Pilot Study of Mepolizumab in Episodic Angioedema with Eosinophilia

NIH Protocol Number: 20-I-0002

Conducted by:
National Institute of Allergy and Infectious Diseases (NIAID)
National Institutes of Health (NIH)

Pharmaceutical Support Provided by:
GlaxoSmithKline (GSK)

Mepolizumab Provided by:
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Version Number: 3.0

March 15, 2021

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Statement of Compliance

The trial will be carried out in accordance with International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP) and the following:

- United States (U.S.) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

NIH-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all subject materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any subject is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from subjects who provided consent, using a previously approved consent form.

List of Abbreviations

AE	Adverse Event
AEC	Absolute Eosinophil Count
ANC	Absolute Neutrophil Count
AR	Adverse Reaction
CBC	Complete Blood Count
CC	Clinical Center
CFR	Code of Federal Regulations
COVID-19	Coronavirus Disease 2019
CPK	Creatine Kinase
CRIMSON	Clinical Research Information Management System of the NIAID
CRP	C-reactive Protein
DAES	Daily Adverse Event Score
EAE	Episodic Angioedema with Eosinophilia
ELISA	Enzyme-linked Immunosorbent Assay
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GSK	GlaxoSmithKline
HES	Hypereosinophilic Syndrome
HRPP	Human Research Protections Program
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
Ig	Immunoglobulin
IL-5	Interleukin 5
IRB	Institutional Review Board
IV	Intravenous
LDH	Lactate Dehydrogenase
LPD	Laboratory of Parasitic Diseases
N	Number (typically refers to number of subjects/sample size)
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
OHSRP	Office of Human Subjects Research Protections
PBMC	Peripheral Blood Mononuclear Cell
RNAseq	RNA sequencing
SAE	Serious Adverse Event
SC	Subcutaneous
TBNK	T-, B-, and Natural Killer–Cell Analysis
UP	Unanticipated Problem
U.S.	United States

Protocol Summary

Full Title:	***Pilot Study of Mepolizumab in Episodic Angioedema with Eosinophilia***
Short Title:	Mepolizumab in EAE
Sample Size:	N = 12
Accrual Ceiling:	15
Study Population:	Adult subjects with confirmed episodic angioedema with eosinophilia (EAE)
Accrual Period:	2 years
Study Design:	This will be an open-label pilot study. Following completion of eligibility assessments, subjects with confirmed EAE will enter a run-in period, during which the periodicity of their cycle will be assessed (if not known) and the optimal timing of the baseline visit will be determined. During this time, subjects will begin daily and monthly patient-reported outcome measures; the daily measures will be continued for about 3 months (through the third mepolizumab dose), and the monthly measures will be continued through the end of the primary study phase. Also, subjects will begin visiting a local lab 2-3X/week for collection of blood for complete blood count (CBC) with differential and serum and urine to be mailed to NIH for research; local lab visits will continue between study visits through the second mepolizumab administration. Once cycling and periodicity is confirmed, the baseline visit will be scheduled at the predicted neutrophil peak. Baseline assessments will include history and physical examination, routine laboratory testing, and whole blood flow cytometric analysis. Subjects will again be evaluated and have bloodwork during their predicted eosinophil peak (may be concurrent with baseline if peak eosinophils and neutrophils occur <1 week apart) and at the next predicted eosinophil nadir; at the nadir visit, they will receive their first administration of mepolizumab (700 mg via intravenous [IV] infusion). Subjects will return to the NIH at the predicted neutrophil peak for clinical evaluation and whole blood flow cytometry.

	After that visit, subjects will return for additional infusions 1 and 2 months after the first, for a total of 3 monthly mepolizumab infusions at a dose of 700 mg. All subjects will be evaluated 1 month after the third mepolizumab infusion at 700 mg for primary and secondary endpoint assessments and to assess clinical benefit. Subjects who receive benefit from mepolizumab will undergo protocol-specified de-escalation of drug to monthly doses of 500 mg and then 300 mg over the course of 6 months. In-person or remote study visits will continue monthly during de-escalation to assess symptoms and collect blood for clinical and exploratory research evaluations. An end of study visit will occur 1 month after the last study dose. Clinical responders may be able to receive mepolizumab at the completion of the study.
Study Duration:	Start Date: January 2021 End Date: September 2023 Subjects will be on study for approximately 6 to 10 months
Study Agent/ Intervention Description:	Mepolizumab 700 mg IV given monthly for a total of 3 doses. Subjects who undergo dose de-escalation will receive 3 additional doses of mepolizumab at 500 mg (in-person by IV or remotely by subcutaneous [SC] injection), followed by 3 additional doses at 300 mg by SC injection.
Primary Objective:	To determine the effect of mepolizumab on the number and severity of symptoms of EAE.
Secondary Objectives:	1) To determine if blocking interleukin 5 (IL-5) with mepolizumab will reduce eosinophilia in subjects with EAE. 2) To assess the durability of eosinophil reduction with approximately monthly mepolizumab administration (at Visit 8).
Exploratory Objectives:	1) To investigate the etiology of cycling in EAE by: exploring the role of cell lineages in EAE including eosinophil reduction on other cell lineages, leukocyte

	activation markers, serum cytokines and chemokines, and other immunologic mediators. 2) To investigate response to 500 mg and 300 mg of mepolizumab during dose de-escalation.
Primary Endpoint:	Reduction in the number and severity of clinical symptoms associated with EAE after 3 doses of mepolizumab (as compared to symptoms logged in the approximately monthly cycle preceding initiation of mepolizumab).
Secondary Endpoints:	1) A ≥75% reduction in peak blood eosinophil count following the first dose of mepolizumab (as compared to the peak eosinophil count in the cycle preceding initiation of mepolizumab therapy). 2) Sustained reduction of eosinophilia after administration of mepolizumab (at Visit 8).
Exploratory Endpoints:	1) Effect of mepolizumab on cycling of cells other than eosinophils (e.g., neutrophils and lymphocytes) following mepolizumab administration. 2) Effect of mepolizumab on markers of eosinophil activation and other biomarkers of disease activity in EAE, including whole blood transcriptomics, levels of eosinophil and neutrophil granule proteins, serum immunoglobulin (Ig) G and IgM levels, serum cytokine and chemokine levels, and serum tryptase levels. 3) Reduction of symptoms and severity of clinical symptoms of EAE on mepolizumab at doses of 500 mg and 300 mg SC.

Précis

Episodic angioedema with eosinophilia (EAE), also known as Gleich's Syndrome, is a rare disorder characterized by recurrent episodes of urticaria, fever, angioedema, weight gain and dramatic eosinophilia that occur at 3- to 6-week intervals and resolve with spontaneous diuresis in the absence of therapy. Although the syndrome is often classified in the broad category of idiopathic hypereosinophilic syndrome (HES), EAE is a distinct eosinophilic syndrome that is remarkably homogeneous in clinical presentation. More recently, it has become apparent that there is multilineage cycling, involving lymphocytes and neutrophils in addition to eosinophils. Early studies described cyclic elevations of serum interleukin 5 (IL-5) preceding the rise in eosinophilia, and additional studies have shown cyclic elevations in other type II cytokines as well as in eosinophilic chemokines. Aberrant T cells with a CD3-CD4+ surface phenotype have also been detected in the majority of subjects with EAE. The cyclic nature of the disorder and the involvement of multiple cell lineages have made it difficult to determine the underlying cause of EAE.

We hypothesize that IL-5–driven eosinophilia is central to the pathogenesis of EAE. Suppression of eosinophil cycling by blocking IL-5 would help determine whether eosinophils are indeed the main drivers of the symptoms of angioedema and urticaria and pave the way for future mechanistic studies investigating the etiology of this unusual disorder. The purpose of this pilot study is to evaluate the effect of mepolizumab, a humanized antibody to IL-5, on eosinophil cycling in 12 subjects with EAE. Subjects with EAE will undergo screening on the National Institutes of Health protocol 94-I-0079 to establish the periodicity of their cycling (if not previously determined) and the optimal timing for the baseline visit. After screening, subjects will be followed closely with signs and symptoms recorded in a daily log and daily and monthly questionnaires, as well as complete blood counts and research blood collected for one cycle prior to administration of mepolizumab. Subjects will receive a total of 3 monthly administrations of mepolizumab at 700 mg, followed by drug de-escalation over 6 additional monthly administrations for subjects who demonstrate benefit from mepolizumab. All subjects will have a follow-up visit about 1 month after the last study administration of mepolizumab.

The primary efficacy endpoint will be reduction of symptoms and severity of symptoms after mepolizumab. Secondary endpoints will include reduction in peak eosinophils after mepolizumab, continued suppression of absolute eosinophil count and reduction in symptoms following monthly dosing of mepolizumab therapy.

1. Background Information and Scientific Rationale

1.1 Background Information

EAE, also known as Gleich's Syndrome, is characterized by recurrent episodes of urticaria, fever, angioedema, weight gain, and dramatic eosinophilia that occur at 3- to 6-week intervals and resolve with spontaneous diuresis in the absence of therapy (1). The dramatic waxing and waning of clinical manifestations, which can include shifts in weight of up to 40 pounds, has a significant impact on the lives of patients with EAE. Although conventional HES therapies, including glucocorticoids, are effective in reducing symptoms, the toxicity of these therapies limits their long-term use in EAE.

EAE is an extremely rare disorder. Among 303 consecutively enrolled subjects on our natural history protocol studying eosinophilic syndromes (94-I-0079), 12 subjects with EAE were identified. The estimated prevalence of HES is 0.036/100,000 persons based on coding in the SEER (Surveillance, Epidemiology, and End Results Program) database, although this is likely underestimated due to the lack of specific International Classification of Diseases codes for HES (2). Assuming that the proportion of referrals to NIH for EAE reflects the proportion of such patients in the general HES population, the prevalence of EAE is estimated to be 0.012-0.2/100,000 persons in the United States. This low prevalence has precluded clinical treatment studies in EAE to date.

Classified in the broad category of idiopathic HES (3), EAE is a distinct eosinophilic syndrome that is remarkably homogeneous in clinical presentation, suggesting a common etiology in affected subjects. Early studies of EAE demonstrated cyclic elevations of IL-5 and eosinophils with eosinophilic deposition and degranulation in the dermis during symptomatic episodes (1,4), suggesting that IL-5–driven eosinophils are the primary orchestrators of the angioedema in EAE. Although activated T cells and an aberrant T-cell population (CD3-CD4+) commonly found in the lymphocytic variant of HES are also present in the blood and skin of patients with EAE (5), the role of the T-cell axis in regulating eosinophilia and the skin manifestations is unknown. Elevated serum IgM levels have also been detected in the majority of subjects with EAE and are of unknown significance. More recent studies have demonstrated multilineage cycling involving eosinophils, lymphocytes, and neutrophils, as well as cyclic elevations in cellular activation markers and a wide variety of cytokines, chemokines, and other mediators (5).

Characterization of the cycles of patients with EAE performed at the NIH have revealed characteristics that are reproducible among subjects. Although cycle lengths may vary in individual subjects, the duration of time between peak neutrophil counts and symptoms to the development of peak eosinophilia is approximately 7 days in many individuals. Likewise, the time from the peak

eosinophilia to the nadir of counts and cytokines is approximately 9 days (see Appendix D: Cycle Length Determination for 3 Patients for examples). As in other cyclic hematologic disorders, including cyclic neutropenia, the etiology of the cycling in EAE is unknown.

1.1.1 Description of the Study Agent

Mepolizumab is a high-affinity, humanized monoclonal antibody (IgG1, kappa) treatment that targets human IL-5, preventing binding to its cognate receptor, IL-5 receptor alpha, on human eosinophils and thus preventing downstream signaling. Effects of mepolizumab include reduction of eosinophil counts and improvement of eosinophil-associated symptoms.

1.1.2 Summary of Relevant Clinical Studies

Mepolizumab was initially developed for the treatment of severe asthma. Although early studies failed their efficacy endpoints despite reduction in blood and sputum eosinophilia, more recent studies focusing on eosinophilic asthma (6,7) have led to the recent U.S. Food and Drug Administration (FDA)–approval of mepolizumab (100 mg subcutaneous injection monthly) for the treatment of severe eosinophilic asthma. Phase 3 placebo-controlled, double-blind studies have also demonstrated the efficacy of mepolizumab in preventing exacerbations in chronic obstructive pulmonary disease and eosinophilic granulomatosis with polyangiitis (8). Most relevant to the proposed study, monthly mepolizumab (750 mg IV) was well tolerated and effective as a steroid-sparing agent in a placebo-controlled, double-blind phase 2 study in 84 subjects with HES (9). An open-label extension confirmed the long-term efficacy in this patient population (10), and the FDA has approved mepolizumab administered at 300 mg (3 separate 100-mg SC injections) once every 4 weeks for HES. There have been no published cases of mepolizumab use in EAE to date.

1.2 Rationale

As described above, eosinophils and eosinophil activation have been implicated in the pathogenesis of EAE. Furthermore, IL-5, a key mediator in the development, maturation, and survival of eosinophils, can be demonstrated by flow cytometry intracellularly in CD3+CD4+ populations in subjects with EAE (3), and has been found in high levels in the serum just prior to the onset of eosinophilia in all EAE subjects studied at NIH, with peak IL-5 levels ranging from 150-5447 pg/mL (median 2787 pg/mL) in 7 subjects studied. These levels are among the highest documented in our cohort of more than 450 subjects with HES. Interestingly, the peak cytokine levels occur at the peak of neutrophil elevation, raising the question of whether other cell populations (such as neutrophils) are involved in the pathogenesis of the disease.

These findings suggest that blocking IL-5 with mepolizumab could prevent the dramatic elevation of eosinophils, trafficking of eosinophils to the skin and soft

tissues, and consequently the symptoms of angioedema, weight gain, and myalgias associated with EAE. The impact of a reduction of eosinophils and eosinophil activation on other features of EAE, including the cycling and activation of other cell lineages, immunoglobulins, and serum mediators, could in turn provide new insights into the role of eosinophils in the pathogenesis of EAE.

2. Study Objectives

2.1 Primary Objective

To determine the effect of mepolizumab on the number and severity of symptoms of EAE.

2.2 Secondary Objectives

1) To determine if blocking IL-5 with mepolizumab will reduce eosinophilia in subjects with EAE.

2) To assess the durability of eosinophil reduction with approximately monthly mepolizumab administration (at Visit 8).

2.3 Exploratory Objectives

1) To investigate the etiology of cycling in EAE by: exploring the role of cell lineages including the effect of eosinophil reduction on other cell lineages, leukocyte activation markers, serum cytokines and chemokines, and other immunologic mediators (Appendix H: Research Studies).

2) To investigate response to 500 mg and 300 mg of mepolizumab during dose de-escalation.

3. Study Design

3.1 Description of the Study Design

Subjects with proven EAE will be invited to participate in the study. The timing of visits for each individual subject will be determined based on the periodicity of cycles in that subject (section 3.2, Figure 1). Screening will be performed on NIH protocol 94-I-0079. Potential study subjects with EAE who have not previously been studied at NIH or who are on a new treatment regimen may be asked to undergo blood draws 2-3 times per week for several weeks on protocol 94-I-0079 to a) confirm the diagnosis and cycling with peak absolute eosinophil count (AEC) >1500/mm^3 and b) to determine presence of symptoms associated with EAE since subjects will need to have symptoms to enroll. Subjects who no longer cycle or who have controlled disease on prednisone or other agents may taper their current therapy 3 months prior to screening under the direction of their primary doctor to meet entry criteria before enrolling in this study.

If an EAE patient has eosinophilia and symptoms on current therapy and agrees to join the study, they will be brought in for an enrollment visit (Visit 1) for initial evaluation. After the enrollment visit, subjects will begin a daily symptom and weight log (Appendix E: Patient Symptom Log) and daily and monthly assessments of angioedema symptoms using validated angioedema activity and quality of life scales (Appendix F). Logs and assessments will be collected electronically using REDCap and continued as described in section 7.1.2. Also, between study visits, subjects will have 2-3X/week collection of blood for CBC with differential and serum and urine for cytokine and mediator analysis. These blood and urine collections will continue between study visits until Visit 6.

Once cycling and periodicity is confirmed, the baseline visit (Visit 2) will be scheduled at the predicted peak absolute neutrophil count (ANC), and ideally while eosinophils have not yet risen. Baseline assessments will include history and physical examination, routine laboratory testing, and whole blood flow cytometric analysis.

During their predicted AEC peak subjects will have Visit 3 to receive clinical evaluation and laboratory testing including whole blood flow cytometry. If the subject has peak eosinophils and neutrophils within a week of each other, Visits 2 and 3 may be combined. At the time of the next predicted AEC nadir (Visit 4), subjects will receive mepolizumab (700 mg IV). History and physical examination, routine laboratory testing, and research samples will be repeated at this visit. At the predicted ANC peak (Visit 5), subjects will have clinical evaluation and laboratory testing research samples. Subjects will also return to the NIH for mepolizumab infusions 1 and 2 months after the first (Visits 6 and 7) and will undergo clinical and laboratory evaluations and receive mepolizumab.

All subjects will be evaluated 1 month after the third mepolizumab infusion at 700 mg for primary and secondary endpoint assessments and to assess clinical benefit. Subjects who receive benefit from mepolizumab (clinical improvement as determined by the investigator) will undergo protocol-specified de-escalation of drug during the Dose De-escalation Phase. Subjects will receive monthly doses of 500 mg (in-person by IV or remotely by SC injection) and then 300 mg SC over the course of 6 months. In-person or remote study visits will continue monthly during de-escalation to assess symptoms and collect blood for clinical and exploratory research evaluations. Subjects may additionally be requested to obtain bloodwork during a symptomatic flare in between monthly visits for safety reasons. Subjects will continue to complete weekly symptom logs, weekly angioedema questionnaires, and monthly quality of life questionnaires during de-escalation.

For all subjects, the end of study visit will be about 1 month after the final study mepolizumab administration and will include safety and research procedures and evaluations.

During the coronavirus disease 2019 (COVID-19) pandemic, subjects may be unable to attend study visits due to travel limitations or safety concerns. In these circumstances, study visits may be rescheduled or modified to allow for remote evaluations (see section 6).

Clinical responders may be eligible to receive compassionate use mepolizumab at the discretion of the investigator and GSK (NCT00244686).

Justification for study dosing: This study will begin by administering mepolizumab at a higher dose (700 mg) and by a different delivery method (IV rather than SC injection) than the FDA-approved administration. The dose and administration method was chosen because it was the dose used in prior studies of HES (9) as well as in our experience treating HES patients at the NIH CC, which involved doses of 500-700 mg IV (11). Pharmacodynamic studies performed in asthma studies have not shown differences in maximal eosinophil reduction in peripheral blood based on dosing; however, experience with lower dosing of mepolizumab in both EAE patients given 100 mg for asthma as well as patients receiving 300 mg for eosinophilic granulomatosis with polyangiitis have demonstrated continued tissue and peripheral eosinophils in some cases. Serologic evaluations of patients with EAE have shown very high levels of IL-5 in the bloodstream that are higher than the levels of most HES patients studied at NIH, hence the chosen dose of 700 mg IV for this study. Adverse reactions (ARs) and side effects from 700 mg IV have not been shown to differ from those noted in studies using lower doses (9,11).

After 3 IV infusions of mepolizumab at 700 mg, subjects who benefit from drug administration will undergo de-escalation to monthly doses of 500 mg IV or SC and then 300 mg SC, the latter of which is the dosage and administration method approved for use in patients with HES.

3.2 Study Schematic

A general schematic for the primary and secondary assessments on the study is given below. Cycle duration varies between subjects, but is approximately 4-6 weeks (examples of cycle length determination based on data from individual subjects are given in Appendix D: Cycle Length Determination for 3 Patients). Study dose de-escalation is not shown.

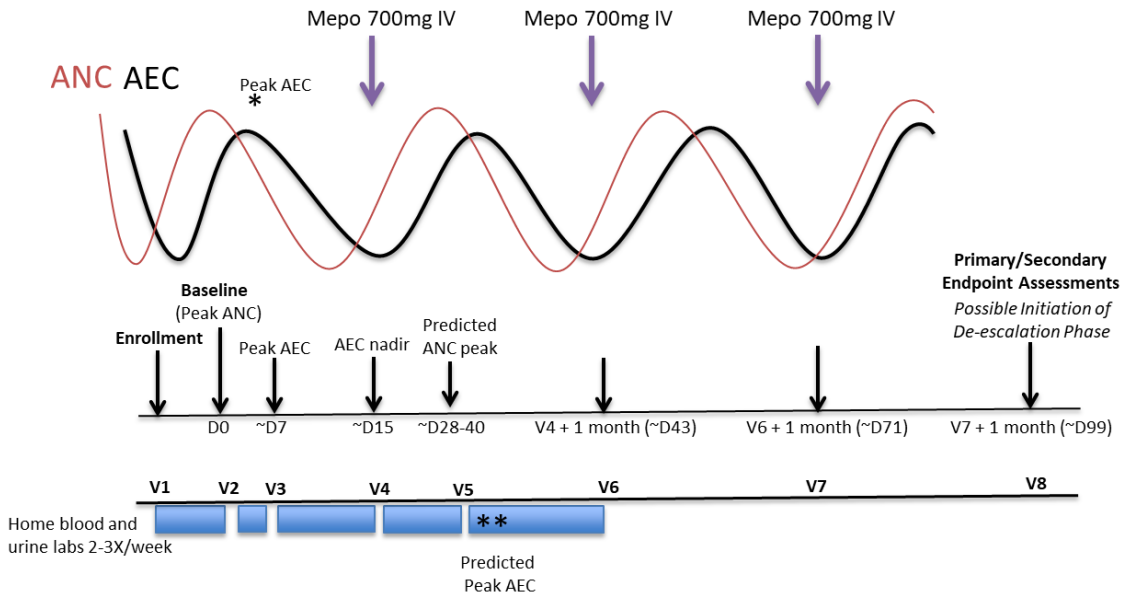

Figure 1. Study Schematic.
The black arrows indicate study visits (V) for blood draws and clinical assessments, the purple arrows indicate mepolizumab infusions, and the blue bars indicate interval blood draws being performed locally. The estimated timing of visits is indicated in approximate days (~D). The asterisks (* and **) define the comparison timepoints for the secondary efficacy endpoint. Subjects will document their weight, temperature, and symptoms and severity every day for about 3 months after enrollment. V8 will begin the Dose De-escalation Phase for subjects who receive benefit from mepolizumab.

3.3 Study Endpoints

3.3.1 Primary Endpoint

Reduction in the number and severity of clinical symptoms associated with EAE after 3 doses of mepolizumab (as compared to symptoms logged in the approximately monthly cycle preceding initiation of mepolizumab).

3.3.2 Secondary Endpoints

1) A ≥75% reduction in peak blood eosinophil count following the first dose of mepolizumab (as compared to the peak eosinophil count in the cycle preceding initiation of mepolizumab therapy).

2) Sustained reduction of eosinophilia after administration of mepolizumab (at Visit 8).

3.3.3 Exploratory Endpoints

1) Effect of mepolizumab on cycling of cells other than eosinophils (e.g., neutrophils and lymphocytes) following mepolizumab administration.

2) Effect of mepolizumab on markers of eosinophil activation and other biomarkers of disease activity in EAE, including whole blood transcriptomics, levels of eosinophil and neutrophil granule proteins, serum IgG and IgM levels, serum cytokine and chemokine levels, and serum tryptase levels.

3) Reduction of symptoms and severity of clinical symptoms of EAE on mepolizumab at doses of 500 mg and 300 mg.

4. Study Population

4.1 Rationale for Subject Selection

Subjects will be selected based on a confirmed diagnosis of EAE and demonstration of ongoing symptoms and cycling. Potential subjects whose peak eosinophil count is <1500/mm^3 in the 3 months prior to enrollment will not be eligible.

4.2 Recruitment Plan

EAE is an extremely rare disorder. A maximum of 12 intention-to-treat subjects with EAE will be enrolled with a ceiling of 15 patients for subjects that drop out prior to receiving the first dose. Subjects who are withdrawn prior to receiving mepolizumab will be replaced. The anticipated recruitment period will be 2 years. Subjects will be recruited from those already enrolled on NIH protocol 94-I-0079 ("Eosinophil activation and function in parasitic infections and other conditions with increased tissue or blood eosinophilia in humans"). The NIH is a major referral center for HES, with >200 patients actively followed on protocol 94-I-0079, of which 12 are known to have EAE, and 1-2 new patients referred for evaluation of eosinophilia weekly.

4.3 Subject Inclusion Criteria

A subject will be eligible for participation in the study only if all of the following criteria apply:

1) The subject is male or female, aged 18 years or older.
2) The subject has a documented diagnosis of EAE.
3) The subject has symptoms of EAE in the cycle prior to screening, including but not limited to fever, swelling, hives or rashes, weight gain, muscle pain, and lymphadenopathy.
4) Cycling of eosinophils is ongoing as indicated by a peak AEC ≥1500/mm^3 during at least one cycle in the prior 3 months.
5) If taking corticosteroids ≤40 mg daily, the subject is able and willing to stay on a stable dose for 6 weeks prior to screening.
6) The subject agrees to storage of study samples.

7) The subject is able to provide informed consent.
8) Females are eligible for this study if they are:
    i. of non-childbearing potential (i.e., women who have had a hysterectomy or tubal ligation or are postmenopausal as defined by no menses in 1 year); OR
    ii. of childbearing potential but willing to practice effective contraception or abstinence during administration of the study drug and for 100 days (5 terminal half-lives) after administration of the study drug.
    iii. Not breastfeeding.

Participation of Women:
Pregnancy: The effects of mepolizumab on the developing human fetus are unknown. For this reason, women of childbearing potential must agree to use adequate contraception (see below for acceptable methods) prior to study entry, for the duration of study participation, and for >5 terminal half-lives (approximately 100 days) after administration of the last dose of study drug. Non-reproductive potential is defined as post-menopausal, male partner who has azoospermia or is surgically sterile (at least 6 weeks before screening) and is the sole sexual partner, surgical sterility, or a congenital or acquired condition that definitely prevents conception. Further, postmenopausal is defined as at least 12 consecutive months with no menses at age 50 or older, or a high follicle-stimulating hormone level in the postmenopausal range at ages 45-50 years in subjects not using hormonal contraception or hormone replacement therapy.

Females with reproductive potential must either practice complete and uninterrupted abstinence from heterosexual activity or use 2 of the following methods of contraception with their partners. The 2 methods must include either 2 barrier methods, or 1 barrier method and 1 non-barrier method, both of which must be consistently used:

<u>Barrier Methods:</u>
1) Diaphragm with spermicide
2) Cervical cap with spermicide or contraceptive sponge (for nulliparous subjects only)
3) Male or female condom (cannot be used together)

<u>Non-Barrier Methods</u>
1) An intrauterine device with a documented failure rate of <1%

2) Hormonal contraception: pill (estrogen/progestin or progestin-only), patch, vaginal ring, rod implanted in the skin, or subcutaneous injection methods

Females of childbearing-potential must have a negative pregnancy test result prior to receiving mepolizumab at each on-site study visit. During the course of the study, if a woman becomes pregnant or suspects she is pregnant, she should inform the study staff and her primary care physician immediately. Subjects who become pregnant will be entered into the mepolizumab pregnancy registry.

Fertility: There is no fertility data in humans. Animal studies showed no adverse effects of anti-IL-5 treatment on fertility.

4.4 Subject Exclusion Criteria

A subject will not be eligible to participate in the study if any of the following conditions are fulfilled at the time of screening:

1) Treatment with immunosuppressive or immunomodulatory agents including but not limited to cyclosporine, interferon-alpha, azathioprine, methotrexate, and cyclophosphamide within the past 3 months.
2) Treatment with biologics including but not limited to mepolizumab, IVIG, anti-TNF agents, rituximab, benralizumab, alemtuzumab, reslizumab, dupilumab, lebrikizumab, and omalizumab within 6 months or 5 half-lives (whichever is longer). Subjects who received rituximab at any time in the past must have normal B-cell numbers to participate.
3) Co-morbid illness, alcohol or substance abuse, or any other condition (e.g., HIV, active hepatitis) that, in the opinion of the investigator, places the subject at undue risk by participating in the study.
4) Treatment with a daily dose of corticosteroids >40 mg.

Co-enrollment Guidelines: Co-enrollment in other trials is restricted, other than enrollment on observational studies or those evaluating the use of a licensed medication (see section 5.6). Study staff should be notified of co-enrollment as it may require the approval of the investigator.

4.5 Justification for Exclusion of Women, Minorities, Children, and Special Populations

Exclusion of Women:

- **Pregnancy:** Pregnant women are excluded from this study because the effects of mepolizumab on the developing human fetus are unknown (see section 4.3).

- **Breastfeeding:** Mepolizumab was excreted into the milk of cynomolgus monkeys at concentrations that were less than 0.5% of those detected in plasma. Because of the unknown but potential risk for adverse events (AEs) in nursing infants secondary to treatment of the mother with mepolizumab, subjects who are breastfeeding are not eligible for enrollment.

Exclusion of Children:
Although mepolizumab is approved for use in children 6 years and older with severe asthma and 12 years and older in HES, there is insufficient data in children with EAE to judge the potential risks. Moreover, the amount of blood required by this study exceeds the volume permitted for children; therefore, children are excluded from this study.

Exclusion of Decisionally Impaired Adults:
Adults who lack decision-making capacity to consent are not eligible for enrollment in this study (section 4.3), and an enrolled subject who permanently loses decision-making capacity to consent during the study will be withdrawn (section 12.8). NIH Policy 403 *Research with Subjects Lacking Capacity to Consent* will be followed.

5. Study Agent/Interventions

5.1 Disposition and Dispensation

Study agent will be distributed via the NIH Central Pharmacy according to standard pharmacy procedures.

5.2 Formulation, Packaging and Labeling

Study drug will be labeled with the drug name, dose, storage conditions, the name and address of the manufacturer, Investigational Use Statement (“Caution: New Drug – Limited by Federal [USA] Law to Investigational Use”) if investigational study agent is sent. For commercial agent labeled for subcutaneous use on the package, the agent may be reconstituted for IV use.

5.3 Study Agent Storage and Stability

Mepolizumab will be stored at temperatures of 2°C-8°C in the pharmacy and protected from light. Maintenance of a temperature log will be required to ensure no excursions.

5.4 Preparation, Administration, and Dosage of Study Agent

Study Agent: *Mepolizumab 700 mg IV; 500 mg IV/SC; and 300 mg SC*

Description
Mepolizumab is a fully humanized monoclonal antibody (IgG1, kappa mAb) supplied for IV infusion as 100 mg of lyophilized powder in sterile, single-dose vials. Mepolizumab for SC injection is supplied as single-dose prefilled autoinjectors containing 100 mg/mL mepolizumab as a clear to opalescent, colorless to pale yellow to pale brown solution.

Dosing and Administration
All subjects will receive mepolizumab 700 mg IV x 3 doses at approximately one-month intervals at study Visits 4, 6, and 7. Subjects in the Dose De-escalation Phase will receive additional monthly doses of 500 mg IV or SC at Visits 8 to 10, and 300 mg SC at Visits 11 to 13.

Dose Preparation
For the IV formulation: Mepolizumab 100 mg vials will be reconstituted with 1.2 mL sterile water for injection (according to the product labeling) and secondarily diluted in 100-250 mL of 0.9% normal saline (NaCl) for infusion (to a final concentration of 0.3-7.5 mg/mL). The reconstituted solution should be used as soon as possible but may be kept for up to 8 hours while refrigerated or at controlled room temperature (up to 25°C). Any unused solution remaining after 8 hours should be discarded.

For the SC formulation: Mepolizumab will be provided in prefilled autoinjectors each containing a single 100 mg/mL dose. The autoinjectors should be stored in the refrigerator and allowed to sit at room temperature for 30 minutes prior to injection. An autoinjector should not be used if it is dropped on a hard surface or if the solution appears cloudy, milky, or containing particular matter.

Route of Administration
For the IV formulation: Mepolizumab will be administered as a peripheral IV infusion over 30 minutes.

For the SC formulation: Mepolizumab administration will occur via an autoinjector and will be given by the subject or a caregiver as multiple injections of 100-mg each. Each injection should be given into the thigh or abdomen (avoiding the 5 cm [2 inches] around the navel) when given by the subject or caregiver; the upper arm may be used if the injection is administered by a caregiver. Each injection site should be separated by at least 5 cm (2 inches). Injections should not be administered into areas of skin that are tender, bruised, red, or hard. If a dose is missed, it should be administered as soon as possible. Thereafter, the subject should resume dosing on the usual day of administration. If the next dose is already due, then it should be administered as planned.

Dose Adjustments/Modifications/Delays
During the Primary Study Phase administering mepolizumab at 700 mg IV, the dose will remain the same without modification unless held or stopped due to a

pause (section 12.5), halt (section 12.6), or withdrawal of the subject (section 12.8). During the Dose De-escalation Phase, the dose will be reduced as outlined in the protocol unless held or stopped due to a pause, halt, or withdrawal of the subject.

Duration of Therapy
A total of 3 doses of 700 mg IV will be given on this protocol over a 3-month duration for all subjects; subjects in the Dose De-escalation Phase will have 6 months of additional de-escalating doses at 500 mg (3 doses) and 300 mg (3 doses). The total duration of therapy for participation in both the Primary Study Phase and the Dose De-escalation Phase will be 9 months.

Tracking of Dose
A record of the number of doses and lots used will be kept in the pharmacy for doses administered in-person at the NIH Clinical Center Day Hospital. For remote visits during the Dose De-escalation Phase, subjects will either have an observed administration via telehealth or will log the dose on a dose tracker.

Limitations on Prior Therapy
Subjects who have received mepolizumab within 6 months or 5 half-lives (whichever is longer) will not be eligible for this protocol.

Use of Ancillary Medications/Over-the-Counter Products/Foods
Only corticosteroids will be permitted for treatment in addition to mepolizumab. There are no restrictions on use of other prescribed medications, over-the-counter products or foods.

Subject Access to Study Agent After End of Study Assessments
All attempts to obtain approved mepolizumab will be pursued by the subject at the completion of the study.

5.5 Concomitant Medications and Procedures

All concomitant prescription medications taken during study participation will be tracked in the Clinical Research Information Management System of the NIAID (CRIMSON). For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in CRIMSON are concomitant prescription medications, over-the-counter medications and non-prescription medications taken at the time of AEs.

5.6 Prohibited Medications and Procedures

Treatment with other biologics or immunosuppressive or cytotoxic agents will not be permitted unless discussed with and approved by the investigator.

6. Study Schedule

All study assessments/procedures required for each study visit are summarized in Appendix B: Schedule of Procedures/Evaluations. Windows for the study timepoints are specified where appropriate. Blood volumes are given in Appendix C: Blood Volumes for Specimen Collection. Research studies are detailed in Appendix H: Research Studies.

If in-person study visits are limited due to the COVID-19 pandemic, study visits may be rescheduled or study procedures may be modified to allow for continued safety monitoring and data collection for study endpoints remotely. If a visit needs to be rescheduled as a remote visit, a member of the study team will contact subjects with sufficient time prior to the study timepoint to make arrangements. In-person visits may be replaced with or modified to include phone calls, virtual telehealth visits or visits with a local healthcare provider for assessment and sample collection. Mandatory study procedures to be performed at remote visits are indicated for applicable visits throughout section 6. For example, EAE symptom logs can be completed electronically, and blood and/or urine samples may be collected with a local specialist or healthcare provider and sent in for clinical and research analyses. However, some research blood cannot be shipped and would be waived based on the discretion of the principal investigator. Regular phone or virtual calls will occur (as per the assessment schedule or more frequently if needed) for safety monitoring and discussion of the subject's health status until the subject can again visit the site.

6.1 Enrollment (Visit 1)

Screening of subjects for this study will occur under NIH protocol 94-I-0079 ("Eosinophil activation and function in parasitic infections and other conditions with increased tissue or peripheral blood eosinophilia in humans").

Subjects with EAE will be brought in for enrollment (Visit 1). Visit 1 may occur over several days.

After signed informed consent is obtained, the following procedures and laboratory tests will be performed:

- Complete history and physical examination, including limb measurements
- Vital signs, including weight
- Photography (with infrared thermography if available)
- CBC with differential
- Routine laboratory testing, including acute care panel, hepatic panel, mineral panel, lactate dehydrogenase (LDH), creatine kinase (CPK), C-reactive protein (CRP), and urinalysis

- Serum tryptase, aldolase, and quantitative immunoglobulin levels (including IgE)
- Urine pregnancy test (females of childbearing potential only)
- Collection of serum or plasma and urine for immunologic analyses and quantification of eosinophil granule proteins
- Collection of whole blood for isolation and storage of plasma and cells for research studies
- Whole blood lymphocyte phenotyping by T-, B-, and natural killer–cell analysis (TBNK) and RNA sequencing (RNAseq)
- Optional bone marrow biopsy and aspiration, if not available under protocol 94-I-0079 or another NIH protocol
- Optional skin punch biopsy
- AE assessment

The subjects will start a log of symptoms of EAE that they will fill out daily to track symptoms, weight, and temperature. They will also start daily and monthly patient-reported outcome measures for angioedema symptoms and quality of life at this time. These logs and measures will be completed electronically using REDCap. Subjects will discontinue daily logs and measures after the third mepolizumab visit (Visit 7) but will continue monthly measures through the end of the primary study phase (section 7.1.2).

After this visit, subjects will also begin 2-3X/week lab blood draws locally to assess cycles and blood counts between visits. Frequency of weekly labs may be adjusted to avoid exceeding NIH CC blood volume limits (2X/week for subjects with shorter cycles or after peak AEC is reached). Blood will be drawn for CBCs (performed at local lab with results sent to NIH), and urine will be collected and mailed to the NIH for research evaluations. These local blood and urine collections will continue between scheduled study visits through Visit 6. Estimated total number of blood draws is approximately 3X/week for 2 months (approximately 24 blood draws) while not at NIH.

6.2 Baseline (Visit 2, Day 0)

The baseline visit (Visit 2) will be scheduled to coincide with the estimated peak ANC based on data from prior cycles. If the ANC begins to rise prior to the scheduled baseline visit, the subject will be brought back to NIH as soon as feasible. For subjects whose ANC peak and AEC peak are closer together than a week, Visits 2 and 3 may be combined.

The following procedures and laboratory tests will be performed at the baseline visit:

- Complete history and physical examination, including limb measurements
- Vital signs, including weight
- Photography (with infrared thermography if available)
- Review of symptom/weight logs and patient-reported outcome measures adherence
- CBC with differential
- Routine laboratory testing, including acute care panel, hepatic panel, mineral panel, CRP, and urinalysis
- Serum tryptase, aldolase, and quantitative immunoglobulin levels (including IgE)
- Collection of serum or plasma and urine for immunologic analyses and quantification of eosinophil granule proteins
- Collection of whole blood for isolation and storage of plasma and cells for research studies
- Collection of whole blood for TBNK and RNAseq
- Optional bone marrow biopsy and aspiration, if not done at the enrollment visit or available under protocol 94-I-0079 or another NIH protocol
- Optional skin punch biopsy, if not done at the enrollment visit (Visit 1)
- AE assessment

6.3 Primary Study Phase

6.3.1 AEC peak (Visit 3)

Subjects will return to the clinic at the predicted AEC peak (denoted by * in Figure 1) as determined by their individual history of EAE cycle times. For a 4-week EAE cycle time, Visit 3 would occur at about Day 7 (±3 days). For subjects whose ANC peak and AEC peak are closer together than a week, Visits 2 and 3 may be combined.

The following procedures and laboratory tests will be performed:

- Targeted history and physical examination, including limb measurements
- Vital signs, including weight
- Review of symptom/weight logs and patient-reported outcome measures adherence

- CBC with differential
- Routine laboratory testing, including acute care, hepatic, and mineral panels, LDH, CPK, CRP, and urinalysis
- Serum tryptase, aldolase, and quantitative immunoglobulin levels (including IgE)
- Collection of serum or plasma and urine for immunologic analyses and quantification of eosinophil granule proteins
- Collection of whole blood for storage of plasma and cells for research studies
- Whole blood lymphocyte phenotyping (TBNK) and RNAseq
- Optional skin punch biopsy, if not done at Visits 1 or 2
- AE assessment

6.3.2 AEC nadir and mepolizumab administration (Visit 4)

Visit 4 will be scheduled to capture the AEC nadir. We anticipate that, for most subjects (assuming a 4-week cycle time), this will fall at about Day 15 (±3 days). The schedule may be adjusted based on results of at-home CBCs.

The following procedures and laboratory tests will be performed:

- Targeted history and physical examination, including limb measurements
- Vital signs, including weight
- Review of symptom/weight logs and patient-reported outcome measures adherence
- CBC with differential
- Routine laboratory testing, including acute care panel, hepatic panel, mineral panel, LDH, CPK, CRP, and urinalysis
- Urine pregnancy test (females of childbearing potential only)
- Serum tryptase, aldolase, and quantitative immunoglobulin levels (including IgE)
- Collection of serum or plasma and urine for immunologic analyses and quantification of eosinophil granule proteins
- Collection of whole blood for storage of plasma and cells for research studies
- Whole blood lymphocyte phenotyping (TBNK) and RNAseq
- AE assessment

Following completion of the evaluations and pregnancy test (if applicable), mepolizumab 700 mg IV will be administered in the 5SW Day Hospital. The drug will be infused over about 30 minutes. Vitals will be checked and subjects will be able to leave 30 minutes after the infusion.

6.3.3 Predicted ANC peak (Visit 5)

Visit 5 will be scheduled based on the individual subject's cycle length to coincide with the predicted ANC peak. It is possible that mepolizumab administration may alter cycle times, which could affect the scheduling of this visit; however, the time between each infusion will be about 1 month. Every effort will be made to schedule Visit 5 in advance of Visit 6; in the event that Visit 5 occurs within 1 day of Visit 6, only collection of whole blood for storage will be performed for Visit 5.

The following procedures and laboratory tests will be performed:

- Targeted history and physical examination, including limb measurements
- Vital signs, including weight
- Photography (with infrared thermography if available)
- Review of symptom/weight logs and patient-reported outcome measures adherence
- CBC with differential
- Routine laboratory testing, including acute care panel, hepatic panel, mineral panel, LDH, CPK, CRP, and urinalysis
- Serum tryptase, aldolase, and quantitative immunoglobulin levels (including IgE)
- Collection of serum or plasma and urine for immunologic analyses and quantification of eosinophil granule proteins
- Collection of whole blood for storage of plasma and cells for research studies
- Whole blood lymphocyte phenotyping (TBNK) and RNAseq
- AE assessment

In order to capture the peak AEC post-mepolizumab (denoted by ** during blood draws in Figure 1), subjects will continue 2-3X/week blood draws for CBC with differential until Visit 6. Additionally, at the time of the predicted peak AEC, urine and blood will be collected for shipment to the NIH.

6.3.4 Mepolizumab administration (Visit 6)

Visit 6 will be scheduled 28 days (±5 days) after Visit 4.

The following procedures and laboratory tests will be performed:

- Targeted history and physical examination, including limb measurements
- Vital signs, including weight
- Review of symptom/weight logs and patient-reported outcome measures adherence
- CBC with differential
- Routine laboratory testing, including acute care panel, hepatic panel, mineral panel, LDH, CPK, CRP, and urinalysis
- Urine pregnancy test (females of childbearing potential only)
- Serum tryptase, aldolase, and quantitative immunoglobulin levels (including IgE)
- Serum or plasma and urine for eosinophil granule protein and mediator levels
- Collection of whole blood for purification and storage for research studies
- AE assessment

Following completion of the evaluations and pregnancy testing (if applicable), mepolizumab 700 mg IV will be administered in the Day Hospital.

There will not be additional at-home blood or urine collections under this protocol after Visit 6.

6.3.5 Mepolizumab administration (Visit 7)

Visit 7 will be scheduled for 28 days (±5 days) after Visit 6. The following procedures and laboratory tests will be performed:

- Targeted history and physical examination, including limb measurements
- Vital signs, including weight
- Review of monthly patient-reported outcome measures adherence
- CBC with differential
- Routine laboratory testing, including acute care panel, hepatic panel, mineral panel, LDH, CPK, CRP, and urinalysis
- Urine pregnancy test (females of childbearing potential only)
- Serum tryptase, aldolase, and quantitative immunoglobulin levels (including IgE)
- AE assessment

Following completion of the evaluation and pregnancy testing (if applicable), mepolizumab 700 mg IV will be administered in the Day Hospital.

6.3.6 Completion of primary and secondary endpoint assessments (Visit 8)

Visit 8 will again be scheduled 28 days (±7 days) after Visit 7. This visit may occur over several days.

The following procedures and laboratory tests will be performed:

- Targeted history and physical examination, including limb measurements
- Vital signs, including weight
- CBC with differential
- Routine laboratory testing, including acute care, hepatic, and mineral panels, CRP, and urinalysis
- Serum tryptase, aldolase, and quantitative immunoglobulin levels (including IgE)
- Collection of serum or plasma and urine for immunologic analyses and quantification of eosinophil granule proteins
- Collection of whole blood for isolation and storage of plasma and cells for research studies
- Whole blood lymphocyte phenotyping (TBNK) and RNAseq
- Optional bone marrow biopsy and aspiration, requested of subjects for whom a sample was collected/available prior to study treatment (may be done once at any time while on study drug)
- AE assessment

After completion of the procedures described above, subjects who have received benefit from mepolizumab administration will begin the Dose De-escalation Phase (Visits 8-14, as outlined in section 6.4).

For subjects who do not participate in the Dose De-escalation Phase, Visit 8 will be the end of study visit.

6.4 Dose De-escalation Phase

6.4.1 Initiation of de-escalation (Visit 8)

For subjects who demonstrated clinical benefit from mepolizumab administration, Visit 8 will begin the Dose De-escalation Phase. The following additional procedures and laboratory tests will be performed:

- Urine pregnancy test (females of childbearing potential only)
- Teaching for autoinjector drug administration (if applicable)

Following completion of these procedures, mepolizumab 500 mg IV will be administered in the Day Hospital. The drug will be infused over about 30 minutes. Vitals will be checked and subjects will be able to leave 30 minutes after the infusion.

The subjects will start a log of symptoms of EAE that they will fill out weekly (rather than daily) to track symptoms and weight during de-escalation. They will also continue patient-reported outcome measures: weekly for angioedema symptoms and monthly for quality of life. These logs and measures will be completed electronically using REDCap (section 7.1.3). Subjects will complete the logs and measures through the end of the De-escalation Phase (Visit 14).

If subjects experience a symptomatic flare between visits at any time during the dose de-escalation phase, they may be requested to obtain additional bloodwork for safety reasons.

6.4.2 Mepolizumab administration at 500 mg (Visits 9 and 10)

Visits 9 and 10 will be scheduled 28 days (±7 days) from the prior visit. In-person visits are preferred, but these visits may be performed remotely if needed due to COVID-19 restrictions and difficulties with travel. In such cases, the pharmacy will provide autoinjectors for SC administration. Visit 10 should be performed at NIH if feasible.

The following procedures and laboratory tests will be performed:

- Targeted history and physical examination, including limb measurements. If the visit is conducted remotely, a targeted history and physical examination will be conducted virtually.
- Vital signs, including weight. The subject will measure and report their weight and temperature if the visit is performed remotely.
- Pregnancy test (females of childbearing potential; not performed at a remote visit)
- Review of symptom/weight logs and patient-reported outcome measures adherence

- CBC with differential
- Collection of serum or plasma and urine for immunologic analyses and quantification of eosinophil granule proteins (not performed at a remote visit)
- AE assessment
- Visit 10 only:
    - Routine laboratory testing, including acute care, hepatic, and mineral panels, LDH, CPK, CRP, and urinalysis (not performed at a remote visit)
    - Serum tryptase, and aldolase, serum immunoglobulins and IgE
    - Collection of whole blood for isolation and storage of plasma and cells for research studies (not performed at a remote visit)
    - Whole blood lymphocyte phenotyping (TBNK) (not performed at a remote visit) and RNAseq

Following completion of the evaluation and pregnancy testing (if applicable), mepolizumab 500 mg will be administered by IV infusion or by SC injection.

6.4.3 Mepolizumab administration at 300 mg (Visits 11 to 13)

Visits 11 to 13 will be scheduled 28 days (±7 days) from the prior visit. These visits may be performed remotely if needed due to COVID-19 restrictions and difficulties with travel. In such cases, the pharmacy will provide autoinjectors for SC mepolizumab administration.

The following procedures and laboratory tests will be performed:

- Targeted history and physical examination, including limb measurements. If the visit is conducted remotely, a targeted history and physical examination will be conducted virtually.
- Vital signs, including weight. The subject will measure and report their weight and temperature if the visit is performed remotely.
- Review of symptom/weight logs and patient-reported outcome measures adherence
- CBC with differential
- Pregnancy test (females of childbearing potential; not performed at a remote visit)
- AE assessment

Following completion of the evaluation and pregnancy test (if applicable), mepolizumab 300 mg SC will be administered.

6.4.4 End of de-escalation phase (Visit 14)

The end of study visit for subjects in the Dose De-escalation Phase will be scheduled 28 days (±7 days) after Visit 13.

The following procedures and laboratory tests will be performed:

- Targeted history and physical examination, including limb measurements
- Vital signs, including weight
- CBC with differential
- Routine laboratory testing, including acute care, hepatic, and mineral panels, LDH, CPK, CRP, and urinalysis
- Serum tryptase, aldolase, and quantitative immunoglobulin levels (including IgE)
- Collection of serum or plasma and urine for immunologic analyses and quantification of eosinophil granule proteins
- Collection of whole blood for isolation and storage of plasma and cells for research studies
- Whole blood lymphocyte phenotyping (TBNK) and RNAseq
- AE assessment

After this visit, participation of subjects in the Dose De-escalation Phase will be complete.

6.5 Unscheduled Visit

Extra visits may be scheduled in between any of the scheduled visits due to need to re-evaluate the subject or conduct additional studies for clinical care of the subject.

6.6 Early Termination Visit

Subjects who desire to withdraw from study and further administration will be brought back for an early termination visit and clinical assessment. The principal investigator may also elect to terminate participation of the subject from the study for lack of adherence to study visits (according to the withdrawal criteria in section 12.8), safety (pausing or halting rules as described in sections 12.5 and 12.6), or lack of efficacy. Reasons for withdrawal and safety labs will be captured during this visit.

6.7 Pregnancy and Follow-up Visit

Subjects who become pregnant during the conduct of this study will be discontinued from the study. They will return to the NIH CC 4 weeks after

discontinuation of mepolizumab for clinical assessment, including physical examination and safety laboratory tests. They will be entered into the mepolizumab pregnancy registry and followed on 94-I-0079 for their disease and eosinophilic process but will be removed from this protocol and will not receive further doses of mepolizumab on this study.

6.8 Recontact of Subjects After Trial Termination

Subjects will not be recontacted on this study after trial termination. Subjects will remain on the screening protocol 94-I-0079 on which they may be recontacted if there are important findings that result from this study.

7. Study Procedures/Evaluations

7.1 Clinical Evaluations

7.1.1 History and Physical Exam

A complete medical history and physical examination will be performed as part of the enrollment and baseline evaluations. Subsequent clinical evaluations will focus on the assessment of new symptoms, signs, or untoward medical events. Vital signs, including blood pressure, heart rate, and body temperature, will be measured as part of all physical examinations, according to standard nursing practice. Weight will be assessed at all visits. Subjects sometimes have demonstrable changes in sizes of limbs (forearms, upper arms, or legs). As such, limb diameter measurements will be taken at all visits. More specifically, maximal forearm and upper arm diameter of both arms will be taken 2 inches above and below the cubital fossa. Similarly, for lower extremities, maximal diameter will be measured in both legs 3 inches above and below the popliteal fossa in all patients. Other measurements may be taken on an individual subject depending on symptoms.

7.1.2 Photography and Infrared Thermography

Full or partial body photographic documentation of skin and musculoskeletal involvement will be obtained at enrollment (Visit 1), baseline (Visit 2), and Visit 5 as well as if clinically indicated. Infrared thermography may be used to assess extent of skin involvement using a gradation of colors to demonstrate emission of heat, or thermal radiation, emitted from a surface, if available. Subjects will be asked to refrain from eating for 4 hours prior to thermography so pictures can be compared in the future. The room may also be kept cooler than usual (about 22°C or 72°F) during infrared thermography. Images will be analyzed, transmitted, and stored securely to maintain confidentiality and privacy of subjects.

7.1.3 Symptom Logs and Patient-reported Outcomes

Patients will fill out a daily log capturing symptoms, weight, and temperature (Appendix E: Patient Symptom Log), and fill out validated patient-reported outcome measures for angioedema (daily) and quality of life (monthly; questionnaires provided as Appendix G). These logs and measures will be completed electronically using REDCap. Subjects will also be encouraged to notify the investigators of any new signs or symptoms that develop between study visits. The daily logs and measures will be completed through the end of study endpoint assessments (Visit 8). For subjects in the de-escalation phase, after Visit 8 until study completion, weekly angioedema and monthly quality of life questionnaires will be continued, and weekly symptoms logs (Appendix F) will be completed.

7.1.4 Optional Bone Marrow Biopsy and Aspiration

Optionally once before starting study treatment or at the time of peak symptoms (Visits 1 or 2), and once while on study drug, subjects may be asked to participate in bone marrow biopsy and aspiration. If bone marrow was already collected at the NIH (e.g., under protocol 94-I-0079), the study team may instead request a portion of that sample for analyses under this protocol.

Bone marrow biopsy and aspiration will be performed using standard sterile techniques. Local anesthesia (lidocaine) will be administered. Any additional anesthetic (e.g., oral benzodiazepine) will be administered at the discretion of the investigator. Each aspirate typically provides 2-3 mL of bone marrow, and 2-10 aspirates will be obtained.

7.1.5 Optional Skin Punch Biopsy

Optionally once before starting study treatment (Visits 1-3) subjects may be asked to undergo skin punch biopsy.

Skin punch biopsy will be performed using standard sterile techniques after administration of local anesthesia (lidocaine). Two to three 3-mm biopsies will be collected for histopathology, transcriptomics, and fibroblast cultures. Punch biopsies will be obtained by credentialed clinicians following standard procedures. One to two sutures will be placed as appropriate.

7.2 Laboratory Evaluations

Routine laboratory evaluations, including CBC with differential, routine chemistries, and urinalysis, will be performed in the Department of Laboratory Medicine at the NIH CC or at outside laboratories. Serum, plasma, whole blood, and urine will be collected at various timepoints for assessment of eosinophil activation, cytokine/chemokine profile, and other immunologic parameters as described in Appendix H: Research Studies. Bone marrow and skin punch biopsy

samples will be stored for future research, including exploratory research of progenitor cells and fibroblasts, respectively.

7.2.1 Specimen Preparation, Handling and Shipping

Research samples collected at the NIH will be either sent to Clinical Support Laboratory, Frederick National Laboratory for Cancer Research, Leidos Biomedical Research, Inc. for processing or will be transported from phlebotomy to Building 4/B1-28 for processing by laboratory staff after collection. No samples will be shipped to other sites.

8. Potential Risks and Benefits

8.1 Potential Risks

8.1.1 Mepolizumab

The risks of mepolizumab and AE profile have been well described in several prior studies of mepolizumab in asthma, eosinophilic granulomatosis with polyangiitis, and HES. ARs with a ≥5% incidence include: headache, injection site reactions (pain, erythema, swelling, itching, burning), back pain, and fatigue. Mepolizumab 100 mg and 300 mg have been approved for SC use. The package insert lists the following risks of mepolizumab use: hypersensitivity reactions including anaphylaxis, angioedema, bronchospasm, hypotension, urticaria, and rash; and herpes zoster infection (12). Other noted findings in studies of mepolizumab include the development of anti-drug antibodies; however, neutralizing antibodies were only identified in 1 of 260 patients with severe asthma receiving mepolizumab 100 mg. Such antibodies are not considered to affect the course of treatment. Additionally, there are unknown risks to the fetus in the case of pregnancy or nursing infants (see section 4.3).

8.1.2 Oral Corticosteroids

Prednisone and other oral corticosteroids are commonly used to treat a wide variety of disorders, including HES. Some subjects enrolled on this study will be on prednisone (or equivalent corticosteroid) for treatment (although subjects could be enrolled off all background therapy). Although the protocol does not administer corticosteroids specifically, the subjects will be required to maintain their current therapy throughout the duration of the protocol without further attempts to taper to ensure that changes in counts and cycles are not a result of alteration of therapy. As such, the risks of continuing on corticosteroids are listed here. The common side effects of corticosteroids are well described and include increased appetite, insomnia, fluid retention, indigestion, headache, facial swelling, menstrual irregularities, and visual changes. When used over extended periods of time, corticosteroids may cause weight gain, cataracts, myopathy, osteoporosis, glucose intolerance, increased risk of infections such as *Pneumocystis jirovecii*, and can affect mood. Subjects will be monitored closely

for these side effects as per usual care. Additionally, evidence-based approaches for glucocorticoid bone loss prevention or treatment will be followed.

8.1.3 Optional Bone Marrow Biopsy and Aspiration

Bone marrow biopsy and aspiration is optional in this protocol. It is a common medical procedure associated with low risk. Risks include the potential for bleeding and a small possibility of infection at the aspirate site that may require antibiotic treatment. There is usually some pressure-like discomfort during needle insertion, and the procedure may result in a bruise and/or some discomfort at the biopsy site or surrounding tissue for 1-2 days after the anesthetic wears off. Standard procedures will be followed for all bone marrow biopsies to minimize pain and the possibility of infection.

8.1.4 Optional Skin Punch Biopsy

The risks of skin punch biopsy include local pain, bleeding, infection, and potential scar and keloid formation. Antibiotics and oral analgesics will be used to manage infection and pain, respectively. The local anesthetic may cause skin irritation. These risks should not be substantially different than those experienced with diagnostic biopsies routinely done on inflamed or infected skin. Any nonroutine complications resulting from the procedure will be addressed in consultation with the NIH CC Dermatology service.

8.1.5 Blood Drawing and IV Insertion

The potential risks of IV catheter insertion and the needle stick for blood drawing include pain, fainting, infection and bruising, or a small hematoma. The bruising may last up to 72 hours. Any hematomas will be treated with local pressure. Infection from the needle puncture is rare, but if this does occur, appropriate treatment will be given. The total blood drawn during the study is within the guidelines of the NIH CC (Medical Administrative Policy 95-9, Guidelines for Limits of Blood Drawn for Research Purposes in the Clinical Center: http://cc-internal.cc.nih.gov/policies/PDF/M95-9.pdf) (see Appendix C: Blood Volumes for Specimen Collection for total blood draw volumes).

8.1.6 Photography and Infrared Thermography

Taking pictures of the face and body may be embarrassing to some people. Infrared thermography, in contrast, would not risk exposing the patients identity. These photographs may be published in medical journals, without identifying the subject. We will attempt to preserve the anonymity of the subject as much as possible while providing the information needed to support the research being published. Subjects may decline photographs or place any restrictions on their use. Subjects will be given the opportunity to discuss this with the principal or associate investigators.

8.2 Potential Benefits

Since the effect of mepolizumab in EAE is unknown, subjects may or may not benefit from the experimental therapy. Findings from this study will help researchers understand the underlying mechanisms of EAE and possibly identify a new therapeutic option for patients.

9. Research Use of Stored Human Samples, Specimens, and Data

- **Intended Use:** Samples, specimens, and data collected under this protocol may be used to study factors related to etiology of EAE. Blood samples from subjects may be used for genetic analysis, including RNA expression (transcriptomics) and analysis of target variants of interest in EAE by real-time polymerase chain reaction. Genetic analyses will be for research purposes only, and no individual genetic results will be returned to subjects. Screening for diseases unrelated to EAE will not be performed, and findings unrelated to eosinophilia and immune function will not be further investigated. Genetic data will be coded and stored securely as described below. Deidentified genetic data (specifically transcriptomic data) may be shared in public databases as described in section 10. The informed consent document will describe the possible future use of stored specimens and data.
- **Storage:** All of the stored study research samples are labeled by a code that only the investigators can link to the subject. Serum will be stored in a −80°C freezer, cells in liquid nitrogen, and tissue samples at room temperature or frozen as appropriate. Data will be kept in password-protected computers. Only investigators or their designees will have access to the samples and data.
- **Tracking:** Samples and data will be tracked using Biological Specimen Inventory software.
- **Disposition:** In the future, other investigators (both at NIH and outside) may wish to use these samples and/or data for research purposes. If the planned research falls within the category of “human subjects research” on the part of the NIH researchers, NIH IRB review and approval will be obtained. This includes the NIH researchers sending out coded and linked samples or data and getting results that they can link back to their subjects.
- **Loss or Destruction:**
    - Any loss or unanticipated destruction of samples (for example, due to freezer malfunction) or data (for example, misplacing a printout of data with identifiers) that meets the definition of a reportable event will be reported to the NIH IRB according to NIH Human Research Protections Program (HRPP) Policy 801.

- Additionally, subjects may decide at any point not to have their samples stored. In this case, the principal investigator will destroy all known remaining samples and report what was done to both the subject and to the IRB. This decision will not affect the subject's participation in other protocols at NIH.

10. Data Sharing Plan

Human data generated in this study will be shared for future research as follows:

- Identified data in the Biomedical Translational Research Information System (BTRIS)
- De-identified transcriptomic data in a public repository such as the National Center for Biotechnology Information Gene Expression Omnibus or Sequence Read Archive
- De-identified data with approved outside collaborators under appropriate agreements
- De-identified data will be shared with the public at the time of publication and/or public presentations

Data will be shared at the time of publication or shortly thereafter.

If this study and future research using samples and data collected in this study are expected to generate genetic data that triggers the NIH Genomic Data Sharing Policy, which applies to all NIH-funded research that generates large-scale human (e.g., transcriptomic data), the NIH Genomic Data Sharing Policy (https://osp.od.nih.gov/scientific-sharing/policies/) will be followed for deposition of data into the appropriate database (e.g., dbGaP).

11. Remuneration Plan for Subjects

Subjects will not be compensated for participation in this study, but will be reimbursed for out-of-pocket expenses related to at-home sample collection and their transportation costs as is permitted by the NIAID travel policy.

If in-person visits are performed remotely due to the COVID-19 pandemic, subjects will be reimbursed for the costs of remote procedures or the costs will be paid directly from study funds (e.g., shipping samples collected remotely).

12. Assessment of Safety

AEs and other reportable events are defined in NIH HRPP Policy 801: Reporting Research Events.

12.1 Toxicity Scale

The investigator will grade the severity of each AE according to the "Common Terminology Criteria for Adverse Events (CTCAE)" (v 5.0), which can be found at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

12.2 Recording/Documentation

At each contact with the participant, information regarding AEs will be elicited by appropriate questioning and examinations. All events, both expected/unexpected and related/unrelated will be recorded on a source document. Source documents will include: progress notes, laboratory reports, consult notes, phone call summaries, survey tools, and data collection tools. Source documents will be reviewed in a timely manner by the research team. All reportable AEs that are identified will be recorded in CRIMSON. The start date, the stop date, the severity of each reportable event, and the principal investigator's judgment of the AE's relationship and expectedness to the study agent/intervention will also be recorded in CRIMSON.

If a diagnosis is clinically evident (or subsequently determined), the diagnosis rather than the individual signs and symptoms or lab abnormalities will be recorded as the AE.

A laboratory abnormality will not be recorded as an AE if ALL of the following criteria are met:

- It is no more than "Grade 1" or "Mild" per the protocol-specified toxicity table (or investigator assessment if not listed on the table); AND
- It does NOT require an intervention (e.g., discontinuation of treatment, dose reduction/delay, additional assessments, or treatment); AND
- It is assessed by the principal investigator as NOT related to the study agent(s); AND
- It is assessed by the principal investigator as NOT clinically significant (e.g., the abnormal value does NOT suggest a disease or organ toxicity).

Laboratory findings that are known to be associated with the disease under study (EAE) will not be recorded as AEs (e.g., elevated Ig, aldolase, leukocytes such as eosinophils, neutrophils, or lymphocytes, and serum tryptase).

All abnormal laboratory findings will be reviewed on a routine basis by the principal investigator to identify potential safety signals. An abnormal lab not included on the toxicity table should be assessed in a similar fashion to the criteria above.

12.3 Pregnancy

Although pregnancy itself is not an AE, events occurring during pregnancy, delivery, or in the neonate (e.g., congenital anomaly/birth defect) may be AEs or serious AEs (SAEs).

In the event of pregnancy, the following steps will be taken:

- Discontinue the study agent and procedures and withdraw from this study, but continue to follow-up for safety until delivery or termination of the pregnancy on 94-I-0079.
- Enroll in pregnancy registry.
- Report to the IRB.
- Advise research participant to notify the obstetrician of study participation and study agent exposure.

12.4 Type and Duration of the Follow-up of Participants after Adverse Events

AEs that have not resolved by the end of the follow-up period will be followed until final outcome is known. If it is not possible to obtain a final outcome for an AE (e.g., the participant is lost to follow-up), the reason a final outcome could not be obtained will be recorded by the investigator in CRIMSON.

12.5 Pausing Rules for an Individual Participant

Pausing is the suspension of administration of study agent to a single participant until a decision is made whether or not to resume administration of the study agent.

The pausing criteria for a single participant in this study include any of the following:

- A participant experiences an SAE that is unexpected and possibly, probably, or definitely related to a study agent;
- A participant experiences 2 Grade 3 or greater AEs that are unexpected and possibly, probably, or definitely related to a study agent;
- Any safety issue that the site investigator determines should pause administration of a study agent to a single participant.

12.5.1 Reporting a Pause

If a pausing criterion is met, a description of the AE(s) or safety issue must be reported by the principal investigator within 1 business day to the IRB by fax or email.

12.5.2 Resumption of a Paused Participant

The principal investigator will determine whether or not it is safe to resume administration of the study agent to the participant. The principal investigator will notify the IRB of the decision on resumption of the study agent.

12.5.2.1 Discontinuation of Study Agent for an Individual Participant

A participant who does not resume study agent will continue to be followed for safety.

12.6 Halting Rules for the Protocol

Halting the study requires immediate discontinuation of study agent administered for all participants and suspension of enrollment until a decision is made whether or not to continue enrollment and study agent administration.

The halting rules are:

- 2 or more participants experience the same or similar SAEs that are unexpected and possibly, probably, or definitely related to the study agent;

OR

- 2 or more of the same or similar AE in different participants that are grade 3 or above and are unexpected and possibly, probably, or definitely related to the study agent;

OR

- any safety issue that the principal investigator determines should halt the study.

The principal investigator will determine if the study should be halted.

12.6.1 Reporting a Study Halt

If a halting rule is met, a description of the AE(s) or safety issue must be reported by the principal investigator within 1 business day to the IRB by fax or email.

12.6.2 Resumption of a Halted Study

The principal investigator will determine if it is safe to resume the study. The principal investigator will notify the IRB of the decision on resumption of the study.

12.6.2.1 Discontinuation of Study Agent

Participants who do not resume study agent will continue to be followed for safety.

12.7 Study Discontinuation

The IRB, the NIAID, GSK, and other oversight bodies as applicable, as part of their duties to ensure that research participants are protected, may discontinue the study at any time. Subsequent review of serious, unexpected, and related AEs by the IRB may also result in suspension of enrollment and further trial interventions/administration of study agent.

12.8 Withdrawal Criteria for an Individual Subject

A study subject will be withdrawn for any of the following:

- An individual subject's decision. (The investigator should attempt to determine the reason for the subject's decision.)
- Non-compliance with study procedures (e.g., changing corticosteroid doses without principal investigator discretion, inability to comply with study visits) to the extent that it is potentially harmful to the subject or to the integrity of the study data.
- Any clinical AE, laboratory abnormality, intercurrent illness, other medical condition or situation that occurs such that continued participation in the study would not be in the best interest of the subject.
- The investigator determines that continued participation in the study would not be in the best interest of the participant.
- A change in the subject's baseline condition after enrollment so that the subject no longer meets the following eligibility criteria:
    - Development of pregnancy.
    - Development of a new malignancy (with the exception of localized skin cancer [basal or squamous] that may be cured by resection).
    - Withdrawal of permission for storage of study samples.
    - Permanent loss of decision-making capacity to provide informed consent.

12.8.1 Replacement of Withdrawn Participants or Participants Who Discontinue Study Agent

Participants who withdraw or are withdrawn from the study prior to receiving mepolizumab will be replaced. If a participant is replaced, all the data collected from that participant will still be included for the safety assessment.

13. Reporting Procedures

13.1 Reporting to the NIH IRB

Reportable events will be tracked and submitted to the NIH IRB according to HRPP Policy 801.

13.2 Reporting to the NIAID Clinical Director

The principal investigator will report unanticipated problems (UPs), major protocol deviations, and deaths to the NIAID clinical director according to institutional timelines.

14. Clinical Monitoring Structure

To help ensure that NIH Office of Research Support and Compliance procedures and GCP are being carried out, a Clinical Trials Management designee within the Office of Clinical Research Policy and Regulatory Operations, Regulatory Compliance and Human Subjects Protection Program will conduct a study initiation visit before study enrollment begins. The purpose of this meeting is to review with the principal investigator and study team designees the roles and responsibilities concerning their commitment to adhere to the requirements of the protocol, especially in terms of NIH OHSRP reporting requirements for AEs, SAEs, and UPs. In addition, the quality management and data management plan for the study will be reviewed.

14.1 Quality Management Plan

During the study, the principal investigator and study team will be responsible for implementing a quality management plan. Additionally, the study team will be responsible for completing and submitting a summary report on the quality plan to the NIAID Clinical Director or designee at least annually as detailed in the quality management plan. A courtesy copy will also be sent to Clinical Trials Management.

14.2 Safety Monitoring Plan

The data gathered during this study will be monitored by the principal investigator for safety and compliance with protocol-specified requirements.

15. Statistical Considerations

15.1 Study Hypotheses, Endpoint and Sample Size Justification

This is a pilot study designed as proof of principle, and the small sample size is dictated both by our ability to recruit sufficient numbers of patients in this rare disease and the complexity of the study design.

First, based on the fact that there are n=12 subjects already in the natural history protocol, we expect to be able to get n=5 subjects to participate in this study.

For the primary endpoint of change in maximum daily AE score (DAES), we use a quasi-Poisson model to estimate the average percent reduction after treatment compared to pre-treatment. This model accounts for within-person correlation of

the repeated maximum DAES by allowing a different (fixed) person effect for each person, and it also allows overdispersion from the Poisson model. Because of the small sample sizes and to be conservative, we allow for overdispersion but not underdispersion and use a t-distribution for inferences. We calculate power to show that the percent reduction is greater than 0. We simulate the maximum DAES values using n=5 individuals measured for 4 cycles using a Poisson model with random effects for each individual (to induce within-person correlation) and additional extra-Poisson variation for each observation. Specifically, for the i^{th} individual at the j^{th} cycle, the DAES is distributed Poisson with mean: $a_i * e_{ij} * f_j$, where a_i is distributed gamma with mean 3 and variance 3 (this models the per-person average baseline maximum DAES such that 8% have values less than 1, 58% are less than 3, and 94% are less than 6), e_{ij} is distributed gamma with mean 1 and variance 0.25, and f_j =1 when j=1 for the baseline cycle and f_j =1-(r/100) for the cycles after treatment, where r is the percent reduction. We first simulate 10,000 data sets under the null hypothesis of 0% reduction. The simulated the type I error rate for the two-sided test is 4.9% (close to the nominal 5%); however, the one-sided type I error rate is slightly inflated at 3.4% (for a nominal 2.5%). This slight inflation is due to the small sample size and because of this, we will make two-sided inferences. Next we simulate 10,000 data sets with 87.5% reduction and show that we have over 91% power to show a significant effect at the two-sided 5% level. For the analysis we will present two-sided 95% confidence intervals which can show larger effects than just the difference from 0. For example, the simulations show that with an 87.5% reduction we have 80% power to show that the percent reduction is at least 30% (i.e., 80% of the simulated 95% confidence intervals show percent reductions of at least 30%).

Now consider the power of the secondary endpoint. Review of cycles occurring in subjects collected to date for whom we had 2 consecutive peaks in the absence of medication changes shows a typical variation of 4-30% from cycle to cycle. One subject had a change of 37% from peak eosinophils from one cycle (AEC 12100/mm^3) to the next (AEC 7600/mm^3). A primary endpoint requiring reduction of eosinophils by 75% would be double the highest variation in any individual and would not be likely to occur without concurrent administration of medications such as glucocorticoids in these individuals based on our experience in managing these patients. As such, we can provide a rough estimate of the power of the study to show that the percent reduction in peak AEC in subjects after receiving mepolizumab is more than 75%.

We model the peak AEC using a linear model on the log transformed peak AEC values. Let Y_{ij} be the log10(peak AEC) for subject i at cycle j, where j=1 is baseline and j=2 is the first cycle after baseline (and the first peak after treatment). The model is $Y_{ij} = m_i + b\ (j-1) + e_{ij}$ where m_i is the random effect for the ith subject distributed normally with mean u and variance v_m, i.e., $m_i \sim N(u, v_m)$ and the errors are $e_{ij} \sim N(0, v)$. We use preliminary data on 5 subjects with 2 peaks to estimate u, v_m, and v. We get u=4.2, v_m=0.05 and v=0.01. We assume

that the effect b=log10(0.125), so that the model predicts a ratio of geometric means of post-treatment peak AEC over baseline peak AEC of 12.5%. This corresponds to an 87.5% reduction in peak AEC. We simulate from this model with 10,000 data sets with n=5 subjects in each data set. The simulated power to show at the one-sided 2.5% level that the percent reduction in peak AEC is more than 75% (i.e., the ratio of post-treatment to baseline is less than 25%) is 94%.

15.2 Description of the Analyses

- **Safety Analysis:** All safety data will be summarized with descriptive statistics (number of subjects [N], mean, standard deviation, median, minimum, and maximum) for continuous variables, and frequency and percentage for discrete variables. The safety summary will include AEs, SAEs, and clinical laboratory data.
- **Efficacy Analysis (change in symptoms):** For the primary endpoint we will use the quasi-Poisson model as described in section 15.1. As a sensitivity analysis, we will model the sum of the DAES during each cycle (instead of the maximum), and perform quasi-Poisson analysis on that. We may also perform analyses on specific symptoms. For binary symptoms (present/absent), we will use a two-sided exact McNemar's test to see if there is a change in the presence of the symptom in the baseline cycle as compared to the first subsequent post-treatment cycle. For ordinal symptom data (i.e., counts of the event during a cycle), we will use a Wilcoxon signed rank tests. For continuous data we can use the methods for the percent change in peak AEC (i.e., confidence intervals based on the t-distribution of the transformed data).
- **Efficacy Analysis (peak AEC):** For the percent change in peak AEC, we will calculate tests and confidence intervals based on a one-sample t-test on the transformed responses. Specifically, we will transform the percent change in peak AEC to the log of the ratio of post-treatment peak AEC over the baseline peak AEC. Notationally, let x_i be the percent change in peak AEC for subject i, then the log of the ratio of post-treatment peak AEC over baseline peak AEC is $W_i = \log(1-[x_i/100])$. Then we will calculate the mean of the W_i and the 95% confidence interval about that mean, and back-transform the results to the percent change in peak AEC. If the resulting confidence interval is completely above 75% reduction, then this shows that, at the 2.5% one-sided level, the percent reduction is greater than 75%.

16. Protection of Human Subjects

16.1 Informed Consent Process

Informed consent is a process where information is presented to enable persons to voluntarily decide whether or not to participate as a research subject. It is an

ongoing conversation between the human research subject and the researchers which begins before consent is given and continues until the end of the subject's involvement in the research. Discussions about the research will provide essential information about the study and include: purpose, duration, experimental procedures, alternatives, risks and benefits. Subjects will be given the opportunity to ask questions and have them answered.

The subjects will sign the informed consent document prior to undergoing any research procedures. The subjects may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the subjects for their records. The researcher will document the signing of the consent form in the subject’s medical record. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

16.2 Subject Confidentiality

All records will be kept confidential to the extent provided by federal, state and local law. The study monitors and other authorized individuals may inspect all documents and records required to be maintained by the Investigator, including but not limited to, medical records. Records will be kept locked and data will be coded. Any personally identifiable information maintained for this study will be kept on restricted-access computers and networks. Personally identifiable information will only be shared with individuals authorized to receive it under this protocol. Individuals not authorized to receive personally identifiable information will be provided with coded information only, as needed. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the IRB, the NIAID, the Office of Human Research Protections, or GSK.

To further protect the privacy of study subjects, a Certificate of Confidentiality has been issued by the NIH. This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research subjects, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to subjects.

17. Data Handling and Record Keeping

17.1 Data Capture and Management

Study data will be maintained in CRIMSON, and collected directly from subjects during study visits and telephone or virtual contacts, or will be abstracted from subjects' medical records. Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary to confirm the data abstracted for this study. Data entry into CRIMSON will be performed by authorized individuals. The Investigator is responsible for assuring that the data collected are complete, accurate, and recorded in a timely manner.

17.2 Record Retention

Study records will be maintained by the principal investigator in accordance with regulatory and institutional requirements, ICH GCP guidelines, and the NIH Intramural Records Retention Schedule. All stored records will be kept confidential to the extent required by federal, state, and local law. No records will be destroyed without the written consent of the principal investigator.

Appendix A: Scientific References

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Appendix B: Schedule of Procedures/Evaluations

Table 1. Schedule of Procedures/Evaluations for the Primary Study Phase.

Evaluations ‖	Enrollment	Home Labs§	Baseline (Peak ANC)	Home Labs§	Peak AEC	Home Labs§	Study Drug 700mg/ AEC Nadir	Home Labs§	Predicted ANC Peak	Home Labs§	Study Drug 700mg	Study Drug 700mg	Primary Assess-ments**	U V
Visit Number	1		2		3		4		5		6	7	8	
Day (assuming 4-week cycle)¶	Day -30 to -15		Day 0		Day 7		Day 15		Will vary		Day 43	Day 71	Day 99	
Window (days)					±3		±3		-		±5	±5	±7	
Consent and clinical assessments														
Informed consent	X													
Complete medical history and physical examination	X		X											
Targeted medical history and physical examination					X		X		X		X	X	X	X
Limb measurements	X		X		X		X		X		X	X	X	X
Vital signs including weight	X		X		X		X		X		X	X	X	X
Photography with/without infrared thermography	X		X						X					
Symptom log and PROs#	X		X		X		X		X		X	X	X	X
Optional bone marrow biopsy and aspiration	X†		X†										X‡	
Optional skin punch biopsy	X†		X†		X†									

Study drug administration														
Mepolizumab							X				X	X		
Clinical and safety laboratory testing														
CBC with differential	X	X	X	X	X	X	X	X	X	X	X	X	X	
Acute care panel	X		X		X		X		X		X	X	X	
Hepatic panel	X		X		X		X		X		X	X	X	
Mineral panel	X		X		X		X		X		X	X	X	
LDH, CPK	X				X		X		X		X	X		
C-reactive protein	X		X		X		X		X		X	X	X	
Urinalysis	X		X		X		X		X		X	X	X	
Serum tryptase	X		X		X		X		X		X	X	X	
Serum aldolase	X		X		X		X		X		X	X	X	
Serum immunoglobulins	X		X		X		X		X		X	X	X	
Serum IgE levels	X		X		X		X		X		X	X	X	
*Urine pregnancy test**	(X)						(X)				(X)	(X)		
Research laboratory studies														
Eosinophil and PBMC purification from whole blood and storage	X		X		X		X		X				X	
Urine for eosinophil granule protein and mediator levels	X	X	X	X	X	X	X	X	X	X	X		X	
Whole blood flow for TBNK cell subsets and RNAseq	X		X		X		X		X				X	
Serum or plasma for eosinophil granule protein and mediator levels	X		X		X		X		X		X		X	

Study assessments														
AE assessments	X		X		X		X		X		X	X	X	

Key: AE- adverse event; AEC- absolute eosinophil count; ANC- absolute neutrophil count; CBC- complete blood count; CPK- creatine kinase; IgE- immunoglobulin E; LDH- lactate dehydrogenase; PBMC- peripheral blood mononuclear cell; PROs- patient-reported outcome measures; TBNK- T, B, and natural killer cell; UV- unscheduled visits; ‖ Study visits may be rescheduled or study procedures may be waived or modified to be performed remotely due to travel limitations or safety concerns during the coronavirus disease 2019 pandemic (as described in protocol section 6); ** All subjects will complete Visit 8. Subjects who experienced benefit from mepolizumab administration will continue in the de-escalation phase (which begins with additional procedures at Visit 8—see Table 2 below); for subjects who do not continue in the de-escalation phase, Visit 8 will be the final study visit. ¶ The exact timing of Visit 1 through Visit 5 may vary from this schedule based on subject cycle times. Visits 2 and 3 may be combined if neutrophils and eosinophils peak <1 week apart. Every effort will be made to schedule Visit 5 in advance of Visit 6; in the event that Visit 5 occurs within 1 day of Visit 6, only collection of whole blood for storage will be performed for Visit 5. Visits 6, 7, and 8 will be scheduled monthly based on the timing of Visit 4. * Urine pregnancy test prior to administration of mepolizumab in women of child-bearing potential; # Performed electronically via REDCap. Symptom logs to be performed daily, PROs daily and monthly, not only at study visits; daily symptom logs and measures will be discontinued after Visit 7, and monthly questionnaires will be continued through Visit 8; § Home labs will be done 2-3X/week, depending on subject cycle times (2X/week in some cases or after peak is obtained); † Optional. Bone marrow will be requested to be performed only once at Visit 1 or 2, if a sample is not available from another NIH protocol. Skin punch biopsy will be requested to beperformed only once on Visits 1, 2, or 3; ‡ May be requested once at any time while on study drug if a pre-treatment sample was collected/available.

Table 2. Schedule of Procedures/Evaluations for the De-escalation Phase.

Evaluations ‖	Study Drug 500 mg	Study Drug 500 mg	Study Drug 500 mg	Study Drug 300 mg	Study Drug 300 mg	Study Drug 300 mg	End of De-escalation	UV
Visit Number	8	9	10	11	12	13	14	
Day (assuming 4-week cycle)	Day 99	Day 127	Day 155	Day 183	Day 211	Day 239	Day 267	
Window (days)	±7	±7	±7	±7	±7	±7	±7	
Clinical assessments and laboratory evaluations								
Targeted medical history and physical examination		X	X	X	X	X	X	X
*Limb measurements^*		X	X	X	X	X	X	X
*Vital signs including weight^*		X	X	X	X	X	X	X
Symptom log and PROs#	X	X	X	X	X	X		X
Autoinjector training (for remote subjects)	X							
Mepolizumab	X	X	X	X	X	X		
CBC with differential		X	X	X	X	X	X	X
Acute care panel			X				X	
Hepatic panel			X				X	
Mineral panel			X				X	
LDH, CPK			X				X	
C-reactive protein			X				X	X
Urinalysis			X				X	X

Serum tryptase			X				X	X
Serum aldolase			X				X	X
Serum immunoglobulins			X				X	X
Serum IgE levels			X				X	X
*Urine pregnancy test**	(X)	(X)	(X)	(X)	(X)	(X)		
Research laboratory studies								
Eosinophil and PBMC purification from whole blood and storage			X				X	
Urine for eosinophil granule protein and mediator levels		X	X				X	
Whole blood flow for TBNK cell subsets and RNAseq			X				X	
Serum or plasma for eosinophil granule protein and mediator levels		X	X				X	
Study assessments								
AE assessments	X	X	X	X	X	X	X	X

Key: AE- adverse event; CBC- complete blood count; CPK- creatine kinase; IgE- immunoglobulin E; LDH- lactate dehydrogenase; PBMC- peripheral blood mononuclear cell; PROs- patient-reported outcome measures; TBNK- T, B, and natural killer cell; UV- unscheduled visits; ‖ Study visits may be rescheduled or study procedures may be waived or modified to be performed remotely due to travel limitations or safety concerns during the coronavirus disease 2019 pandemic (as described in protocol section 6); ^Some vital signs or assessments may not be possible if performed virtually. Visit 8 will begin after completion of procedures listed at Visit 8 in Table 2. Visits 9 to 14 will be scheduled 28 days (±7 days) from the prior visit. * Urine pregnancy test prior to administration of mepolizumab in women of child-bearing potential (not performed at remote visits); # Performed electronically via REDCap. A weekly symptom log will be completed through Visit 14; PROs will be done weekly and monthly through Visit 14.

Appendix C: Blood Volumes for Specimen Collection

Table 3. Blood Volumes for Collection in the Primary Study Phase.

Evaluations		Enrollment V1	Home Labs[2]	Baseline V2	Home Labs[2]	Peak AEC V3	Home Labs[2]	Study Drug/ AEC Nadir V4	Home Labs[2]	Predicted ANC Peak V5	Home Labs[2]	Study Drug V6	Study Drug V7	V8 (± Study Drug)
CBC with differential	Light Lavender, EDTA	3		3		3		3		3		3	3	3
Acute care, hepatic, and mineral panels	Green-Yellow rim	4		4		4		4		4		4	4	4
C-reactive protein	Green-Yellow rim	X		X		X		X		X		X	X	X
Immunoglobulins (quantitative)	Green-Yellow rim	X		X		X		X		X		X	X	X
LDH, CPK	Green-Yellow rim	X				X		X		X		X	X	X
Aldolase, serum	Red-Yellow SST	4		4		4		4		4		4	4	4
Tryptase, serum	Red-Yellow SST	X		X		X		X		X		X	X	X
IgE	Red-Yellow SST	X		X		X		X		X		X	X	X
CBC with differential	Light Lavender, EDTA		12		12		12		12		12			
Whole blood for cells and plasma	Light Lavender, EDTA	40		40		40		40		40				40
Serum for storage	Red-Yellow SST	8		8		8		8		8		8		8
TBNK-eos	Light Lavender, EDTA	3		3		3		3		3				3

RNA PAX	PAXgene	2.5		2.5		2.5		2.5		2.5				2.5
Daily or Interval Volume (mL)		**64.5**	**12**	**64.5**	**12**	**64.5**	**12**	**64.5**	**12**	**64.5**	**12**	**19**	**11**	**64.5**
Cumulative Volume (mL)		**64.5**	**76.5**	**141**	**153**	**217.5**	**229.5**	**294**	**306**	**370.5**	**382.5**	**401.5**	**412.5**	**477**

Abbreviations: AEC- absolute eosinophil count; ANC- absolute neutrophil count; CBC- complete blood count; CPK- creatine kinase; EDTA- ethylenediaminetetraacetic acid; IgE- immunoglobulin E; LDH- lactate dehydrogenase; TBNK- T, B, and natural kill cell; V- visit.
[1] Specific timing of visits is provided in the Schedule of Evaluations (Appendix B) and Study Schematic (section 3.2). Per NIH MEC Policy M95-9, maximum blood volumes drawn for *research purposes* for an adult subject (aged 18 years or older) may not exceed: 10.5 mL/kg or 550 mL, whichever is smaller, over any 8-week period. This table reflects a model 4-week EAE cycle time with Day -15 enrollment, which would result in the highest possible blood volumes per time period. Blood volumes may be adjusted as needed for in-person visits that are changed to remote visits during the coronavirus disease 2019 pandemic.
[2] Home lab blood volumes reflect total volume over interval. Home lab collections may be performed 2 times per week in subjects with 4-week or shorter cycle times or after peak is obtained. For subjects with longer cycle times, home labs will be performed up to 3 times per week without exceeding the NIH CC blood volume limit.
[3] Day 0 evaluations are the baseline for subsequent safety assessments.

Table 4. Blood Volumes for Collection in the Dose De-escalation Phase.

Evaluations (De-escalation phase)		**Study Drug V9**	**Study Drug V10**	**Study Drug V11**	**Study Drug V12**	**Study Drug V13**	**End of De-escalation V14**
CBC with differential	Light Lavender, EDTA	3	3	3	3	3	3
Acute care, hepatic, and mineral panels	Green-Yellow rim		4				4
C-reactive protein	Green-Yellow rim		X				X
Immunoglobulins (quantitative)	Green-Yellow rim		X				X
LDH, CPK	Green-Yellow rim		X				X
Aldolase, serum	Red-Yellow SST		4				4

Tryptase, serum	Red-Yellow SST		X				X
IgE	Red-Yellow SST		X				X
Whole blood for cells and plasma	Light Lavender, EDTA		40				40
Serum for storage	Red-Yellow SST	8	8				8
TBNK-eos	Light Lavender, EDTA		3				3
RNA PAX	PAXgene		2.5				2.5
Daily or Interval Volume (mL)		**22**	**64.5**	**3**	**3**	**3**	**64.5**
Cumulative Volume (mL)		**499**	**563.5**	**566.5**	**569.5**	**572.5**	**637**

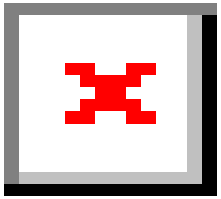
Abbreviations: CBC- complete blood count; CPK- creatine kinase; EDTA- ethylenediaminetetraacetic acid; IgE- immunoglobulin E; LDH- lactate dehydrogenase; TBNK- T, B, and natural kill cell; V- visit.

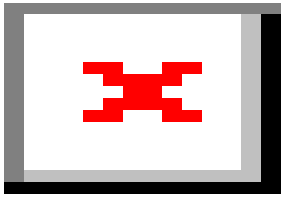
[1] Specific timing of visits is provided in the Schedule of Evaluations (Appendix B) and Study Schematic (section 3.2). Per NIH MEC Policy M95-9, maximum blood volumes drawn for *research purposes* for an adult subject (aged 18 years or older) may not exceed: 10.5 mL/kg or 550 mL, whichever is smaller, over any 8-week period. This table reflects a model 4-week EAE cycle time with Day -15 enrollment, which would result in the highest possible blood volumes per time period. Blood volumes may be adjusted as needed for in-person visits that are changed to remote visits during the coronavirus disease 2019 pandemic.

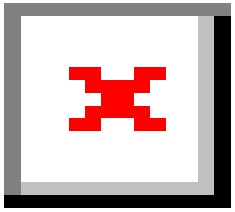
[2] Home lab blood volumes reflect total volume over interval. Home lab collections may be performed 2 times per week in subjects with 4-week or shorter cycle times or after peak is obtained. For subjects with longer cycle times, home labs will be performed up to 3 times per week without exceeding the NIH CC blood volume limit.

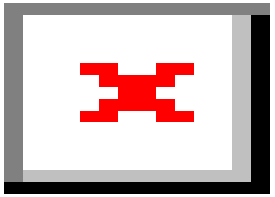
[3] Day 0 evaluations are the baseline for subsequent safety assessments.

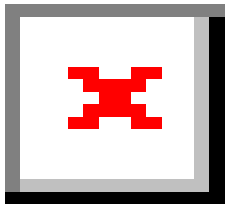
Appendix D: Cycle Length Determination for 3 Patients

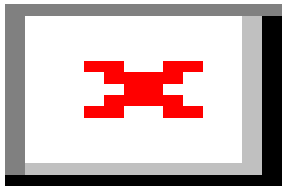












Appendix E: Patient Symptom Log (Daily)

The following information will be captured on an electronic version of this log through REDCap.

Name:

Instructions: Record oral **temperature** and write "C" for degrees Celsius or "F" for degrees Fahrenheit. Take temperature and **weight** around the same time each day, preferably morning.

Rate the severity of **general symptoms** experienced due to your EAE each day. If there were no symptoms on one day, check the "No Symptoms" box and leave blank all listed symptoms.

Record all **medications** taken for any of the above symptoms for each day (name, dose, number of times taken, how often taken, and reason; for example, "Ibuprofen, 200 mg, 2 tabs 4 times today for headache"). If there were no medications on a particular day, the "None" box should be **checked.**

Date (mm/dd)	___/___	___/___	___/___	___/___	___/___	___/___	___/___
Temperature (write °F or °C)							
Weight (write lbs or kg)							
GENERAL SYMPTOMS	**If you have any of the symptoms below, then rate their severity from 1 to 4:** **1** – mild; **2** – moderate; **3** – severe; **4** – severe and leads to doctor/ER visit or hospitalization						
NO SYMPTOMS							
Fever							
Muscle and body aches							
Swelling							
Swollen lymph nodes							
Hives							
Pain, redness, swelling, itching, or burning at injection site (please specify)							
Rash							
Fatigue							
Headache							
Other symptoms (please describe)							
MEDICATIONS OR OTHER EVENT	**NONE**	**NONE**	**NONE**	**NONE**	**NONE**	**NONE**	**NONE**
Were you evaluated by a health professional?							
List all medications taken for any of the above symptoms, or any other event. Also list any changes in medication.							

If you experience new symptoms or have questions, contact Dr. Paneez Khoury at 301-402-3673, khouryp@niaid.nih.gov; or Thomas Brown at 301-402-4834, browntho@mail.nih.gov. For after hours or weekend emergencies, call the NIH page operator at (301)-496-1211 to page Dr. Khoury.

Appendix F: Patient Symptom Log (Weekly)

Pilot Study of Mepolizumab in Episodic Angioedema with Eosinophilia

PARTICIPANT WEEKLY SYMPTOM LOG

Name:

Instructions: Record symptoms you have experienced in the past week due to your EAE.

For any other symptoms attributed to your EAE please indicate the number of days you experienced the symptoms (zero if didn't experience) or number of days experienced and how severe the symptoms were at their worst. **1 –** mild; **2 –** moderate; **3 –** severe; **4 –** severe and leads to doctor/ER visit or hospitalization.

☐ **No EAE symptoms in the prior week**

Fever

Frequency (days)	Severity
0 1 2 3 4 5 6 7	1 2 3 4

Muscle and body aches

Frequency (days)	Severity
0 1 2 3 4 5 6 7	1 2 3 4

Swelling

Frequency (days)	Severity
0 1 2 3 4 5 6 7	1 2 3 4

Swollen lymph nodes

Frequency (days)	Severity
0 1 2 3 4 5 6 7	1 2 3 4

Hives

Frequency (days)	Severity
0 1 2 3 4 5 6 7	1 2 3 4

Rash

Frequency (days)	Severity
0 1 2 3 4 5 6 7	1 2 3 4

Fatigue

Frequency (days)	Severity
0 1 2 3 4 5 6 7	1 2 3 4

Headache

Frequency (days)	Severity
0 1 2 3 4 5 6 7	1 2 3 4

Other symptoms (please describe)

Frequency (days)	Severity
0 1 2 3 4 5 6 7	1 2 3 4

Did you experience any pain, redness, swelling, or burning at injection site (please specify)

Appendix G: Quality of Life Scores and Disease Activity Scales

The following measures will be captured electronically in REDCap.

AE-QoL (Angioedema Quality of Life Assessment)

AE-QoL

Quality of Life Questionnaire for Patients with Recurrent Swelling Episodes

Patient name: _____

Date questionnaire completed (dd mmm yyyy): ____ ____ _____

Instructions: This questionnaire asks a number of questions. Please read each question carefully and choose from the five answers the one that fits best for you. Please do not think too long about the questions; be sure to answer all of the questions and to give only one answer to each question, i.e., to check only one box for each question.

Indicate how often within the **last 4 weeks** you have been restricted in the areas of your daily life listed below because of swelling episodes (angioedema). (regardless of whether or not you have actually experienced swelling episodes during that time period)	**Never**	**Rarely**	**Occasionally**	**Often**	**Very often**
1. Work	☐	☐	☐	☐	☐
2. Physical activity	☐	☐	☐	☐	☐
3. Leisure time	☐	☐	☐	☐	☐
4. Social relations	☐	☐	☐	☐	☐
5. Eating and drinking	☐	☐	☐	☐	☐
In the following questions we would like to get more details about the difficulties and problems that may be associated with your recurrent swelling episodes (angioedema) (during the **last 4 weeks**)	**Never**	**Rarely**	**Occasionally**	**Often**	**Very often**
6. Do you have difficulty falling asleep?	☐	☐	☐	☐	☐
7. Do you wake up during the night?	☐	☐	☐	☐	☐
8. Are you tired during the day because you are not sleeping well at night?	☐	☐	☐	☐	☐
9. Do you have trouble concentrating?	☐	☐	☐	☐	☐

Angioedema Quality of Life Questionnaire – American English Version 2012

	Never	Rarely	Occasionally	Often	Very often
10. Do you feel depressed?	☐	☐	☐	☐	☐
11. Do you have to limit your choices of food or beverages?	☐	☐	☐	☐	☐
12. Do the swelling episodes place a burden on you?	☐	☐	☐	☐	☐
13. Are you afraid that a swelling episode could occur suddenly?	☐	☐	☐	☐	☐
14. Are you afraid that the frequency of the swelling episodes might increase?	☐	☐	☐	☐	☐
15. Are you ashamed to go out in public because of the swelling episodes?	☐	☐	☐	☐	☐
16. Do the swelling episodes make you embarrassed or self-conscious?	☐	☐	☐	☐	☐
17. Are you afraid that the treatment of the swelling episodes could have negative long-term effects for you?	☐	☐	☐	☐	☐

Instructions for evaluation of the AE-QoL

The structure of AE-QoL

The AE-QoL consists of four domains and a total score:

Domain	Item (Question)
Functioning	1. Impairment of work
	2. Impairment of physical activity
	3. Impairment of spare time activities
	4. Impairment of social relations
Fatigue/Mood	6. Difficulties of falling asleep
	7. Waking up during the night
	8. Feeling tired during the day
	9. Difficulties in concentrating
	10. Feeling downhearted
Fears/Shame	12. Feeling burdened at having swellings
	13. Fear of new suddenly appearing swellings
	14. Fear of increased frequency of swellings
	15. Ashamed to visit public places
	16. Embarrassed by the apppearence of swellings
	17. Fear of long term negative drug effects
Nutrition	5. General limitations in foods and eating
	11. Limitations in the selection of food and beverages
Total Score	Items 1 to 17

How to calculate AE-QoL domain scores and the AE-QoL total score

AE-QoL is meant to be evaluated by determining its four individual domain scores (application as a profile instrument) but it may also be used to determine a total score (application as an index instrument):

Each item answered by the patient scores between 0 and 4 points depending on the answer option chosen by the patient. The 1st answer option gets 0 points, the 2nd option 1 point, the 3rd

option 2 points, etc. The AE-QoL domain scores as well as the AE-QoL total score are calculated by using the following formula:

$$\frac{\text{Sum of all completed items}}{\text{Max. possible sum of all completed items}} \times 100 = \text{AE-QoL Score}$$

Computation of Total Score:

Example 1: All items were completed (Max. possible sum: 68 points)
Sum of all 17 completed items: 41 points

$$\frac{41}{68} \times 100 = 60 \rightarrow \text{AE-QoL Total Score} = 60 \text{ out of possible } 100 \text{ points}$$

Example 2: 2 items were not completed (Max. possible sum: 60 points).
Sum of all 15 completed items: 41 points

$$\frac{41}{60} \times 100 = 68 \rightarrow \text{AE-QoL Total Score} = 68 \text{ out of possible } 100 \text{ points}$$

Computation of Domain Scores (Example: Fears/Shame):

Example: Sum of all 6 completed items: 14 points
Max possible sum: 24 points

$$\frac{14}{24} \times 100 = 58 \rightarrow \text{Fears/Shame Score} = 58 \text{ out of possible } 100 \text{ points}$$

Remarks

Since only answered items are included in the computation (and the calculated domain and total scores are not raw scores but linear transformations to a 0 to 100 scale), the calculated scores are not or only little influenced by missing items.

An AE-QoL domain score should not be calculated if more than one item is left unasnwered in that domain. The AE-QoL total score should not be calculated if more than 25% of items (>4 items) are left unanswered.

The minimal and highest possible domain and total scores are 0 and 100, respectively.

References:

Weller K, Groffik A, Magerl M, Tohme N, Martus P, Krause K, Metz M, Staubach P, Maurer M. Development and construct validation of the angioedema quality of life questionnaire. Allergy. 2012; 67(10): 1289-98.

Instructions for evaluation of the AE-QoL

AAS (Angioedema Activity Scale)

AAS

(Angioedema Activity Score)

Angioedema activity documentation

Patient name: _____

Date questionnaire completed (dd mm yyyy): ____ ____ _____

Week 1:

Instructions: Please document your symptoms retrospectively once a day. Refer to the last 24 hours in each case. Please answer all questions as fully as possible

		Day						
		1	2	3	4	5	6	7
Have you had a swelling episode in the last 24 hours?	no							
	yes							
⇩ **Please answer the questions below about this swelling episode during the last 24 hours. If you did not have a swelling episode, leave them blank.**								
At what time(s) of day was this swelling episode(s) present? (please select all applicable times)	midnight – 8 a.m.							
	8 a.m. – 4 p.m.							
	4 p.m. - midnight							
How severe is / was the physical discomfort caused by this swelling episode(s) (e.g., pain, burning, itching?)	no discomfort							
	slight discomfort							
	moderate discomfort							
	severe discomfort							
Are / were you able to perform your daily activities during this swelling episode(s)?	no restriction							
	slight restriction							
	severe restriction							
	no activities possible							
Do / did you feel your appearance is / was adversely affected by this swelling episode(s)?	no							
	slightly							
	moderately							
	severely							
How would you rate the overall severity of this swelling episode?	negligible							
	mild							
	moderate							
	severe							

AAS

(Angioedema Activity Score)

Scoring Template

The AAS consists of 5 questions as well as an opening question. A score between 0 and 3 is assigned to every answer field. The question scores are summed up to an AAS day sum score, 7 AAS day sum scores to an AAS week sum score (AAS7), and 4 ASS week sum scores may be summed up to an AAS 4-week sum score (AAS28). Accordingly, the minimum and maximum possible AAS scores are 0-15 (AAS day sum score), 0-105 (AAS7), and 0-420 (AAS28).
The opening question may be used to count the number of angioedema affected days during the AAS documentation period but has no score.

Days of week 1						
1	**2**	**3**	**4**	**5**	**6**	**7**
n	n	n	n	n	n	n
y	y	y	y	y	y	y
1	1	1	1	1	1	1
1	1	1	1	1	1	1
1	1	1	1	1	1	1
0	0	0	0	0	0	0
1	1	1	1	1	1	1
2	2	2	2	2	2	2
3	3	3	3	3	3	3
0	0	0	0	0	0	0
1	1	1	1	1	1	1
2	2	2	2	2	2	2
3	3	3	3	3	3	3
0	0	0	0	0	0	0
1	1	1	1	1	1	1
2	2	2	2	2	2	2
3	3	3	3	3	3	3
0	0	0	0	0	0	0
1	1	1	1	1	1	1
2	2	2	2	2	2	2
3	3	3	3	3	3	3

Days of week 2						
1	**2**	**3**	**4**	**5**	**6**	**7**
n	n	n	n	n	n	n
y	y	y	y	y	y	y
1	1	1	1	1	1	1
1	1	1	1	1	1	1
1	1	1	1	1	1	1
0	0	0	0	0	0	0
1	1	1	1	1	1	1
2	2	2	2	2	2	2
3	3	3	3	3	3	3
0	0	0	0	0	0	0
1	1	1	1	1	1	1
2	2	2	2	2	2	2
3	3	3	3	3	3	3
0	0	0	0	0	0	0
1	1	1	1	1	1	1
2	2	2	2	2	2	2
3	3	3	3	3	3	3
0	0	0	0	0	0	0
1	1	1	1	1	1	1
2	2	2	2	2	2	2
3	3	3	3	3	3	3

Days of week 3						
1	**2**	**3**	**4**	**5**	**6**	**7**
n	n	n	n	n	n	n
y	y	y	y	y	y	y
1	1	1	1	1	1	1
1	1	1	1	1	1	1
1	1	1	1	1	1	1
0	0	0	0	0	0	0
1	1	1	1	1	1	1
2	2	2	2	2	2	2
3	3	3	3	3	3	3
0	0	0	0	0	0	0
1	1	1	1	1	1	1
2	2	2	2	2	2	2
3	3	3	3	3	3	3
0	0	0	0	0	0	0
1	1	1	1	1	1	1
2	2	2	2	2	2	2
3	3	3	3	3	3	3
0	0	0	0	0	0	0
1	1	1	1	1	1	1
2	2	2	2	2	2	2
3	3	3	3	3	3	3

Days of week 4						
1	**2**	**3**	**4**	**5**	**6**	**7**
n	n	n	n	n	n	n
y	y	y	y	y	y	y
1	1	1	1	1	1	1
1	1	1	1	1	1	1
1	1	1	1	1	1	1
0	0	0	0	0	0	0
1	1	1	1	1	1	1
2	2	2	2	2	2	2
3	3	3	3	3	3	3
0	0	0	0	0	0	0
1	1	1	1	1	1	1
2	2	2	2	2	2	2
3	3	3	3	3	3	3
0	0	0	0	0	0	0
1	1	1	1	1	1	1
2	2	2	2	2	2	2
3	3	3	3	3	3	3
0	0	0	0	0	0	0
1	1	1	1	1	1	1
2	2	2	2	2	2	2
3	3	3	3	3	3	3

Day sum scores						
Week sum score						

Day sum scores						
Week sum score						

Day sum scores						
Week sum score						

Day sum scores						
Week sum score						

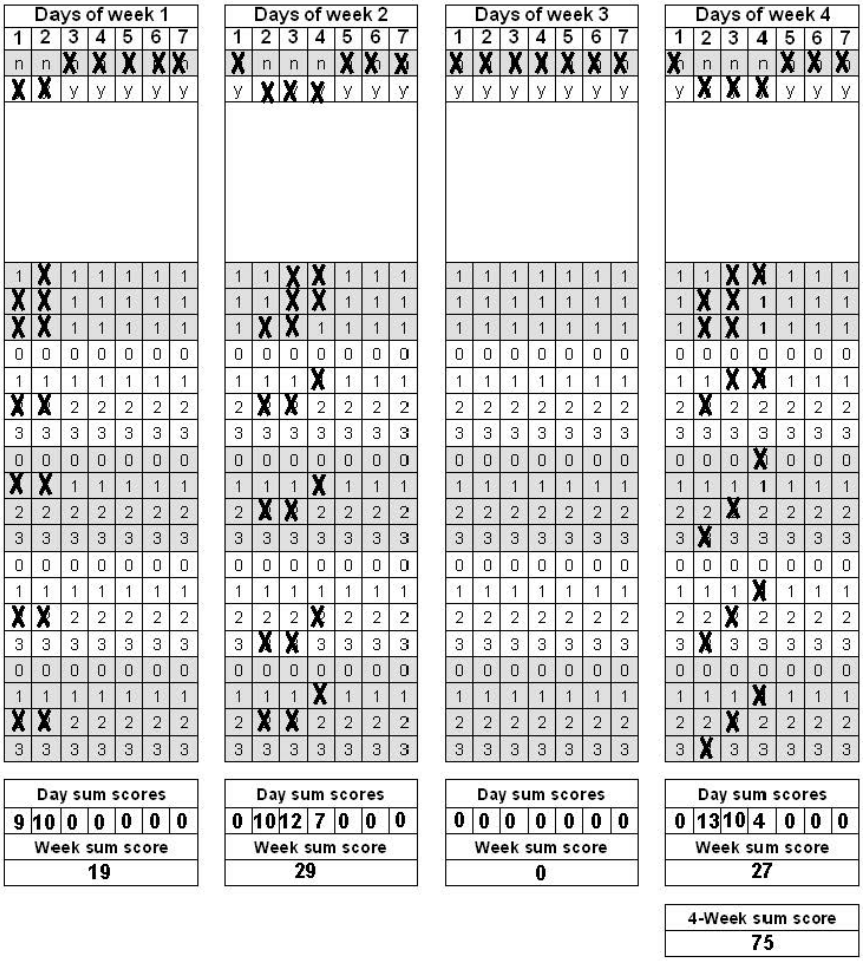
4-Week sum score

AAS

(Angioedema Activity Score)

Scoring Template

Example for AAS scoring:



Appendix H: Research Studies

Despite eosinophils being touted as central to the etiology of EAE, the pathophysiology of EAE is still not fully understood. Multiple cell lineages cycle, but the order of cycling and whether various cellular or serum factors influence the cycling is not known. Because the etiology of EAE is not fully understood, research blood and urine samples will be collected to assess the effects of mepolizumab on 1) eosinophil activation, 2) other potential biomarkers of disease activity in EAE, and 3) cell lineages other than eosinophils that may play a role. With eosinophils reduced or removed from the circulation, this trial will afford an opportunity to study the individual effects of other cell populations that may play a role in EAE pathogenesis.

1) Eosinophil activation

   Eosinophil activation will be assessed by measurement of serum and urine levels of eosinophil degranulation products (MBP, ECP, EDN, EPO) using a suspension array assay in multiplex.

2) Biomarkers of disease activity in EAE

   Although validated biomarkers of disease activity in EAE have not been identified to date, a number of serum mediators, including cytokines (IL-5), chemokines (i.e., TARC, eotaxin), soluble receptors (i.e., soluble IL-5 receptor alpha, soluble CD25), and serum tryptase, have been shown to cycle and correlate with disease activity. Blood samples obtained in the cycle prior to mepolizumab and those obtained during the cycle following the first mepolizumab infusion will be used to explore the effects of blocking IL-5 (and hopefully eosinophilia) on production and secretion of these mediators using a variety of assays, including enzyme-linked immunosorbent assay (ELISA), suspension array assays in multiplex, intracellular flow cytometry, and RNA expression analysis. Peripheral blood mononuclear cells (PBMCs) will also be viably frozen from the peak and nadir pre- and post-mepolizumab for future studies on selected cell populations. Finally, PBMCs, eosinophils, and neutrophils will be purified and stored in Trizol from the same time points for assessment of changes in RNA expression profiles in response to mepolizumab. Whole blood RNAseq will be performed at key timepoints.

3) Other lineages

   - Lymphocytes: In addition to assessing total lymphocyte numbers in the CBC with differential, lymphocyte phenotyping will be performed in the Department of Laboratory Medicine, NIH on whole blood samples using a standard panel that includes CD3, CD4, CD8,

CD25, HLA-DR, CD20, and CD16/CD56. In addition to standard TBNK cell phenotyping, the numbers of CD3-CD4+ T cells will be assessed. Serum TARC levels, an indirect measure of lymphocyte activation, will be measured by ELISA.

- Basophils: Due to the low numbers of circulating basophils in peripheral blood, basophil numbers and activation will be assessed by whole blood flow cytometry using CCR3 and CD203c, a specific marker of basophil activation.