1. To determine whether the following patient characteristics/biomarkers predict response to mepolizumab:
    - BMI percentile
    - Peripheral blood eosinophils
    - Bronchodilator responsiveness at baseline
    - Exacerbation history in year prior to study (2, 3 or 4+)
    - Total serum IgE
    - Fractional exhaled nitric oxide
    - Nasal mucus eosinophil cationic protein (ECP)
2. To determine whether mepolizumab improves other important clinical outcomes, including rate of virus-induced asthma exacerbations, time to first virus-induced asthma exacerbation, Asthma Control Test (ACT) scores, maximum number of asthma symptom days, and bronchodilator response
3. To use transcriptomics to identify immunologic pathways in the airways and peripheral blood that are characteristic of exacerbations prevented with and persistent despite anti-IL5 therapy.
4. To measure levels of antibody to mepolizumab

3 Study Design

3.1 Description of Study Design

Protocol ICAC-30 MUPPITS-2 is a multicenter, double-blind, placebo-controlled, randomized trial of mepolizumab adjunctive therapy for the prevention of asthma exacerbations in urban children and adolescents 6 to 17 years with exacerbation-prone asthma and peripheral blood eosinophilia. Approximately 290 participants will be randomized 1:1 to one of two study arms: 1) guidelines-based asthma treatment + placebo, or 2) guidelines-based asthma treatment + mepolizumab. All participants will be followed for one year.

There will be a Screening Visit followed by a 4-week run-in period. After the run-in, children who continue to meet eligibility criteria will be randomized to one of the 2 treatment arms and receive their first injection. Participants will receive monthly injections of either 40 mg or 100 mg of mepolizumab/placebo administered subcutaneously, according to their age. Participants 6 to 11 years of age and weighing ≥40 kg who were enrolled in Protocol ICAC-30 under the previous versions of the protocol and initially assigned a 100 mg dose will have their dose reduced to 40 mg. Participants who receive the 40 mg dose at randomization will increase to the 100 mg dose if they become 12 years old during the study. All participants will have Evaluation and Management (E&M) visits every 3 months where their asthma will be assessed and adjustments made to their medications based on asthma guidelines.

Participants will be asked to monitor and report cold symptoms. Participants will come to the clinic at the time of a cold, up to three times, for collection of blood and nasal secretions for associated mechanistic studies.

Participants will also be asked to come into the clinic following an asthma exacerbation for collection of blood and nasal secretions for associated mechanistic studies, assessments of airway physiology, and to collect information for ongoing asthma management.

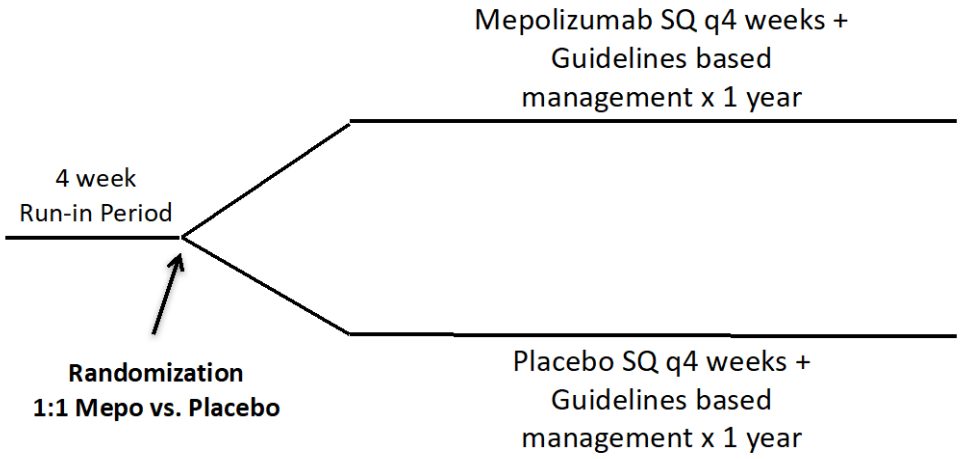

Figure 3.1. Protocol ICAC-30 MUPPITS-2 Study Design

3.2 Primary Endpoint

The primary endpoint is the number of asthma exacerbations during the 12-month treatment period. Exacerbations will be defined as a prescription of a course of systemic corticosteroids by a clinician, initiation of a course of systemic corticosteroids by a participant, or as a hospitalization for asthma. If a participant initiates and completes a course of systemic corticosteroids without clinician involvement, this course will be counted only if the study clinician agrees the treatment was warranted, and it meets the following dosage: the course for prednisone, prednisolone, or methylprednisolone will be at least 20 mg daily dose for 3 of 5 consecutive days. The course for dexamethasone will be at least a 10 mg single daily dose. If a corticosteroid burst for the treatment of an asthma exacerbation is prescribed by a non-ICAC clinician, it will be counted regardless of dose.

3.3 Secondary Endpoint(s)/Outcome(s)

1. CASI during the treatment period
2. Quality of life, as measured by physician-patient global assessment
3. Lung function as assessed by spirometry and IOS during the treatment period
4. Time to first asthma exacerbation during the treatment period
5. Number of reported adverse events and serious adverse events, including their severity and treatment relatedness

3.4 Exploratory Endpoint(s)/Outcome(s)

1. Time to first respiratory virus-induced exacerbation as measured by an exacerbation associated with a respiratory virus detected using nasal mucus samples obtained at the time of an exacerbation, during the treatment period

2. Number of respiratory virus-induced exacerbations as measured by an exacerbation associated with a respiratory virus detected using nasal mucus samples obtained at the time of an exacerbation, during the treatment period
3. Childhood ACT/ACT during the treatment period
4. Maximum number of asthma symptom days, defined as the highest value among the following three variables over a two-week period: number of days with wheezing, tightness in the chest, or cough; number of nights with disturbed sleep as a result of asthma; and number of days on which the participant had to slow down or discontinue play/physical activities
5. Bronchodilator responsiveness at the end of the treatment period
6. Gene expression using whole genome transcriptomics of nasal lavage samples during treatment period
7. Gene expression using whole genome transcriptomics of whole blood RNA samples during treatment period
8. Levels of antibody to mepolizumab during treatment period
9. Other biomarkers (banking plasma, banking nasal sample, RNA, DNA)

3.5 Stratification, Randomization, and Blinding/Masking

Randomization will be performed using a validated system created by the DAIT Statistical and Clinical Coordinating Center (SACCC) called RhoRAND™ that automates the random assignment of treatment groups to study ID numbers. The randomization scheme will be reviewed and approved by a statistician at the DAIT SACCC. Participants will be randomized using a 1:1 ratio of active study drug and control (placebo) participants. A covariate adaptive randomization algorithm developed by Pocock and Simon (1975) [32] will be used to maintain a balance between treatment arms with respect to site, number of previous exacerbations (2 or 3+), peripheral blood eosinophils (< or ≥400 cells/µl), BMI (< or ≥95th percentile for age), total serum IgE (< or ≥540 kUA/L) and treatment dose (40 mg or 100 mg). This strategy was chosen to enhance our ability to balance the distribution of covariates that are known to be highly associated with our primary endpoint (e.g., number of previous exacerbations and peripheral blood eosinophils) as well as covariates that determine if a participant fits the FDA-approved dosing tables for omalizumab therapy (e.g., BMI and total serum IgE), which is related to Secondary Objective 5.

Randomization data will be kept strictly confidential, accessible only to authorized persons, until the time of unblinding. When the study has been completed, the data files verified, and the protocol deviations determined, the investigational agent codes will be broken and made available for data analysis.

The clinical site's research pharmacy will dispense the study drug as per the randomization schedule provided by RhoRAND™. The injections will be administered by trained clinical research staff. Prior to injection, an unblinded site pharmacist will confirm the expiration date, the dose, and randomization assignment. The product will remain blinded to blinded clinical research staff and the participant.

3.5.1 Procedure for Unblinding/Unmasking

Unblinding must be approved by the DAIT NIAID Medical Monitor unless an immediate life threatening condition has developed and the Medical Monitor is not accessible. If unblinding is performed through RhoRAND™, a notification will be sent to the protocol chair, the DAIT NIAID Medical Monitor, and the DAIT SACCC team of the unblinding event as soon as unblinding occurs. Otherwise, the site principal

The study clinician may use their discretion as to whether to administer a nasal decongestant prior to the nasal collections. The nasal decongestant may be associated with temporary discomfort such as burning, stinging, sneezing, or an increase in nasal discharge.

The nasosorption procedure may produce mild discomfort such as a tickle and/or tearing of the eyes. If a participant is unable to come to a cold visit (Section 8.11) or an exacerbation visit (Section 8.12), he/she will be asked to perform the nasosorption procedure at home. It is unknown whether the nasosorption procedure will increase the risk of infection to household members. Because we do not know if there is an increased risk of infection transmission, the study will provide gloves and masks for each participant as a protection to those assisting with the nasosorption procedure at home.

5.4.6 Sputum Induction

Sputum induction may result in wheezing, coughing or chest tightness. Participants will be pre-medicated with albuterol to minimize this risk.

5.4.7 Nasal Epithelial Cell Collection

The risks associated with the nasal cell collection procedure include discomfort or pain, transient nosebleed, sneezing, tearing of the eyes, runny nose, and postnasal drip.

5.4.8 Questionnaires

There is a possibility that participants and caretakers may find the questions too personal. Participants and caretakers may refuse to answer any questions that make them feel uncomfortable.

5.5 Potential Benefits

Whereas the participant's asthma may or may not improve while in this study, participants may directly benefit from study participation by receiving study-based asthma assessments and care from an asthma specialist. Participants will receive information about their spirometry and allergy skin testing and relevant asthma education consistent with recommendations in the national asthma guidelines for patients with asthma. Furthermore, these participants will receive close monitoring and direct management of asthma exacerbations on a 24/7 basis throughout the study. Participant families will receive contact numbers for the study site and a tailored asthma action plan and education. A supply of prednisone will be provided in concert with this management.

6 Investigational Agent

6.1 Investigational Agent: Mepolizumab

Mepolizumab (SB-240563) is the investigational agent and will be provided to the clinical research sites by the IND Sponsor (DAIT NIAID). Mepolizumab will be obtained by the IND Sponsor free of charge from the manufacturer (The Glaxo-Smith Kline Research and Development Ltd., Brentford, Middlesex, UK).

6.1.1 Formulation, Packaging, and Labeling

Mepolizumab is a humanized monoclonal antibody (IgG1, kappa) that blocks interleukin-5 (IL-5) from binding to the IL-5 receptor complex expressed on the eosinophil cell surface and thus inhibits signaling, resulting in the reduction in production and survival of eosinophils.

Mepolizumab for injection (100 mg/vial) is supplied as a sterile, white to off-white, preservative-free, lyophilized powder. Mepolizumab is presented in a 10 mL Type I clear glass, stoppered, single-use vial. The drug product must be stored in a refrigerator at a temperature of 2°C to 8°C (36°F to 46°F), protected from light.

6.1.2 Dosage, Preparation, and Administration

Mepolizumab is licensed for add-on maintenance treatment for severe eosinophilic asthma at a dose of 100 mg every 4 weeks and is available as a powder for solution for subcutaneous (SC) injection. In the United States, the approved dose of Nucala® for children age 12 years and above is 100 mg administered once every 4 weeks by subcutaneous injection into the upper arm, thigh, or abdomen [34]. For children between 6 and 11 years of age, the approved dose is 40 mg every 4 weeks.

Mepolizumab for injection (100 mg/vial) is reconstituted in the vial with 1.2 mL Sterile Water for Injection, USP, preferably using a 2- or 3-mL syringe and a 21-G needle. The reconstituted solution will contain a concentration of approximately 100 mg/mL mepolizumab. Participants assigned to the 100 mg treatment group will receive 1.0 mL of reconstituted mepolizumab. Participants assigned to the 40 mg treatment group will receive 0.4 mL of reconstituted mepolizumab.

The reconstituted solution should be kept at refrigerated or controlled room temperature (up to 25°C). It should be used as soon as possible and any unused solution remaining after 8 hours should be discarded. Complete reconstitution instructions are available in the GSK Investigator's Brochure for mepolizumab.

6.2 Placebo

Placebo for mepolizumab will be 0.9% sodium chloride (normal saline) for injection. Supplies of normal saline for injection will be obtained locally by the clinical site research pharmacists.

6.3 Drug Accountability

Under Title 21 of the Code of Federal Regulations (21CFR §312.62), the investigator will maintain adequate records of the disposition of the investigational agent, including the date and quantity of the drug received, to whom the drug was dispensed (participant-by-participant accounting), and a detailed accounting of any drug accidentally or deliberately destroyed.

Records for receipt, storage, use, and disposition will be maintained by the study site. A drug-dispensing log will be kept current for each participant. This log will contain the identification of each participant and the date and quantity of drug dispensed.

All remaining unused investigational product will be returned to the sponsor (DAIT, NIAID) or sponsor's representative after study termination, or destroyed with the permission of the sponsor in accordance with applicable law and study site procedures. If investigational product is to be destroyed locally, the investigator will provide documentation in accordance with sponsor's specifications.

All records regarding disposition of the investigational product will be available for inspection by the clinical trial monitor.

6.4 Assessment of Participant Compliance with Investigational Agent

taking the medication prior to study enrollment. Montelukast will not affect the algorithm for prescribing asthma treatment.

All participants are required to have medical insurance at randomization that will cover, at least in part, the costs of their asthma medications. It is likely, however, that some participants may lose this coverage during the time that they are in the study. To maintain participant safety and avoid a loss of these participants to the study, Protocol ICAC-30 MUPPITS-2 will fund the cost of these participants' asthma medications during the remainder of the study or until the participant regains insurance coverage during the study. Medications will be prescribed in the same manner using the same algorithms as all insured participants. Each research site will develop plans to use local pharmacies in a cost efficient and timely manner. This design is in place to mimic the support typical insured participants receive, i.e. they receive a prescription and have to fill it at a pharmacy. To limit the time that Protocol ICAC-30 MUPPITS-2 is required to fund the cost of asthma medications for a participant who has lost coverage, each research site will develop a plan for evaluation of insurance options and referral to appropriate support systems to obtain coverage for medication costs. In addition to addressing potential insurance from employers and governmental sources, this may include free clinics and support programs available through pharmaceutical firms.

7.1.1.1 Initial Regimen

During Visit 0, the study clinician will determine the appropriate asthma control regimen that will be prescribed for the participant. Along with the participant's medical history and physical examination results, the medication regimen is determined by the participant's symptoms, number of systemic corticosteroid bursts in the past 6 months, percent-predicted forced expiratory volume in 1 second (FEV_1), and current level of asthma controller therapy.

First, the study clinician will determine the highest "control level" of the participant's morbidity across the 4 categories in Table 7.1.1.1a. For example, if the participant has 8 days with symptoms (control level 2), 1 night with symptoms (control level 1), is at 75% predicted FEV_1 (control level 3), and has 0 systemic steroid bursts, (control level 1), then the participant is at control level 3 because that is the highest of these 4 categories.

Table 7.1.1.1a Asthma Symptoms for Determining Run-in Asthma Medication Regimen

Control Level	**Maximum of 1) # days with asthma symptoms/ two weeks and 2) # days with rescue albuterol or levalbuterol use/ two weeks***	**Maximum of 1) # nights of sleep disruption due to asthma/ 2 weeks and 2) # nights use of albuterol or levalbuterol for awakening / 2 weeks***	**FEV_1 (% pred)****	**Courses of systemic steroids in the last 6 months*****
1	0-3 days	0-1 night	≥ 85	0
2	4-9 days	2 nights	80 - < 85	1
3	10-13 days	3-4 nights	70 - < 80	2
4	14 days	5-14 nights	< 70	>2

after 4 weeks of observation during the assumption care. As such, participants will not be stepped down at Visit 1. At Visit 1, a participant may be stepped up as per the protocol algorithm.

Participants will undergo pre-bronchodilator spirometry and an assessment of symptoms, rescue bronchodilator usage, recent exacerbations and medication adherence. Bronchodilator use as a preventive measure prior to exercise will not count toward the assessment of rescue bronchodilator use. Based on these data, a study clinician will determine the participant's current control level from Table 7.1.1.2a below. The control levels originated from the published national guidelines, Guidelines for the Diagnosis and Management of Asthma (EPR-3, NAEPP, 2007).

For all participants, the highest control level for days with symptoms or albuterol/levalbuterol use, nights with symptoms or albuterol/levalbuterol use, percent personal best FEV_1, and exacerbation history will be determined by the study clinician. The day and night symptoms, albuterol/levalbuterol use, adherence to prescribed asthma controller medications by self-report, and exacerbation history will be determined from questionnaires. The FEV_1 personal best is defined in the Protocol ICAC-30 MOP. If the quality of the spirometry maneuver at the current visit is unacceptable according to the technician's judgment, FEV_1 in Table 7.1.1.2a will not be used, and the control level will be based on day and night symptoms, albuterol/levalbuterol use, and exacerbation history. For example, if a participant has 0 days with symptoms (control level 1), 4 nights with symptoms (control level 3), FEV_1 at 85% of personal best (control level 1), and 1 exacerbation (control level 2), then the participant's overall control level is 3 because that is the maximum value of control levels 1, 3, 1, and 2.

Table 7.1.1.2a Control Levels of Symptoms, Bronchodilator Usage, FEV_1 (% personal best), and Exacerbations

Control Level	**Maximum of 1) # days with asthma symptoms/ 2 weeks and 2) # days with rescue albuterol/levalbuterol use/ 2 weeks***	**Maximum of 1) # nights of sleep disruption due to asthma/ 2 weeks and 2) # nights use of albuterol/levalbuterol for awakening/ 2 weeks***	**FEV_1 (% personal best)**	**Courses of Systemic Steroids since the Last Medication Management Visit****
1	0-3 days	0-1 night	≥ 85	0
2	4-9 days	2 nights	80-84	1
3	10-13 days	3-4 nights	70-79	-
4	14 days	5-14 nights	< 70	-

FEV_1 = forced expiratory volume in 1 second

* Determined from participant recall, based on the 2-week interval directly preceding the study visit.

**Defined as a prescription of a course of systemic steroids by a clinician or initiation of a course of systemic steroids by a participant to prevent a serious asthma outcome. If a participant initiates and completes a course of systemic steroids without clinician involvement, this course will be counted only if it meets the following dosage: the course for prednisone, prednisolone, or methylprednisolone will be at least 20 mg daily dose for 3 of 5 consecutive days. The course for dexamethasone will be at least a 10 mg single daily dose. If a corticosteroid burst for the treatment of an asthma exacerbation is prescribed by a non-ICAC clinician, it will be counted regardless of dose.

Note: Shading in the table corresponds to the example described in the text above.

8.1.6 Questionnaire Assessments

Questionnaires administered by site staff will collect inclusion/exclusion criteria information, participant contact information, demographic characteristics, asthma and allergy history and medication use, asthma exacerbation history, asthma symptoms and health care utilization, cold symptoms, concomitant medications, AE information, and primary care provider (PCP) contact information.

8.2 Clinical and Research Laboratory Evaluations and Specimen Collection

8.2.1 Blood Sample Collection

Blood will be collected by peripheral venipuncture for transcriptomics, to preserve DNA/RNA, to assess a complete blood count (CBC) and differential and blood chemistry, as well as total and allergen-specific serum IgE. With the participant's consent, blood will be stored for use in the future for unplanned tests. Blood volume will be indicated in the informed consent document along with any possibility for a repeat blood draw, if needed. An eosinophil value obtained as part of a participant's participation in another ICAC protocol within 6 months of the screening visit will determine the requirement for Protocol ICAC-30 MUPPITS-2 eligibility.

Table 8.2.1 CBC and Blood Chemistry Components

CBC (and differential)	Blood Chemistry
Platelets	BUN
Hematocrit	CO2
Hemoglobin	Creatinine
Erythrocytes	Glucose
Leukocytes	Serum chloride
Monocytes	Serum potassium
Eosinophils	Serum sodium
Basophils	Bilirubin
Neutrophils	Aspartate aminotransferase
Lymphocytes	Alanine aminotransferase

8.2.2 Nasal Sample Collection

Nasal mucus samples will be collected as per the Protocol ICAC-30 MOP for respiratory virus/bacteria assessment. If a participant is unable to come to an exacerbation visit (Section 8.12), he/she will be asked to collect the nasal mucus sample at home. Training and supplies will be provided to the participant by the site staff.

If a participant is unable to come to a cold visit (Section 8.11) or exacerbation visit (Section 8.12), he/she will be asked to collect a nasal swab sample at home. Training and supplies will be provided to the participant by the site staff as per the Protocol ICAC-30 MOP.

As per the Protocol ICAC-30 MOP, nasal lavage samples will be collected by instilling preservative-free buffered saline into the nostrils and allowing fluid to return passively into collection specimens. If the nostrils are occluded, a nasal decongestant may be administered at the discretion of the study clinician. Samples will be split and processed for both cell differentials and RNA extraction. Site clinical research staff who are trained, study-authorized, and certified will perform the nasal lavage and processing procedure as specified in the Protocol ICAC-30 MOP.

Nasosorption is a non-invasive technique for sampling of undisturbed mucosal lining fluid from the upper airways through which quantification of in situ levels of immune mediators, such as cytokines and chemokines, will be performed. The sample will be obtained using the size-appropriate single use Nasosorption™ FX·i device (Hunt Developments, UK) consisting of a synthetic absorptive matrix strip (SAM) attached to an applicator handle. Details of the nasosorption sample collection and processing procedures are described in the Protocol ICAC-30 MOP. If a participant is unable to come to a cold visit (Section 8.11) or an exacerbation visit (Section 8.12), he/she will be asked to perform the nasosorption procedure at home. Training and supplies will be provided to the participant by the site staff.

8.2.3 Urine Pregnancy Testing

All females who have reached menarche will be required to have a urine pregnancy test before completing study activities. If the result is positive, the participant will not undergo any procedures. Results of all pregnancy tests will be given to the participant or caretaker as per site-specific guidelines and relevant state laws.

8.2.4 Urine Cotinine

Urinary cotinine (a metabolite of nicotine) will be measured to determine exposure to environmental tobacco smoke. A urine sample will be collected following procedures described in the Protocol ICAC-30 MOP. The urine sample will be stored long-term for future research in the field of asthma. Participants will be asked to give permission for long-term storage and future use during the consent process.

8.2.5 Induced Sputum

Sputum will be induced by inhalation of hypertonic saline solution using the method that was used in the Asthma Clinical Research Network (ACRN)[35]. Safety monitoring per spirometry and symptom report will be performed during and after sputum induction. Slides will be read at a central site for cellular determinations. Sputum cells will be processed for CyTOF analysis and RNA and DNA isolation for expression and epigenetic studies. Sputum supernatants will be collected, aliquoted, and stored pending further analysis. This procedure will be performed in a subset of children ≥10 years of age.

8.2.6 Nasal Epithelial Cell Collection

Nasal epithelial cell samples will be obtained by using a cytology brush. A trained study clinician will conduct the procedure. The inferior turbinate of one nasal passage will be sampled to obtain an adequate number of epithelial cells for mRNA and DNA isolation. The sampled area will be observed for hemostasis. Procedural details and instructions for processing the samples are included in the Protocol ICAC-30 MOP. This procedure will be performed in a subset of children ≥10 years of age.

8.3 Recruitment

The research site may use any Institutional Review Board (IRB)-approved means to identify potential participants. Examples include hospital, clinic, or emergency department admission records; study clinicians' specialty clinic records; and advertising (in public locations and on the radio). Potential participants will be screened and recruited using a standardized questionnaire that collects contact information and inclusion/exclusion criteria information. Participants may be recruited by phone or in person.

Retention methods involve a number of different approaches. We will use an appointment reminder system that consists of phone calls several days and one day prior to scheduled appointments for

- Exhaled nitric oxide (see Section 8.1.4)
- Nasal mucus sample collection (see Section 8.2.2)
- Nasal lavage (see Section 8.2.2)
- Nasosorption (see Section 8.2.2)
- Blood sample collection (see Section 8.2.1)
    - CBC with differential
    - Blood chemistry
    - Total and allergen-specific IgE
    - Blood markers (plasma/DNA/RNA)
    - Antibody to drug
- Sputum induction for a subset of children ≥10 years of age
- Nasal epithelial cell collection for a subset of children ≥10 years of age

8.11 Cold Visits

Participants will be asked to report cold symptoms and come to the clinic at the start of up to three colds over the course of the 12-month follow-up. At this visit, the following activities will take place:

- Urine cotinine (see Section 8.2.4)
- Vital signs (see Section 8.1.1)
- Questionnaires (see Section 8.1.6)
- AE assessment (see Section 12)
- Concomitant medication assessment (see Sections 8.1.6 and 7.1.2)
- Spirometry and IOS (see Section 8.1.4)
- Targeted pulmonary exam (see Section 8.1.2)
- Exhaled nitric oxide (see Section 8.1.4)
- Nasal swab sample collection (see Section 8.2.2)
- Nasal lavage (see Section 8.2.2)
- Nasosorption (see Section 8.2.2)
- Blood sample collection (see Section 8.2.1)
    - CBC with differential
    - Blood markers (plasma/DNA/RNA)

8.12 Exacerbation Follow-Up Visits

Over the course of the 12-month follow-up, participants who exacerbate will be asked to come in for a follow-up visit approximately 7 days following the start of systemic corticosteroids. As described in Section 3.2, exacerbations will be defined as a prescription of a course of systemic corticosteroids by a clinician, initiation of a course of systemic corticosteroids by a participant, or as a hospitalization for asthma. This visit will include:

- Urine cotinine (see Section 8.2.4)
- Vital signs (see Section 8.1.1)
- Questionnaires (see Section 8.1.6)
- AE assessment (see Section 12)
- Concomitant medication assessment (see Sections 8.1.6 and 7.1.2)

- Spirometry and IOS (see Section 8.1.4)
- Targeted pulmonary exam (see Section 8.1.2)
- Nasal swab sample collection (see Section 8.2.2)
- Nasal lavage (see Section 8.2.2)
- Nasosorption (see Section 8.2.2)
- Blood sample collection (see Section 8.2.1)
    - CBC with differential
    - Blood markers (plasma/DNA/RNA)

8.13 Unscheduled Visits and Telephone Follow-up

Participants who experience asthma-related problems or concerns between scheduled study visits will be seen by a study clinician as warranted by the situation. If the site staff are notified by the participant, his/her family, an ED, a hospital, or clinic that the participant was seen for an unscheduled visit elsewhere, the site staff will contact the participant within 2 weeks of discharge to obtain an update on the participant's clinical status. A follow-up visit at the study center will be scheduled if needed. For details on the management plan, please refer to Section 7.1 and the Protocol ICAC-30 MOP.

Participants who are at Control Level 4 at a study visit will either be seen in the clinic or will receive a follow-up phone call within 1-2 weeks of the visit to check the status of their symptoms. Participants prescribed systemic corticosteroids who do not have an exacerbation study visit will receive a follow-up phone call within 1-2 weeks of the visit to check the status of their symptoms. If systemic corticosteroids were prescribed by a non-ICAC clinician and the participant does not come in for a study exacerbation visit, the participant will be seen in the clinic or will receive a follow-up phone call within 1-2 weeks to check the status of their symptoms. If this prescribing is uncovered more than two weeks after initiation of the corticosteroids, and the participant is doing well at the time of notification, then no follow up phone call is required.

Participants who have 2 exacerbations within a 1-month period must be seen in a face-to-face visit to determine if continuation in the study is in the best interest of the participant.

8.14 Visit Windows

Mepolizumab/placebo injections should be scheduled as close as possible to the every 4 week target date. See the Protocol ICAC-30 MOP for details regarding visit scheduling.

9 Mechanistic Assays

To identify the inflammatory pathways affected by mepolizumab treatment, we will examine airway gene expression using whole genome transcriptomics of nasal lavage and peripheral blood. Blood and nasal lavage samples for these studies will be collected pre-treatment (V1), early in the treatment period (V3), at the end of the study (V14), as well as during cold visits as described in Section 8.11 and exacerbation visits as described in Section 8.12. The quality of nasal lavage samples will be monitored regularly throughout the course of the study. Specifically, RNA will be extracted from samples for assessment of RNA concentration as well as integrity.

10 Biospecimen Storage

Unused samples of biological specimens collected during the course of the study will be stored (described in the Protocol ICAC-30 MOP) for future use for tests that may or may not be planned. These tests may or may not be related to the study of asthma and allergy. Participants will be asked to give permission for long-term storage and future use during the consent process. With the participant's approval and as approved by the Central IRB, de-identified biological samples will be stored indefinitely at the central repository.

11 Criteria for Participant and Study Completion and Premature Study Termination

11.1 Participant Completion

Participants are considered to have completed the study once they have completed the Final Study Visit.

11.2 Participant Stopping Rules and Withdrawal Criteria

Participants may be prematurely terminated from the study for the following reasons:

1. The participant elects to withdraw consent from all future study activities, including follow-up.
2. The participant is "lost to follow-up" as defined in the Protocol ICAC-30 MOP.
3. The participant dies.
4. Development of any serious medical illness whose natural history, sequela, or treatment would be worsened or impaired by continuation in the protocol.
5. The study clinician no longer believes participation is in the best interest of the participant.
6. The participant has an SAE related to investigational agent.
7. The participant becomes pregnant.
8. The participant has a need for > 3 bursts of systemic corticosteroids for asthma exacerbations during the treatment period OR has a need for > 2 bursts of systemic corticosteroids for asthma exacerbations within 6 months during the treatment period. If prescribed by an ICAC study clinician, a burst is defined as a course for prednisone, prednisolone, or methylprednisolone will be 2 mg/kg/day as a single daily dose for 4 days with a maximum dose of 60 mg per day. The course for dexamethasone will be 0.3 to 0.6 mg/kg/day as a single daily dose for 2 days with a maximum dose of 16 mg per day. If a corticosteroid burst for the treatment of an asthma exacerbation is prescribed by a non-ICAC clinician, it will be counted regardless of dose. If a participant initiates and completes a course of systemic corticosteroids without clinician involvement, this course will be counted only if the study clinician agrees the treatment was warranted, and it meets the following dosage: the course for prednisone, prednisolone, or methylprednisolone will be at least 20 mg daily dose for 3 of 5 consecutive days. The course for dexamethasone will be at least a 10 mg single daily dose.
9. The participant has a need for > 14 days of prednisone (or prednisolone or methylprednisolone; defined as a minimum of 5 mg/day) over a 21 day period from Visit 1 through Visit 14.
10. The participant has a hypoxic seizure due to asthma.
11. The participant requires intubation due to asthma.
12. The participant has intolerable adverse experiences.

Principal Investigator from reporting to the Study Sponsor any other events, associated or not with these procedures, as AEs.

Allergen Skin Testing

- Prolonged (>24 hours) itching at test site
- Swelling (> 10 cm) at site of test lasting more than 24 hours
- Nasal allergic symptoms within 30 minutes of the procedure
- Fainting/Vasovagal event within 30 minutes of the procedure
- Anaphylaxis

Blood Draws

- Fainting/Vasovagal events
- Bruising at puncture site larger than 2 cm diameter
- Bleeding from puncture site lasting more than 30 minutes
- Swelling at puncture site larger than 2 cm

Pulmonary Function Testing

- Wheezing or bronchoconstriction requiring treatment with bronchodilators within 30 minutes from the procedure
- Coughing requiring treatment with bronchodilators within 30 minutes from the procedure

Sputum Induction

- Wheezing or bronchoconstriction requiring treatment with bronchodilators within 30 minutes from the procedure
- Coughing requiring treatment with bronchodilators within 30 minutes from the procedure

Nasal Lavage

- Acute sinusitis diagnosed by study clinician or based upon assessment of outside health records within 72 hours after the procedure

Nasal Blow / Nasal Mucus Sample Collection

- Acute sinusitis diagnosed by study clinician or based upon assessment of outside health records within 72 hours after the procedure

Nasal Epithelial Cell Collection

- Epistaxis within 24 hours of the procedure

Nasal Swab Sample Collection

Exacerbations of asthma are ordinary, anticipated complications of asthma observed in patients receiving standard of care. An asthma exacerbation that requires hospitalization and is determined to be a SAR (Section 12.2.1.1) will be considered a SAE. All other asthma hospitalizations will not be considered SAEs and will be entered only on the appropriate CRFs. The date of onset of the asthma exacerbation SAE will be the date of hospital admission and the date of resolution will be the discharge date. The underlying condition will be followed as per protocol.

12.3 Grading and Attribution of Adverse Events

Information in this section complies with *ICH Guideline E-6: Guidelines for Good Clinical Practice* and applies the following standards:

- Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (published September 2007) for local reactions to study procedures.
- Grading System of Severity of Anaphylaxis adapted, from the grading scale of Brown et al. ([36]) for anaphylaxis and systemic reactions to study procedures.
- National Cancer Institute *Common Terminology Criteria for Adverse Events Version 4.03* (published June 14, 2010) for all other reactions. (This document is referred to herein as the "NCI-CTCAE manual.")

12.3.1 Grading Criteria

12.3.1.1 Adverse Events Related to Skin Testing and Injection Site Reactions

Skin Testing

Excessive local reactions to skin testing not associated with systemic signs or symptoms will be graded according to Table 12.3.1.1a below. All AEs should be recorded on the eCRF.

Table 12.3.1.1a Grading of Local Reactions to Study Procedures

Grade	1 (Mild)	2 (Moderate)	(Severe)
Skin testing	Meets the minimum criteria listed in section 12.2.1, but requiring no medication other than topical corticosteroids or antihistamines.	Interfering with usual daily activities or sleep and requiring oral steroids.	Requiring a visit to a health care provider for treatment

Anaphylaxis

Criteria from [37]Sampson, et al. will be used to determine whether systemic allergic reactions constitute anaphylaxis (see Table 12.3.1.1b Clinical Criteria for Diagnosing Anaphylaxis). To be considered anaphylaxis, the event must involve at least two organ systems (e.g. not be simply a skin reaction). All anaphylaxis events, regardless of severity grade, will be recorded as Adverse Events and will be graded according to Table 12.3.1c Grading System of Severity of Anaphylaxis, adapted from the grading scale of [36]Brown et al.

Table 12.3.1.1b Clinical Criteria for Diagnosing Anaphylaxis

Anaphylaxis is highly likely when any <u>one</u> of the following 3 criteria are fulfilled:

1) Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (e.g., generalized hives, pruritus or flushing, swollen lips-tongue-uvula)
   AND AT LEAST ONE OF THE FOLLOWING:
   a) Respiratory compromise (e.g., dyspnea, wheeze-bronchospasm, stridor, reducted PEF, hypoxemia
   b) Reduced BP or associated symptoms of end-organ dysfunction (e.g., hypotonia [collapse], syncope, incontinence)
2) Two or more of the following that occur rapidly after exposure *to a <u>likely</u> allergen for that patient* (minutes to several hours):
   a) Involvement of the skin-mucosal tissue (e.g., generalized hives, itch-flush, swollen lips-tongue-uvula)
   b) Respiratory compromise (e.g., dyspnea, wheeze-bronchospasm, stridor, reducted PEF, hypoxemia)
   c) Reduced BP or associated symptoms (e.g., hypotonia [collapse], syncope, incontinence)
   d) Persistent gastrointestinal symptoms (e.g., crampy abdominal pain, vomiting)
3) Reduced BP after exposure to *<u>known</u> allergen for that patient* (minutes or several hours):
   a) Infants and children: low systolic BP (age specific) or greater than 30% decrease in systolic BP*
   b) Adults: systolic BP of less than 90 mm Hg or greater than 30% decrease from that person's baseline

PEF, Peak expiratory flow; *BP,* blood pressure.
*Low systolic blood pressure for children is defined as less than 70 mm Hg from 1 month to 1 year, less than (70 mm Hg + [2 x age]) from 1 to 10 years, and less than 90 mm Hg from 11 to 17 years.

Table 12.3.2 Attribution of Adverse Events

Code	Descriptor	Relationship (to primary investigational product and/or other concurrent mandated study therapy or study procedure)
UNRELATED CATEGORY		
1	Unrelated	The adverse event is clearly not related; there is insufficient evidence to suggest a causal relationship.
RELATED CATEGORIES		
2	Possible	The adverse event has a reasonable possibility to be related; there is evidence to suggest a causal relationship.
3	Definite	The adverse event is clearly related.

12.4 Collection and Recording of Adverse Events

12.4.1 Collection Period

Adverse events will be collected from the time of consent until a participant completes study participation or until 30 days after he/she prematurely withdraws (without withdrawing consent) or is withdrawn from the study.

12.4.2 Collecting Adverse Events

Adverse events (including SAEs) may be discovered through any of these methods:

- Observing the participant
- Interviewing the participant
- Receiving an unsolicited complaint from the participant
- In addition, an abnormal value or result from a clinical or laboratory evaluation can also indicate an adverse event, as defined in Section 12.3, Grading and Attribution of Adverse Events

12.4.3 Recording Adverse Events

Throughout the study, the study clinician will record adverse events and serious adverse events as described previously (Section 12.2, Definitions) on the appropriate AE/SAE eCRF regardless of the relationship to study therapy regimen or study procedure.

Once recorded, an AE/SAE will be followed until it resolves with or without sequelae, or until the end of study participation, or until 30 days after the participant prematurely withdraws (without withdrawing consent)/or is withdrawn from the study, whichever occurs first. The DAIT NIAID Medical Officer may request continued follow-up of an adverse event beyond the stated timeframe if deemed clinically important.

12.5 Reporting of Serious Adverse Events and Adverse Events

12.5.1 Reporting of Serious Adverse Events to the IND Sponsor (DAIT NIAID)

This section describes the responsibilities of the Principal Investigator to report serious adverse events to the IND sponsor via SAE eCRF. Timely reporting of adverse events is required by 21 CFR and ICH E6 guidelines.

Principal Investigators will report all serious adverse events (see Section 12.2.3, Serious Adverse Event), regardless of relationship or expectedness within 24 hours of discovering the event.

For serious adverse events, all requested information on the AE/SAE eCRF will be provided. The following minimal criteria must be included in the initial SAE eCRF:

- AE Term
- Study Drug Treatment
- Relationship to study drug
- Reason why the event is serious

However, unavailable details of the event will not delay submission of the known information. As additional details become available, the AE/SAE eCRF will be updated and submitted.

12.5.2 Reporting to FDA

After an adverse event requiring 24 hour reporting (per Section 12.5.1, Reporting of Serious Adverse Events to Sponsor) is submitted by the Principal Investigator and assessed by the IND sponsor (DAIT NIAID), there are two options for DAIT NIAID to report the adverse event to the FDA: annual reporting and expedited safety reporting.

12.5.2.1 IND Annual Reporting

DAIT NIAID will include in the IND Annual Report to the FDA all adverse events classified as:

- Serious, expected, suspected adverse reactions (see Section 12.2.1.1, Suspected Adverse Reaction, and Section 12.2.2, Unexpected Adverse Event).
- Serious and not a suspected adverse reaction (see Section 12.2.1.1, Suspected Adverse Reaction).
- Pregnancies.

Note that all adverse events (not just those requiring 24-hour reporting) will be reported in the IND Annual Report.

12.5.2.2 Expedited Safety Reporting

This option, with 2 possible categories, applies if the adverse event is classified as one of the following:

Category 1: Serious and unexpected suspected adverse reaction [SUSAR] (see Section 12.2.1.1, Suspected Adverse Reaction and Section 12.2.2, Unexpected Adverse Event and 21 CFR 312.32(c)i).

The sponsor shall report any suspected adverse reaction that is both serious and unexpected. The sponsor shall report an adverse event as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study drug and the adverse event, such as:

1. A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure (e.g., angioedema, hepatic injury, or Stevens-Johnson Syndrome)

- Appearance, pulse, grimace, activity, and respiration (APGAR) score at 1 minute, 5 minutes, and 24 hours after birth, if available
- Any abnormalities

All pregnancy complications that result in a congenital abnormality, birth defect, miscarriage, and medically indicated abortion - an SAE shall be submitted to the DAIT SACCC and the IND sponsor (DAIT NIAID) using the SAE reporting procedures described above.

12.7 Reporting of Other Safety Information

A Principal Investigator shall promptly notify the Central IRB as well as the DAIT SACCC and the IND sponsor (DAIT NIAID) via email when an "unanticipated problem involving risks to subjects or others" is identified, which is not otherwise reportable as an adverse event.

12.8 Review of Safety Information

12.8.1 Medical Monitor Review

The DAIT NIAID Medical Monitor shall receive monthly reports from the DAIT SACCC compiling new and accumulating information on AEs, SAEs, and pregnancies recorded by the study site(s) on appropriate eCRFs.

In addition, the DAIT NIAID Medical Monitor shall review and make decisions on the disposition of the SAE and pregnancy reports received by the DAIT SACCC (See Sections 12.5 and 12.6).

12.8.2 DSMB Review

12.8.2.1 Planned DSMB Reviews

The NIAID Allergy and Asthma Data and Safety Monitoring Board (DSMB) shall review safety data at least yearly during planned DSMB Data Review Meetings. Data for the planned safety reviews will include, at a minimum, a listing of all reported AEs and SAEs.

The DSMB will be informed of an Expedited Safety Report as determined by the DAIT NIAID Medical Monitor. An SAE which the DAIT NIAID Medical Monitor determines to be an unexpected safety risk will be sent to the DSMB immediately.

12.8.2.2 Ad hoc DSMB Reviews

In addition to the pre-scheduled data reviews and planned safety monitoring, the DSMB may be called upon for *ad hoc* reviews. The DSMB will review any event that potentially impacts safety at the request of the protocol chair or the IND sponsor (DAIT NIAID). In addition, occurrences described in Section 11.5 Study Stopping Rules will trigger an ad hoc comprehensive DSMB Safety Review.

After review of the data, the DSMB will make recommendations regarding study conduct and/or continuation.

A temporary halt in enrollment and study treatment will be implemented if an ad hoc DSMB safety review is required.

13 Statistical Considerations and Analytical Plan

13.4 Analysis Plan

13.4.1 Analysis Populations

The following groups of participants will define samples for endpoint analysis:

- Modified Intent-to-treat (ITT) sample: All participants who are randomized and receive at least one dose of study treatment. Participants will be analyzed according to the treatment arm to which they were randomized, regardless of the medication they actually received.
- Safety sample: All participants who are randomized and receive at least one dose of study treatment. Participants will be analyzed according to the medication they actually received, regardless of the treatment arm to which they were randomized. Non-treatment emergent adverse events (e.g., any adverse event that occurs before the first injection) will be summarized in all participants who are enrolled, while treatment emergent adverse events (e.g., any adverse event that occurs on or after the first injection) will be summarized in the safety sample.
- Per-protocol (PP) sample: All participants who are randomized, received the correct medication and dosage to which they were randomized, do not have a major protocol deviation and cannot have missed 3 or more consecutive injections or 4 or more scheduled injections.

13.4.2 Primary Analysis of Primary Endpoint(s)/Outcome(s)

The primary analysis will compare the rate of exacerbations during the treatment period using a negative binomial model applied to the modified intent-to-treat sample. The model will include an offset term to account for differential follow-up among participants. To account for adaptive randomization, the model will also be adjusted for study site, number of exacerbations in year prior to study (2 or 3+), peripheral blood eosinophils (< or ≥400 cells/µl), BMI (< or ≥95th percentile for age), total serum IgE (< or ≥540 kUA/L) and treatment dose (40 mg or 100 mg). Such a model will allow us to estimate the adjusted relative rate and corresponding 95% CI between the mepolizumab and placebo arms. A two-sided significance level of 0.05 will be used for this analysis. The reduction of the dose of mepolizumab from 100 mg to 40 mg in children 6 to 11 years of age and >40 kg will not affect the primary outcome as data from the GSK-sponsored pediatric trial 200363, NCT02377427 is therapeutically equivalent.

13.4.3 Supportive Analyses of the Primary Endpoint(s)/Outcome(s)

Analyses for the primary endpoint may be supported by several analyses.

One disadvantage of a covariate adaptive randomization strategy is that Type I error may be inflated in certain circumstances (e.g., time trends). Consequently, rather than adjusting the model for the variables used in the adaptive randomization as discussed in Section 13.4.2, a permutation test will be used for inference instead as it involves fewer assumptions. Under a permutation model, the observed relative rate between the mepolizumab and placebo arms will be assessed with reference to all possible permutations that the treatment arms could have been assigned to participants.

Rather than adjusting the model for the variables used in the adaptive randomization, the model will instead adjust for study site (as a random or fixed effect), number of exacerbations in year prior to study (2, 3 or 4+), BMI percentile (treated as a continuous variable), total serum IgE (treated as a continuous variable and log-transformed, if appropriate) peripheral blood eosinophils (treated as a continuous variable and log-transformed, if appropriate), and treatment dose (40 or 100 mg).

To test if the effect of mepolizumab on the rate of asthma exacerbations during the treatment period is modified by particular sub-groups, separate negative binomial models of the rate of asthma exacerbations during the treatment period will be developed to test the two-way interaction between treatment arm and:

- Indicator for whether or not a participant fits the FDA-approved dosing table for omalizumab therapy
- BMI percentile at baseline (continuous or categorical)
- Peripheral blood eosinophils at baseline (continuous or categorical and log-transformed, if appropriate)
- Bronchodilator responsiveness at baseline (continuous or categorical)
- Number of exacerbations in year prior to study (2, 3 or 4+)
- Total serum IgE at baseline (continuous or categorical and log-transformed, if appropriate)
- Dose at baseline (40 mg or 100 mg)
- FeNO (continuous or categorical) at baseline
- Nasal ECP at baseline

To test if the effect of mepolizumab on the rate of asthma exacerbations during the treatment period is modified by both number of exacerbations in year prior to study as well as peripheral blood eosinophils, a separate negative binomial model of the rate of asthma exacerbations during the treatment period will be developed to test the three-way interaction between treatment arm, peripheral blood eosinophils (treated as either a piecewise linear spline or non-linear spline and log-transformed, if appropriate) and number of exacerbations in year prior to study (2, 3 or 4+).

13.4.4 Analyses of Secondary and Other Endpoint(s)/Outcome(s)

Generalized linear mixed models will be used to model CASI and lung function during the treatment period. A generalized logit model will be used to analyze the physician-patient global assessment at Visit 14. Kaplan-Meier curves and a Cox PH model will also be used to model the time to first asthma exacerbation during the treatment period.

As with the primary analyses, generalized linear mixed models, the generalized logit model, as well as the Cox PH model will include treatment arm as the primary exposure but will also adjust for study site, number of exacerbations in year prior to study (2 or 3+), peripheral blood eosinophils (above or below 400 cells/µl), BMI (above or below 95th percentile for age) and total serum IgE (above or below 540 kUA/L) to account for adaptive randomization.

To determine if mepolizumab reduces the rate of asthma exacerbation in participants who do not fit the FDA-approved dosing table for omalizumab therapy or alternatively, if mepolizumab reduces the rate of asthma exacerbation in participants who fit the FDA-approved dosing table, a separate negative

binomial model similar to that used for the primary analysis will be applied to all participants in the modified intent-to-treat sample in each of the 2 sub-groups. Because the goal of this objective is to estimate the effect of mepolizumab on the rate of asthma exacerbation in each sub-group, rather than compare these two effects using an interaction test, these effects will be reported regardless of the results obtained from the interaction test, which will be performed as part of the supportive analyses for the primary endpoint (see Section 13.4.3).

Safety will be summarized by arm in the safety sample by the number and percentage of serious and non-serious adverse events stratified by grade and relationship to treatment as well as the number and percentage of participants who have a serious and non-serious adverse event stratified by grade and relationship to treatment. Levels of antibody to mepolizumab will be analyzed in the safety sample using generalized linear mixed models as stated above in the mepolizumab arm only.

13.4.5 Analyses of Exploratory Endpoint(s)/Outcome(s)

To determine whether mepolizumab improves other important clinical outcomes, including rate of virus-induced asthma exacerbations, ACT/cACT scores, maximum number of asthma symptom days or bronchodilator response, negative binomial models, generalized linear mixed models, generalized logit models, Kaplan-Meier curves and Cox PH models will be applied as appropriate to each endpoint in a manner similar to that described in Sections 13.4.2 to 13.4.4.

To identify immunologic pathways in the airways and peripheral blood that are characteristic of exacerbations prevented with and persistent despite anti-IL5 therapy, general linear models appropriate for RNA-sequencing count data will be used. Between group comparisons will be treated as a two independent group comparison and within group comparisons will use a mixed effect model with individual as a random effect. For comparisons where we are testing whether the two groups behave differently with a cold, an exacerbation, or over time, a general linear model will be created adding in these variables as main effects in the model and interaction terms between each effect. The general linear models will be expanded to include cell enumeration data as additional variables. Similarly, other salient variables (eg virus PCR data, collection site, collection time) that explain heterogeneity seen in the gene expression pattern, will be added to the model as appropriate. When relevant, predefined genes and gene sets of interest identified through Protocol ICAC-29 MUPPITS-1 results or previously published data will be tested separately without stringent multiple testing correction. Gene expression differences will be assigned to specific cell types using gene expression deconvolution algorithms appropriate for bulk cell population expression data. Predefined subgroups including PCR virus results, age, sex, race/ethnicity, obesity, timing of onset of asthma exacerbation relative to onset of cold symptoms, systemic corticosteroid use, season, nasal corticosteroid use, tobacco smoke exposure, and eNO will also be compared.

13.4.6 Descriptive Analyses

Descriptive analyses will be reported separately for mepolizumab and placebo arms. Continuous baseline measures will be reported 1) means (or geometric means) with 95% confidence intervals, or 2) median with first and third quartiles, as appropriate. Categorical baseline and demographic characteristics and study disposition will be reported as frequencies and proportions.

13.5 Interim Analyses

13.5.1 Interim Analysis of Efficacy Data

No formal interim analysis of efficacy data will be performed for this study.

13.5.2 Interim Analysis of Safety Data

The DSMB will receive periodic safety reports on enrolled participants. However, no formal interim analysis of safety data will be conducted.

13.5.3 Futility Analysis

No formal futility analysis will be performed for this study.

13.6 Statistical Hypotheses

All primary and secondary objectives will be based on two-sided superiority tests. For instance, the null and alternative hypotheses for the primary objective are:

H0: The rate of asthma exacerbations during the treatment period in the mepolizumab arm is equal to the rate of asthma exacerbations during the treatment period in the placebo arm.

Ha: The rate of asthma exacerbations during the treatment period in the mepolizumab arm is not equal to the rate of asthma exacerbations during the treatment period in the placebo arm.

Additionally, Secondary Objective 5 will separately determine whether mepolizumab reduces the rate of asthma exacerbations in the sub-group of study participants who do not fit the FDA-approved dosing table for omalizumab therapy and in study participants who fit the FDA-approved dosing table for omalizumab therapy. In this case, the null and alternative hypotheses for this objective are:

H0: The rate of asthma exacerbations during the treatment period in the mepolizumab arm is equal to the rate of asthma exacerbations during the treatment period in the placebo arm in study participants who do not fit the FDA-approved dosing table for omalizumab therapy.

Ha: The rate of asthma exacerbations during the treatment period in the mepolizumab arm is not equal to the rate of asthma exacerbations during the treatment period in the placebo arm in study participants who do not fit the FDA-approved dosing table for omalizumab therapy.

H0: The rate of asthma exacerbations during the treatment period in the mepolizumab arm is equal to the rate of asthma exacerbations during the treatment period in the placebo arm in study participants who fit the FDA-approved dosing table for omalizumab therapy.

Ha: The rate of asthma exacerbations during the treatment period in the mepolizumab arm is not equal to the rate of asthma exacerbations during the treatment period in the placebo arm in study participants who fit the FDA-approved dosing table for omalizumab therapy.

13.7 Sample Size Considerations

Based on Pavord et al,[14] the estimated relative rate of clinically significant asthma exacerbations between 75 mg, 250 mg, and 750 mg Mepolizumab arms and the Placebo arm was 0.52, 0.61, and 0.48 respectively. In Protocol ICAC-19 APIC, we found that among 94 enrolled participants that were difficult-to-treat at screening, had at least 2 exacerbations in the previous year at screening and peripheral blood eosinophils ≥150 cells/µl, the rate of asthma exacerbations per patient –year was approximately 1.43

15.1.2 Major Protocol Deviation

A protocol violation is a deviation from the IRB-approved protocol that may affect the participant's rights, safety, or well-being and/or the completeness, accuracy and reliability of the study data. In addition, protocol violations include willful or knowing breaches of human subject protection regulations, or policies, any action that is inconsistent with the NIH Human Research Protection Program's research, medical, and ethical principles, and a serious or continuing noncompliance with federal, state, local or institutional human subject protection regulations, policies, or procedures.

15.1.3 Non-Major Protocol Deviation

A non-major protocol deviation is any change, divergence, or departure from the study design or procedures of a research protocol that does not have a major impact on the participant's rights, safety or well-being, or the completeness, accuracy and reliability of the study data.

15.2 Reporting and Managing Protocol Deviations

The Principal Investigator has the responsibility to identify, document and report major and non-major protocol deviations as directed by the study Sponsor. However, major and non-major protocol deviations may also be identified during site monitoring visits or during other forms of study conduct review.

Upon determination that a protocol deviation (major or minor) has occurred, the study staff will a) notify the Principal Investigator (PI), b) notify the DAIT SACCC, and c) complete the Protocol Deviation form. The Protocol Deviation form will document at a minimum the date the protocol deviation (PD) occurred, the date PD identified, a description of event, whether the deviation resulted in SAE/AE, the signature of PI, report to Central IRB, and documentation of a corrective action plan. The DAIT SACCC and the IND sponsor (DAIT NIAID) may request discussion with the PI to determine the effect of the protocol deviation on the study participant and his/her further study participation, the effect of the protocol deviation on the overall study, and corrective actions. The PI will complete and sign the Protocol Deviation form and submit it to the DAIT SACCC and to the Central IRB, per IRB regulations. Major protocol deviations will be reported to the DSMB by the DAIT NIAID Medical Monitor at the Medical Monitor's discretion.

16 Ethical Considerations and Compliance with Good Clinical Practice

16.1 Quality Control and Quality Assurance

The Principal Investigator is required to keep accurate records to ensure that the conduct of the study is fully documented. The Principal Investigator is required to ensure that all CRFs are completed for every participant entered in the trial.

The sponsor is responsible for regular inspection of the conduct of the trial, for verifying adherence to the protocol, and for confirming the completeness, consistency, and accuracy of all documented data.

The CRFs will be completed online via a web-based electronic data capture (EDC) system that has been validated and is compliant with Part 11 Title 21 of the Code of Federal Regulations. Study staff at the site will enter information into the electronic CRFs, and the data will be stored remotely at a central database. Data quality will be ensured through the EDC system's continuous monitoring of data and real-time detection and correction of errors. All elements of data entry (i.e., time, date, verbatim text,

Nasal blow		+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Nasal resp virus		+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Nasal ECP		+	+	+	+			+							+		
Nasal swab collection^																+	+
Nasal epithelial cell collection															+**		
Sputum Induction															+**		
Vital signs and growth parameters	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adverse event assessment	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Concomitant medication assessment	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

* may use CBC results collected in another ICAC protocol if within past 6 months and allergen skin tests performed in another ICAC protocol if within past 12 months – skin tests can be performed at V0, V2, V3 or V4 if not previously obtained in past 12 months
+ up to two with an optional third cold visit will occur for each participant
# as needed exacerbation visits to occur 7 days after start of systemic corticosteroids
** to be performed in participants 10 years and older
^Nasal swab collection replaced the nasal blow procedure at cold and exacerbation visits in Protocol Version 8.0

NIAID	National Institute of Allergy and Infectious Diseases
MedDRA	Medical Dictionary for Regulatory Activities
mITT	Modified Intent-to-Treat
PD	Protocol Deviation
PT	Preferred Term
SACCC	Statistical and Clinical Coordinating Center
SD	Standard Deviation
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Suspected Adverse Reaction
SOC	System Organ Class
TEAE	Treatment-Emergent Adverse Event

8. ENDPOINT EVALUATION

8.1. Overview of Efficacy Analysis Methods

8.1.1. Multicenter Studies

Although this is a multicenter study, the data is intended to be collected and analyzed as a whole. There are too few participants enrolled in the study relative to the number of sites to perform formal analyses stratified by site.

8.1.2. Assessment Time Windows

Mepolizumab/placebo injections were scheduled as close as possible to the every 4-week target date as outlined in the Schedule of Events (Section 15.1). Any data collected outside of a visit window will still be included in any analyses of the scheduled visit.

At the onset of the COVID-19 pandemic, the following procedures were restricted, and were site-specific: Impulse oscillometry, spirometry, FeNO, nasal lavage samples, nasal blow samples, nasal epithelial cell collection, and sputum samples. Exacerbation visits were conducted only via telephone and cold visits were suspended. If participants were unable to come into the study center for visits, the visits were conducted via telephone.

8.1.3. Multiple Comparisons/Multiplicity

Adjustment for multiplicity will not be performed for the primary or secondary endpoints.

8.2. Primary Endpoint

8.2.1. Computation of the Primary Endpoint

For participants who completed the study, the primary endpoint is the number of asthma exacerbations between randomization and the end of participant follow-up (Visit 14), or four weeks from the final injection--whichever comes first. For participants who terminated from the study early, the primary endpoint is number of exacerbations between randomization and their last known completed visit.

Exacerbations are defined as a prescription of a course of systemic corticosteroids by a clinician, initiation of a course of systemic corticosteroids by a participant, or as a hospitalization for asthma. If a participant initiated and completed a course of systemic corticosteroids without clinician involvement, this course will be counted only if the study clinician agrees the treatment was warranted, and it meets the following dosage: the course for prednisone, prednisolone, or methylprednisolone will be at least 20 mg daily dose for 3 of 5 consecutive days. The course for dexamethasone will be at least a 10 mg single daily dose. If a corticosteroid burst for the treatment of an asthma exacerbation was prescribed by a non-ICAC clinician, it was counted regardless of dose.

8.2.2. Primary Analysis of the Primary Endpoint

The primary analysis will compare the rate of exacerbations during the treatment period using a negative binomial model applied to the mITT sample. The model will include an offset term to account for differential follow-up among participants. To account for adaptive randomization, the model will also be adjusted for study site, number of exacerbations in year prior to study (2 or 3+), peripheral blood eosinophils (< or ≥400 cells/μl), BMI (< or ≥95th percentile for age), total serum IgE (< or ≥540 kUA/L) and treatment dose (40 mg or 100 mg). Such a model will allow us to estimate the adjusted relative rate and corresponding 95% CI between the mepolizumab and placebo arms. A two-sided significance level of 0.05 will be used for this analysis.

Although the primary version of the primary analysis will be conducted on the mITT sample, the same analysis of the primary endpoint will be performed for the PP population.

8.2.3. Sensitivity Analyses of the Primary Analysis

At the time of the COVID-19 pandemic and subsequent quarantine period, observable exacerbation rates decreased relative to observed rates in the prior years of the study. In order to account for the unknown effects of the pandemic on the primary endpoint, the exacerbation rate will be analyzed using the same model as specified in section 8.2.2, but only including participants who were randomized, received at least one injection, and who had at least one follow-up visit prior to March 16, 2020. For participants who met this definition, only exacerbations that occurred between randomization and March 16, 2020 will be analyzed in the model. All subsequent exacerbations and follow-up time will be censored after March 16, 2020. The offset variable used in the model will be defined as the length of time, in years, from randomization to the end of a participant's follow-up, or March 16, 2020, whichever occurs first.

The same analysis will be applied to the PP population.

8.2.4. Secondary Analyses of the Primary Endpoint

Analyses for the primary endpoint may be supported by several analyses.

One disadvantage of a covariate adaptive randomization strategy is that Type I error may be inflated in certain circumstances (e.g., time trends). Consequently, rather than adjusting the model for the variables used in the adaptive randomization as discussed in Section 8.2.2, a permutation test will be used for inference instead as it involves fewer assumptions. Under a permutation model, the observed relative rate between the mepolizumab and placebo arms will be assessed with reference to all possible permutations that the treatment arms could have been assigned to participants, or up to 5000 iterations.

Rather than adjusting the model for the variables used in the adaptive randomization, the model will instead adjust for study site (as a random or fixed effect), number of exacerbations in year prior to study (2, 3 or 4+), BMI percentile (treated as a continuous variable), total serum IgE (treated as a continuous variable and log-transformed, if appropriate) peripheral blood

eosinophils (treated as a continuous variable and log-transformed, if appropriate), and treatment dose (40 or 100 mg).

8.3. Secondary Endpoints

8.3.1. Endpoint 1

Variable: Composite Asthma Severity Index (CASI) at Visits 4, 7, 10, 13, and 14.

Analysis: A generalized linear mixed model will be used to model CASI during the treatment period. The model will include a fixed effect for treatment arm as the primary exposure but will also adjust for baseline CASI, study site, number of exacerbations in year prior to study (2 or 3+), peripheral blood eosinophils (above or below 400 cells/µl), BMI (above or below 95th percentile for age) and total serum IgE (above or below 540 kUA/L) to account for adaptive randomization. Visit will be treated as a categorical variable in the model, and an interaction term between treatment arm and visit will be used to estimate the least square mean CASI for each treatment arm for each timepoint of measurement. The difference in least square mean, the corresponding 95% CI and the p-values will be provided for the comparison of mepolizumab vs placebo. The model will be fit using restricted maximum likelihood, and each fixed effect will be tested using an F-test statistic, the Kenward-Roger approximation for the degrees of freedom, an unstructured covariance matrix, and a two-sided significance level of 0.05. If the model assuming an unstructured covariance matrix does not converge, other covariance structures, such as a spatial power covariance structure, will be considered. Descriptive statistics including the number of participants, mean, and standard deviation will also be presented. This analysis will be conducted on the mITT population.

As sites were restricted to limited visit assessments due to COVID-19, lung function assessments were not performed and FEV1 (% predicted), a domain of the CASI score, was not collected. In these cases, the last known FEV1 (% predicted) value, from Visit 4 or onward, will be used to calculate a participant's CASI at subsequent visits. Additionally, sites were discouraged from stepping down participants' asthma controller therapy during the pandemic, another domain of the CASI score, which could bias the results towards the null.

8.3.2. Endpoint 2

Variable: Quality of life, as measured by physician-patient global assessment, at Visit 14

Analysis: A generalized logit model will be used to analyze the physician-patient global assessment at Visit 14 for the mITT population. The model will include treatment arm as a fixed effect as the primary exposure but will also adjust for study site, number of exacerbations in year prior to study (2 or 3+), peripheral blood eosinophils (above or below 400 cells/µl), BMI (above or below 95th percentile for age) and total serum IgE (above or below 540 kUA/L). A table will summarize the observed quality of life measurements, as recorded in the CRF at Visit 14, by treatment arm. No imputation of missing data will be used for participants who were not assessed for quality of life measurements at Visit 14.

8.3.3. Endpoint 3

Variable: Lung function as assessed by spirometry and impulse oscillometry

Analysis: A generalized mixed model as described in section 8.3.1 will be used to analyze each spirometry and impulse oscillometry parameter, separately, at each visit where the lung function was collected for the mITT population. It is noted that lung function data was not collected during the limited assessment visits during the COVID-19 pandemic and is considered missing completely at random. No imputation of missing data will be used in the analysis of lung function data.

8.3.4. Endpoint 4

Variable: Time to first asthma exacerbation

Analysis: Kaplan-Meier curves and a Cox PH model will also be used to model the time to first asthma exacerbation during the treatment period. The Cox PH model will include treatment arm as the primary exposure but will also adjust for study site, number of exacerbations in year prior to study (2 or 3+), peripheral blood eosinophils (above or below 400 cells/µl), BMI (above or below 95th percentile for age) and total serum IgE (above or below 540 kUA/L). The analysis will be conducted for the mITT population ignoring the COVID-19 intercurrent event

8.3.5. Endpoint 5

Variable: Rate of exacerbations (mepolizumab vs. placebo) during the treatment period for participants in the mITT sample who do not fit the FDA-approved dosing table for omalizumab therapy and for participants who do fit the FDA-approved dosing table.

Analysis: A negative binomial model, as specified in section 8.2.2, will be used to estimate the effect of mepolizumab on the rate of exacerbation, as compared to the placebo group, in the omalizumab-eligible and omalizumab-ineligible sub-groups. A treatment and a treatment by omalizumab-eligibility interaction term will be used to estimate the exacerbation rates.

8.3.6. Endpoint 6

Variable: Adverse Events

Analysis: Safety will be summarized treatment arm in the safety sample by the number and percentage of serious and non-serious adverse events stratified by grade and relationship to treatment as well as the number and percentage of participants who have a serious and non-serious adverse event stratified by grade and relationship to treatment. See Section 9 for further details and definitions.

8.4. Other Endpoints

8.4.1. Exploratory Analysis of the Primary Endpoint

To test if the effect of mepolizumab on the rate of asthma exacerbations during the treatment period is modified by particular sub-groups, separate negative binomial models of the rate of asthma exacerbations during the treatment period will be developed to test the two-way interaction between treatment arm and:

- BMI percentile at baseline (continuous or categorical)
- Peripheral blood eosinophils at baseline (continuous or categorical and log-transformed, if appropriate)
- Bronchodilator responsiveness at baseline (continuous or categorical)
- Number of exacerbations in year prior to study (2, 3 or 4+)
- Total serum IgE at baseline (continuous or categorical and log-transformed, if appropriate)
- Dose at baseline (40 mg or 100 mg)
- FeNO (continuous or categorical) at baseline

To test if the effect of mepolizumab on the rate of asthma exacerbations during the treatment period is modified by both number of exacerbations in year prior to study as well as peripheral blood eosinophils, a separate negative binomial model of the rate of asthma exacerbations during the treatment period will be developed to test the three-way interaction between treatment arm, peripheral blood eosinophils (treated as either a piecewise linear spline or non-linear spline and log-transformed, if appropriate) and number of exacerbations in year prior to study (2, 3 or 4+).

8.4.2. Exploratory Endpoints

Analyses of exploratory endpoints and outcomes will be conducted as described in section 13.4.5 of the protocol.

9. SAFETY EVALUATION

9.1. Overview of Safety Analysis Methods

The safety analyses will be performed in the safety population defined in Section 5. Safety assessment summaries will include:

- AEs, defined in Protocol Section 12.2.1.
- Suspected Adverse Reactions (SARs), defined in Protocol Section 12.2.1.1.
- Unexpected Adverse Event, defined in Protocol Section 12.2.2
- AEs leading to discontinuation of study drug
- AEs leading to discontinuation from study
- Serious adverse events (SAEs), defined in Protocol Section 12.2.3.
- Deaths
- Clinical laboratory results
- Vital signs

Adverse events were collected from the time of consent until a participant completes study participation or until 30 days after he/she prematurely withdraws (without withdrawing consent) or is withdrawn from the study.

For this analysis, a treatment-emergent adverse event (TEAE) will be defined as any AE not present prior to the initiation of study drug or any AE already present that worsens in either intensity or frequency following exposure to study drug. TEAEs will be summarized in the safety population.

Partial dates will be imputed for the purposes of defining TEAEs as follows:

- For a missing start day where the month and year are present, the start day will be set to the first day of the month, unless: 1) the first day of the month is before the date of administration of study drug and the month and year are the same as the month and year of the date of administration of study drug; and 2) the end date is on or after the date of administration of study drug or the end date is completely missing, in which case the start day will be set to the day of administration of study drug.
- For a missing start day and month where the year is present, the start day and month will be set to January 1st, unless: 1) January 1st is before the date of administration of study drug and the year is the same as the year of the date of administration of study drug; and 2) the end date is on or after the date of administration of study drug or the end date is completely missing, in which case the start day and month will be set to that of the date of administration of study drug.

- For a missing end day where the month and year are present, the end day will be set to the last day of the month, unless the month and year are the same as the month and year of the last contact date for the participant, in which case the end day will be set to that of the participant's last contact date.
- For a missing end day and month where the year is present, the end day and month will be set to the participant's last contact date, unless the year of the participant's last contact date is greater than the end year, in which case the end day and month will be set to December 31st.

Unless otherwise specified, a data listing for all measurements in Section 9 will be provided for the safety population and sorted in order of treatment arm, participant ID, and time of assessment (e.g., visit, time, and/or event).

9.2. Adverse Events

All AEs will be classified by system organ class (SOC) and preferred term, according to a standardized thesaurus (Medical Dictionary for Regulatory Activities [MedDRA]). MedDRA V20.0 or later will be used to classify all AEs.

The severity of AEs will be classified, as applicable, using the following criteria and complies with *ICH Guideline E-6: Guidelines for Good Clinical Practice*:

- Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (published September 2007) for local reactions to study procedures.
- Grading System of Severity of Anaphylaxis adapted, from the grading scale of Brown et al. (3) for anaphylaxis and systemic reactions to study procedures.
- National Cancer Institute *Common Terminology Criteria for Adverse Events Version 4.03* (published June 14, 2010) for all other reactions. (This document is referred to herein as the "NCI-CTCAE manual.")

The relationship, or attribution, of an adverse event to the study therapy regimen or study procedure(s) will initially be determined by the study clinician and recorded on the appropriate AE/SAE eCRF. Final determination of attribution for safety reporting will be determined by DAIT NIAID. The relationship of an adverse event to study therapy regimen or procedures will be determined using the descriptors and definitions provided in Table 12.3.2 of the protocol.

Overall summary tables will be developed using the safety population to report the number of events and the number and percentage of participants having at least one treatment-emergent event in the following categories:

• AEs

• AEs by maximum grade for each of the following:

- Skin Testing
- Anaphylaxis

- Injection Site Reactions
- CTCAE

TEAEs classified by treatment arm, MedDRA SOC, and preferred term will be summarized in the Safety Population for each of the following categories:

- AEs
- AEs by relationship to study drug

Summary tables will present the total number of events as well as the number and percentage of participants experiencing the events. If a participant experiences the same AE on multiple occasions, the event will be counted once for each occurrence when reporting the number of AEs. When reporting the number of participants experiencing the events, a participant will only be counted once if they experience an event within the particular SOC or preferred term. Percentages will be based on the number of participants in the safety population.

Similar summaries will be generated for SARs (defined in Protocol Section 12.2.3), AEs leading to discontinuation of study drug, and AEs leading to study withdrawal separately.

Data listings will be provided separately for AEs, SARs, AEs leading to discontinuation of study drug, and cases of COVID-19.

9.3. Deaths, Serious Adverse Events

SAEs will be listed and summarized in the same manner described in Section 9.2. Separate displays listing death, including time to death and primary cause of death, will also be created.

9.4. Clinical Laboratory Evaluation

Clinical laboratory measurements will be performed at Screening and at various visits throughout the study and include a complete blood count with differential (CBC). Results will be converted to standardized units where possible. For numeric laboratory results, descriptive statistics of laboratory values will be presented for each treatment arm and overall. For categorical laboratory results, the number and percentage of participants reporting each result will be presented for each treatment arm and overall. A data listing of all laboratory measurements including an indication of any abnormal values and clinical significance will be provided.

9.5. Vital Signs and Growth Parameters, Physical Findings, and Targeted Pulmonary Exam Results

9.5.1. Vital Signs and Growth Parameters

Vital sign measurements (including temperature, heart rate, respiratory rate, systolic blood pressure, and diastolic blood pressure) and growth parameters will be collected at each clinic visit.

A data listing of all vital sign and growth parameter measurements will be provided.

9.5.2. Physical Examinations

A study clinician performed a physical examination at Visit 0. Additional examinations may have been completed at the remainder of the visits, but at the study clinician's discretion. Significant findings that met the definition of an adverse event (AE) were recorded on the AE form.

9.5.3. Targeted Pulmonary Exam

Targeted pulmonary exams were conducted at randomization; Asthma Evaluation and Management Visits 4, 7, 10, 13; Cold Visits; Exacerbation Follow-up Visits; and at the Final Visit. Chest auscultation results, including inspiratory wheeze, retractions, rhonchi, and crackles, will be listed by ID and study visit. Evidence of oral candidiasis, any significant findings, and participant's discontinuation from the study will also be reported in the listing.

	Scree-ning	Rand omiz ation	Treatment Phase (12 months)														
		Asthma Management															
Week	0	4	8	12	16	20	24	28	32	36	40	44	48	52	56	PRN	PRN
Visit	V0	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	Cold+	EX#
Consent/Assent	+																
Medical history	+																
Asthma Eval & Mgt	+	+			+			+			+			+	+		
Asthma Counseling	+	+			+			+			+			+	+		
Spirometry	+	+			+			+			+			+	+	+	+
IOS		+			+									+		+	+
Bronchodilator reversibility (Spiro & IOS)		+			+									+			
CASI	+	+			+			+			+			+	+		
ACT/c-ACT	+	+			+			+			+			+	+		
Physician-patient global assessment tool															+		
Physical Exam	+																
Targeted pulmonary exam		+			+			+			+			+	+	+	+
Lungs assessment			+	+		+	+		+	+		+	+				
FeNO		+		+				+			+				+	+	
SQ study drug/placebo		+	+	+	+	+	+	+	+	+	+	+	+	+			
Pregnancy test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Urinary cotinine		+		+											+	+	+
Blood collection	+	+		+							+				+	+	+
CBC with differential	+ *	+		+							+				+	+	+
Blood chemistry	+			+											+		
Serum total IgE & specific IgE	+														+		
Blood markers (plasma/RNA/DNA)		+		+											+	+	+
Antibodies to drug		+		+											+		
Skin Testing	+*		+*	+*	+*												

investigator will notify the protocol chair and the DAIT SACCC team of the unblinding event on the next business day. The emergency unblinding will also be reported to the NIAID Allergy and Asthma Data and Safety Monitoring Board (DSMB).

A full account of the event will be recorded, including the date and time of the unblinding, the reason for the decision to unblind, and the name of the individual who made the decision to unblind and the names of the DAIT NIAID Medical Monitor and others who were notified. The reasons for unblinding of a participant's treatment will be included in the final clinical study report.

Unblinding the study due to an approved interim analysis, final analysis, or study termination will require written approval from the DAIT NIAID Medical Monitor.

4 Selection of Participants and Clinical Sites/Laboratories

4.1 Rationale for Study Population

The population selected for this trial is urban asthma patients aged 6-17 years with difficult-to-control, exacerbation-prone asthma and a peripheral blood eosinophil count ≥150 cells/µl. Previous DAIT NIAID-sponsored inner-city asthma clinical trials have shown that high proportions of children who meet Protocol ICAC-30 MUPPITS-2 inclusion criteria continue to experience exacerbations despite receiving guidelines-based asthma management and maximum levels of treatment with inhaled steroids.[7,8] Omalizumab is an effective add-on treatment, but up to 50% of children and adolescents identified with difficult-to-control, exacerbation-prone asthma may be ineligible for omalizumab due to their high weight, high (or low) total serum IgE level, or lack of aeroallergen sensitization. Given the existing shortcomings in the effectiveness of current treatments for a significant proportion of children and adolescents with asthma, a new approach for their management is needed to improve disease control and reduce morbidity, focusing on exacerbations.

4.2 Inclusion Criteria

1. Participant and/or parent guardian must be able to understand and provide informed consent and age-appropriate assent
2. Are male and female aged 6-17 years, inclusive at randomization
3. Have a primary place of residence in one of the pre-selected recruitment census tracts as outlined in the Protocol ICAC-30 Recruitment MOP

   3a. Participants who do not live in the pre-selected census tracts but live within the Office of Management and Budget (OMB) defined Metropolitan Statistical Area and have publicly-funded health insurance will qualify for inclusion.
4. Have had a diagnosis of asthma made > 1 year prior to recruitment; participants who received an asthma diagnosis by a clinician ≤ 1 year prior to recruitment must report that their respiratory symptoms were present for more than 1 year prior to recruitment
5. Have had at least two asthma exacerbations in the prior year (defined as a requirement for systemic corticosteroids and/or hospitalization)
6. At Visit 0 (Screening), have the following requirement for asthma controller medication: for participants aged 6 to 11 years, treatments with at least fluticasone 250 mcg dry powder inhaler

As participants will receive their mepolizumab/placebo injections in the clinic, compliance will be monitored by assessing the number of completed and missed clinic visits.

6.5 Toxicity Prevention and Management

Mepolizumab/placebo doses will not be modified. Protocol Section 8.5 describes safety measures to monitor potential signs and symptoms post injection.

6.6 Premature Discontinuation of Study Participation

Study participation may be prematurely discontinued for any participant for any of the reasons identified in Section 11.2 Participant Stopping Rules and Withdrawal Criteria. Participants who are prematurely discontinued will follow end of study procedures as described in Section 11.4 Follow-up After Early Study Withdrawal.

Study participation may also be prematurely discontinued for any participant if the study clinician believes that the study treatment is no longer in the best interest of the participant.

6.7 Premature Discontinuation of Investigational Product

Participants who are discontinued from investigational product for any reason after receiving at least one injection, and who agree to remain in the study, will have an altered visit schedule. These participants will be seen in clinic every 3 months for asthma management and evaluation of protocol defined outcomes and endpoints (i.e. Visits 3, 4, 7, 10, 13 and 14 and cold and exacerbation visits). The participants will not attend the monthly protocol defined injection visits which do not involve outcome evaluation and asthma management. These participants will not receive any injections of the investigational product during the visits they do attend. During the visits the participant attends, they will undergo all other scheduled procedures, evaluations, and blood draws.

7 Other Medications

7.1 Concomitant Medications

7.1.1 Protocol-mandated

All participants will receive asthma step care according to the Guidelines for the Diagnosis and Management of Asthma (Expert Panel Report 3 [EPR-3], National Asthma Education and Prevention Program [NAEPP], 2007). Treatment will be supervised by a study clinician. The study clinician will prescribe initial appropriate concomitant asthma therapies (see Section 7.1.1.1) and will provide a written Asthma Action Plan for the participant at Visits 0, 1, 4, 7, 10 and 13 (see Section 7.1.1.4). The regimen will then be adjusted as necessary at those visits throughout the duration of the trial based on the principles of the EPR-3 guidelines (see Table 7.1.1.2a, below).

The study clinician will prescribe the appropriate step controller therapy according to the participant's insurance/health maintenance organization guidelines. The preferred controller steps and alternate equivalent medications may be modified or updated based on changes in standard clinical practice, discontinuation of current medications, or the introduction of new medications. For the standardized treatment of asthma exacerbations, the clinical research staff will provide oral prednisone and written instructions for use. Montelukast may be prescribed by study clinicians if the participant was already

FEV_1 = forced expiratory volume in 1 second; pred = predicted
* Determined from participant recall, based on the 2-week interval directly preceding the study visit.
**Predicted references are provided in the Protocol ICAC-30 MOP.
*** Defined as a prescription of a course of systemic steroids by a clinician or initiation of a course of systemic steroids by a participant to prevent a serious asthma outcome. If a participant initiates and completes a course of systemic steroids without clinician involvement, this course will be counted only if it meets the following dosage: the course for prednisone, prednisolone, or methylprednisolone will be at least 20 mg daily dose for 3 of 5 consecutive days. The course for dexamethasone will be at least a 10 mg single daily dose. If a corticosteroid burst for the treatment of an asthma exacerbation is prescribed by a non-ICAC clinician, it will be counted regardless of dose.

Next, the study clinician will determine the current level of controller therapy that the participant is using. Table 7.1.1.1b shows the asthma regimens that will be used during the treatment phase of Protocol ICAC-30 MUPPITS-2. If the participant is currently on one of the therapies below, then the step level of that therapy will be noted. Otherwise, the study clinician must refer to the Protocol ICAC-30 MOP for equivalency tables for other asthma therapies. From the information in the Protocol ICAC-30 MOP, the study clinician will determine the corresponding step level of therapy that the participant is currently using. For example, if the participant is currently taking Flovent® Diskus® 250 mcg twice per day (bid), then he/she is receiving Step 3 therapy.

Table 7.1.1.1b Asthma Regimen by Step Level of Therapy

Step	Medication Equivalent*
0	No controller medication; albuterol or levalbuterol prn
1	fluticasone 50 mcg bid
2	fluticasone 100 mcg bid
3	fluticasone 250 mcg bid
4	fluticasone 250 mcg bid plus LABA
5	fluticasone 500 mcg bid plus LABA

bid = twice per day; LABA = long-acting beta agonist
*Equivalent medication covered by participant's insurance formulary – see Protocol ICAC-30 MOP.

Based on the participant's current control level (from Table 7.1.1.1a) and the participant's current step level of therapy (from Table 7.1.1.1b and the Protocol ICAC-30 MOP), the study clinician will find the corresponding row in Table 7.1.1.1c below that includes both the current regimen and the current control level. The last column of that row gives the corresponding regimen as a step level, which refers back to Table 7.1.1.1b. The study clinician will prescribe this regimen. Study eligibility in terms of treatment step level will be as follows:

- For participants ages 6 to 11 years, who have a requirement for at least Step Level 3 (defined in Table 7.1.1.1b) at Visit 0
- For participants ages 12 years and older, who have a requirement for at least Step Level 4 (defined in Table 7.1.1.1b) at Visit 0

Based on the overall control level and the participant's estimated level of adherence, the study clinician will use Table 7.1.1.2bb below to determine how the participant's current therapy should be modified. At E&M Visits, the participant will be considered to have acceptable adherence if he/she reports having taken 50% or more of the prescribed doses in the last 2 weeks. Participants with unacceptable adherence will not be stepped up in the same way as those with acceptable adherence. Continuing with the same example, since this participant is at control level 3 and assuming he/she has acceptable adherence, the study clinician would recommend a 2-step increase in therapy (or an increase to Step 5 if on Step 4 or maintenance on Step 5 if already on Step 5). In all cases, consideration will be given to a burst of prednisone according to the suggestions in the table.

Table 7.1.1.2b Treatment Adjustment Based on Control Levels and Adherence

Control Level	Treatment Algorithm for Participants with Unacceptable Adherence	Treatment Algorithm for Participants with Acceptable Adherence
1	Continue same controller regimen	If on Step 0, maintain at Step 0 If on Steps 1-5, decrease controller regimen by 1 step ** Do not decrease controller regimen if at Visit 1
2	Continue same controller regimen or place on Step 2 therapy, whichever is higher	Increase controller regimen by 1 step, or continue Step 5 therapy if already on Step 5
3	Continue same controller regimen or place on Step 2 therapy, whichever is higher	**No systemic steroids since the Last Medication Management visit*** If on Steps 0-4 increase controller regimen by 1 step If on Step 5 continue Step 5 **or** treat with Step 5 and a 4 day burst of prednisone or equivalent; **≥1 course(s) of systemic steroids since the last E&M visit*** If on steps 0-3 increase controller regimen by 2 steps If on treatment Step 4 increase controller regimen to Step 5 **or** treat with Step 5 and a 4 day burst of prednisone or equivalent If on treatment Step 5 continue Step 5 **or** treat with Step 5 and a 4 day burst of prednisone or equivalent
4	Continue same controller regimen or place on Step 3 therapy, whichever is higher OR Treat with 4-day prednisone burst and continue same controller regimen or place on Step 3 therapy, whichever is higher	If on Steps 0-3 increase controller regimen by 2 steps If on Step 4, increase to Step 5 **or** treat with Step 5 and a 4-day prednisone burst or equivalent If already on Step 5, continue on Step 5 **or** treat with Step 5 and a 4-day burst of prednisone or equivalent

*Defined as a prescription of a course of systemic steroids by a clinician or initiation of a course of systemic steroids by a participant to prevent a serious asthma outcome. If a participant initiates and completes a course of systemic steroids without clinician involvement, this course will be counted only if it meets the following dosage: the course for prednisone, prednisolone, or methylprednisolone will be at least 20 mg daily dose for 3 of 5 consecutive days. The course for dexamethasone will be at least a 10 mg single daily dose. If a corticosteroid burst for the treatment of an asthma exacerbation is prescribed by a non-ICAC clinician, it will be counted regardless of dose.
Note: Shading in the table corresponds to the example described in the text above.

Based on the participant's step level assigned at the last E&M visit and the change in steps determined above, the new regimen will be prescribed according to Table 7.1.1.2b above. In the example above, if

confirmation. To facilitate telephone contact with participants whose phone service may change during the study, up to three telephone contact numbers (relatives, neighbors, friends) will be collected for each participant. This has proven to be an effective strategy in previous DAIT NIAID-sponsored ICAC trials. Those who have no obvious characteristics making them ineligible and who are interested will be invited to the clinic for a Screening Visit.

8.4 Screening/Baseline Visit

This research study will be explained in lay terms to the parent/legal guardian of each potential participant. Written informed consent and Health Insurance Portability and Accountability Act (HIPAA) authorization will be obtained from all parents/legal guardians at the beginning of this visit and before the child undergoes any screening study procedures. Children age 12 years and older will sign a written assent form. When the informed consent has been signed, the participant is considered enrolled in the study and will be assigned a unique participant number. Study procedures will be stopped, and the participant will be deemed ineligible for the study at any point during the Screening Visit if and when he/she fails to meet eligibility criteria.

The purpose of the screening period is to confirm eligibility to continue in the study. After the consent (and assent, if applicable) is signed, participants will undergo screening study procedures as listed below:

- Vital signs (see Section 8.1.1)
- Urine collection for pregnancy test for female participants who have reached menarche (see Section 8.2.3)
- Medical history and a physical examination by study clinician (see Section 8.1.2)
- Concomitant medication assessment (see Sections 8.1.6 and 7.1)
- Questionnaires (see Section 8.1.6)
- Weight and height measurement, using a calibrated stadiometer (see Section 8.1.1)
- Spirometry (see Section 8.1.5)
- Assignment of asthma medication regimen by study clinician (see Section 7.1.1)
- Blood sample collection (see Section 8.2.1.1)
    - CBC with differential
    - Blood chemistry
    - Total and allergen-specific IgE
- Asthma counseling (see Section 7.1.1) (not completed if participant is a screen fail)
- AE assessment (see Section 12)

Participants will also undergo skin prick tests (see Section 8.1.3) to a panel of allergens detailed in the Protocol ICAC-30 MOP to characterize the study population. Participants who have valid skin test results within the previous year under another ICAC protocol will not need to be re-tested. Clinical research staff may choose to perform the skin prick tests at Visits 2, 3, or 4 instead of Screening.

Participants may be re-screened per study clinician discretion. Participants who meet any of the following criteria are not eligible for re-screening:

- Have a known, pre-existing clinically important lung condition other than asthma
- Have known, pre-existing, unstable liver disease

13. There is a major violation of the clinical trial protocol (defined as any variation from the protocol-directed conduct of the study that affects the safety of a participant or the ability of the trial to evaluate efficacy of the study agent).
14. The participant begins immunotherapy during the study period.
15. The participant has a new diagnosis of malignancy.
16. The participant has a new diagnosis requiring regular treatment with systemic corticosteroids, immunosuppressives, or any other drug that would interfere with participation in the study.
17. The participant has a serious parasitic infection and does not respond to treatment.

Note: If investigational product is discontinued by a study clinician, participants may continue on-study if agreed upon by the participant and study clinician (see Section 8.13).

11.3 Participant Replacement

Participants who are discontinued prior to completing the 12-month follow up will not be replaced. All reasons for withdrawal will be captured on the appropriate electronic case report form (eCRF).

11.4 Follow-up after Early Study Withdrawal

Participants who withdraw or are dropped from the study between visits will be contacted by phone and will be invited to the study center for a final assessment. This assessment will include:

- Urine pregnancy test, where indicated (see Section 8.2.3)
- Questionnaires (see Section 8.1.6)
- AE assessment (see Section 12)
- Concomitant medication assessment (see Sections 8.1.6 and 7.1.2)
- Vital signs (see Section 8.1.1)
- Spirometry (see Section 8.1.5)
- Targeted pulmonary exam (see Section 8.1.2)
- Assignment of asthma medication regimen by study clinician (see Section 7.1.1)
- Asthma education/counseling (see Section 7.1.1.5)
- Blood sample collection (see Section 8.2.1)
    - CBC with differential
    - Blood chemistry

Procedures contraindicated by the medical condition of the participant will not be conducted at the discretion of the study clinician. For participants who refuse to come to the clinic, the final clinical assessment as described above may be conducted over the phone. Besides this visit, no further follow-up will be made, except for participants who are discontinued due to pregnancy or SAEs. These participants will be contacted to determine the outcome of the pregnancy or the SAE. Refer to Section 12.6 for more information on AE reporting for pregnancy.

11.5 Study Stopping Rules

Study enrollment will be temporarily suspended pending expedited review of all pertinent data after the occurrence of one death or SAE which is unexpected and related to the investigational agent.

- Epistaxis within 24 hours of the procedure
- Headache, earache or rhinorrhea lasting longer than 24 hours

12.2.1.1 Suspected Adverse Reaction

Any adverse event for which there is a reasonable possibility that the investigational drug [or investigational study therapy regimen] caused the adverse event. For the purposes of safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug (21 CFR 312.32(a)).

12.2.2 Unexpected Adverse Event

An adverse event or suspected adverse reaction is considered "unexpected" when its nature (specificity), or severity, or rate of occurrence is not consistent with applicable product information as described in the safety information provided in the current GSK Investigator's Brochure for mepolizumab.

"Unexpected" also refers to adverse events or suspected adverse reactions that are mentioned in the current GSK Investigator's Brochure for mepolizumab as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation (21 CFR 312.32(a)).

12.2.3 Serious Adverse Event

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or the IND sponsor (DAIT NIAID), it results in any of the following outcomes (21 CFR 312.32(a)):

1. Death.
2. A life-threatening event: An AE or SAR is considered "life-threatening" if, in the view of either the Principal Investigator or IND Sponsor, its occurrence places the participant at immediate risk of death. It does not include an AE or SAR that, had it occurred in a more severe form, might have caused death.
3. Inpatient hospitalization or prolongation of existing hospitalization.
4. Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
5. Congenital anomaly or birth defect.
6. Important medical events that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

Elective hospitalizations, hospitalizations for pre-existing conditions that do not worsen during the course of the trial, or hospital admissions for the purpose of conduct of protocol mandated procedures are not to be reported as an SAE unless hospitalization is prolonged due to complications.

Table 12.3.1.1c Grading System of Severity of Anaphylaxis

Grade	Defined By
1. Mild (skin & subcutaneous tissues, GI, &/or mild respiratory)	Flushing, urticaria, periorbital or facial angioedema; mild dyspnea, wheeze or upper respiratory symptoms; mild abdominal pain and/or emesis
2. Moderate (mild symptoms + features suggesting moderate respiratory, cardiovascular or GI symptoms)	Marked dysphagia, hoarseness, and/or stridor; shortness of breath, wheezing & retractions; crampy abdominal pain, recurrent vomiting and/or diarrhea; and/or mild dizziness
3. Severe (hypoxia, hypotension, or neurological compromise)	Cyanosis or SpO2 $\leq$ 92% at any stage, hypotension, confusion, collapse, loss of consciousness; or incontinence

Injection Site Reactions (ISRs)

This study will grade injection site reactions as defined by the following table, from the Guidance for Industry – Toxicity Grading Scale for healthy Adult and Adolescent Volunteers Enrolled in Preventative Vaccine Clinical Trials, Food and Drug Administration, September 2007[38].

Table 12.3.1.1d Grading System of Severity of Injection Site Reactions

Local Reaction to Injectable Product	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Pain	Does not interfere with activity	Repeated use of non-narcotic pain reliever >24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	Emergency room (ER) visit or hospitalization
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	ER visit or hospitalization
Erythema/Redness*	2.5 – 5 cm	5.1 – 10 cm	>10 cm	Necrosis or exfoliative dermatitis
Induration/Swelling**	2.5 – 5 cm and does not interfere with activity	5.1 – 10 cm or interferes with activity	>10 cm or prevents daily activity	Necrosis

*In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.

**Induration/swelling should be evaluated and graded using the functional scale as well as the actual measurement.

2. One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the drug (e.g., tendon rupture)
3. An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group

Category 2: Any findings from studies that suggests a significant human risk

The sponsor shall report any findings from other epidemiological studies, analyses of adverse events within the current study or pooled analysis across clinical studies or animal or in vitro testing (e.g. mutagenicity, teratogenicity, carcinogenicity) that suggest a significant risk in humans exposed to the drug that would result in a safety-related change in the protocol, informed consent, investigator brochure, or other aspects of the overall conduct of the study.

DAIT NIAID must notify the FDA and all participating Principal Investigators of expedited Safety Reports within 15 calendar days; unexpected fatal or immediately life-threatening suspected adverse reaction(s) shall be reported as soon as possible or within 7 calendar days.

12.5.3 Reporting of Adverse Events to IRBs

All Principal Investigators must report adverse events, including expedited reports, in a timely fashion to the Central IRB in accordance with applicable regulations and guidelines. All IND Safety Reports to the FDA shall be distributed by the IND sponsor (DAIT NIAID) or designee to all participating institutions for Central IRB submission.

12.6 Pregnancy Reporting

The Principal Investigator shall be informed immediately of any pregnancy in a study participant occurring from time of consent through 30 days after the participant completes study participation. A pregnant participant shall be instructed to stop taking study medication. The investigator shall counsel the participant and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the pregnant participant shall continue until the conclusion of the pregnancy.

The investigator shall report to the DAIT SACCC and the IND sponsor (DAIT NIAID) all pregnancies within 1 business day of becoming aware of the event using the Pregnancy eCRF. All pregnancies identified during the study shall be followed to conclusion and the outcome of each must be reported. The Pregnancy eCRF shall be updated and submitted to the DAIT SACCC when details about the outcome are available.

Information requested about the delivery shall include:

- Gestational age at delivery
- Birth weight, length, and head circumference
- Gender

13.1 Overview

The primary research question of this study is whether mepolizumab will lead to a reduced rate of asthma exacerbations in urban children and adolescents with difficult-to-control, exacerbation-prone asthma and peripheral blood eosinophils ≥150 cells/µl. This objective will be addressed using a multicenter, double-blind, placebo-controlled, randomized trial of mepolizumab adjunctive therapy for the prevention of asthma exacerbations in urban children and adolescents ages 6 to 17 years with difficult-to-control, exacerbation-prone asthma and peripheral blood eosinophilia.

13.2 Endpoints/Outcomes

The primary endpoint is the number of asthma exacerbations between randomization and the end of participant follow-up or 12 months since randomization, whichever comes first. Exacerbations will be defined as a prescription of a course of systemic corticosteroids by a clinician, initiation of a course of systemic corticosteroids by a participant, or as a hospitalization for asthma. If a participant initiates and completes a course of systemic corticosteroids without clinician involvement, this course will be counted only if the study clinician agrees the treatment was warranted, and it meets the following dosage: the course for prednisone, prednisolone, or methylprednisolone will be at least 20 mg daily dose for 3 of 5 consecutive days. The course for dexamethasone will be at least a 10 mg single daily dose. If a corticosteroid burst for the treatment of an asthma exacerbation is prescribed by a non-ICAC clinician, it will be counted regardless of dose.

Key secondary endpoints include:

1. Asthma burden, as measured by a composite score (CASI) of asthma symptoms, medication usage, and exacerbation risk measured at Visits V1, V4, V7, V10, V13 and V14.
2. Patient reported outcomes, as measured by physician-patient global assessment tool measured at Visit V14.
3. Lung function, as assessed by spirometry measured at Visit V1, V4, V7, V10, V13, and V14 and IOS measured at Visit V1, V4, and V13.
4. Time between randomization and first asthma exacerbation or the end of participant follow-up, whichever comes first. Participants that do not have an asthma exacerbation will be right-censored at the end of follow-up.

13.3 Measures to Minimize Bias

To minimize bias, randomization will be performed using a validated system created by the DAIT SACCC that automates the random assignment of treatment groups to study ID numbers. The randomization scheme will be reviewed by a statistician at the DAIT SACCC and locked after approval. Participants will be randomized using a 1:1 ratio of active and control (placebo) participants. A covariate adaptive randomization algorithm developed by Pocock and Simon (1975)[32] will be used to maintain a balance between treatment arms with respect to study site, number of previous exacerbations (2 or 3+), peripheral blood eosinophils (< or ≥400 cells/µl), BMI (< or ≥95th percentile for age), total serum IgE (< or ≥540 kUA/L) and treatment dose (40 mg or 100 mg). This strategy was chosen to enhance our ability to balance the distribution of covariates that are known to be highly associated with our primary endpoint (e.g., number of previous exacerbations and peripheral blood eosinophils) as well as covariates that determine if a participant fits the FDA-approved dosing tables for omalizumab therapy (e.g., BMI and total serum IgE), which is related to Secondary Objective 5.

with an associated dispersion parameter of 0.47. Assuming that we will observe a relative rate similar to that seen in Pavord et al (2012) of 0.60 and that the event rate in the placebo arm is assumed to be 1.20 asthma exacerbations per patient-year with corresponding dispersion parameter of 0.50 in both arms, a total sample size of 256 participants (128 participants per arm) is required to detect such a relative rate with 90% power, based on a two-sided significance level of 0.05 and the work outlined in Zhu & Lakkis,[39] using maximum likelihood (method 3) for estimating the variance under the null hypothesis. Assuming approximately 20% of randomized participants do not meet criteria for modified intent-to-treat and are not replaced, we originally proposed to enroll 320 participants (160 participants per arm). However, our observed post-randomization dropout rate has been lower than expected (11%), and therefore the sample size was revised to 290.

Secondary Objective 5 will also determine if mepolizumab reduces the rate of asthma exacerbation in participants who do not fit the FDA-approved dosing table for omalizumab therapy and in participants who fit the FDA-approved dosing table for omalizumab therapy. In Protocol ICAC-19 APIC participants meeting Protocol ICAC-30 MUPPITS-2 eligible criteria, approximately 50% of participants did not fit the FDA-approved dosing table for omalizumab therapy and so we would expect approximately 128 participants (64 participants per arm) in each of these sub-groups in Protocol ICAC-30 MUPPITS-2. Assuming an event rate in the placebo arm of 1.20 and dispersion parameter of 0.50 in both arms, such a sample size would allow us to detect a relative rate between arms of 0.53 with 80% power.

14 Identification and Access to Source Data

14.1 Source Data

Source documents and source data are considered to be the original documentation where participant information, visits consultations, examinations and other information are recorded. Documentation of source data is necessary for the reconstruction, evaluation and validation of clinical findings, observations and other activities during a clinical trial. All clinical site laboratory reports, spirometry reports, and any paper and electronic CRFs will be maintained as source data.

14.2 Access to Source Data

The Principal Investigators and site staff will make all source data available to the IND sponsor (DAIT NIAID), as well as to the FDA. Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that may be linked to identified individuals.

15 Protocol Deviations

15.1 Protocol Deviation Definitions

15.1.1 Protocol Deviation

The Principal Investigators and site staff will conduct the study in accordance to the protocol; no deviations from the protocol are permitted. Any change, divergence, or departure from the study design or procedures constitutes a protocol deviation. As a result of any deviation, corrective actions will be developed by the site and implemented promptly.

and the name of the person performing the data entry) will be recorded in an electronic audit trail to allow all changes in the database to be monitored and maintained in accordance with federal regulations.

16.2 Statement of Compliance

This clinical study will be conducted using good clinical practice (GCP), as delineated in *Guidance for Industry: E6 Good Clinical Practice Consolidated Guidance*, and according to the criteria specified in this study protocol. Before study initiation, the protocol and the informed consent documents will be reviewed and approved by the Central IRB. Any amendments to the protocol or to the consent materials will also be approved by the Central IRB before they are implemented.

The Principal Investigator will ensure that this study is conducted in full conformity with Regulations for the Protection of Human Subjects of Research codified in 45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, and/or the ICH E6.

16.3 Informed Consent Process

The consent process will provide information about the study to a prospective participant and parent(s)/legal guardian(s) and will allow adequate time for review and discussion prior to his/her decision. The Principal Investigator or designee listed on the FDA 1572 will review the consent and answer questions. The consent designee must be listed on the delegation log and have knowledge of the study. The prospective participant and parent(s)/legal guardian(s) will be told that being in the trial is voluntary and that he or she may withdraw from the study at any time, for any reason. All participants (or their legally acceptable representative) and parent(s)/legal guardian(s) will read, sign, and date a consent form before undergoing any study procedures. Assent will be obtained as per instruction by the Central IRB. Consent materials will be presented in participants' primary language. A copy of the signed consent form will be given to the participant.

The consent process will be ongoing. The consent form will be revised when important new safety information is available, the protocol is amended, and/or new information becomes available that may affect participation in the study.

16.4 Privacy and Confidentiality

A participant's privacy and confidentiality will be respected throughout the trial. Each participant will be assigned a unique identification number and these numbers rather than names will be used to collect, store, and report participant information. Site personnel will not transmit documents containing personal health identifiers (PHI) to the study sponsor or their representatives.

17 Publication Policy

Presentations and publication of the results of this trial will be governed by the ICAC Publication Policy.

Appendix B: Nasal Lavage Quality Control Methods

1 Study Population

1.1 Population Description

Clinical research staff performing nasal lavage will be required to demonstrate proficiency in performing the procedure within Protocol ICAC-30 MUPPITS-2. The proficiency testing will be conducted by collecting and processing nasal lavage samples from individuals that have signed a consent specific to this procedure. Up to 200 participants may be enrolled across the research sites to ensure all study staff members are trained.

1.1.1 Inclusion Criteria

Participants to be included in this training are those:

1. who are male or female age 6-17, inclusive
2. have a history of asthma
3. who are willing and able to provide informed consent
4. who are willing and able to provide assent, if applicable

1.2 Requirements

All clinical research staff performing the nasal lavage collection and processing must perform at least two proficiency tests. After collection and processing, the site staff will send the samples to a central lab for review. The central lab will determine whether the samples are sufficient. If a sample is deemed not sufficient, then the staff member may be required to repeat the proficiency testing.

1.3 Procedures

The nasal lavage collection and processing procedures are detailed in the Protocol ICAC-30 MOP; however, the study clinician will not have the option to use a nasal decongestant prior to performing the nasal lavage for the quality control procedure (as described in Section 8.2.2).

2. PURPOSE OF THE ANALYSES

The purpose of this statistical analysis plan (SAP) is to describe the planned analyses and data displays to be included in the Clinical Study Report (CSR) for Protocol ICAC-30 MUPPITS-2. This document provides details on study populations, how the variables will be derived, how missing data will be handled and details on statistical methods to be used to analyze the safety and efficacy data.

The statistical analysis plan (SAP) is based on ICH guidelines E3 and E9 (Statistical Principles for Clinical Trials).

The document may evolve over time, for example, to reflect the requirements of protocol amendments or regulatory requests. However, the final SAP must be finalized, approved by the Sponsor, and placed on file before database is locked. Deviations from the final approved plan will be noted in the clinical study report.

10. OTHER ANALYSES

No formal interim analyses of efficacy data were performed for this study.

Nasal lavage		+		+										+	+	+
Nasosorption														+	+	+
Nasal blow		+	+	+	+	+	+	+	+	+	+	+	+	+		
Nasal resp virus		+	+	+	+	+	+	+	+	+	+	+	+	+		
Nasal ECP		+	+	+	+			+						+		
Nasal swab collection^															+	+
Nasal epithelial cell collection														+**		
Sputum Induction														+**		
Vital signs and growth parameters	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adverse event assessment	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Concomitant medication assessment	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+