parallel group study evaluating the effectiveness of 4 doses of mepolizumab for the treatment of refractory chronic cough in patients with eosinophilic airways disease (Figure 1). We plan to complete 15 subjects in each arm of the clinical trial. The duration of intervention will be 12 weeks, during which

subjects will receive 4 doses at days 0, 28, 56 and 84. The effects of mepolizumab on objective cough frequency will be tested at week 14, 2 weeks following the last dose of mepolizumab/placebo.

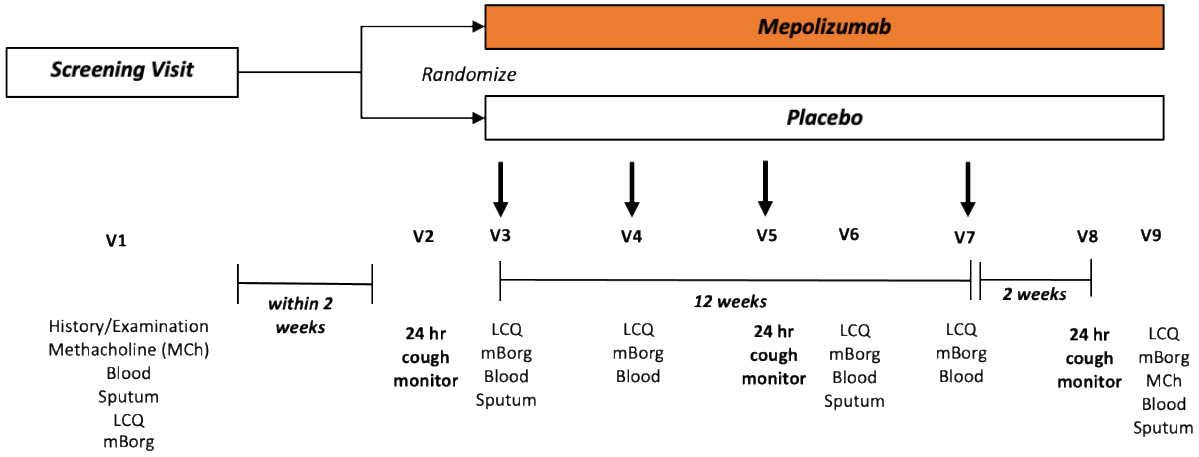

Figure 1. Study flow diagram detailing the study visits and procedures per visit per subject following enrolment.

5.2. Duration of Study

The total length of a subject's participation in the trial is up to 16 weeks;

- Up to 2 weeks screening period
- 14-week intervention period

5.3. Number of Sites

1. McMaster University, 1200 Main St West, Suite 3U31
   Hamilton, Ontario, Canada, L8N 3Z5

5.4. Study Period

Estimated date of first subject Q2 2021

Estimated date of last subject Q4 2022

5.5. Study Population

Male and female patients ≥ 18 years of age assessed in a secondary care clinic for chronic cough due to asthma and/or NAEB.

5.6. Inclusion Criteria

The following inclusion criteria must be met for patients to enter the screening phase of the study:

1. Male sex and female sex patients
2. Aged ≥18
3. Subjects with a history of chronic cough (cough lasting for >8 weeks)
4. Evidence of airway eosinophilia (sputum eosinophilia≥2%)
5. FEV1 ≥ 70% of predicted
6. Normal chest x-ray (within the last 2 years)
7. At least one dose of a COVID-19 vaccine a minimum of 2 weeks prior to enrollment

5.7. Exclusion Criteria

Patients should not enter the study if any of the following exclusion criteria are fulfilled:

1. Symptoms of upper respiratory tract infection in the last 1 month which have not resolved.
2. Lower respiratory tract infection or pneumonia in the last 1 month.
3. Subjects with a positive covid-19 test within 2 weeks of screening
4. Subjects with seasonal allergic rhinitis that affects their asthma control
5. Current smoker or ex-smoker with ≥20 pack year smoking history and abstinence of ≤6 months
6. Symptoms of uncontrolled asthma at screening defined as: an exacerbation in the previous month requiring oral prednisone or antibiotics.
7. Use of regular maintenance oral corticosteroids or long-acting muscarinic antagonist within 4 weeks prior to enrolment into the study.
8. A previous asthma exacerbation requiring Intensive Care Unit admission.
9. Significant other primary pulmonary disorders in particular; pulmonary embolism, pulmonary hypertension, interstitial lung disease, lung cancer, cystic fibrosis, emphysema or bronchiectasis.

10. Any history or symptoms of uncontrolled cardiovascular disease, particularly coronary artery disease, uncontrolled arrhythmias, uncontrolled hypertension, or uncontrolled congestive heart failure.
11. Any history or symptoms of significant neurologic disease, including transient ischemic attack, stroke, seizure disorder, or behavioral disturbances
12. Uncontrolled diabetes
13. End stage kidney or liver disease
14. Clinically significant abnormalities in laboratory test results during the screening period (including complete blood count, coagulation, electrolytes, liver function tests) unless deemed not significant by the investigator.
15. Any history or symptoms of clinically significant autoimmune disease
16. History of anaphylaxis to any biologic therapy or vaccine
17. History of Guillain-Barre Syndrome
18. A helminth parasitic infection diagnosed within 24 weeks prior to the date of informed consent is obtained that has not been treated with or has failed to respond to standard of care therapy.
19. Positive hepatitis B surface antigen, or hepatitis C virus antibody serology, or a positive medical history for hepatitis B or C. Subjects with a history of hepatitis B vaccination without history of hepatitis B can enroll.
20. A history of immunodeficiency disorders including a positive human immunodeficiency virus test (HIV)
21. Pregnancy or breast-feeding.
22. Women of childbearing potential (WOCBP) must not be actively seeking pregnancy, and must use an effective form of birth control (confirmed by the Investigator). Effective forms of birth control include: true sexual abstinence, a vasectomized sexual partner, Implanon, female sterilization by tubal occlusion, any effective IUD intrauterine device/IUS levonorgestrel Intrauterine system, Depo-Provera™ injections, oral contraceptive, and Evra Patch™ or Nuvaring™. WOCBP must agree to use an effective method of birth control, as defined above, from enrolment, throughout the study duration and within the 8 treatment weeks. They must demonstrate a negative serum pregnancy test at screening and demonstrate a negative urine pregnancy test immediately before

each dose of study drug or placebo. Women not of childbearing potential are defined as women who are either permanently sterilized (hysterectomy, bilateral oophorectomy, or bilateral salpingectomy), or who are postmenopausal. Women will be considered postmenopausal if they have been amenorrheic for 12 months prior to the planned date of randomization without an alternative medical cause. The following age-specific requirements apply:

i. Women <50 years old would be considered postmenopausal if they have been amenorrheic for 12 months or more following cessation of exogenous hormonal treatment and follicle stimulating hormone (FSH) levels in the postmenopausal range.
ii. Women ≥50 years old would be considered postmenopausal if they have been amenorrheic for 12 months or more following cessation of all exogenous hormonal treatment.

23. Male patients not using an acceptable method of contraception. All male patients who are sexually active must agree to use an acceptable method of contraception (condom with or without spermicide, vasectomy) from the first dose of study drug until their last dose.
24. Use of angiotensin-converting-enzyme inhibitors
25. Use of immunosuppressive medication (including but not limited to: methotrexate, cyclosporine, azathioprine, intramuscular long-acting depot corticosteroid, oral corticosteroid, or any experimental anti-inflammatory therapy) within 3 months prior to the date informed consent is obtained
26. Use of any other biological within 4 months or 5 half-lives prior to randomization, whichever is longer.
27. Any centrally acting medication within the last 2 weeks which in the view of the investigator could influence the coughing*
28. History of psychiatric illness, drug or alcohol abuse which may interfere in the participation of the trial.

**Any participant who is taking amitriptyline, dextromethorphan, pregabalin, gabapentin or opioids will not be eligible to take part in this study unless they are willing and medically able to*

withdraw from such medication for the duration of the study. The reason for this is that centrally acting medications may influence coughing rates.

5.8. Recruitment Process

Participants will be recruited by clinicians from clinics at either of the following sites:

a) McMaster University Medical Centre, Department of Respiratory Medicine, 1280 Main St. West, Hamilton, Ontario, Canada, L8S 4K1
b) Firestone Institute for Respiratory Health, St Joseph's Healthcare, 50 Charlton Ave E, Hamilton, ON L8N 4A6

All study visits will take place at McMaster University, Cardio-Respiratory Research Laboratory, Faculty of Health Sciences, 3U31, 1280 Main St. West, Hamilton, Ontario, Canada, L8S 4K1.

5.9. Informed Consent

Patients recruited to participate in the study will meet with study staff and receive the participant information sheet. Patients will be given the opportunity to ask study staff questions about the study. Patients who express an interest in participating in the study will provide their fully informed written and verbal consent to be enrolled in the study. Informed consent will be obtained at the screening visit before any study procedures are conducted. Patients will also be informed that mepolizumab is currently licensed in Canada for treatment of patients with severe eosinophilic asthma only. If a patient's chronic cough improves whilst on the active treatment, mepolizumab is currently not licensed from the treatment of chronic cough.

5.9.1. Associated Risks and Benefits to Subjects

Benefits

Subjects will benefit from regular assessment of lung physiology, blood and airway inflammation and cough frequency. Along with helping to advance our understanding of the mechanisms causing chronic cough and to develop potentially improved treatments, the subject will benefit from being reviewed by a chest specialist.

Risks

The common risk of treatment with mepolizumab from placebo-controlled studies is local injection site reaction. In 2 placebo-controlled studies the incidence of local injection site reactions with mepolizumab 100 mg subcutaneous and placebo was 8% and 3%, respectively. These events were all non-serious, mild to moderate in intensity and the majority resolved within a few days. Local injection site reactions occurred mainly at the start of treatment and within the first 3 injections with fewer reports on subsequent injections. The most common manifestations reported with these events included pain, erythema, swelling, itching, and burning sensation. A full list of adverse events are tabulated in section 8.3 below.

It is possible that subjects may develop an increase in their asthma symptoms during the with-holding of asthma medication before the methacholine challenge on visit 1. Should this happen, patients will be advised to re-start their medication and inform the principle investigator. There will be an on-call study physician who is contactable outside of working hours should advice be needed.

The main risk of performing a methacholine challenge is that of developing asthma symptoms, due to the fall in FEV_1. The American Thoracic Society (ATS) has published a detailed guideline for performing methacholine challenge, which we have adopted for our local standard operating procedure (SOP) to maintain international standards (31).

Sputum induction using hypertonic saline carried a small risk of a fall in lung function along with a salty unpleasant taste at the back of the mouth.

Precautions will be taken for patient safety such as the availability of monitoring equipment (spirometer, pulse oximeter, blood pressure monitor) and short acting bronchodilators (salbutamol and ipratropium via inhaler/nebuliser), oxygen, and resuscitation equipment.

Risk of contracting COVID-19 infection during study participation

As long as the COVID-19 pandemic is ongoing, there is a risk of contracting a SARS-CoV-2 infection during the time period of study participation. Participants will be asked about

symptoms and risks of COVID-19 infection prior to each study visit and on the day of the study visit. Participants who are at high risk will be asked to have a COVID-19 test performed at a local public health lab and must have received a negative test prior to attending the study site. Participants will be asked further information regarding symptoms suggestive of COVID-19 infection as part of the screening visit and subsequent visits (as applicable in accordance with guidance from the relevant regional and local public health authorities). Participants must not have had a positive test within 2 weeks of the screening visit. If a participant received a COVID-19 positive test during study participation, the investigator will have to decide whether remaining in the study is compatible with participant's and site personnel's safety and well-being.

5.10. Visit schedule

5.10.1. Pre-Study Screening and Baseline Evaluation

Prior to visit 1, all subjects will be provided with the Information Letter related to COVID-19 and asked to complete the Ontario Covid-19 Assessment Tool 24 hours and 1 hour prior to scheduled study visit.

Visit 1: Screening

Recruited patients will be invited to attend Visit 1 to undergo study screening. All subjects will undergo Ontario Covid-19 Assessment Tool and Information Letter. Patient eligibility will be assessed against the study inclusion/exclusion criteria and patients will undergo informed consent. Subjects who provide informed consent and are enrolled into the study will undergo screening procedures: complete medical history, physical examination, methacholine challenge, spirometry sputum induction, and blood sampling. Subjects will complete the Leicester Cough Questionnaire, the modified Borg Scale, and Cough Severity Visual Analogue Scale (VAS, 0-100mm) to assess severity and intensity of cough sensations including urge to cough, throat itch, throat tickle, and throat irrigation. If the sputum quality obtained during the screening visit is deemed of poor quality, then the subject will be requested to repeat the sputum induction procedure within 14 days of visit 1 to ensure an adequate sample is obtained for assessment. Following the screening visit, subjects will be booked into the 8 remaining study visits and randomized into the intervention (mepolizumab) arm or placebo arm. Subjects will be scheduled to return for Visit 2 within 2 weeks of the screening visit.

5.10.2. Treatment/Assessment Visits

Visit 2: Baseline Cough Monitoring

All subjects will undergo Ontario Covid-19 Assessment Tool and Information Letter prior to study visit. Enrolled subjects will wear an ambulatory cough monitor for 24 hours and be scheduled to return the following day for Visit 3.

Visit 3: Week 0 Administration of Mepolizumab/Placebo (1st Injection)

All subjects will undergo Ontario Covid-19 Assessment Tool and Information Letter prior to study visit. Subjects will return with the cough monitor 24 hours following Visit 2. If no recording was obtained during the past 24 hours due to recording device malfunction (such as battery or card failure) then the subject will be asked to repeat the recording within 24-48 hours of prior attempt at recording. Subjects will undergo spirometry, sputum induction, and blood sampling and complete the Leicester Cough Questionnaire, the modified Borg Scale, and the Cough Severity VAS to measure the severity and intensity of cough sensations. Urine will be collected in WOCBP to screen for pregnancy. The first injection of mepolizumab/placebo will be administered in the clinical research facility by a study physician. The subject will be observed for a minimum of 1 hour for the appearance of any acute drug reactions.

Visit 4: Week 4 Administration of Mepolizumab/Placebo (2nd Injection)

All subjects will undergo Ontario Covid-19 Assessment Tool and Information Letter prior to study visit. Subjects will return to the clinical research facility 4 weeks following Visit 3. Subjects will undergo blood sampling and complete the Leicester Cough Questionnaire, the modified Borg Scale, and the Cough Severity VAS. Urine will be collected in WOCBP to screen for pregnancy. The second injection of mepolizumab/placebo will be administered in the clinical research facility by a study physician. The subject will be observed for a minimum of 1 hour for the appearance of any acute drug reactions.

Visit 5: Week 8 Cough Monitoring and Administration of Mepolizumab/Placebo (3rd Injection)

All subjects will undergo Ontario Covid-19 Assessment Tool and Information Letter prior to study visit. Subjects will return to the clinical research facility 4 weeks following Visit 4. Urine will be collected in WOCBP to screen for pregnancy. The third injection of mepolizumab/placebo will be administered in the clinical research facility by a study physician. The subject will be observed for a minimum of 1 hour for the appearance of any acute drug reactions. Subjects will wear an ambulatory cough monitor for 24 hours and be scheduled to return the following day for Visit 6.

Visit 6: Assessment of Blood, Sputum and Questionnaires

All subjects will undergo Ontario Covid-19 Assessment Tool and Information Letter prior to study visit. Subjects will return with the cough monitor 24 hours following Visit 5. If no recording was obtained during the past 24 hours due to recording device malfunction or if the recording quality is deemed poor, then the subject will be asked to repeat the recording within 24-48 hours of prior attempt at recording. Subjects will undergo spirometry, blood sampling and sputum induction and complete the Leicester Cough Questionnaire, the modified Borg Scale, and the Cough Severity VAS.

Visit 7: Week 12 Administration of Mepolizumab/Placebo (4th Injection)

All subjects will undergo Ontario Covid-19 Assessment Tool and Information Letter prior to study visit. Subjects will return to the clinical research facility 4 weeks following Visit 6. Subjects will undergo blood sampling and complete the Leicester Cough Questionnaire, the modified Borg Scale, and the Cough Severity VAS. Urine will be collected in WOCBP to screen for pregnancy. The fourth injection of mepolizumab/placebo will be administered in the clinical research facility by a study physician. The subject will be observed for a minimum of 1 hour for the appearance of any acute drug reactions.

Visit 8: Week 14 Cough Monitoring

All subjects will undergo Ontario Covid-19 Assessment Tool and Information Letter prior to study visit. Subjects will wear an ambulatory cough monitor for 24 hours and be scheduled to return the following day for Visit 9.

Visit 9: Final Visit: Assessment of Blood, Sputum and Questionnaires

All subjects will undergo Ontario Covid-19 Assessment Tool and Information Letter prior to study visit. Subjects will return with the cough monitor 24 hours following Visit 8. If no recording was obtained during the past 24 hours due to recording device malfunctionthen the subject will be asked to repeat the recording within 24-48 hours of prior attempt at recording. Subjects will undergo spirometry, methacholine challenge, blood sampling and sputum induction and complete the Leicester Cough Questionnaire, the modified Borg Scale, and the Cough Severity VAS.

5.11. Study procedures

All study procedures will be conducted by appropriately trained and experienced staff and supervised by a medical doctor familiar with the protocol and the experimental techniques and who will be available at all times. Participants will be asked to refrain from consuming caffeine at least 2 hours prior to each visit, and to refrain from taking any salbutamol 4-6 hours before study visits. All procedures listed will be performed according to local standard operating procedures (SOPs)(Table 1). All data produced from study procedures will be documented on CRFs, which will be considered source documents.

A. Spirometry:

During a spirometry manoeuvre, participants are asked to inhale maximally then blow out with maximal force into the spirometer until all air from the lungs has been expelled. The two main physiological measures derived from spirometry are FEV_1 and FVC, both expressed in litres. FEV_1 is the volume of air exhaled in the first second of forced expiration. FVC is the total volume of air exhaled with maximally forced effort. Volumes will be recorded at body temperature–prevailing atmospheric pressure–water vapour saturation. Spirometry in this study will be performed according to ATS and European Respiratory Society (ERS) standards.

B. Blood Sampling:

Blood will be collected from subjects during Visits 1, 3, 4, 6, 7 and 9. At visits 1 blood will be taken to assess for complete blood count for blood eosinophils and to ensure no

other clinically significant liver, electrolyte disorders or infections (HIV, Hepatitis B and C). Blood will be collected at Visits 3, 4, 6, 7 and 9 for complete blood count, liver enzymes, and electrolytes. Blood will be collected at Visits 3 (pre-treatment) and 9 (post-treatment) for total, activated mature eosinophils, eosinophil progenitors (assessed by flow cytometry) and related transcriptomics. Remaining plasma from blood processing and sputum at Visit 1 will be analysed to fully characterise type 2 inflammation.

C. Objective Cough Monitoring:

Participants will be asked to wear an ambulatory cough monitor (VitaloJAK™, Vitalograph, UK) for 24 hours, 3 times during the study: Visits 2, 5 and 8. This involves attaching an air microphone to the lapel of the clothing and a chest sensor on the sternum. The VitaloJAK cough recorder is worn around the waist of the subject. Subjects will wear the cough monitor for a continuous 24 hours and return the following day for the procedures for the next visit. Subjects will be informed not to have a shower during this period to prevent the air microphone and chest sensor being damaged by water.

The VitaloJAK cough monitor is a non-invasive, battery operated, and custom-built validated recording device and microphone intended to acquire, record and store ambulatory cough sounds from participants for 24 hours. Briefly, this consists of a digital data logger recording sounds at a sample rate of 8 kHz, with 16-bit resolution and in Waveform Audio File format, which is a commonly used uncompressed sound file format. Recordings will be transferred to a personal computer; silences and background noise will be removed by using validated, custom-written software, and cough sounds will be counted by using an audio editing package. The number of coughs will be expressed as coughs per hour and added up to give a cough count for a 24-hour period, awake cough frequency, and night-time cough frequency.

The cough monitor is an audio recording device and will record all sound including conversations. The cough monitor microphones capture speech in the local vicinity in addition to cough sounds. Therefore, it is possible that despite processing of the cough recording by customised software, some of the participant's conversations are also

captured in addition to their cough sounds. The recordings will be processed using an algorithm which compresses the recording and removes any non-cough sounds. On occasion the recordings may capture non-cough sounds which will require sound analysis by a cough analyst. The recordings will be sent outside of McMaster to be processed by an analyst who will be bound by a strict duty of confidentiality not to disclose any information heard. A central analyst is required for data consistency and accuracy. Vitalograph Ltd. (Maids Moreton Bukingham, England, Mk18 1SW) will act as the external vendor responsible for processing the cough monitor audio recordings. Prior to enrolment in the trial, this possibility will be explained to the subjects. Any conversations recorded would be treated with the usual standards of confidentiality as per GCP guidelines. Confidentially will be maintained so long as the analyst does not hear any recordings suggesting the participant may harm themselves or someone else. The cough monitor has a mute button to allow sensitive personal conversations to be muted. If any personally sensitive information become apparent during the cough counting process, then these will be deleted out.

D. Methacholine Challenge Test

Methacholine is a synthetic form of acetylcholine and therefore causes smooth muscle contraction. It is commonly used in clinical practice to exclude asthma, but also as a measure of airway hyper-responsiveness. It involves giving nebulised methacholine in doubling concentrations from 0.0625 mg/mL up to 16 mg/ml via the 2-minute tidal breathing method. At each concentration, FEV_1 is measured 30s and 90s after the end of each inhalation. The % fall in FEV_1 from baseline is calculated after each dose using the best FEV_1 and the test is terminated once a 20% fall in FEV_1 (PC20) is documented or the maximum concentration of 32 mg/mL is reached. Patients with a methacholine PC20<16mg/ml will be considered to have asthma. The methacholine PC20 will be assessed at Visit 1 and Visit 9. Information about which medication to stop prior to the test will be given to the patient.

E. Sputum Induction:

Prior to performing the sputum induction, spirometry will be repeated to ensure FEV_1 has returned to within 10% of the baseline prior to the methacholine challenge test. This may require 200-400mcg of salbutamol in addition to the post-methacholine challenge test salbutamol. The purpose is to induce sputum from the airways by giving nebulised hypertonic saline (3%/5%/7%) in incremental doses for a 7 min interval, provided the FEV_1 does not fall by more than 20% drop from pre-sputum baseline. If there is a drop in FEV_1 between 10-20% then a lower saline concentration (0.9%, 3%, 5%) will be administered. If the FEV_1 drops by ≥20% then sputum induction will be stopped and salbutamol via inhaler or nebuliser will be given to bring the FEV1 to at least 90% of the baseline pre-sputum FEV_1. Once sputum is collected in a sterile pot, it will be put on ice and sent to the laboratory for total, differential cell count, eosinophil free granules. Remaining cells from sputum processing on Visits 3 and 9 will be used to determine total and activated mature eosinophils by flow cytometry. Remaining supernatant from sputum processing at Visit 1 will be stored and used to analyse neuro-inflammatory cytokines and protein biomarkers at a later date.

F. Questionnaires:

i) Ontario Covid-19 Assessment Tool and Information Letter

In light of the current global COVID-19 pandemic, Canadian public health authorities have strongly recommended that everyone take additional precautions, including those outlined in the Information Letter, which will be provided to every participant via email before they start this study, and in person at every visit to the research laboratory. This Information Letter will be signed by each participant at every visit before any procedures take place. The day before, and one hour before every study visit, a member of the study team will ask each participant to complete the Ontario Covid-19 self-assessment tool and confirm that the result indicates that "you do not seem to have symptoms or be part of an at-risk group", using the participants preferred contact method (phone, text, email). If this is not the case, the participant will be asked to contact the research team to reschedule their appointment. McMaster University is attempting to limit the risk of exposure to COVID-19 by using reasonable efforts to follow the health and safety guidelines recommended by the provincial and federal health authorities. Nevertheless, there

remains a risk that by attending McMaster University campus or any of the McMaster University study sites, the participant may contract COVID-19. COVID-19 can result in severe illness, medical expenses, loss of income and death.

ii) The Leicester Cough Questionnaire is a patient-reported questionnaires conducted on Visits 1, 3, 4, 6, 7 and 9. The LCQ is a 19-item questionnaire on a 7-point Likert scale ranging from 1 (all of the time) to 7 (none of the time) used to assess the impact of chronic cough on patients using a recall period of 2 weeks. The 19 questions assess impact on psychological, social and physical domains (Appendix 1).

iii) The modified Borg Scale is a patient-reported questionnaire reported on Visits 1, 3, 4, 6, 7 and 9 and will be used to assess the intensity of sensations and self-perceived cough severity related to cough over a 2-week period; cough severity, urge to cough, throat itch, tickle, and irritation. The scale ranges from 0-10, where words are linked with numbers on a logarithmic scale (Appendix 2).

iv) The Cough Severity Visual Analogue Scale (0-100mm) is a single item that assesses a patient's severity of cough over a recall period of 1 week using a 0 to 100mm horizontal visual analogue scale (VAS). Participants will rate their severity of cough on the scale which has a minimum value of 0 equalling "no cough" and a maximum value of 100 equalling "worst cough". A higher score indicates greater cough severity.

Table 1. Visit and procedure schedule.

Visit	1	2	3	4	5	6	7	8	9
Study Day	1	13±7 *	14±3†	42±3†	70±3 †	71±3 †	99±3†	113±7†	114±3†
Post-Randomization	*-14*	*-1*	*0*	*28*	*56*	*57*	*84*	*98*	*99*
Week	*-2*		*0*	*4*	*8*		*12*	*14*	
Procedure									
Ontario Covid-19 Assessment Tool	X	X	X	X	X	X	X	X	X
Informed Consent	X								
Medical History	X								
Adverse Events		X	X	X	X	X	X	X	X
Prior and Con Meds		X	X	X	X	X	X	X	X
Examination	X								
Vital Signs	X	X	X	X	X	X	X	X	X

Urine B-HCG¥	X		X	X	X		X		
Spirometry	X		X			X			X
Cough Monitoring		X			X			X	
Methacholine PC_{20}	X								X
Induced Sputum	X≠		X			X			X
Blood Sampling	X		X	X		X	X		X
LCQ	X		X	X		X	X		X
mBorg Scale	X		X	X		X	X		X
Cough Severity VAS	X		X	X		X	X		X
Mepolizumab/Placebo			X	X	X		X		

* May be shortened to 7 days or extended to 21 days depending on availability of sputum cell counting to confirm eligibility of sputum eosinophilia >2%.
†Flexibility provided to allow visits cough monitoring on Friday and subsequent visit on Monday.
¥ Urine pregnancy test will be done for women of childbearing potential (WOCBP) prior to administration of IP.
≠Sputum induction at visit 1 could be repeated after 24-48 hours if insufficient sample at visit 1.

5.11.1. Safety of Study procedures

Methacholine challenge testing has been used extensively both as a clinical tool in diagnosing asthma but also as a research tool to measure airway hyper-responsiveness. Methacholine acts directly on bronchial smooth muscle and causes bronchoconstriction. Most patients have no symptoms at all or transient symptoms whilst performing this challenge which improve within 24 hours. In a 1000 patient multi-centre COPD trial using methacholine challenge, 25% had cough, 21% had dyspnoea, 10% had wheezing, 6% had dizziness, and 2% had headache after the test, with two-thirds reporting no symptoms. They were asymptomatic on leaving the trial centre on the visit day and only 0.3% had symptoms such as chest soreness in the days after the test (32).

Vlachos-Mayer H et al (33) retrospectively analyzed the safety of sputum induction in 304 patients with asthma and 25 with smoking related chronic airflow obstruction. Sputum induction protocols varied according to baseline FEV_1 (<70% or ≥70%) but they found it was safe even amongst patients with an FEV_1 of <60% and <1 L, provided that safety precautions similar to that of methacholine challenge testing are taken. Fahy et al (34) also showed the safety and reproducibility of combining methacholine challenge testing with sputum induction immediately afterwards twice within 1 week with moderate to severe asthmatics. Furthermore, Mulder et al

(35) showed repeatedly performing sputum induction at 4, 7 and 24hrs after a MCT was safe and well tolerated.

5.12. Washout Period

This study does not have a washout period because this is a parallel group study design.

5.13. Blinding and Randomization

Subjects will be assigned to one of the two possible treatment arms generated by a computer-generated randomisation schedule prepared by McMaster University, with a ratio of 1:1, mepolizumab or placebo. As there is a known imbalance in the prevalence and cough rates, the study will include sex as a randomisation factor. This will mitigate the possibility of the intervention being confounded by sex differences in the 2 arms of the study. Blinded study drug supplies will be provided in sequentially numbered identical syringes in accordance with the randomisation schedule and dispensed by a pharmacist who shall not be delegated any other role in the study. Subjects, investigators, research staff (with the exception of the pharmacist) and the sponsor will be masked to the treatment sequence assignment. A sealed code-break envelope for each subject containing details of the treatment allocated will be kept in a locked safe at the study site.

5.14. Premature Withdrawal/Discontinuation Criteria

During the process of informed consent, participants will be informed that they may withdraw from the study at any time voluntarily without giving any reason and without their medical care or legal rights being affected. If a participant decides to withdraw from the study early, the data collected will be retained with their permission. If the participant declines to give permission for the data collected to be retained, it will be destroyed.

It is also possible that patients may develop an upper respiratory tract infection and as a result the subjects will be withdrawn. If this occurs, then the participant will be offered a re-screen after 4 weeks from the end of symptoms.

The study may be terminated if there is a determination of futility.

6. Investigational Product

6.1. Formulation

All investigational products will be manufactured in accordance with Good Manufacturing Practice (GMP). Mepolizumab and placebo will be supplied by GSK in 1ml pre-filled syringes (Table 2). Mepolizumab 100mg or a normal (0.9%) saline placebo subcutaneous injection will be administered at the study centre on days 0, 28, 56 and 84 during the 12 week treatment period. The placebo will have an appearance identical to that of the study drug. Those administering treatment, any study staff involved in subject evaluations, and the subjects will remain blind to treatment assignment for the duration of the study.

Table 2. Intervention, dose, form, strength, and manufacturer per intervention.

Intervention	Dose	Form	Strength	Manufacturer
Mepolizumab	100mg	pre-filled (1ml) solution in syringe	100 mg/ml	GSK
Placebo - 0.9% normal saline	N/A	pre-filled (1ml) solution in syringe	N/A	GSK

6.2. Dosage Regimen

Patients will be randomized in 1:1 to receive mepolizumab 100mg monthly (4 doses) or matched placebo (4 doses) over 12 weeks.

6.3. Administration

The investigational product will be administered by an authorized study physician at McMaster University on study visits and within visit windows as described in study visits summary in section 5.6.1. Prior to administration urine pregnancy test will be done in women of childbearing potential to confirm the test is negative; the investigational product will only be administered when the result of the test is negative. The investigational product will be removed from the study refrigerator and sit at room temperature 30 minutes prior to administration.

Injections sites will be rotated such that the subjects receive the investigational product at different anatomical sites (Figure 2). The injection will be recorded in the source document at each treatment visit. After investigational product administration the subjects will be observed for a minimum of 1 hour for the appearance of any acute drug reactions. Appropriate medications such as epinephrine, antihistamines, and medical equipment to treat acute anaphylaxis must be available. Study personnel must be trained to recognise and treat anaphylaxis.

Figure 2. Injection sites for investigational product administration.

6.4. Labelling

Labelling will be performed according to local regulations in Canada by pharmacist or another authorised person not involved in the project. Labels will include:

- Study code
- Investigational product/study drug dosage form, route of administration, and quantity of dosage units
- Kit ID
- Lot ID
- Expiry date
- Investigator name (to be written on the label)
- Sponsor name

- Directions for use
- Storage conditions
- Standard statements required by Regulatory Authorities
- Sponsor name and contact details

6.5. Storage

Mepolizumab/placebo will be stored at McMaster University, Cardio-Respiratory Research Facility, in a secure facility with limited access and controlled temperature. The temperature will be monitored on a daily basis and documented in a temperature monitoring log.

The investigational product must be kept in the original outer container and under conditions specified on the label between 2–8°C (36–46°F) and protected from the light. The recommended storage condition and expiry dates will be provided on the product labels.

6.6. Accountability

The study drug provided for this study will be used only as directed in the study protocol. The study personnel will account for all study drugs dispensed to the patient. The study personnel will account for all study drugs received at the centre, unused study drugs, and for appropriate destruction. Certificates of delivery, destruction, and/or return should be signed.

6.7. Concomitant Medications

Patients will be allowed to have the COVID-19 vaccination before or during the whole study duration. Any adverse effects related to vaccination will be assessed for severity by the study physician.

Patients with long standing asthma on stable doses of ICS or a combination ICS/long-acting-beta agonists (LABA) will be allowed to continue using these medications so long as the dose does not change during the study. Patients with asthma who experienced an asthma exacerbation during the treatment period will be allowed oral prednisone, with or without antibiotics, as is the current standard of care. Patients with non-asthmatic eosinophilic bronchitis who have tried ICS or a leukotriene receptor antagonist (LTRA) but for whom cough has not

improved will not be allowed to continue using ICS and LTRA and will require a washout period of 28 days prior to screening. Patients with non-asthmatic eosinophilic bronchitis with partial relief on ICS will be allowed to continue using these medications so long as the dose does not change during the study. Patients on a long-acting muscarinic antagonist (LAMA) will be required to undergo a washout period for 28 days prior to screening. Patients using SABA or LABA (in an ICS combination inhaler) will be required to withhold from using the inhaler 8 and 48 hrs prior to any methacholine challenges tests as per local SOP.

Medications not allowed are as followed: opioids (including codeine, tramadol, morphine), neuromodulators (including pregabalin, gabapentin, amitriptyline), ACE inhibitors, immunosuppressive medication (including but not limited to: methotrexate, cyclosporine, azathioprine, intramuscular long-acting depot corticosteroid, oral corticosteroid, or any experimental anti-inflammatory therapy) within 3 months of date of informed consent, and other biologics within 4 months or 5 half-lives prior to date of randomization. If the subject has been on an opiod or an opiod like medication for a long period of time for a longstanding medical condition other than chronic cough, such as but not restricted to chronic pain, neuropathic pain or fibromyalgia, then they will be allowed to continue on their usual dosage.

7. Assessment of Efficacy

7.1. Primary Outcome

In patients with refractory chronic cough with airway eosinophilia to measure the change in objective 24-hour cough frequency (coughs/hour) from baseline after 14 weeks of treatment with mepolizumab compared with placebo.

7.2. Secondary Outcomes

In patients with refractory chronic cough with airway eosinophilia to measure changes from baseline after 8 and 14 weeks of treatment with mepolizumab compared with placebo of:

1. Awake cough frequency (coughs/hr)
2. Night-time cough frequency (coughs/hr)
3. Percentage of participants with a reduction in 24-hour cough frequency of ≥30%

4. All cough outcomes by disease group (asthma and NAEB)
5. Cough severity and intensity of sensations (itch, tickle, irritation, urge to cough on a modified Borg Scale)
6. Quality of life measured using the Leicester Cough Questionnaire (LCQ)
7. Blood eosinophils
8. Sputum Eosinophilia (% cell count)

To determine changes from baseline after 14 weeks of treatment with mepolizumab compared with placebo of:

1. Methacholine PC20
2. Total, activated mature eosinophils, eosinophil progenitors in blood and sputum (Flow cytometry) and related transcriptomics.

8. Assessment of Safety

8.1. Definitions

8.1.1. Adverse Events

Adverse Events (AEs) are defined as an adverse change in health that occurs while a patient is taking part in a study. All AEs will be documented in the source documents and assessed for whether or not it is serious (see below) or expected. If not a serious adverse event (SAE), then the AE will be reported to McMaster University when copied into the Annual Progress Report if the AE was related to the study drug or a study procedure and if the event was unexpected.

8.1.2. Serious Adverse Events

Any adverse event, adverse reaction or unexpected adverse reaction, respectively, that:

- Results in death
- Is life threatening
- Requires admission to hospital or prolongation of hospitalisation
- Results in persistent or significant disability/incapacity

- Is a congenital anomaly or birth defect
- Is otherwise medically significant

8.2. Reporting Procedures for SAEs

Any SAE occurring between enrolment and end of protocol follow up should be recorded in the subject's hospital notes and recorded in the source documents and reported to the REB according to the current REB guidelines. The majority of SAEs will be expected complications associated with the underlying respiratory condition such as an exacerbation of asthma requiring hospital admission.

The study drug, mepolizumab, is an off-label indication for subjects recruited for this study. The Principal Investigator will therefore submit expedited reports, as indicated/necessary for reporting of serious adverse drug reactions, by fax, directly to Health Canada.

Adverse drug reactions that are *both serious and unexpected* are subject to expedited reporting to Health Canada. Expedited reporting is not required for serious events from clinical investigations that are considered unrelated to the study product, whether or not the event is expected.

In accordance with Health Canada requirements, an Adverse Drug Reaction Report will be filed in the cases

- where the adverse drug reaction is neither fatal nor life-threatening, within 15 days after becoming aware of the information.
- where adverse drug reactions is fatal or life-threatening, immediately where possible and, in any event, within 7 days after becoming aware of the information;
- within 8 days after having informed Health Canada of the adverse drug reactions, submit as complete as possible, a report which includes an assessment of the importance and implication of any findings

8.2.1. GSK Reporting Procedures

All safety reports and associated information, including the investigator's causality assessment, as presented in Table 3 that occur during the study in subjects exposed to the IP will be sent by email to GSK's Case Management Group (Americas), marked for the attention of the Case Administrator: OAX37649@GSK.com or Fax +1-919-483-5404 and to Kirill Nikitin : kirill.d.nikitin@gsk.comor Fax: 919-256-5152. All safety reports will be submitted within 1 working day of the Principal Investigator becoming aware of the event.

Any SAEs reported by study subjects during the study which the investigator considers there to be a reasonable possibility of a causal association to any other known GSK product taken concurrently with the IP will be forwarded to GSK within 24 hours of the investigator becoming aware of the event, regardless of investigator/sponsor expectedness assessments.

Further detailed information about safety reports will be submitted to GSK within 24 hours of follow-up information becoming available.

Events exempt from reporting to GSK include any unblinded reports for subjects exposed only to placebo during the study.

The safety reports and information that are subject to the presented GSK reporting provisions are those that occur following the 1st dose of the IP through to 28 days or 5 terminal phase half-lives (whichever is longer) following discontinuation of the IP.

Table 3. GSK reporting safety events and information procedure

Safety Data	Format	Time Line
All SAEs that are unblinded during the course of the study, for which the investigator considers there to be a reasonable possibility of causal association with the GSK IP (as specified by the protocol), regardless of Investigator/designee/Sponsor expectedness assessments against GSK IP	Copies of Case Report Form pages	Within 24 hours of Sponsor first unblinding the event

All SAEs that remain blinded on the Sponsor database during the course of the study, where the investigator considers there to be a reasonable possibility of causal association with the GSK IP (as specified by the protocol), regardless of Investigator/designee/Sponsor expectedness assessments against GSK IP.	Copies of regulatory reports (e.g., CIOMS 1 or MedWatch)	Retrospectively within five working days of the Sponsor unblinding the study database

8.3. Adverse Events with Mepolizumab

Local injection site reactions: In 2 placebo-controlled studies the incidence of local injection site reactions with mepolizumab 100 mg subcutaneous and placebo was 8% and 3%, respectively. These events were all non-serious, mild to moderate in intensity and the majority resolved within a few days. Local injection site reactions occurred mainly at the start of treatment and within the first 3 injections with fewer reports on subsequent injections. The most common manifestations reported with these events included pain, erythema, swelling, itching, and burning sensation.

Serious adverse events: One SAE occurred in >1 patient and more frequently with mepolizumab than placebo: herpes zoster (2 vs 0 patients).

Systemic reactions: In the 3 clinical trials, the percentages of subjects who experienced systemic (allergic and nonallergic) reactions were 3% for mepolizumab and 5% for placebo. Manifestations included rash, flushing, pruritus, headache, and myalgia. A majority of the systemic reactions were experienced on the day of dosing.

Table 4. A pooled summary of adverse events in the first 24 weeks of 2 trials (MENSA and SIRIUS).

Adverse Event	Mepolizumab (n=263)	Placebo (n=257)
Headache	19%	18%
Injection site reaction	8%	3%

Back pain	5%	4%
Fatigue	5%	4%
Influenza	3%	2%
Urinary tract infection	3%	2%
Abdominal pain upper	3%	2%
Pruritus	3%	2%
Eczema	3%	<1%
Muscle spasms	3%	<1%

8.4. AEs of Study Procedures

Events that may be regarded as expected complications associated with the investigations are shown in the table below.

Table 5. Known complications associated with study procedures conducted in this study.

Procedure	Known complications
Spirometry	Cough, breathlessness
Blood Sampling	Fainting, vasovagal events, bruising or swelling at site of venepuncture
Bronchodilators	Tachycardia, tremor, cough, nausea, headache
Methacholine test	Headaches, throat irritation, light headedness, chest tightness, breathlessness cough, wheezing
Sputum Induction	Nausea, vomiting, salty taste, wheeze, breathlessness, cough

9. Statistical Plan

9.1. Sample Size Estimation

It is assumed that 60 subjects will be screened to randomize 30 subjects, and 15 will be randomized to complete each treatment arm in 1:1 ratio. This is a proof-of-concept study investigating the effectiveness of mepolizumab on objective cough rates in patients with

eosinophilic asthma or NAEB. As such, no formal power calculation can be currently performed. The sample size is therefore based on previous similar pilot studies using cough monitoring as an endpoint (36, 37).

With 15 patients per arm, this study will have 80% power with an alpha of 5% to detect an estimated 20% greater reduction in 24hr cough frequency compared with the placebo arm, assuming a log standard deviation of 0.60. A clinically relevant reduction in cough frequency of 20% was established following a recent phase 3 studies of gefapixant in refractory chronic cough which showed a 20% reduction over placebo as significant (38). The data from this study will be used to estimate the sample size required for any future multi-centre studies.

9.2. Statistical Analysis

9.2.1. General Considerations, Assumptions, and Definitions

This study will generate data of demographics, LCQ scores, modified Borg scores for sensations and cough, methacholine (PC20), blood eosinophils, sputum eosinophil, total and activated mature eosinophils in sputum and blood. These continuous variables will be presented and analysed as either mean (S.D), median (IQR) or geometric mean (geometric S.D).
It is generally assumed that cough frequency data will not be normally distributed, and hence cough frequency data will be log transformed and shown as geometric mean (geometric S.D).
Statistical significance will be set at $p<0.05$.

For the purposes of analysis, the populations for analyses will be defined as:

1. Full analysis set (FAS): consists of all subjects randomized to a treatment arm, who received at least one dose of investigational product irrespective of their protocol adherence and performed at least one period of 24-hr cough monitoring before and one after treatment.

2. Per Protocol Set (PPS): consists of all patients in who did not have any major protocol deviations, and provided cough frequency recording at baseline, 4 weeks and 8 weeks after treatment.

9.2.2. Primary Endpoint

The primary endpoint in this study will be the change from baseline in 24-hour cough frequency (geometric mean) after 14 weeks of intervention over placebo (day 98, visit 8) adjusted for baseline cough frequency (day 13, visit 2). The primary efficacy endpoint will be analysed using a generalised estimating equation modelling (GEE) analysis including the baseline cough frequency as a co-variate. Both FAS and PPS populations will be analysed.

9.2.3. Secondary Endpoint

Comparison of cough frequency, LCQ, mBorg, sputum and blood eosinophilia, will be performed using the same statistical approach as for the primary outcome including data after week 8 and week 14 of intervention. The proportion of subjects with a reduction in cough frequency more than 30% in the mepolizumab and placebo groups will be analysed using a chi-squared test.

The effect of intervention on changes in inflammatory cells in blood and sputum and methacholine PC20 will be compared to baseline measurements in the mepolizumab/placebo group. This will be analysed using either an independent t-test or Mann-Whitney test depending on the normality of data.

Changes in objective cough frequency will be correlated with changes in serum and sputum eosinophilia and all subjective patient reported outcomes.

9.3. Missing, Unused, Spurious Data

Spurious data will be re-analyzed with analysis of the primary efficacy endpoints using the primary efficacy analysis set, excluding any spurious data. If the results of this sensitivity analyses confirmed that these participants were influential to the primary endpoint point estimates and treatment effect estimates, the primary efficacy analysis set might be amended to exclude these participants and all endpoints evaluated in this revised analysis set for conclusion purposes.

9.4. Statistical Plan Deviations

Any deviations from the original statistical plan would require a protocol amendment and would be described in the final study report.

10. Data Handling and Recordkeeping

10.1. Confidentiality and Security

All data collected during the study will be stored on a secure database with limited access and password entry. A unique identifier made up of numbers and letters will be allocated and used on patient case notes and investigation results. Data will be collected and stored at McMaster University. All personal data collected during the study will be handled in accordance with the GCP code of practice for confidential patient information and the data protection act. Source data collected in Canada will be stored and archived in Canada according to local research standards policies and procedures. A database of anonymized data will be shared including anonymized 24-hour cough recordings with Professor Jacky Smith. The latter will be transferred using a secure password encrypted file transfer protocol (FTP) to a secure server at the University of Manchester and stored as per local SOP.

10.2. Training

All staff working on this study will have suitable qualifications, training and experience to carry out the procedures listed in section 5.6.4. A medical doctor will always be available during study visits should a participant become unwell and require medical attention. It will be ensured that all members of the research team are trained in local health and safety policies, emergency procedures and policies governing the confidentiality and security of the data collected during the study.

10.3. Records Retention

All research related documentation including the site file, case record forms etc will be kept with the Principal Investigator until all the data has been analysed. Local SOPS will apply for archiving in Canada. All study records will be stored for 25 years following study completed. Per local Manchester procedures all cough recordings will be stored electronically for 25 years on an

encrypted hard drive and archived along with paper documentation. A log will be made of all the documents that are being destroyed.

11. Direct Access to Source Data/Documents

Source documents are considered to be all information in original records and certified copies of original records of clinical findings, observations, data, or other activities in a clinical study necessary for the reconstruction and evaluation of the study. The investigator will provide direct access to source documents and/or source data in the facilitation of trial-related monitoring, audits, review by REB, and regulatory inspections.

The investigator/institution will maintain adequate and accurate source documents and trial records that include all pertinent observations on each of the site's trial participants. Source data will be attributable, legible, contemporaneous, original, accurate, and complete. Changes to source data will be traceable, not obscure the original entry, and be explained if necessary.

The study will be monitored according to the Sponsor's monitoring plan to ensure that it is conducted and documented properly according to the protocol, GCP, and all applicable regulatory requirements. Monitoring visits will be outlined in the study monitoring plan. The PI will assure she and adequate site personnel are available throughout the study to collaborate with clinical monitors. Clinical monitors must have direct access to source documentation in order to check the completeness, clarity, and consistency of the data recorded in the eCRFs for each participant.

The investigator will make available to the clinical monitor all source documents and medical records necessary to review protocol adherence and eCRFs. In addition, the investigator will work closely with the clinical monitor and, as needed, provide them appropriate evidence that the study is being conducted in accordance with the protocol, applicable regulations, and GCP guidelines.

12. Quality Control and Quality Assurance

On behalf of the Sponsor, the Investigators will perform obligations of the sponsor under GCP-ICH, including but not limited to being responsible for (i) overseeing the overall conduct and management of the study and handling of the study data at McMaster; (ii) the design of the study process and protocol; (iii) implementing and maintaining quality assurance and control to facilitate the overall study being conducted in compliance with the protocol.

13. Regulatory

13.1. Ethical Considerations

We do not anticipate any major ethical issues with this study. All patients will be informed that testing for blood borne infections will be performed at screening as part of assessing eligibility of the study. All research methods employed in this study have been previously applied in both healthy volunteers, patients with chronic cough and asthma with no reports of serious adverse events. Mepolizumab is currently approved by Health Canada for use in patients with severe eosinophilic asthma.

Patients who have been prescribed and currently taking amitriptyline, dextromethorphan, pregabalin, gabapentin or opioids for chronic cough, but have demonstrated no clinical benefit, would be withdrawn off those medication. The patients would be eligible for the study after waiting at least 5 half-lives to clear the drug.

The cough monitor is an audio recording device and will record all sound including conversations. It is possible that despite processing of the cough recording by customised software, some of the participant's conversations are also captured in addition to their cough sounds. Prior to enrolment in the trial, this possibility will be explained to the subjects. Any conversations recorded would be treated with the usual standards of confidentiality as per GCP guidelines. Confidentiality would only be broken if it is in the best interest of the patient or members of the public, e.g. if a subject describes serious intent of harming themselves or others. The cough monitor also has a mute button to allow sensitive personal conversations to be muted.

13.2. Research Ethics Board

The Investigator will provide the Research Ethics Board with all appropriate material, including the informed consent document. The study will not be started until appropriate REB approval of the protocol, the informed consent and all recruiting materials is obtained. Appropriate reports on the progress of the study will be made to the Research Ethics Board by the Investigator in accordance with applicable institutional regulations.

13.3. Ethical Conduct of the Study

This study will be conducted in accordance with the protocol, Good Clinical Practice (GCP) according to International Conference on Harmonization (ICH) guidelines, and regulatory requirements for the participating institution. The study will be conducted under a protocol reviewed by a Research Ethics Board; the study will be conducted by scientifically and medically qualified persons; the benefits of the study are in proportion to the risks; the rights and welfare of the subjects will be respected; the physicians conducting the study do not find the hazards outweigh the potential benefits; and each subject will give his or her informed consent without duress.

13.4. Subject Information and Consent

A properly executed, written, informed consent form, in compliance with GCP according to ICH guidelines will be submitted by the investigator to the Research Ethics Board for review and approval prior to the start of the study. Before entering subjects into the study, a copy of the Research Ethics Board-approved informed consent will be reviewed with the potential participant and signed and dated. The investigator will provide a copy of the signed informed consent form to each subject and will maintain a copy in the subject's study file.

13.5. Health Canada

This study will be reviewed by Health Canada and must receive a No Objection Letter prior to commencement. The study will conform to all Health Canada regulations.

13.6. Protocol Amendments

Amendments to the protocol that entail corrections of typographical errors, clarifications of confusing wording, changes in study personnel, and minor modifications that have no effect on

the safety of participants or the conduct of the study will be classed as administrative amendments and will be submitted to the REB for information only. The investigator will ensure that acknowledgement is received and filed. Amendments that are classed as substantial amendments must be submitted to the appropriate regulatory authorities and the REBs for approval and will not be implemented at sites until such approvals are received other than in the case of an urgent safety measure.

13.7. Protocol Deviations

Protocol deviations and/or violations and the reasons they occurred will be included in the clinical study report. Reporting of protocol deviations to the REB and in accordance with applicable regulatory authority mandates is an investigator responsibility. All protocol deviations will be identified, evaluated, and closed before the respective database lock (final analysis) and will be described in the clinical study report. Protocol deviations incurred as a direct result of the COVID-19 pandemic should be specifically recorded as a "COVID-19" deviation so that they can be easily identified and incorporated into the clinical study report.

14. Study Administration

14.1. Organization and Participating Centre

This is a single centre study based at McMaster University, Department of Medicine, Division of Respiratory Medicine, Cardio-Respiratory Research Facility, HSC 3U31, 1280 Main St. West, Hamilton, Ontario, Canada, L8S 4K1.

14.2. Investigators

All study visits and data collection will be conducted at McMaster University.

McMaster University:

Dr. Paul O'Byrne MB
Dr. Imran Satia MD PhD
Dr. Gail Gauvreau, PhD
Dr. Roma Sehmi, PhD
Dr. Kieran Killian, MB

University of Manchester:

Dr. Jacky Smith, MD PhD

Dr. Jacky Smith will be responsible for analysing and providing reports for the objective cough frequency endpoints.

15. Financing

15.1. Funding Source

This study is funded by GSK Canada with an investigator-initiated grant to Dr. Paul O'Byrne and Dr. Imran Satia.

16. Publication Policy and Disclosure of Data

Both the use of data and the publication policy are detailed within the clinical study agreement. Intellectual property rights (and related matters) generated by the investigator and others performing the clinical study will be participant to the terms of a clinical study agreement that will be agreed between the institution and the Sponsor or their designee. With respect to such rights, the Sponsor or its designee will solely own all rights and interests in any materials, data, and intellectual property rights developed by investigators and others performing the clinical study described in this protocol, participant to the terms of any such agreement. In order to facilitate such ownership, investigators will be required to assign all such inventions either to their institution or directly to the Sponsor or its designee, as will be set forth in the clinical study agreement.

The study will be registered on clinicaltrials.gov. We also aim to publish this study in a peer-reviewed scientific journal and present at national and international meetings.

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Appendix 1. Leicester Cough Questionnaire

This questionnaire is designed to assess the impact of cough on various aspects of your life. Read each question carefully and answer by CIRCLING the response that best applies to you. Please answer ALL questions, as honestly as you can.

1. In the last 2 weeks, have you had chest or stomach pains as a result of your cough?

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

2. In the last 2 weeks, have you been bothered by sputum (phlegm) production when you cough?

1	2	3	4	5	6	7
Every time	Most times	Several times	Some times	Occasionally	Rarely	Never

3. In the last 2 weeks, have you been tired because of your cough?

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

4. In the last 2 weeks, have you felt in control of your cough?

1	2	3	4	5	6	7
None of the time	Hardly any of the time	A little of the time	Some of the time	A good bit of the time	Most of the time	All of the time

5. How often during the last 2 weeks have you felt embarrassed by your coughing?

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

6. In the last 2 weeks, my cough has made me feel anxious

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

7. In the last 2 weeks, my cough has interfered with my job, or other daily tasks

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

8. In the last 2 weeks, I felt that my cough interfered with the overall enjoyment of my life

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

9. In the last 2 weeks, exposure to paints or fumes has made me cough

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

10. In the last 2 weeks, has your cough disturbed your sleep?

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

11. In the last 2 weeks, how many times a day have you had coughing bouts?

1	2	3	4	5	6	7
All of the time (continuously)	Most times during the day	Several times during the day	Some times during the day	Occasionally through the day	Rarely	None

12. In the last 2 weeks, my cough has made me feel frustrated

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

13. In the last 2 weeks, my cough has made me feel fed up

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

14. In the last 2 weeks, have you suffered from a hoarse voice as a result of your cough?

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

15. In the last 2 weeks, have you had a lot of energy?

1	2	3	4	5	6	7
None of the time	Hardly any of the time	A little of the time	Some of the time	A good bit of the time	Most of the time	All of the time

16. In the last 2 weeks, have you worried that your cough may indicate serious illness?

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

17. In the last 2 weeks, have you been concerned that other people think something is wrong with you, because of your cough?

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

18. In the last 2 weeks, my cough has interrupted conversation or telephone calls

1	2	3	4	5	6	7
Every time	Most times	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

19. In the last 2 weeks, I feel that my cough has annoyed my partner, family or friends

1	2	3	4	5	6	7
Every time I cough	Most times when I cough	Several times when I cough	Some times when I cough	Occasionally when I cough	Rarely	Never

Thank you for completing this questionnaire.

Appendix 2. Modified Borg Scale

The modified Borg Scale will assess your self-perceived frequency and intensity of your cough and associated sensations including urge to cough, throat itch, throat tickle, and throat irritation. Please use the scale to rate the severity of your cough sensations.

0 or "Nothing at all" means no cough sensations.
10 or "Maximal" means the most severe cough sensations you have experienced.

Scale	Cough Frequency	Cough Intensity	Urge to cough	Throat itch	Throat tickle	Throat irritation
0 - Nothing at all						
0.5 - Very, very slight (just noticeable)						
1 - Very slight						
2 - Slight						
3 - Moderate						
4 - Somewhat severe						
5 - Severe						
6						
7 - Very severe						
8						
9 - Very, very severe						
10 - Maximal						

Appendix 3. Cough Severity Visual Analogue Scale

How severe has your cough been over the last week?

Please place a single vertical line on the scale that best describes the severity of your cough over the past week (7 days).