Title

A Phase 2 Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Safety and Efficacy of Subcutaneous Benralizumab in Reducing Eosinophilia in Subjects with Hypereosinophilic syndrome (HES)

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List of Abbreviations

ACQ	Asthma Control Questionnaire
ADA	Anti-Drug Antibodies
ADCC	Antibody-dependent Cellular Cytotoxicity
AE	Adverse Event/Adverse Experience
AEC	Absolute Eosinophil Count
AER	Asthma Exacerbation Rate
AHR	Airway Hyperresposiveness
APFS	Accessorized Prefilled Syringe
AR	Adverse Reaction
CBC	Complete Blood Count
CC	Clinical Center
CFR	Code of Federal Regulations
COPD	Chronic Obstructive Pulmonary Disease
CPT	Center Point Titer
CRF	Case Report Form
CRP	C-Reactive Protein
CRIMSON	Clinical Research Information Management System of the National Institute of Allergy and Infectious Diseases
CS	Corticosteroid
CSO	Clinical Safety Officer
CTCAE	Common Terminology Criteria for Adverse Events
DCR	Division of Clinical Research
DHHS	Department of Health and Human Services
DSMC	Data and Safety Monitoring Committee
ECG	Electrocardiogram
ePPND	enhanced pre- and post-natal development
ESR	Erythrocyte Sedimentation Rate
FDA	Food and Drug Administration
FEV	Forced Expiratory Volume
FIP1L1	Fip-1-like-1
FWA	Federal Wide Assurance
GCP	Good Clinical Practice
HCG	Human Chorionic Gonadotropin
HES	Hypereosinophilic Syndrome
HGB	Hemoglobin
HIV	Human Immunodeficiency Virus
IB	Investigator’s Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonization
ID	Identification
IEC	Independent or Institutional Ethics Committee
IG	Immunoglobulin
IL	Interleukin

IL-5R	Interleukin 5 receptor
IND	Investigational New Drug
IRB	Institutional Review Board
ISM	Independent Safety Monitor
IUD	Intrauterine Device
IV	Intravenous
KLH	Keyhole Limpet Hemocyanin
LHES	Lymphocytic Variant Hypereosinophilic Syndrome
LPD	Laboratory of Parasitic Diseases
LVEF	Left Ventricular Ejection Fraction
MAb	Monoclonal Antibody
MRI	Magnetic Resonance Imaging
N	Number (typically refers to number of subjects/sample size)
NCI	National Cancer Institute, NIH
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NK	Natural Killer
NOAEL	No-Observed Adverse-Effect-Level
NYHA	New York Heart Association
OCRPRO	Office of Clinical Research Policy and Regulatory Operations
OHRP	Office for Human Research Protections
OHSRP	Office of Human Subjects Research Program
OTC	Over-the-Counter
PBMC	Peripheral Blood Mononuclear Cells
PDGFRA	Platelet-derived growth factor receptor alpha
PHI	Protected Health Information
PI	Principal Investigator
PK	Pharmacokinetics
PT	Prothrombin Time
PTT	Partial Thromboplastin Time
QA	Quality Assurance
QC	Quality Control
RBC	Red Blood Cell Count
RCHSPP	Regulatory Compliance and Human Subjects Protection Program
SAE	Serious Adverse Event/Serious Adverse Experience
SAR	Serious Adverse Reaction
SC	Subcutaneous
SD	Standard Deviation
SMC	Safety Monitoring Committee
SMM	Sponsor Medical Monitor
SOP	Standard Operating Procedure
SRCP	Safety Review and Communications Plan
SUSAR	Suspected and Unexpected Serious Adverse Reaction
TARC	Thymus and activation-regulated chemokine
Tdap	Tetanus-diphtheria-acellular pertussis
Th2	T helper 2

TCR	Tissue cross-reactivity
TEAE	Treatment-Emergent Adverse Events
TK	Toxicokinetics
UP	Unanticipated Problem
UPnonAE	Unanticipated Problem that is not an Adverse Event

Protocol Summary

Full Title:	A Phase 2 Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Safety and Efficacy of Subcutaneous (sc) Benralizumab in Reducing Eosinophilia in Subjects with Hypereosinophilic syndrome (HES)
Short Title:	Benralizumab for treatment-refractory HES
Clinical Phase:	2
IND Sponsor:	OCRPRO/NIAID/NIH
Conducted by:	NIAID/LPD
Principal Investigator:	Amy Klion, MD
Sample Size:	N=20
Accrual Ceiling:	N=25
Study Population:	Adult subjects aged 18-75 with *PDGFRA*-negative HES who are are symptomatic with AEC >1000/μL on stable HES therapy for at least 1 month
Accrual Period:	2 years
Study Design:	This will be a randomized, placebo-controlled, double-blind study followed by an open-label extension. A maximum of 20 subjects will be randomized (1:1) to receive sc injections of 30 mg of benralizumab or placebo at 0, 4 and 8 weeks. Eosinophil counts for a subject will be blinded and background HES therapy will not be tapered until that subject has reached 13 weeks on study. Beginning at the 12 week visit, all subjects will receive open-label benralizumab (30 mg sc) every 4 weeks for 3 doses. Subjects with an AEC<1000/μL and improvement in clinical symptoms without an increase in background HES therapy (responders) at the 24 week visit will continue to receive benralizumab every 4 weeks for the duration of the study (1 year). Non-responders will not receive additional doses of benralizumab. Following the initial dose of benralizumab or placebo and the first open-label (at 12 weeks) dose of benralizumab, subjects will be followed daily for 3 days, weekly for 4 weeks, and every 2 weeks for 8 weeks. Subsequent visits will be at 4 weeks intervals for responders and at 12 week intervals for non-responders for the duration of the study. For subjects who complete the study and for whom benralizumab provides sustained clinical benefit, drug may be provided on an open-label extension. After 2 years of stable and complete response to benralizumab, the dosing interval may be increased to every 8 weeks. Subjects will receive diphtheria-

	tetanus-acellular pertussis (TdaP) booster immunization at the 22 week visit. Titers will be measured 6 weeks after immunization. Subjects with a sustained and complete response to benralizumab with a stable dose for another 2 years will be transitioned to the FasenraPEN autoinjector for at-home self-administration (or by a local provider), with monthly blood counts and visits to the NIH once every 6 months.
Study Duration:	Start Date: March 2014 End Date: June 2025 Subjects will be on study for 12 months (or longer if enrolled on the open label extension).
Study Agent/ Intervention Description:	Benralizumab (30 mg sc) every 4 weeks
Primary Objective:	To determine the efficacy of 3 doses of sc benralizumab in reducing eosinophilia in subjects with *PDGFRA*-negative HES
Secondary Objectives:	To assess the safety of sc benralizumab in reducing eosinophilia in subjects with HES To assess the effect of benralizumab on end organ manifestations of HES To investigate the effects of benralizumab on bone marrow eosinophils, mast cells and their precursors To assess the long-term safety and efficacy of sc benralizumab in reducing eosinophilia in subjects with HES To evaluate the pharmacokinetics (PK) and immunogenicity of benralizumab in HES To determine the efficacy of every 8 week dosing in HES
Exploratory Objectives:	To determine predictors of response to benralizumab in subjects with HES To assess the effects of eosinophil depletion on vaccine responses and glucose homeostasis in humans
Endpoints:	Primary Endpoints: - 50% reduction in peripheral blood eosinophilia on stable HES background therapy at 12 weeks post-initiation of study drug Secondary Endpoints: - Percent reduction in peripheral blood eosinophilia at 12 weeks post-treatment - Frequency and severity of AEs - Reduction in signs and symptoms of HES

- Tissue eosinophilia
- Numbers of eosinophils, mast cells and their precursors in bone marrow
- Levels of markers of eosinophil and mast cell activation
- Eosinophil count and background HES therapy at 1 year
- PK and anti-drug antibody (ADA) levels
- Eosinophil count after 24 weeks of every 8 week benralizumab dosing

Exploratory Endpoints:

- Correlation between IL5 receptor levels and reduction in peripheral blood eosinophilia at 1, 4 and 12 weeks
- Change in anti-tetanus antibody titers at 6 weeks post-immunization
- Differences between weight and hemoglobin (Hgb) A1c levels at baseline and after 12 and 24 weeks of benralizumab therapy

Précis

Hypereosinophilic syndrome (HES) is a rare group of heterogeneous disorders characterized by marked peripheral eosinophilia (>1500/μL) and evidence of eosinophil-associated tissue damage. Although a high proportion of patients respond initially to corticosteroid therapy, high doses are often necessary to control the eosinophilia and clinical symptoms, and many patients become relatively refractory to therapy and/or develop serious side effects. IL-5 receptor α (IL-5Rα) expression in humans is restricted to eosinophils, basophils, mast cells and their precursors and is, therefore, an ideal target for the therapy of HES. Benralizumab was approved in the US in 2017 for treatment of severe asthma in patients 12 years or older and with eosinophilic phenotype. The product has a well-established safety profile based on several years of post-market experience for severe asthma since its approval. In order to explore the safety and efficacy of benralizumab in the treatment of HES, 20 adults (men and non-pregnant women, 18-75 years of age) with HES who are symptomatic with AEC >1000/μL on stable HES therapy for at least 1 month will be recruited for this randomized, placebo-controlled, double-blind phase 2 trial. Benralizumab (30 mg) or placebo will be administered sc at weeks 0, 4, and 8. Eosinophil counts will be blinded for a subject and background HES therapy will not be tapered until that subject has been on study for 13 weeks. At weeks 12, 16, and 20, all subjects will receive a sc injection of benralizumab. Subjects demonstrating a response at the 24 week visit (eosinophil count <1000/μL and stable or improved clinical symptoms without an increase in background HES therapy) will continue to receive additional 30 mg sc injections every 4 weeks. Following the initial dose of benralizumab or placebo and the first open-label dose of benralizumab, subjects will be followed daily for 3 days, weekly for 4 weeks, and every 2 weeks for 8 weeks. Subsequent visits will be at 4 weeks intervals for responders and 12 weeks intervals for non-responders for a minimum of two years. Subjects with stable and complete response for ≥2 years may be eligible to receive benralizumab at a dosing interval of every 8 weeks. Subjects will receive diphtheria-tetanus-acellular pertussis (TdaP) booster immunization at the 22 week visit. Titers will be measured 6 weeks after immunization.The primary endpoint of the study is a 50% reduction in peripheral blood eosinophilia on stable background therapy at 12 weeks post-initiation of study drug. Secondary endpoints will include absolute eosinophil count, the frequency and severity of adverse events, reduction in signs and symptoms of HES, tissue eosinophilia, numbers of eosinophils, mast cells and their precursors in bone marrow, levels of markers of eosinophil and mast cell activation, eosinophil count and background HES therapy at 1 year, pharmacokinetics and anti-drug antibody (ADA) levels and eosinophil count after 24 weeks of every 8 week dosing. Exploratory endpoints will address predictors of response to benralizumab and the impact of eosinophil depletion on vaccine responses and glucose homeostasis. Subjects who complete the study and for whom benralizumab provides sustained clinical benefit, may be eligible to receive drug on an open-label extension protocol until regulatory approval and commercial availability of the marketed drug to prescribing physicians (for any indication), or until development of benralizumab is discontinued by AstraZeneca.

1 Background Information and Scientific Rationale

1.1 Background Information

Hypereosinophilic syndrome (HES) is a rare group of heterogeneous disorders characterized by marked peripheral eosinophilia (>1500/μL) and evidence of eosinophil-associated tissue damage. Treatment is directed at reducing peripheral blood and tissue eosinophilia. With the exception of Fip1-like-1/platelet derived growth factor receptor alpha (*FIP1L1/PDFGRA*)-positive chronic eosinophilic leukemia, first line therapy for all of the varied forms of HES is corticosteroids (CS). Although a high proportion of patients respond initially to CS therapy, high doses are often necessary to control the eosinophilia and clinical symptoms, and many patients become relatively refractory to therapy and/or develop serious side effects (1). Second line agents, including hydroxyurea and interferon, are effective in only a subset of patients and are associated with considerable toxicity. Despite the recent success with mepolizumab, a monoclonal antibody to IL-5, as a steroid-sparing agent in steroid-responsive subjects with HES (2, 3), 15% of steroid-responsive patients did not respond and the response rate in steroid-refractory patients is likely lower. Furthermore, early studies in asthma suggested that anti-IL5 therapy is associated with maturational arrest in the bone marrow (4) that can lead to rebound eosinophilia and symptoms when the drug is discontinued (5). This rebound has been seen with both of the anti-IL-5 antibodies currently in clinical trials and is associated with increased serum levels of biologically active IL-5. Clearly, additional targeted therapies are needed.

1.1.1 Description of the Study Agent

Expression of the human IL-5Rα chain is restricted to eosinophils, basophils, and mast cells and their precursors (6, 7). Benralizumab is a humanized, afucosylated monoclonal antibody (mAb) that binds specifically to the alpha subunit of the human interleukin-5 receptor (IL-5Rα). It specifically targets and depletes IL-5Rα-bearing cells by inducing apoptosis via enhanced antibody-dependent cell-mediated cytotoxicity (ADCC) (7-9). The parental murine clone (KM1259) was humanized by grafting of the corresponding complementary determining regions onto human framework sequences. Benralizumab is formatted as a human immunoglobulin of the G1/kappa isotype (IgG_{1k}). It comprises 2 heavy chains (G1) and 2 light chains (k) and has an overall molecular weight of approximately 150 kDa. The afucosylated form of this recombinant humanized IgG1 is produced in a CHO-DG44 BioWa-afuc cell line deficient in α1,6-fucosyl transferase (FUT8).Appendix_A:_Scientific

1.1.2 Summary of Previous Pre-Clinical Studies

Nonclinical pharmacologic and safety studies conducted in cynomolgus monkeys with benralizumab included a 9-week repeated-dose intravenous (IV) study, a single-dose sc study; a 15-week repeated-dose SC study, a 9-month repeated-dose IV and SC study, and a reproductive toxicology study (enhanced pre- and post-natal development [ePPND]) (10). As expected, eosinophil counts were markedly decreased in all benralizumab-treated animals throughout the dosing and recovery periods. In the 9-

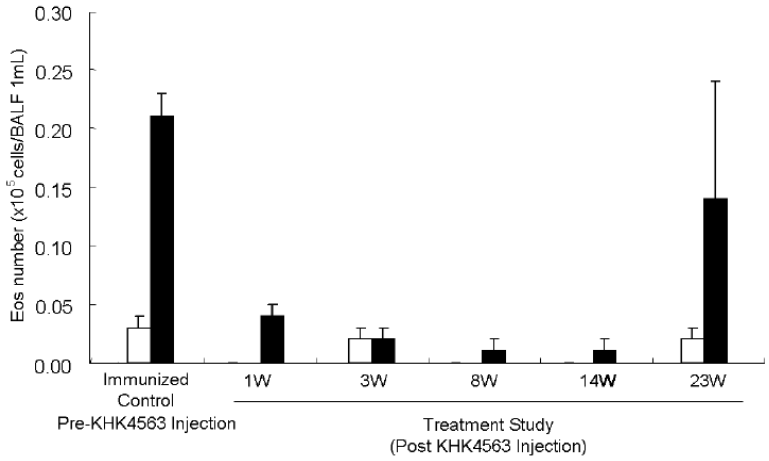
week repeated-dose IV study, transient decreases in leukocytes, resulting from differential decreases in neutrophils counts, were seen in 2 of 10 animals (1 male and 1 female) treated with the highest dose (30 mg/kg) resulting in a no observed adverse effect level (NOAEL) of ≤30.0 mg/kg. In the 9-month repeated-dose IV and SC study, there were no benralizumab-related adverse effects (AEs) observed, including for male and female fertility parameters, resulting in NOAELs of 25 mg/kg for IV and 30 mg/kg for SC, the highest doses tested. In a rabbit local tolerance study with SC administered benralizumab, no benralizumab-related adverse effects were observed. Finally, the tissue cross-reactivity (TCR) of benralizumab was assessed in a panel of normal human and cynomolgus monkey tissues. Both the human and cynomolgus monkey TCR profiles were generally consistent with those in literature.

Findings from the ePPND reproductive toxicology study indicated no adverse maternal effects of benralizumab administration, and the growth and development of the infants were within normal limits for infant cynomolgus monkeys, including immune assessments (keyhole limpet hemocyanin [KLH] immunization; immunoglobulin M [IgM] and immunoglobulin G [IgG] center point titer (CPT) values; and serum IgG, IgM, and immunoglobulin A [IgA] levels), resulting in a NOAEL of 30 mg/kg/dose, the highest dose tested.

Toxicokinetic(s) (TK) and immunogenicity evaluations of benralizumab were investigated in cynomolgus monkeys following single- and repeated-dose IV and SC administration of benralizumab up to the highest dose of 30 mg/kg. The TK of benralizumab in cynomolgus monkeys were linear with dose in the 1-30 mg/kg dose range among various TK studies. The absorption of benralizumab was slow, with the peak serum concentration occurring approximately 3 days after dosing. The TK of benralizumab was similar in male and female cynomolgus monkeys. Distribution of benralizumab from maternal plasma to neonates was observed and was consistent with placental transfer from maternal circulation into fetal circulation. The TK parameter values for benralizumab in cynomolgus monkeys were typical of an IgG1 mAb without an antigen sink. Anti-drug antibodies (ADA) were detected in some benralizumab-treated animals. High-titer ADA was associated with reduced TK exposure in cynomolgus monkeys.

The clinical efficacy of benralizumab was tested in cynomolgus monkeys receiving recombinant IL-5 sc and in a monkey asthma model, in which 5 cynomolgus monkeys that had no skin reaction to SC injection of *Ascaris* antigen were immunized with 6 injections of antigen, administered 4 times intramuscularly and on 2 occasions by inhalation (8). In the first study, benralizumab (0.01 mg/kg or 0.3 mg/kg) dramatically reduced the IL-5 induced peripheral eosinophilia in 4/6 animals. In the second study, the effect of the anti-IL-5R mAb on reactivity to inhaled antigen was evaluated by injecting a single IV dose of benralizumab (1 mg/kg) into the animals at Week 24 after the first immunization. The number of accumulated eosinophils following antigen inhalation in the cynomolgus monkeys who received benralizumab was dramatically decreased over a period of 14 weeks after monoclonal antibody (mAb) administration (Figure 1).

Figure 1 **Effect of benralizumab on the accumulation of eosinophils following *Ascaris suum* antigen inhalation in sensitized cynomolgus monkeys**



Benralizumab (KHK4563); W Week.
Open bars depict the mean number of eosinophils in BAL fluids of the 5 monkeys before antigen challenge. Filled bars indicate the mean number of eosinophils in BAL fluids of the 5 monkeys after antigen challenge. Results are expressed as mean±SD of number of eosinophils in BAL fluids for each time point.

Airway hyperresponsiveness (AHR) was evaluated in the 2 animals that tested positive for antigen reactivity. Tests were performed 1 week after mAb injection and then 3, 8, 18, and 23 weeks after benralizumab injection. Benralizumab suppressed the development of AHR for over 23 weeks in one of these animals.

Additional pre-clinical information can be obtained from the Investigator Brochure.

1.1.3 Summary of Relevant Clinical Studies

Benralizumab has been or is being investigated in adult subjects and adolescents with asthma (20 studies), adults with chronic obstructive pulmonary disease (COPD) (3 studies) and nasal polyposis (1 study). A full list of clinical trials can be found in the Investigator's Brochure. Kyowa Hakko Kirin Co, Ltd is developing benralizumab/KHK4563 in Asia and has completed 2 Phase 1 studies (Studies 4563-001 and 4563-002) in healthy adult Japanese subjects and a Phase 2a study (Study 4563-003) in adult subjects with asthma (18). A phase 2 study in rhinosinusitis is ongoing. Overall, benralizumab has been administered to more than 40 healthy Japanese men, 2500 subjects with asthma and 250 subjects with COPD.

Safety.

Results of the AstraZeneca-sponsored asthma studies (Studies MI-CP158, MICP166, MI-CP186, MI-CP197, and MI-CP220) showed no emerging safety findings. As reflected in the analysis of study MI-CP220, a placebo controlled study of sc multiple dose benralizumab in 609 adults with asthma, treatment-emergent adverse events (TEAE),

regardless of causality, occurred at an approximate 10 percentage point higher frequency in the combined benralizumab group compared with the placebo group with events classified as Infections, General Disorders and Administration Site Conditions, and Musculoskeletal and Connective Tissue Disorders largely accounting for this difference.

Treatment-emergent adverse events within each of these classifications that occurred at least 5 percentage points higher in the combined benralizumab group than in the placebo group were as follows:

- Infections: Nasopharyngitis, upper respiratory tract infection, and pharyngitis
- General Disorders and Administration Site Conditions: Asthenia, contusion, hypotension, injection site erythema, injection site reaction, and pyrexia
- Musculoskeletal and Connective Tissue Disorders: Muscle spasm and pain

Treatment-emergent adverse events classified as Respiratory, Thoracic, and Mediastinal disorders were the most frequently occurring in both the placebo and combined benralizumab groups, with asthma being the most frequently occurring TEAE.

No apparent dose response on the incidence of TEAEs was observed across the individual benralizumab dose groups. The majority of these TEAEs were mild or moderate in severity. Severe TEAEs, regardless of causality, occurred in 20/221 subjects (9.0%) in the placebo group and 47/385 subjects (12.2%) in the combined benralizumab group.

Infusion-related reactions may be defined as any signs or symptoms experienced by subjects during the infusion of pharmacologic or biologic agents or any event occurring on the first day of drug administration. Such reactions may occur after administration of benralizumab. Clinical manifestations of these reactions vary. Anaphylactic or anaphylactoid reactions are the most severe forms of infusion-related reactions and may result in death. Infusion-related reactions usually develop within 2 hours of the start of investigational product administration and often resolve within 24 hours after completion of investigational product administration. Signs/symptoms may include the following: urticaria, arthralgia, bronchospasm, wheeze, cough, dizziness, dyspnea, fatigue, headache, hypertension, hypotension, myalgia, and vomiting.

The ALIZE trial was designed as a randomized, double-blind, parallel group, placebo-controlled study designed to investigate the potential efficacy, safety, PK, and immunogenicity effect of a fixed dose of benralizumab on the humoral immune response following seasonal influenza virus vaccination in adolescent or young adult patients with severe asthma. Antibody titers at 12 weeks post-vaccination were similar across strains and between treatment groups (ranged from 78.0% to 100.0% for the benralizumab treatment group and from 79.6% to 100.0% for the placebo treatment group).

Suppression of eosinophilia.

Benralizumab exhibited pharmacological activity as evidenced by suppression of peripheral blood eosinophil counts in all clinical studies, and in Study MI-CP166, suppression of eosinophil counts (including eosinophils undergoing apoptosis) in airway mucosal biopsies after single IV and multiple SC doses of benralizumab. In study MI-CP158, a dose escalation safety study of single dose IV benralizumab in 44 adult subjects with mild asthma, mean time to eosinophil recovery following a single IV dose of benralizumab ranged from 5.8 days (0.0003 mg/kg) to 190 days (3.0 mg/kg) (9). Similar results were observed in study MI-CP186, a Phase 2 study of single dose benralizumab (0.3 or 1 mg/kg IV) vs. placebo in 110 adults with asthma exacerbation, in which depletion of blood eosinophil counts was observed through the last scheduled visit on Day 84 in all 72 subjects who received benralizumab and in study MI-CP197, a Phase 2 study of 3 doses of benralizumab (0, 25, 100 or 200 mg sc every 4 weeks) in 25 adults with asthma, in which peripheral blood eosinophils had decreased by at least 95% at all benralizumab dose levels by Day 7 and remained <20% of baseline for 57-239 days.

MI-CP220, a Phase 2 double-blind, placebo-controlled dose-ranging study of benralizumab (2, 20 or 100 mg sc every 4 weeks for 3 doses followed by every 8 weeks for 4 doses) in 609 adult subjects with uncontrolled eosinophilic or non-eosinophilic asthma requiring medium to high dose inhaled corticosteroids. Blood eosinophil depletion was seen across all benralizumab dose groups regardless of eosinophilic phenotype. No blood eosinophil depletion was observed in the placebo group. Median blood eosinophil counts have remained ≤ 100 cells/μL through Week 52. A similar study (4563-003) was conducted in Japan with comparable results (18).

Study MI-CP166, a multicenter, double-blind placebo controlled study in 27 subjects with eosinophilic asthma, examined the effect of single dose iv or multiple dose sc benralizumab on eosinophil counts in airway mucosa/submucosa, sputum, bone marrow and peripheral blood (11). In cohort 1, intravenous benralizumab produced a median decrease from baseline of 61.9% in airway mucosal eosinophil counts (Day 28; placebo: +19.6%; P = .28), as well as an 18.7% decrease (Day 21) in sputum and a 100% decrease (Day 28) in blood counts. Eosinophils were not detectable in bone marrow of benralizumab-treated subjects (Day 28, n = 4). In cohort 2 sc benralizumab demonstrated a combined (100 + 200 mg) median reduction of 95.8% in airway eosinophil counts (Day 84; placebo, 46.7%; P = .06), as well as an 89.9% decrease (Day 28) in sputum and a 100% decrease (Day 84) in blood counts.

Clinical efficacy in asthma.

The effect of benralizumab on measures of asthma clinical disease activity and quality of life were exploratory endpoints in Studies MI-CP158, MI-CP166, and MI-CP197 and were difficult to interpret because of the small sample size, mild asthmatic population, and variability of baseline disease characteristics.

In Study MI-CP186, a statistically significant reduction in the rate of asthma exacerbations and asthma exacerbations that resulted in hospitalizations (p=0.007 and p=0.022, respectively) among adult subjects who required an urgent healthcare visit for treatment of an acute asthma exacerbation was observed in benralizumab-treated subjects compared with placebo. In Study MI-CP220, the primary endpoint of the study was met as there was a statistically significant 41% reduction in the annual asthma exacerbation rate (AER) in the EOS+ 100 mg benralizumab group versus the EOS+ placebo group (p = 0.096). Similar results were seen for patient-reported outcome (Asthma Control Questionnaire 6 [ACQ6]) and spirometry measures.

Three large phase 3 studies in asthma (SIROCCO, CALIMA and ZONDA) have been completed and confirmed that benralizumab is well-tolerated and effective in reducing asthma exacerbations in adult and adolescent patients with eosinophilia (16, 17,19). Benralizumab (30 mg every 8 weeks) was approved (November 14 2017) by the FDA as add-on therapy for patients 12 years or older with severe eosinophilic asthma. AppendixA

Additonal trials have demonstrated the efficacy of benralizumab in mild to moderate asthmatics (BISE) and the ability of the majority (93%) of subjects with severe asthma to self-administer benralizumab at home using a pre-filled syringe (GREGALE).

Clinical efficacy in COPD.

Results from Study MI-CP196, performed in patients with sputum eosinophils >3%, indicated that benralizumab was not superior to placebo in exacerbation rate reduction. In a prespecified subgroup, analysis of the primary endpoint was perform.ed using the baseline blood eosinophil count cutpoints of ≥/<150, ≥/<200, and ≥/< 300 cells/μL); these analyses did suggest an increasing treatment effect for benralizumab compared to placebo on the moderate or severe AECOPD rate through Day 393 for the benralizumab group compared with the placebo group for subjects with a baseline blood eosinophil count of ≥ 200 cells/μL (AECOPD rate 1.06 placebo and 0.73 benralizumab) and ≥ 300 cells/μL (AECOPD rate 0.93 placebo and 0.39 benralizumab). This finding was not replicated in those subjects with a blood eosinophil count of < 200 cells/μL, where the exacerbation rate was lower in the placebo group. IN view of these data, additional studies were initated and are ongoing.

Pharmacokinetics and drug metabolism.

Based on data from studies MI-CP158, MI-CP166, MI-CP186, MI-CP197, and MI-CP220, benralizumab PK was dose-proportional in the dose range investigated in humans (0.03-3.0 mg/kg IV and 25-200 mg SC). The $t_{1/2}$ was approximately 2-3 weeks. From population PK meta-analysis, the systemic clearance and the distribution volumes were typical for human IgG. The SC bioavailability was approximately 52%. Both drug clearance and volume of distribution increased with body weight. Age, gender, race,

and tobacco smoking history had no apparent impact on PK of benralizumab in asthma subjects.

Anti-drug antibodies (ADA) were associated with reduced PK exposure in some subjects. In MI-CP220, ADA (titers ≥50) were detected in 15/220 subjects (6.8%) in the placebo group and 89/382 subjects (23.3%) in the combined benralizumab group, with little increase in incidence beyond Week 24. The presence of ADA was highest in the lowest benralizumab dose, but not more frequent in those subjects with blood eosinophils ≥300 cells/μL (20.7%) as compared to those with blood eosinophils <300 cells/μL (21.7%). Samples from the 23 subjects who were ADA-positive also tested positive for neutralizing antibodies, and the presence of ADA titers ≥400 was associated with a decrease in benralizumab concentrations and an increase in the number of eosinophils present in the blood.

Pregnancy. To date, one female study patient receiving open-label benralizumab in the current study became pregnant. Her daughter was delivered at term without complications. CBC revealed 0 eosinophils/mm^3 at birth 1, and 4 month of age and a count of 280/mm^3 at 9 months of age. Her growth has been normal for age, and she has met all developmental milestones. A registry of pregnant women receiving benralizumab has been established by the Organization of Teratology Information Specialists (https://mothertobaby.org/ongoing-study/fasenra/). No data is available at this time.

1.2 Rationale

IL-5Rα expression in humans is restricted to eosinophils, basophils, mast cells and their precursors (6,7) and is, therefore, an ideal target for the therapy of HES. Benralizumab, a humanized afucosylated antibody to IL-5Rα, has been shown to be safe in Phase 1, 2 and 3 trials in humans (9, 16-18 and Investigator's Brochure). Furthermore, data from animal models and asthma studies in humans suggest that eosinophilia is suppressed for up to 6 months, consistent with an effect on precursors in the bone marrow (9-11). Finally, benralizumab is unlikely to cause rebound eosinophilia and symptoms when the drug is discontinued since it acts by inducing apoptosis of IL-5Rα bearing cells and, in contrast to mepolizumab, causes a decrease in eosinophils and CD34+IL5Rα+ eosinophil precursors in the marrow (11). Although patients with HES do have a mild increase in bone marrow mast cells, marked increases in numbers and activation status of mast cells are seen only in patients with *PDGFRA*-associated myeloproliferative neoplasms. Furthermore, rapid reduction of eosinophil or mast cell numbers using targeted therapies such as imatinib (in *PDGFRA*-associated myeloproliferative neoplasms and imatinib-sensitive systemic mastocytosis) or mepolizumab (in HES) has not been associated with signs or symptoms consistent with a tumor lysis syndrome. Thus, benralizumab is anticipated be a safe and effective eosinophil-lowering agent in patients with HES.

Since murine eosinophils have been shown to play a role in the maintenance of plasma cells in the bone marrow (12) and alternatively activated macrophages in adipose tissue (13) that are involved in glucose homeostasis and responses to immunization, respectively, prolonged depletion of eosinophils in the blood and bone marrow following benralizumab treatment provides a unique opportunity to examine the relevance of these findings in humans. It should be noted, however, that eosinophil-deficient mice do not demonstrate increased susceptibility to diabetes, obesity or infection (14, 15).

2 Study Objectives

2.1 Primary Objective

The primary objective of the study will be to determine the efficacy of 3 monthly sc doses of benralizumab in reducing eosinophilia in adult subjects with *PDGFRA*-negative HES.

2.2 Secondary Objectives

The secondary objectives will be:

1) To assess the safety of sc benralizumab in reducing eosinophilia in subjects with HES
2) To assess the effect of benralizumab on end organ manifestations of HES
3) To investigate the effects of benralizumab on bone marrow eosinophils, mast cells and their precursors
4) To assess the long-term safety and efficacy of sc benralizumab in reducing eosinophilia in subjects with HES
5) To assess the pharmacokinetics of benralizumab and the development of ADA levels in HES
6) To assess the efficacy of every 8 week dosing of benralizumab in HES

2.3 Exploratory Objectives

Exploratory objectives will include 1) identification of predictors of response to benralizumab in subjects with HES and 2) assessment of the effects of eosinophil depletion on vaccine responsiveness and glucose homeostasis.

3 Study Design

3.1 Description of the Study Design

This study will be a single center trial with 5 parts: 1) randomized, double-blind sc benralizumab vs. placebo every 4 weeks for 12 weeks, 2) open-label sc benralizumab every 4 weeks for 12-36 weeks, 3) open-label sc benralizumab extension for benralizumab responders, with doses every 4 weeks for 6 months, 4) open label substudy of sc benralizumab administration every 8 weeks in complete responders, and 5) open-label extension of self-administered sc benralizumab using an autoinjector for subjects with a sustained and complete response, for a minimum of 2 years. Eligible subjects enrolled on study protocol 94-I-0079 (Activation and function of eosinophils in conditions with blood or tissue eosinophilia) or referred from outside physicians for evaluation of hypereosinophilia will be invited to participate. A maximum of 20 subjects will be enrolled.

Baseline evaluation will include a complete history and physical examination, routine laboratory testing and assessment of end organ involvement (see Appendix A). Baseline research studies will include bone marrow aspirate and biopsy to assess eosinophil and mast cell numbers and activation, storage of peripheral blood mononuclear cells (PBMC) for future studies, assessment of baseline eosinophil activation and T/B/NK subsets by flow cytometry, and measurement of serum markers of eosinophil and mast cell activation. Potential predictors of response to benralizumab, including eosinophil surface and soluble IL-5Rα levels and plasma cytokine profiles, will also be explored.

Part 1: Benralizumab (30 mg) or placebo will be administered sc every 4 weeks for 3 doses (at weeks 0, 4 and 8). Eosinophil counts will be blinded during this time and background HES therapy will not be tapered.

Part 2: Following the placebo-controlled portion of the trial, all subjects will receive an sc injection of benralizumab at weeks 12, 16, and 20 . Eosinophil counts will be unblinded after the first week of open-label therapy (study week 13) and HES therapy may be tapered if clinically indicated. Subjects demonstrating a response at the 24 week visit (eosinophil count <1000/μL and stable or improved clinical symptoms without an increase in background HES therapy) will receive additional 30 mg sc injections every 4 weeks for 6 doses. Non-responders will receive no further doses of benralizumab.

Subjects will be followed daily for 3 days following the initial dose of benralizumab or placebo, weekly for 4 weeks and every 2 weeks for 8 weeks. At 12 weeks, the baseline evaluation will be repeated prior to initiation of open-label benralizumab therapy. After the first dose of open-label benralizumab, subjects will again be followed for 3 days, weekly for 4 weeks and every 2 weeks for 8 weeks to ensure subject safety. Subsequent visits will occur every 4 weeks for responders and every 12 weeks for non-

responders for the remainder of the trial. Assessment at followup visits other than the baseline, 12, 24, 36 and 48 week visits, will be limited to a targeted history and physical examination, including AE/SAE evaluation, routine laboratory testing and collection of blood and urine samples for research studies. In order to assess the potential impact of eosinophil depletion on vaccine responses, all eligible subjects will receive Tdap vaccine at the 22 week visit with assessment of anti-tetanus antibody titers 6 weeks later.

Part 3: For subjects who complete the study and for whom benralizumab provides sustained clinical benefit, drug (accessorized prefilled syringe (APFS) may be provided on an open-label extension of benralizumab administered every 4 weeks for responders until regulatory approval and commercial availability of the marketed drug to prescribing physicians (for HES) or until AstraZeneca ceases development of the drug for this indication. This open-label extension may involve administration of sc benralizumab by local providers in the setting of the COVID19 epidemic due to the increased risk associated with travel to the NIH.

Part 4: For subjects with a sustained and complete response to benralizumab on the open-label extension for a minimum of 2 years, the dosing interval may be increased to every 8 weeks (the FDA-approved dosing interval for severe eosinophilic asthma).

Part 5: Subjects participating in part 3 or part 4 with a sustained and complete response to benralizumab for a minimum of 2 years will be transitioned to the single-dose autoinjector (FASENRA PEN™) until regulatory approval and commercial availability of the marketed drug to prescribing physicians (for HES) or until AstraZeneca ceases development of the drug for this indication.

3.2 Study Endpoints

3.2.1 Primary Endpoint

The primary endpoint is a 50% reduction in peripheral blood eosinophilia on stable background therapy at 12 weeks post-initiation of study.

3.2.2 Secondary Endpoints

Secondary endpoints include: 1) Reduction in AEC at 12 weeks, 2) Frequency and severity of AEs, 3) Reduction in signs and symptoms of HES, 4) Tissue eosinophilia, 5) Numbers of eosinophils, mast cells and their precursors in bone marrow, 6) Levels of markers of eosinophil and mast cell activation, 7) Eosinophil count and background HES therapy at the 48 week visit, 8) PK and ADA levels, 8) Eosinophil count after 24 weeks of every 8 week dosing.

3.2.3 Exploratory Endpoints

Exploratory endpoints include 1) correlation between IL5 receptor levels and reduction in peripheral blood eosinophilia at 1, 4 and 12 weeks, 2) the rise in anti-tetanus antibodies at 6 weeks post-immunization and 3) the change in weight and HgbA1c levels after 12 and 24 weeks of benralizumab.

4 Study Population

4.1 Rationale for Subject Selection

Adult subjects aged 18-75 with *PDGFRA*-negative HES who are are symptomatic with AEC >1000/μL on stable HES therapy for at least 1 month will be selected for this study. Because benralizumab is of unknown benefit in this patient population, subjects with life-threatening disease will be excluded. Subjects with imatinib-sensitive mutations, including *FIP1L1/PDGFRA*, will also be excluded even if they are resistant to or intolerant of imatinib, since therapeutic alternatives targeting these mutations are currently available and would be the preferred treatment.

4.2 Recruitment Plan

Subjects will be recruited from patients referred from outside physicians for evaluation of hypereosinophilia and those already evaluated on protocol 94-I-0079 (“Eosinophil activation and function in parasitic infections and other conditions with increased tissue or blood eosinophilia in humans”). In addition, a small number of physicians who study this disorder will be contacted directly and informed of this study.

4.3 Subject Inclusion Criteria

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

1) Male or female subject ≥18 and ≤75 years of age at screening.

2) A female subject is eligible for this study only if she is not pregnant or breast-feeding and one of the following:

    a. Of childbearing potential but agrees to practice effective contraception, as determined by the PI, or abstinence throughout the study and for 3 months after administration of the last dose of investigational study drug

b. Of non-child-bearing potential

Females of non-child-bearing potential are defined as females with functioning ovaries with a documented history of tubal ligation or hysterectomy or females who are post-menopausal, as defined by 12 months of spontaneous amenorrhea with an appropriate clinical profile, e.g. age appropriate, >45 years, in the absence of hormone replacement therapy. In questionable cases, a blood sample for follicle stimulating hormone and estradiol will be obtained to confirm child-bearing potential.

Acceptable methods of contraception may include a male partner who is sterile prior to the female subject's entry into the study and is the sole sexual partner for the female subject; or 2 or more of the following: 1) implants of levonorgestrel; 2) injectable progestogen, 3) an intrauterine device with a documented failure rate of <1%; 4) double barrier methods including diaphragm or condom with a spermicide.

3) A male subject is eligible for this study only if he is one of the following:

   a. Surgically sterile
   b. Agrees to practice effective contraception (see above) or abstinence throughout the study and for 3 months after the last administration of the investigational study drug

4) Documented diagnosis of HES (history of persistent eosinophilia >1500/μL without secondary cause and evidence of end organ manifestations attributable to the eosinophilia)

5) Signs or symptoms of HES and AEC >1000/μL on stable HES therapy for ≥ 1 month at the time of enrollment

6) Participation in protocol 94-I-0079 (Activation and function of eosinophils in conditions with blood or tissue eosinophilia)

7) Agrees to storage of samples for study

Participation of Women:

Contraception: The effects of benralizumab on the developing human fetus are unknown. For this reason, men and women of childbearing potential must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Females of childbearing-age must have a pregnancy test result prior to receiving benralizumab. 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.

In the event of pregnancy, the status of the subject and the overall risks and benefits to BOTH mother and fetus with regard to continuing or discontinuing the study agent will be assessed by the PI. The PI will review data that may be available through the manufacturer and that may be in any pregnancy registry. The IRB will also be notified per steps outlined in this protocol.

The investigational drug may be continued if the overall risk and benefit assessment for BOTH the mother and the fetus favors continuation, AND if the subject and the study team agree to this course of action, after a documented informed consent discussion. The IRB and the clinical safety office/sponsor medical monitor will be notified as to the outcome of such a discussion and the subject's decision.

- The pregnant subject will be enrolled in the AstraZeneca pregnancy registry.
- Blood testing will be limited to safety labs, blood draw volumes will be minimized per PI discretion to avoid exacerbation of hemodilution/anemia related to pregnancy.
- The PI will provide the subject with pertinent information to provide to their pregnancy care provider, and will be counseled to consult with a Maternal Fetal Medicine specialist [High Risk Obstetric provider] due to the underlying condition as well as the ongoing study drug exposure.
- All procedures, other than those required for clinical reasons, will be deferred until after the pregnancy.
- The subject will be considered enrolled through at least the end of the pregnancy so as to facilitate collection of outcome data.

4.4 Subject Exclusion Criteria

A subject will be excluded from participation in the study if any of the following criteria apply at the time of enrollment:

1) Subjects with life-threatening or other serious illness or clinical manifestation of HES deemed inappropriate for inclusion in study per the principal investigator, including but not restricted to severe cardiac involvement and prior thromboembolic disease.

2) Human immunodeficiency virus (HIV) or other known immunodeficiency

3) Positive hepatitis B surface antigen, or hepatitis C virus antibody serology, or a positive medical history for hepatitis B or C. Patients with a history of hepatitis B vaccination without history of hepatitis B are allowed to enroll

4) Presence of *FIP1L1/PDGFRA* or another known imatinib-sensitive mutation

5) Diagnosis of systemic mastocytosis or serum tryptase level >40 ng/mL

6) Known lymphoma, hematological malignancy, advanced and metastatic solid tumors and/or subjects who are under chemotherapy, radiotherapy or interleukin 2 treatment

7) Known history of allergic or anaphylactic reaction to previous antibody therapy, including intravenous immunoglobulin and licensed or experimental monoclonal antibodies.

8) A helminth parasitic infection diagnosed within 24 weeks prior to the date informed consent is obtained

9) Acute bacterial or viral infection (subjects may be enrolled once the acute infection has resolved)

10) Receipt of intravenous immunoglobulin (IVIG) within 30 days prior to the date informed consent is obtained

11) Receipt of any marketed (eg omalizumab) or investigational biologic within 4 months or 5 half-lives prior to the date informed consent is obtained, whichever is longer

12) Receipt of live attenuated vaccines 30 days prior to the date of randomization

13) Receipt of inactive/killed vaccinations (e.g. inactive influenza) are allowed provided they are not administered within 1 week before/after any study visit

14) Receipt of any investigational nonbiologic within 30 days or 5 half-lives prior to the date informed consent is obtained, whichever is longer

15) History of alcohol or drug abuse within 12 months prior to the date informed consent is obtained

16) Previous treatment with benralizumab

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. Study staff should be notified of co-enrollment as it may require the approval of the Investigator.

4.5 Justification for Exclusion of Children

Because there are insufficient data regarding dosing or adverse events available in adults with HES to judge the potential risk in children, children are excluded from this study.

5 Study Agent/Interventions

5.1 Disposition and Dispensation

Benralizumab or placebo will be distributed via the NIH Central Pharmacy according to standard pharmacy procedures.

5.1.1 Formulation, Packaging and Labeling

Benralizumab Drug Product is a sterile liquid solution presented in an accessorized prefilled syringe (APFS) or single-dose autoinjector (FASENRA PEN™) for sc injection. Each syringe contains 30 mg of benralizumab in a 1.0 mL volume (nominal). The Drug Product is formulated at 30 mg/mL in 20 mM histidine/histidine-HCl, 0.25 M trehalose dihydrate, and 0.006% (w/v) polysorbate 20, pH 6.0 (Table 1).

Table 1 Benralizumab composition

Ingredient	Concentration	Unit formula per 30 mg syringe (nominal)
Active ingredient		
Benralizumab	30 mg/mL	30 mg
Other ingredients		
L-Histidine	9 mM	1.4 mg
L-Histidine HCl monohydrate	11mM	2.3 mg
Trehalose dihydrate	0.25 M	95 mg
Polysorbate 20	0.006% (w/v)	0.06 mg

During the double-blind period, placebo solution will be visually matched with benralizumab solution. Both benralizumab and placebo will be provided in an accessorized pre-filled syringe.

Each accessorized pre-filled syringe will be individually labeled by the NIH pharmacy with the patient ID number, dosing instructions, recommended storage conditions, the name and address of the manufacturer, Investigational Use Statement (“Caution: New

Drug – Limited by Federal [USA] law to Investigational Use") and that the agent should be kept out of reach of children.

Drug may be administered at a collaborating site under an OHSRP-approved reliance agreement once the site is approved by the NIH IRB. In this case, the NIH pharmacy will provide the individually labeled syringes to the pharmacy at the collaborating site and documentation, including receipt date and condition, storage conditions, and date and time of administration, will be provided by the collaborating site to the NIH Central pharmacy.

FASENRA PEN may be administrated by patients/caregivers during the open-label extension instead of benralizumab in an APFS. Patients/caregivers may inject FASENRA PEN after proper training in subcutaneous injection technique and observation of patient/caregiver administration by the PI or their delegate. Documentation of training and administration will be recorded in CRIMSON.

Each FASENRA PEN carton will be individually labeled by the NIH pharmacy with the patient ID number, dosing instructions, recommended storage conditions, the name and address of the manufacturer, Investigational Use Statement ("Caution: New Drug – Limited by Federal [USA] law to Investigational Use") and that the agent should be kept out of reach of children.

5.2 Study Agent Storage and Stability

Benralizumab must be stored in the original outer container refrigerated at 2-8°C (36°F-46°F), protected from the light, and must not be frozen.

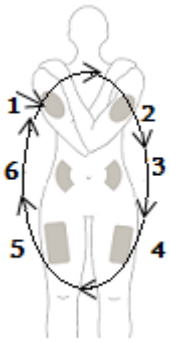
5.3 Preparation, Administration, and Dosage of Study Agent

5.3.1 Study Drug Administration

Benralizumab (30 mg dose) or placebo APFS will be administered by SC injection by the principal investigator or qualified designee using aseptic technique via a 29-gauge 0.5 inch needle over at least 5 seconds.

It is advised that the site of injection be rotated such that the patient receives the study drug at a different anatomical site at each treatment visit. A suggested injection site rotation sequence is presented below (see Figure 2). The injection site must be documented at each treatment visit. If rotation of the site according to the recommended sequence is not possible, a reason for this must be given.

Figure 2 Injection sites and rotation scheme



Administration of the study drug will be rescheduled if the subject has an intercurrent illness that, in the opinion of the PI, may compromise the safety of the subject or if the subject reports a fever (≥38°C; ≥100.4°F) within 72 hours prior to planned administration of the study drug.

The subject will be observed for a minimum of 30 minutes after administration of the study drug for the appearance of any acute drug reactions. Appropriate drugs, such as epinephrine and H1 and H2 antihistamines, corticosteroids, etc, and medical equipment to treat acute anaphylactic reactions must be immediately available, and study personnel must be trained to recognize and treat anaphylaxis.

Anaphylaxis will be defined as serious reaction that is rapid in onset and may cause death. Anaphylaxis typically manifest as one of 3 clinical scenarios:

1. The acute onset of a reaction (minutes to hours) with involvement of the skin, mucosal tissue or both and at least one of the following: a) respiratory compromise; b) or reduced blood pressure or symptoms of end-organ dysfunction;

2. Two or more of the following that occur rapidly after exposure: involvement of the skin/mucosal tissue, respiratory compromise, reduced blood pressure or associated symptoms and/or persistent gastrointestinal symptoms; or

3. Reduced blood pressure after exposure.

In order to help understand the potential drug-relatedness of any acute reaction, a blood sample should be drawn during the event for additional ADA testing (if not already scheduled for this visit).

Refer to the FASENRA PEN ‘Instructions for Use’ for more detailed instructions on the preparation and administration of FASENRA PEN (

Appendix F: FASENRA PEN: Instructions for Use).

5.3.2 Study Drug Dosing

Population modelling of clinical efficacy endpoints (Asthma Exacerbation Rate (AER), Asthma Control Questionnaire (ACQ), and Forced Expiratory Volume in 1 second (FEV_1)) from the interim analysis for the Phase 2 asthma study, MI-CP220, and stochastic simulations identified the optimal dose and regimen for the pivotal Phase 3 studies with benralizumab in adult subjects with asthma as 30 mg every 4 weeks for 3 doses then every 8 weeks thereafter. This dosing regimen corresponds to the ED_{90} for AER and ACQ, and maintains a steady-state PK exposure close to EC_{90} levels for FEV_1 and ACQ. With a steady-state PK exposure 50% higher than 20 mg every 8 weeks, the 30 mg dose every 8 weeks is expected to reduce the impact of PK variability related to factors such as higher body weight. The incidence of ADA (especially high-titer ADA) and the impact on PKPD were dose-dependent with decreased incidence and impact at higher doses. However, the ADA response was not more frequent in those subjects with blood eosinophils ≥300 cells/μL (20.7%) as compared to those with blood eosinophils <300 cells/μL (21.7%). Since doses higher than 20 mg are expected to reduce the impact of ADA without modifying the dose interval, and there are no identified safety risks that would warrant consideration of a lower dose, subjects will receive 30 mg every 4 weeks for 3-12 doses depending on whether they receive drug or placebo during the first part of the study and whether they demonstrate a clinical and hematologic response at study week 24.

Subjects who complete the study and for whom benralizumab appears to provide a sustained clinical benefit, may be eligible to receive drug under an open-label extension until regulatory approval and commercial availability of the marketed drug to prescribing physicians (for HES), or until AstraZeneca ceases development of benralizumab for this indication. Complete responders may be eligible to extend the dosing interval to every 8 weeks after sustained and complete remission of eosinophilia for ≥2 years at the investigator's discretion.

5.4 Study Product Accountability Procedures

The site-designated pharmacist is responsible for investigational product accountability, reconciliation and record maintenance. In accordance with all applicable regulatory requirements, the designated site pharmacist must maintain investigational product accountability records throughout the course of the study. The person(s) will document the amount of investigational product received from AstraZeneca and the amount administered to study subjects. At the end of the study, all unused supplies will be disposed of or destroyed per the policies of the sponsor.

Upon receiving investigational product, the pharmacist will sign a Drug Receipt Form that will specify supply or lot numbers, expiration date, quantity shipped/delivered, and the date of receipt. All investigational products received, dispensed, destroyed or

returned will be recorded on a Drug Accountability Form. At the end of the study, reconciliation must be made between drug received, dispensed, returned and destroyed, and all discrepancies must be accounted for.

For drug sent to outside physicians during the COVID19 epidemic and FASENRA PENs provided to study subjects, receipt, maintenance of cold chain (refrigerator temperature log) and administration will be confirmed by the study team and the pharmacy will be notified of any deviations.

5.5 Concomitant Medications and Procedures

All concomitant prescription medications taken during study participation will be recorded in 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 adverse events (all grades).

5.6 Prohibited Medications and Procedures

HES medications, including but not limited to prednisone, will be allowed during the trial, but must be maintained at a constant dose beginning 1 month prior to enrollment until completion of the 12 week time point (primary endpoint assessment). Tapering or discontinuation of HES medications during this time will be permitted only in the event of drug toxicity.

Treatment with the following agents will not be permitted unless discussed with and approved by the principal investigator:

1) Systemic immunosuppressive medications other than HES medications initiated prior to enrollment
2) Xolair or other monoclonal antibodies
3) Investigational agents
4) Non-drug therapies (i.e. herbal products, alternative therapies)

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 time points are as follows: “Days” ± 2 days; “Weeks” ± 4 days, unless otherwise specified in section 6.5. The study window in the instance of the open-label extension of

benralizumab injection is to occur every 28 days +/- 7 days (since the previous injection). Blood volumes are given in Appendix C.

6.1 Screening

Potential subjects will undergo screening assessments as part of protocol 94-I-0079. At a minimum, the following procedures will be performed within the month prior to enrollment:

- Complete medical history, including year of HES diagnosis, clinical evidence that led to the diagnosis of HES and prior/current HES therapies
- Physical examination
- Complete blood count and routine chemistries
- Serum tryptase level
- HIV 1/2 serology
- Hepatitis B and C serology
- *FIP1L1/PDGFRA* testing (if results of prior testing at NIH are not available)
- Serum or urine pregnancy test (for women of childbearing potential)
- Additional testing as clinically indicated to assess end organ manifestations

Those subjects who meet the eligibility criteria (Section 4.3) will be offered enrollment in the study.

6.2 Enrollment/Baseline

Signed informed consent will be obtained prior to any study procedures being performed. Baseline studies will be performed no more than 14 days prior to study drug administration and will include:

- Complete history and physical examination
- Vital signs, including weight
- CBC with differential
- Routine laboratory testing, including electrolytes, liver panel, mineral panel, LDH, CPK, uric acid, troponin, ESR, CRP, PT/PTT and urinalysis
- Serum tryptase, B12 and quantitative immunoglobulin levels (including IgE)
- HgbA1c
- Serum pregnancy testing in women of child-bearing potential
- Whole blood flow cytometry to assess eosinophil activation, IL-5R expression, basophil numbers and activation, T/B/NK cell subsets
- ECG
- Echocardiogram
- Pulmonary function testing including spirometry with bronchodilator response, evaluation of lung volumes and diffusion capacity

- Additional clinical testing as determined by pattern of clinical involvement
- Tissue biopsies of affected organs (where possible)
- Bone marrow aspirate and biopsy, including
    - quantification of eosinophils, mast cells, and their precursors
    - assessment of mast cell activation status (CD69 and CD63 surface expression)
    - assessment of IL-5R expression on eosinophils, basophils and their precursors
    - viable storage of bone marrow mononuclear cells for future studies
- T and B cell receptor rearrangement studies
- Collection of 60 cc whole blood for isolation and storage of plasma and PBMC for research studies
- Collection of serum, plasma and urine for immunologic analyses
- Measurement of benralizumab and anti-benralizumab antibody levels

6.3 Randomization and Blinding

The subjects, the investigators, and all who are involved in the evaluation of the study subjects including study monitors and central laboratory personnel are blinded to a subject's treatment assignment until all subjects have completed the week 13 visit. The NIH research pharmacist is identified as the unblinded third party who will be in charge of treatment assignment, maintaining study supplies/inventory list, and preparing the blinded study medication for each subject. The NIH research pharmacist will be independent of any study assessments. To maintain blinding, any discussion of the treatment assignment between the clinicians and the pharmacy staff is prohibited until the study is unblinded.

Subjects will be assigned a screening number after completion of the eligibility checklist for enrollment in the study in CRIMSON. The study statistician or NIH research pharmacist will generate the randomization codes. These codes will be maintained by the NIH research pharmacist. To reduce the potential for participant dropouts during the period between enrollment and initial dosing of the study agent, randomization will be defined to occur on the day that the first dose of study drug is administered. A study allocation number (1 through 20) will be assigned at this time.

Because benralizumab (but not placebo) is expected to decrease peripheral and bone marrow eosinophil counts, the results of white blood cell count, peripheral eosinophil count, lymphocyte phenotyping, and bone marrow aspirate and biopsy collected after the initial dose of benralizumab or placebo until week 13 of study treatment will be blinded to the study team and subjects until all subjects have completed week 13 of the study. Results of blood tests performed after this time (week 13 until the end of the study) will not be blinded since all subjects will be receiving benralizumab and knowledge of the results would not allow the study team to determine whether the subject had received benralizumab or placebo during the double-blind portion of the study. The NIH Clinical Research Information System (CRIS) by which laboratory

results are reported does not permit blinding of selected portions of the complete blood count and differential. A qualified NIH pathologist will monitor, in real time on a day to day basis, the results of the complete blood cell count and differential (including peripheral eosinophil count) during this period and will review the results of the bone marrow performed at 12 weeks. The physician who monitors these results will not be involved in the evaluation of study subjects or in making any study assessments. The NIH physician monitoring the results will notify the study team of critical or abnormal values (for example, low absolute neutrophil counts or abnormal hemoglobin or platelet values), but this will not result in unblinding of the randomization unless the absolute eosinophil count is revealed, intentionally (if medically indicated) or unintentionally.

Subjects will be instructed to inform outside physicians that they are participating in this study and should not be informed of the results of eosinophil counts performed at an outside facility during the first 13 weeks of the study unless withholding this information compromises their medical care. The outside physician will also be contacted by the study team regarding the protocol requirements. Results or reports received from outside laboratories or physicians that could lead to unblinding of the study team will be handled by a study coordinator designated to fulfill this role. This study coordinator will not be involved in decisions pertaining to the medical management of the study subjects and will redact information that could inadvertently lead to unblinding of the study team.

6.3.1 Maintenance of Safety in the Face of Blinding

Clinical management of subjects with HES is typically guided by a combination of signs and symptoms and the peripheral absolute eosinophil count (AEC). Changes in AEC in the absence of clinical manifestations would not provoke a change in therapy unless the change were dramatic (change >2 fold or AEC >5000/μL) and confirmed with a second CBC. In order to ensure safety of the participants, the NIH physician monitoring the counts will be instructed to notify the PI if a change of this magnitude occurs. As discussed above, the PI will be informed of all abnormal values in CBC parameters other than the AEC. Although we do not anticipate bone marrow changes at week 12 that would lead to changes in therapy, should abnormalities be detected that require therapeutic intervention, the NIH pathologist will notify the PI.

6.3.2 Implications of Blinding on Safety Reporting

As described above (Section 6.3), the study team will be blinded to the complete blood count and differential during the placebo-controlled portion of the study. It is anticipated that, during this time, some blinded laboratory values will meet the threshold of an AE. For laboratory tests other than the absolute eosinophil count, the NIH pathologist monitoring the tests in real time will inform the PI of critical or abnormal results, and these will be recorded as described in Section 11.14.4. For absolute eosinophil counts that fall outside the normal range but do not reach the threshold for breaking the blind,

reporting will be deferred until the blind has been lifted. Reporting of bone marrow abnormalities at week 12 will also be deferred until the blind is lifted except in cases where therapeutic intervention is indicated on the basis of the findings.

SAEs, and AEs of grade 3 or greater will continue to be forwarded to and assessed by the PI, and will be reported in real time, as will any events that are part of a concerning clinical picture, or that present a scenario requiring intervention.

6.4 Study Phase

Within 14 days of completion of baseline studies, subjects will begin treatment with study drug.

Benralizumab (30 mg) or placebo will be administered sc (as described in Section 5.4) in the OP11 clinic, the 5 SW Day Hospital, or the 5 SE inpatient nursing unit. Vital signs will be checked immediately prior to and at 30 minutes after the injection.

Subjects will be admitted to the inpatient ward for overnight observation following the first dose of study drug to facilitate monitoring of AEs. The inpatient stay may be extended if clinically indicated. Subjects will be seen daily (in OP11 or on the inpatient ward) for 3 days following the first dose of study drug, and then at weeks 1, 2, 3, 4, 6, 8, 10, and 12. Re-dosing of benralizumab or placebo will occur every 4 weeks for a total of 3 doses (at weeks 0, 4, and 8). Subjects who develop AEs after the first dose of study drug may be monitored during subsequent doses at the discretion of the investigator. Subjects who develop grade 3 or greater AEs that are considered by the investigator to be possibly, probably, or definitely related to study drug will promptly, and prior to subsequent dosing, be discussed in writing or verbally with a Sponsor Medical Monitor in the Clinical Safety Office.

At the 12 week visit, all subjects will receive benralizumab (30 mg) sc. Since the subjects who received placebo during the first 12 weeks of the study will be receiving their first dose of benralizumab at this visit, all subjects will be admitted to the inpatient ward for overnight observation following the first dose of study drug and followed daily (in OP11 or on the inpatient ward) for 3 days, every week for 4 weeks and and then at weeks 1, 2, 3, 4, 6, 8, 10, and 12, 13, 14, and 15. Re-dosing of open-label benralizumab will occur every 4 weeks for a total of 3 doses (at weeks 12, 16 and 20), after which subjects demonstrating a clinical response will continue to receive additional doses every 4 weeks for one year. Non-responders will receive no further doses of benralizumab. Subsequent visits will occur at 4-week intervals for responders and at 12-week intervals for non-responders for the duration of the study. After a minimum of 2 years of complete response on the open-label extension, the dosing interval (and visits) may be increased to every 8 weeks (the FDA-approved dosing interval for severe eosinophilic asthma).

Subjects who have have a sustained and complete reduction in eosinophilia and stable clinical signs and symptoms for ≥2 years and are on a stable dosing interval of benralizumab may be eligible to self-administer study drug monthly or every other month using the FASENRA PEN autoinjector. Subjects who self-administer benralizumab will have a CBC drawn monthly at NIH or a local laboratory. They will return to NIH for routine followup visits every 6 months. An interim visit will be scheduled if they develop clinical symptoms of HES and/or their AEC rises above 100/μL.

Women of childbearing potential will undergo serum pregnancy testing prior to each administration of benralizumab.

At the 22 week visit, subjects who have not received tetanus immunization in the prior 5 years and have no contraindication to Tdap immunization will receive Tdap vaccine. The vaccine will be administered by a study nurse in the OP11 clinic according to standard clinical protocol (0.5 mL intramuscularly).

6.5 Follow-up

Follow up visits will be scheduled daily for 3 days, every 7 days (+/- 2 days) for 4 weeks, every 14 days (+/- 3 days) for 8 weeks after the initial dose of benralizumab vs. placebo and after the first open-label dose of benralizumab. Subsequent visits will be scheduled every 4 weeks (+/- 4 days, up to Week 48) for the remainder of the study for responders and at 12 weeks intervals for non-responders. Subjects going on to the open-label extension (after Week 48) will be scheduled every 4 weeks post last study drug administration (+/- 7 days). After a minimum of 2 years in complete remission, subjects may be scheduled every 8 weeks post last study drug administration (+/- 7 days) and/or every 6 months for subjects self-administering benralizumab using the auto-injector (FASENRA PEN).

The following studies will be performed at every followup visit until (and including) week 48:

- Vital signs, including weight
- Targeted history and physical examination
- AE/SAE assessment
- CBC with differential
- Routine chemistries (electrolyte, hepatic and mineral panels)
- LDH
- Creatine Kinase (CPK)
- Serum tryptase level
- Urinalysis
- Collection of urine (only up to Week 48), serum and plasma for research studies

The following additional studies will be performed at the monthly visits until (and including) week 48, prior to administration of study drug:

- ESR, CRP
- Total Protein
- Uric Acid
- Serum pregnancy testing for women of child-bearing potential
- Serum for PK and ADA studies

The following studies will be performed at monthly/bimonthly/every 6 month visits after week 48:

- Vital signs, including weight
- Targeted history and physical examination
- AE/SAE assessment
- CBC with differential
- Routine chemistries (electrolyte, hepatic and mineral panels; only at NIH visits)
- Serum pregnancy testing for women of child-bearing potential

NOTE: if the subject is receiving drug under an OHSRP-approved reliance agreement or by an outside physician during the COVID19 epidemic, these studies may be performed at the outside site. Subjects receiving drug every 8 weeks will continue to have monthly CBC performed at the NIH or an outside site for 24 weeks after the dosing interval increase.

The following additional studies will be performed at the 12, 24, 36 and 48 week followup visits, and every 4-6 months on the open-label extension except as indicated:

- PT/PTT (week 12 only)
- Troponin (yearly after week 48)
- ESR, CRP (yearly after week 48)
- Total Protein (yearly after week 48)
- Uric Acid (yearly after week 48)
- Serum B12 (yearly after week 48)
- Quantitative immunoglobulin levels (including IgE)
- HbA1c (12 and 24 weeks only)
- ECG (yearly after week 96 unless clinically indicated)
- Echocardiogram (yearly after week 96 unless clinically indicated)
- Pulmonary function testing including spirometry with response, evaluation of lung volumes and diffusion capacity bronchodilator (yearly after week 96 unless clinically indicated)
- Additional clinical testing as determined by pattern of clinical involvement
- Tissue biopsies of affected organs (if clinically indicated)
- Bone marrow aspirate and biopsy (at 12 weeks only), including

  - quantification of eosinophils, mast cells, and their precursors
  - assessment of mast cell activation status (CD69 and CD63 surface expression)
  - assessment of IL-5R expression on eosinophils, basophils and their precursors
  - viable storage of bone marrow mononuclear cells for future studies
- Collection of 60 cc whole blood for isolation and storage of plasma and PBMC for research studies (yearly after week 96)
- Collection of serum for immunologic analyses
- Collection of urine for immunologic analyses (until and including week 48)
- Whole blood flow for IL-5 receptor expression, enumeration of basophil numbers and activation (at weeks 0, 12, 24, 36, 48 only)
- Assessment of T/B/NK cell subsets
- T and B cell receptor rearrangements (weeks 12 and 48 only)

Tetanus antibody titers will be assessed at 22 (pre-immunization) and 28 weeks (post-immunization).

6.6 Final Study Visit/Early Termination Visit

The final study visit will occur 48 weeks after the first dose on study drug (either benralizumab or placebo). The studies to be performed are listed in Section 6.5.

Subjects who are not eligible to continue receiving benralizumab, who elect not to participate in the open-label extension, or who withdraw for any reason prior to completing the study will be asked to return for followup visits every 4 weeks until week 12 (with a scheduled visit at week 12 included) and every 12 weeks therafter for the planned duration of their study participation.

6.7 Open-label Extension

For subjects who complete the study and for whom benralizumab appears to provide sustained clinical benefit, drug may be provided on an open-label extension until regulatory approval and commercial availability of the marketed drug to prescribing physicians (for HES) or until AstraZeneca ceases development of the drug in HES. All subjects continuing to receive drug monthly on the open-label extension will be scheduled for followup visits and drug administration every 3 months at the NIH. Intervening monthly followup and drug administration will occur either at the NIH or at a collaborating site under an OHSRP-approved reliance agreement. All collaborating sites will submit a local context form and be approved by the NIH IRB prior to site initiation.

Subjects who have a sustained and complete reduction in eosinophilia for ≥2 years may be eligible to extend the dosing interval to 8 weeks at the investigator's discretion. If at any time following extension of the dosing interval to 8 weeks, the eosinophil count rises

and/or symptoms worsen, the dosing interval may be reduced to every 4 weeks for that subject for the duration of the study. Subjects will be asked to fill out weekly patient-reported outcome questionnaires to assess pain, fatigue, dyspnea and gastrointestinal symptoms (PROMIS v1.0; http://www.healthmeasures.net) for 2-4 weeks prior to and 4 months following the change to every 8 week dosing to assist in the objective determination of symptom worsening.

Subjects who have a sustained and complete reduction in eosinophilia and stable clinical signs and symptoms for ≥2 years and are on a stable dosing interval of benralizumab may be eligible to self-administer benralizumab using the FASENRA PEN autoinjector. After subjects undergo training in the use of the autoinjector by credentialed OP8 or LPD nursing staff and are provided with FASENRA PEN Instructions for Use (see Appendix F), FASENRA PEN autoinjectors will be provided to the study subject in a cold transport box or sent by the NIH Pharmacy to the subject's home. Subjects will be instructed to obtain monthly CBC (with results sent to the study team) and to contact the study team within 24 hours of each injection to report the date, time, and site of the injection as well as any adverse events. Followup visits will occur every 6 months unless clinical signs or symptoms develop or the AEC rises to >100/μL, in which case an interim visit will be arranged.

6.8 Recontact of Subjects After Trial Termination

After study termination, subjects will be contacted yearly as part of the required study visits for Protocol #94-I-0079. In settings where subjects request to withdraw consent for protocol #94-I-0079, they will be asked whether they would be willing to be re-contacted in the future.

7 Study Procedures/Evaluations

7.1 Clinical Evaluations

7.1.1 History and Physical Examination

A complete medical history and physical examination will be performed as part of the baseline evaluation. 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. Tympanic measurements of body temperature will be performed.

7.1.2 Symptom Questionnaire

An HES symptom questionnaire will be administered at all study visits (see

Appendix E: HES Questionnaire) . Subjects will also be encouraged to notify the Investigators of any new signs or symptoms that develop between follow-up visits. Newly emergent or worsening symptoms, clinically significant physical examination findings, and laboratory abnormalities will be recorded and scored as AEs using the "Common Terminology Criteria for Adverse Events (CTCAE)" (v 4.0): http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

As described above, subjects who elect to increase the dosing interval to every 8 weeks will fill out patient-reported outcome questionnaires to assess pain, fatigue, dyspnea and gastrointestinal symptoms (PROMIS v1.0; http://www.healthmeasures.net).

7.1.3 End Organ Assessment

ECG, echocardiography, and pulmonary function testing will be prior to initiation of benralizumab or placebo, after 12 weeks of therapy and at the end of study visit. Quantification of other end organ manifestations will be individualized and may include physical examination findings, laboratory testing (ex. serum aldolase for eosinophilic myositis, serum transaminase levels for eosinophilic hepatitis, urine cytology for eosinophilic cystitis), imaging (such as fibroscan for liver involvement, MRI with stir images for eosinophilic myositis/fasciitis), and/or other minimally-invasive tests. All end organ assessments will be performed at the NIH Clinical Center using standard protocols.

7.2 Laboratory Evaluations

7.2.1 Routine laboratory testing

Routine safety laboratory evaluations, including complete blood count with differential, routine chemistries and urinalysis, will be performed in the Department of Laboratory Medicine at the NIH Clinical Center or at outside laboratories. The study team will be blinded to the results of complete blood counts with differential and flow cytometry studies during the first 13 weeks of study drug administration until all subjects have completed the 13 week time point (as described in Section 6.3).

7.2.2 Bone marrow biopsy and aspirate

Bone marrow aspirates and biopsies from the iliac crest will be performed using standard sterile techniques prior to and at 12 weeks following the first dose of benralizumab. Local anesthesia will be obtained using lidocaine. Each aspirate typically provides 2-3 ml of bone marrow. Two to 10 aspirates will be obtained to assess bone marrow morphology and the effects of benralizumab on the numbers and activation status of eosinophils, neutrophils, mast cells and their precursors. The results

of the 12 week bone marrow examinations will be blinded until all subjects have completed week 13 of the study (as described in Section 6.3).

7.2.3 Exploratory assessments

Serum, plasma, whole blood and urine will be collected at various time points for assessment of eosinophil activation, cytokine/chemokine profile and other immunologic parameters as described in Appendix D: Research Studies.

Serum will also be collected for assessment of antibodies to benralizumab (ADA) and benralizumab drug levels. After centrifugation, the serum will be divided into 2 aliquots of at least 1.5 mL each, frozen, and then stored at -20 ºC until shipped on dry ice to AstraZeneca for analysis.

If tissue biopsies are obtained for clinical indications during benralizumab therapy and sufficient material is available, immunohistochemical staining for eosinophil granule protein deposition may be performed according to a standard protocol developed by the Laboratory of Pathology, NCI.

8 Potential Risks and Benefits

8.1 Potential Risks

8.1.1 Blood Drawing

The potential risks of 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 Clinical Center. (See Appendix C for total blood draw volumes).

8.1.2 Benralizumab

Based on data from the clinical trials to date, the most common AEs that were more frequently seen in subjects receiving benralizumab (>2% increased incidence compared to placebo) were nasopharyngitis, upper respiratory tract infection, pharyngitis, injection site erythema, injection site reaction, pyrexia, arthralgia and myalgia. Although AEs related to rapid lowering of eosinophil, basophil or mast cell numbers are not anticipated, subjects will be monitored as outpatients daily for 3 days after the initial dose of benralizumab. Long-term theoretical risks related to depletion of eosinophils in

the bone marrow and tissues include diminished responses to immunization and alterations in glucose homeostasis.

Two subjects in the current trial have developed urinary stones. In the first case, analysis was not performed. Consequently, it is possible that this was a uric acid stone due to eosinophil lysis by benralizumab. The second subject had a prior history of a calclium oxalate stone, 3 years prior to enrolling on the trial. Analysis of her stone revealed that it was a calcium oxalate stone and thus, unlikely to be related to study drug. Urinary stones have not been reported in any of the other clinical trials.

Benralizumab has not been tested in women who are pregnant; therefore the risk to a developing fetus is unknown. Pregnant women will be excluded from the trial and all women of child-bearing age will be required to undergo pregnancy testing prior to and during the study and to practice contraception for the duration of the trial and for 3 months after the last administration of the study drug. In the event of pregnancy, the status of the subject and the overall risks and benefits to BOTH mother and fetus with regard to continuing or discontinuing the study agent will be assessed by the PI. The investigational drug may be continued if the overall risk and benefit assessment for BOTH the mother and the fetus favors continuation, AND if the subject and the study team agree to this course of action, after a documented informed consent discussion.

Development of anti-drug antibodies (ADA) to benralizumab has been documented. Theoretical risks of developing ADA include decreased drug efficacy and hypersensitivity reactions (e.g. anaphylaxis or immune complex disease).

Eosinophils are a prominent feature of the inflammatory response to helminthic parasitic infections, and the presence of infiltrating eosinophils has been circumstantially associated with a positive prognosis in certain solid tumors. Therefore, there is a theoretical risk that prolonged eosinophil depletion may diminish the ability to defend against helminthic parasites, or negatively impact the natural history of certain malignant tumors. Risk minimization measures include exclusion of patients with untreated parasitic infection and active or recent malignancy, in conjunction with the performance of routine pharmacovigilance activities.

8.1.3 Tdap

Any routine immunization can be associated with injection site reactions, allergic reactions and rarely, Guillain Barre syndrome. The current Tdap vaccine on formulary at the Clinical Center is Boostrix (GlaxoSmithKline). Common solicited adverse events (≥15%) in adults (19 to 64 years of age) following administration of Boostrix were pain, redness, and swelling at the injection site, headache, fatigue, and gastrointestinal symptoms. The most common solicited adverse event (≥15%) in the elderly (65 years of age and older) was pain at the injection site. Detailed information can be found in the package insert

(https://www.gsksource.com/pharma/content/dam/GlaxoSmithKline/US/en/Prescribing_Information/Boostrix/pdf/BOOSTRIX.PDFhttp://us.gsk.com/products/assets/us_boostrix.pdf).

8.1.4 Bone marrow biopsy

Bone marrow aspirates and biopsies from the iliac crest will be performed using standard sterile techniques just prior to initiation of benralizumab treatment and after 12 weeks of benralizumab therapy. Local anesthesia will be obtained using lidocaine. Each aspirate typically provides 2-3 ml of bone marrow. Two to 10 aspirates will be obtained to assess bone marrow morphology and the effects of dexpramipexole on the numbers and activation status of eosinophils and their precursors.

8.1.5 Tissue biopsy

Patients with eosinophilic tissue involvement that is accessible (skin, gastrointestinal tract, lung or sinus) may undergo biopsies as part of standard of care. In this case, 1-2 additional tissue samples may be obtained for research purposes. The risks of obtaining additional biopsies represent a minimal increase in risk over the risks of the clinically-indicated procedure and include bleeding, scarring (skin), and rarely, perforation (gastrointestinal).

8.1.6 Other research procedures

Other procedures performed for research purposes include electrocardiogram, echocardiogram, pulmonary function testing and urine collection. These are associated with minimal (if any) risk.

8.2 Potential Benefits

Individual participants in the study will benefit from a thorough medical evaluation. Since the effect of benralizumab on eosinophilia in HES is unknown, subjects may or may not benefit from the experimental therapy. In the event that a beneficial effect is found, a larger efficacy study will likely be performed that may benefit subjects with HES. Since Tdap is currently recommended for adults aged 18-65, subjects who have not been previously immunized with this vaccine will derive benefit from this procedure. Subjects will not be compensated for participation in this study, but will be reimbursed for their transportation costs as is permitted by the NIAID travel policy.

9 Research Use of Stored Human Samples, Specimens or Data

- **Intended Use:** Samples and data collected under this protocol may be used to study eosinophilic disorders. Genetic testing will be performed. Any other

research or experimental treatments will be done under this or other protocols for which separate signed informed consent documents will be obtained.

- **Storage:** Access to stored samples will be limited using either a locked room or a locked freezer. Samples and data will be stored using codes assigned by the investigators. Data will be kept in password-protected computers. Only investigators will have access to the samples and data.
- **Tracking:** Samples and data acquired during this study will be tracked using BioSpecimen Inventory (BSI).
- **Disposition at the Completion of the Protocol:**
    - 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, 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.
    - At the completion of the protocol (termination), samples and data will either be destroyed, or after IRB approval, transferred to another existing protocol.
- **Reporting the Loss or Destruction of Samples/Specimens/Data to the IRB:**
    - 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 and/or compromises the scientific integrity of the data collected for the study, will be reported to the NIH IRB.
    - 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 this protocol or any other protocols at NIH.

10 Remuneration Plan for Subjects

Subjects will not be remunerated for participation in this study.

11 Assessment of Safety

11.1 Definitions

The NIAID Clinical Safety Office (CSO) is responsible for sponsor safety oversight of this study, and the definitions below comply with CSO requirements.

Adverse Event (AE)
An adverse event is any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (e.g., abnormal physical exam or laboratory

finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the research.

Adverse Reaction (AR)
An adverse reaction is an adverse event that is caused by an investigational agent (drug or biologic).

Suspected Adverse Reaction (SAR)
An adverse event for which there is a reasonable possibility that the investigational agent caused the adverse event. 'Reasonable possibility' means that there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction which implies a high degree of certainty.

Serious Adverse Event (SAE)
A Serious Adverse Event is an AE that results in one or more of the following outcomes:

- death
- a life threatening (i.e., an immediate threat to life) event
- an inpatient hospitalization or prolongation of an existing hospitalization
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- a congenital anomaly/birth defect
- a medically important event*

* Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life threatening or result in death or hospitalization but they may jeopardize the subject or may require intervention to prevent one of the other outcomes listed above.

Unexpected Adverse Event
An AE is unexpected if it is not listed in the Investigator's Brochure or Package Insert (for marketed products) or is not listed at the specificity or severity that has been observed. It is the responsibility of the IND Sponsor to make this determination.

Serious and Unexpected Suspected Adverse Reaction (SUSAR)
A SUSAR is a Suspected Adverse Reaction that is both Serious and Unexpected.

Unanticipated Problem (UP)
An Unanticipated Problem is any event, incident, experience, or outcome that is

1. unexpected in terms of nature, severity, or frequency in relation to
   a. the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents; and

   b. the characteristics of the subject population being studied; and
2. possibly, probably, or definitely related to participation in the research; and
3. places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized. (Per the IND Sponsor, an AE with a serious outcome will be considered increased risk.)

Serious Unanticipated Problem (UP)
A UP that meets the definition of a Serious Adverse Event or compromises the safety, welfare or rights of subjects or others.

Unanticipated Problem that is not an Adverse Event (UPnonAE)

Unanticipated problem that is not an Adverse Event (UPnonAE): An unanticipated problem that does not fit the definition of an adverse event, but which may, in the opinion of the investigator, involve risk to the subject, affect others in the research study, or significantly impact the integrity of research data. Such events would be considered a non-serious UP. For example, we will report occurrences of breaches of confidentiality, accidental destruction of study records, or unaccounted-for study drug

Protocol Deviation: Any change, divergence, or departure from the IRB-approved research protocol.

1. **Major Deviations:** Deviations from the IRB-approved protocol that have, or may have the potential to, negatively impact the rights, welfare, or safety of the subject, or to substantially negatively impact the scientific integrity or validity of the study.
2. **Minor Deviations:** Deviations that do not have the potential to negatively impact the rights, safety, or welfare of subjects or others, or the scientific integrity or validity of the study.

Non-compliance: Failure of investigator(s) to follow the applicable laws, regulations, or institutional policies governing the protection of human subjects in research, or the requirements or determinations of the IRB, whether intentional or not.

1. **Serious non-compliance:** Non-compliance, whether intentional or not, that results in harm or otherwise materially compromises the rights, welfare and/or safety of the subject. Non-compliance that materially affects the scientific integrity or validity of the research may be considered serious non-compliance, even if it does not result in direct harm to research subjects.
2. **Continuing non-compliance:** A pattern of recurring non-compliance that either has resulted, or, if continued, may result in harm to subjects or otherwise materially compromise the rights, welfare and/or safety of subjects, affect the scientific integrity of the study or validity of the results. The pattern may comprise repetition of the same non-compliant action(s), or different noncompliant events. Such non-compliance may be unintentional (e.g. due to lack of understanding,

knowledge, or commitment), or intentional (e.g. due to deliberate choice to ignore or compromise the requirements of any applicable regulation, organizational policy, or determination of the IRB).

11.2 Documenting, Recording and Reporting Adverse Events

All AEs occurring from the time the informed consent is signed through the specified 1 year study follow-up period will be documented, recorded, and reported.

At each contact with the subject, information regarding AEs will be elicited by appropriate questioning and examinations and will be:
• immediately documented in the subject's medical record/source document,
• recorded in CRIMSON and
• reported as outlined below (e.g., IND Sponsor, Institutional Review Board [IRB], and Food and Drug Administration [FDA]).

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 reported as an adverse event 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 PI as NOT related to the study agent(s); AND
- It is assessed by the PI as NOT clinically significant (e.g., the abnormal value does NOT suggest a disease or organ toxicity)

All abnormal laboratory findings will be reviewed on a routine bases by the PI 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.

Blinded laboratories, other than absolute eosinophil counts, that meet criteria for AEs will be reported to the study team by the unblinded NIH pathologist and included in the subject's medical record and will be reported as above. Reporting of absolute eosinophil counts during the placebo-controlled portion of the study and/or bone marrow abnormalities at week 12 that meet criteria for an AE will be deferred until the blind is lifted except as specified in Section 6.3.

11.3 Investigator Assessment of Adverse Events

All AEs occurring from the time the informed consent is signed through the end of study visit will be documented, recorded, and reported. The Investigator will evaluate all AEs with respect to **Seriousness** (criteria listed above), **Severity** (intensity or grade), and

Causality (relationship to study agent and relationship to research) according to the following guidelines.

11.3.1 Severity

The “Common Terminology Criteria for Adverse Events (CTCAE)” (v 4.0): http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

11.3.2 Causality

Causality (likelihood that the event is related to the study agent) will be assessed considering the factors listed under the following categories:

Definitely Related
- reasonable temporal relationship
- follows a known response pattern
- clear evidence to suggest a causal relationship
- there is no alternative etiology

Probably Related
- reasonable temporal relationship
- follows a suspected response pattern (based on similar agents)
- no evidence of a more likely alternative etiology

Possibly Related
- reasonable temporal relationship
- little evidence for a more likely alternative etiology

Unlikely Related
- does not have a reasonable temporal relationship

  OR
- good evidence for a more likely alternative etiology

Not Related
- does not have a temporal relationship

  OR
- definitely due to an alternative etiology

Note:
Other factors (e.g., dechallenge, rechallenge) should also be considered for each causality category when appropriate. Causality assessment is based on available information at the time of the assessment of the AE. The investigator may revise the causality assessment as additional information becomes available.

11.4 Investigator Reporting Responsibilities to the Sponsor

11.4.1 Adverse Events

Line listings, frequency tables, and other summary AE data will be submitted to the IND Sponsor when needed for periodic safety assessments, review of IND annual reports, review of IND safety reports, and preparation of final study reports.

11.4.2 Serious Adverse Events

SAEs whether or not they are also UPs) must be reported on the Safety Expedited Report Form (SERF) and sent to the Sponsor Clinical Safety Office (CSO) by fax or e-mail attachment. Deaths and immediately life threatening SAEs must be reported within 1 business day after the site becomes aware of the event. All other SAEs must be reported within 3 business days of site awareness.

SPONSOR CLINICAL SAFETY OFFICE CONTACT INFORMATION:

OCRPRO Clinical Safety Office
5705 Industry Lane
Frederick, MD 21704
Phone 301-846-5301
Fax 301-846-6224
E-mail: rchspsafety@mail.nih.gov

11.4.3 Unanticipated Problems

Unanticipated Problems that are also adverse events must be reported to the CSO and sent by fax or e-mail attachment no later than 7 calendar days of site awareness of the event. UPs that are not AEs are not reported to the Sponsor CSO.

Report all UPs that are also adverse events to the CSO on the NIH Problem Report Form.

11.4.4 Pregnancy

Pregnancy itself is not an AE. However, complications of pregnancies are AEs and may be SAEs. Pertinent obstetrical information for all pregnancies will be reported to the CSO via fax or email within 3 business days from site awareness of the pregnancy.

Pregnancy outcome data (e.g., delivery outcome, spontaneous or elective termination of the pregnancy) will be reported to the CSO within 3 business days of the site's awareness.

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

- The study drug may be discontinued
- The subject will be advised to notify their obstetrician of the study agent exposure
- The subject will be followed up for safety until delivery or termination of the pregnancy
- The event will be reported to the DSMB and IRB

11.5 Investigator Reporting Responsibilities to the IRB and NIAID

11.5.1 NIH IRB Reporting

UPs, non-compliance, and other reportable events will be reported to the NIH IRB according to NIH Human Research Protections Program (HRPP) Policy 801.

11.5.2 Reporting to the NIAID Clinical Director

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

11.6 Investigator Reporting Responsibilities to the Drug Supplier

As per their requirements, AEs, SAEs, pregnancy, and unanticipated problems reported to the sponsor (OCRPRO) will also be reported to the drug supplier (AstraZeneca) within the same time frames described in Section 11.4.

11.7 Follow-up of Adverse Events and Serious Adverse Events

AEs that occur following enrollment of the subject (by signing the informed consent) are followed until the final outcome is known or until the end of the study follow-up period.

SAEs that have not resolved by the end of the follow-up period are followed until final outcome is known. If it is not possible to obtain a final outcome for an SAE (e.g., the subject is lost to follow-up), the reason a final outcome could not be obtained will be recorded by the investigator within CRIMSON and the SERF.

SAEs that occur after the study follow-up period (last study visit) that are reported to and are assessed by the Investigator to be possibly, probably, or definitely related must be reported to the CSO, as described above.

11.8 Sponsor's Reporting Responsibilities

Serious and unexpected suspected adverse reactions (SUSARs) as defined in 21 CFR 312.32 and determined by the IND Sponsor will be reported to FDA and all participating Investigators as IND Safety Reports.

The IND Sponsor will also submit an IND Annual Report of the progress of the investigation to the FDA as defined in 21 CFR 312.33.

11.9 Halting Rules for the Protocol

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

The halting criteria (as determined by the site investigators) for an individual site include:

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

OR

- any safety issue that the site investigators determine should halt the study

The IRB/EC, the IND Sponsor, the DSMB or the FDA may halt the study at any time following review of any safety concerns

11.9.1 Reporting of Study Halting

If a halting requirement is met, a description of the event(s) or safety issue must be reported by the PI within one business day to the Sponsor CSO, DSMB, and AstraZeneca by fax or email. The PI must also inform the IRB that a halting rule has been met.

11.9.2 Resumption of a Halted Study

The IND Sponsor, in collaboration with the PI and DSMB will determine if it is safe to resume the study. The IND Sponsor will notify the PI of this decision. The conditions for resumption of the study will be defined in this notification. The PI will notify the IRB and AstraZeneca of the decision to resume the study.

11.10 Pausing Criteria for a Subject or Group

The decision to suspend administration of the study agent(s) for a single subject or for all subjects in a specific group requires discontinuation of study agent administrated for

the study subject(s) or group until a decision is made whether or not to continue study agent administration.

The pausing criteria for a single subject or for the subjects in a specific group in this study include:

- A subject experiences an SAE or Grade 3 or greater AE that is unexpected (as determined by the IND Sponsor) and is possibly, probably, or definitely related to the study agent;

OR

- Any safety issue that the Site Investigator determines should pause administration of the study agent to a single subject or to all subjects in a specific group.

The IND Sponsor, in collaboration with the PI, may also pause for an individual subject or entire group if a safety concern is identified during routine aggregate data analysis.

11.10.1 Reporting of Pausing for a Subject or Group

If a pausing requirement is met, a description of the adverse event(s) or safety issue must be reported by the PI by fax or email within one business day to the Sponsor CSO, the IRB, the DSMB, and AstraZeneca.

11.10.2 Resumption of a Paused Study

The IND Sponsor in collaboration with the PI and DSMB will determine if it is safe to resume administration of the study agent to the subject/group. The IND Sponsor will notify the PI of this decision. The PI will notify the IRB and AstraZeneca of the decision to resume administration of the study agent prior to resumption.

11.11 Withdrawal Criteria for an Individual Subject

An individual 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 to the extent that it is potentially harmful to the subject or to the integrity of the study data.
- A change in the subject's baseline condition after enrollment so that the subject no longer meets the following exclusion criteria.

    - Malignancy (other than a resectable skin cancer)
    - HIV infection or any other known immunodeficiency.
    - Hepatitis B or C infection
    - Positive test for *FIP1L1/PDGFRA* fusion gene
    - Suspicion of drug or alcohol abuse
    - Treatment with another investigational agent

11.12 Replacement of a Subject Who Discontinues Study Treatment

Subjects who discontinue study treatment after the first dose will not be replaced.

11.13 Unblinding of the study

11.13.1 Scheduled unblinding

The study drug assignments will be unblinded after all 20 subjects have enrolled and completed the 13 week visit. At that time, efficacy and safety data collected through this time point will be analyzed.

11.13.2 Intentional Unscheduled Unblinding for Treatment Conditions

Prior to breaking the treatment blind, the PI will be consulted. The research pharmacist will keep the treatment code list locked in a secure area and can be reached 24 hours a day to rapidly access subject unblinding codes if necessary. If a subject's treatment assignment is unblinded, the information will be provided only to the individuals needing it for treatment decisions, with documentation of the event and the reason for unblinding recorded in the subject's research record.

11.13.3 Reporting of Unblinding

The PI must report all cases of unblinding (whether intentional or unintentional) in writing to the IRB, DSMB and AstraZeneca within 2 business days after the unblinding. If a serious adverse event (SAE) has resulted in unblinding, this information will be included in the SERF. Incidences of unblinding in and of themselves will be reported to the OCRPRO Clinical Safety Office. A detailed report of the event that resulted in the unblinding will be sent to the DSMB, AstraZeneca and the IRB.

11.14 Safety Oversight

11.14.1 Safety Review and Communications Plan (SRCP)

A Safety Review and Communication Plan (SRCP) has been developed for the protocol. The SRCP is an internal communications document between the PI and the CSO, which delineates the safety oversight responsibilities of the PI, the CSO, and other stakeholders. The SRCP also includes the overall plan for conducting periodic safety surveillance assessments.

11.14.2 Sponsor Medical Monitor (SMM)

A Medical Monitor, representing the IND Sponsor (OCRPRO), has been appointed for oversight of safety in this clinical study. The Sponsor Medical Monitor will be

responsible for performing safety assessments as outlined in a Safety Review and Communications Plan (SRCP).

11.14.3 Data and Safety Monitoring Board (DSMB)

The NIAID Intramural DSMB will review the study prior to initiation and twice a year thereafter. The Board may convene additional reviews as necessary. The Board will review the study data to evaluate the safety, efficacy, study progress, and conduct of the study. All serious adverse events, all unanticipated problems, and all IND Safety Reports will be reported by the PI to the DSMB at the same time they are submitted to the IRB or IND Sponsor. The PI will notify the DSMB of any cases of intentional or unintentional unblinding as soon as possible. The PI will notify the Board at the time pausing or halting criteria are met and obtain a recommendation concerning continuation, modification, or termination of the study. The PI will submit the written DSMB summary reports with recommendations to the IRB.

11.14.4 Unblinded Pathologist

An NIH pathologist who is not involved in the clinical care of the study subjects will review all blinded CBCs and differentials obtained during the placebo-controlled portion of the study within 24 hours of the blood draw. Significant abnormalities (i.e., laboratory values that meet criteria for a grade 3 or 4 AE, SAE or that the pathologist deems clinically significant),will be reported to the PI and study coordinator immediately. Other laboratory abnormalities that represent grade 1 or 2 AEs and require no specific medical intervention, with the exception of the absolute eosinophil counts, will be reported to the PI and study coordinator in a weekly summary table.

12 Clinical Monitoring Structure

12.1 Site Monitoring Plan

As per ICH-GCP 5.18 and FDA 21 CFR 312.50, clinical protocols are required to be adequately monitored by the study sponsor. This study monitoring will be conducted according to the “NIAID Intramural Clinical Monitoring Guidelines. Monitors under contract to the NIAID/OCRPRO will visit the clinical research site to monitor aspects of the study in accordance with the appropriate regulations and the approved protocol. The objectives of a monitoring visit will be: 1) to verify the existence of signed informed consent documents and documentation of the ICF process for each monitored subject; 2) to verify the prompt and accurate recording of all monitored data points, and prompt reporting of all SAEs; 3) to compare CRIMSON data abstracts with individual subjects’ records and source documents (subjects’ charts, laboratory analyses and test results, physicians’ progress notes, nurses’ notes, and any other relevant original subject information); and 4) to help ensure investigators’ are in compliance with the protocol. The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements (Office for Human Research Protections-OHRP), *FDA,* and applicable

guidelines (ICH-GCP) are being followed. During the monitoring visits, the investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit.

The investigator (and/or designee) will make study documents (e.g., consent forms, CRIMSON data abstracts and pertinent hospital or clinical records) readily available for inspection by the local IRB, the FDA, the site monitors, and the NIAID staff for confirmation of the study data.

A specific protocol monitoring plan will be discussed with the Principal Investigator and study staff prior to enrollment. The plan will outline the frequency of monitoring visits based on such factors as study enrollment, data collection status and regulatory obligations.

13 Statistical Considerations

13.1 Study Hypotheses

This randomized, placebo-controlled, double-blind Phase 2 study followed by an open-label extension is designed to test the hypothesis that benralizumab can safely reduce eosinophilia in patients with HES.

13.2 Sample Size Justification

In addition to potentially eligible subjects already followed on protocol 94-I-0079, we receive 1-2 new patient referrals/week for evaluation and treatment of HES. Furthermore, based on prior experience, we anticipate an increase in referrals with the availability of a new clinical trial. Thus, it is likely that recruitment of 20 subjects will be feasible in a 2 year time frame.

The primary endpoint is a binary response (50% reduction in peripheral eosinophil count at 12 weeks). To compare the 2 groups, we will perform a one-sided Fisher's exact test at the 2.5% level (this is essentially the same as a two-sided central Fisher's exact test at the 5% level). For the power calculations, we make some assumptions. First, we assume that we have 10 subjects in each arm of the study. We expect that at 12 weeks virtually none of the subjects randomized to placebo will have a response, so we set that value to 1%. We calculate the power for different levels of response for the subjects on benralizumab. The results are listed inTable 2. Thus, we need 60% responders in the benralizumab group in order to get 79% power to reject at the one-sided 2.5% level (or equivalently at the two-sided 5% level) if we have 10 in each group as planned. If we are only able to recruit 6 in each group by the end of the 2 year accrual, then we need 90% responders to get over 86.4% power.

Table 2 Power Estimates

Percent Responders	Percent Responders on	Power to Reject (in Percent)	Power to Reject (in Percent)	Power to Reject (in Percent)

on Placebo	Benralizumab	N=10 per group	N=8 per group	N=6 per group
1	50	57.9	34.6	10.4
1	60	79.0	57.2	22.2
1	70	92.3	78.5	40.2
1	80	98.2	93.2	63.2
1	90	99.8	99.2	86.4

13.3 Description of the Analyses

- **Safety Analysis**: All safety data will be summarized with descriptive statistics (number of subjects [N], mean, standard deviation [SD], median, minimum, and maximum) for continuous variables, and frequency and percentage for discrete variables. The safety summary will include AEs, serious adverse events (SAEs), clinical laboratory data, vital signs, physical examination, and ECG data. All individual subject data will be listed by subject.
- **Efficacy Analysis**: The primary analysis compares the proportion of subjects with a ≥50 % change in AEC at 12 weeks in the 2 randomized arms. If there is dropout, the primary analysis will be the intent-to-treat analysis, which includes all subjects who received the Day 0 treatment. We will compare with a one-sided Fisher's exact test at the 2.5% level of significance, or equivalently a two-sided central Fisher's exact test at the 5% level.
- For the secondary analysis comparing the 2 groups on the post-treatment AEC at 12 weeks as a percentage of the pre-treatment AEC, we use a Wilcoxon-Mann-Whitney test.
- There are other secondary endpoints with continuous responses that measure a change from pre-treatment to after 12 weeks of treatment: for example, % bone marrow eosinophils, and % bone marrow myeloid precursors. These will be analyzed similarly to the percentage of pretreatment AEC.
- As exploratory analyses, we may use a Mixed-Effect Model Repeated Measure (MMRM) model to analyze changes in AEC over time. The dependent variable will be the percentage of baseline AEC at post-baseline protocol-specified visits (up to the 12 week visit). Treatment group will be fitted as the explanatory variable, together with visit, the interaction between visit and treatment. Visit will be fitted as a categorical variable, and the variance-covariance matrix will be assumed to be unstructured. If the procedure does not converge, then a compound symmetric variance-covariance matrix will be used instead.
- **Calculation or derivation of pharmacokinetic variables**
  The PK analyses will be performed at (or under the guidance of) AstraZeneca Research and Development.

  Due to the limited sampling schedule, the PK assessment will be primarily based on the observed steady-state serum trough (predose) concentrations, C_{trough}. Empirical evaluation of potential impact of demographic covariates and ADA on C_{trough} will be conducted. Serum concentrations of benralizumab, summary statistics, empirical covariate analysis results and PK profiles will be provided in a clinical PK report (an addendum to the CSR).

To further characterize the pharmacokinetic properties of benralizumab in HES, the PK data will be merged with those from other clinical studies for a population-based, which will be presented in a separate pharmacometrics report outside of the CSR.

- **Calculation or derivation of immunogenicity variables**
  ADA assessments will be conducted utilizing a tiered approach (screen, confirm, titer). Evaluations will be made at baseline (prior to benralizumab administration), and at specified time points during the treatment and follow up phases of the study or discontinuation. ADA titers by treatment group and visit will be summarized. The association of ADA status (positive or negative) with treatment-emergent adverse events (TEAEs)/ treatment-emergent serious adverse events (TESAEs), benralizumab concentration and blood eosinophil levels will be evaluated. In addition, the association of ADA titers (≥median titer vs. < median titer) with TEAEs/ TESAEs, benralizumab concentration and blood eosinophil levels will be evaluated for ADA positive subjects only.

13.4 Final Analysis Plan

The primary endpoint is the number of subjects with a ≥50% change in AEC. The details of the statistical tests used for this endpoint is described in Section 13.3. For this test and all tests, we define significance using a two-sided 5% level or equivalently a one-sided 2.5% level. If the study shows that there is a significant reduction in AEC, this would merit further study of benralizumab in HES. This design relies on the assumption that the type of HES patients included in this study would not have 50% improvement in their eosinophil count in the absence of a change in therapy. This is a well-established assumption in the HES research community.

This single center trial has 2 parts: 1) a randomized, double-blind comparison of sc benralizumab vs. placebo every 4 weeks for 12 weeks, 2) an open-label extension of sc benralizumab every 4 weeks for 12-36 weeks. The primary endpoint and secondary and exploratory endpoints based on the data from the first part of the study will be analyzed once all 20 subjects have completed the 13 weeks visit and data is unblinded.

Since there are only 20 patients in the study, both an intent-to-treat and a per-protocol analysis may be done. For the intent-to-treat analysis, we will impute a “worst case” post-treatment AEC, whereby we will use the maximum of the baseline AEC or the last AEC as the post-treatment AEC for analysis. If a patient does not complete treatment, that patient may be removed from the per-protocol analysis.

Although we do not specify the exploratory analyses in advance, we do note that care will be taken in making inferences when many analyses are undertaken. If needed multiple comparison adjustments will be made.

14 Ethics/Protection of Human Subjects

14.1 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 on-going 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.

14.2 Subject Confidentiality

All records will be kept confidential to the extent provided by federal, state and local law. The study monitors and other authorized representatives of the Sponsor 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 all computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by IRB, the FDA, the NIAID, the OHRP, AstraZeneca or the sponsor's designee.

15 Data Handling and Record Keeping

15.1 Data Capture and Management

Study data will be maintained in CRIMSON and collected directly from subjects during study visits and telephone calls, 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.

If a subject receives drug at an outside site on the long-term extension,(after week 48), the outside PI will provide documentation of drug administration, AE/SAEs and laboratory testing as required by the protocol. These will be entered into CRIMSON by the NIH study team.

15.2 Record Retention

The investigator is responsible for retaining all essential documents listed in the ICH Good Clinical Practice Guideline. Study records will be maintained by the PI for a minimum of 3 years and in compliance with institutional, IRB, state, and federal medical records retention requirements, whichever is longest. All stored records will be kept confidential to the extent required by federal, state, and local law.

Should the investigator wish to assign the study records to another party and/or move them to another location, the investigator will provide written notification of such intent to OCRPRO/NIAID with the name of the person who will accept responsibility for the transferred records and/or their new location. Destruction or relocation of research records will not proceed without written permission from NIAID/OCRPRO.

Appendix A: Scientific References

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Appendix B: Schedule of Procedures/Evaluations

Events	Baseline	Placebo-controlled Phase							Open Label Phase													EXTENSION	
	Day 0 to -14	Day 0	Days 1,2,3	Wk 1,2, 3	Wk 4	Wk 6	Wk 8	Wk 10	Wk 12	Days 85,86, 87	Wk 13,14 ,15	Wk 16	Wk 18	Wk 20	Wk 22	Wk 24	Wk 28	Wk 32	Wk 36	Wk 40, 44	Wk 48	Q 6 Mon ths	Q12 Mont hs
	Window			± 2d	± 2d	± 3d	± 3d	± 3d	± 3d		± 2d	± 2d	± 3d	± 3d	± 3d	± 3d	± 4d	± 4d	± 4d	± 4d	± 4d	± 7d	± 7d
Informed consent	X																						
Complete medical history and physical examination	X																						
Targeted medical history, including HES symptom questionnaire		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Targeted physical examination		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital signs, including weight	X	X[6]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Study Drug																							
Study Drug administration[1]		X			X		X		X			X		X		X[3]	X[3]	X[3]	X[3]	X[3]			
tudy Procedures																							
Tdap vaccine															X[2]								
ECG (12-lead)	X								X							X			X		X		X
Echocardiogram	X								X							X			X		X		X
Pulmonary function tests	X								X							X			X		X		X
Bone marrow aspirate and	X								X														

biopsy																						
Tissue biopsy of affected organs	X[4]								X[4]							X[4]			X[4]		X[4]		X[4]
Clinical and safety laboratory testing																							
Electrolyte Panel	X	X[6]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Hepatic Panel	X	X[6]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Mineral Panel	X	X[6]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CBC w/ differential	X	X[6]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PT/PTT	X								X														
ESR, CRP	X				X		X		X			X		X		X	X	X	X	X	X	X	X[7]
LDH, CPK	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Total Protein	X				X				X			X		X		X	X	X	X	X	X	X	X[7]
Troponin	X								X							X			X		X		X[7]
Uric acid	X				×		×		X			×		×		X	×	×	X	×	X	×	X[7]
HbgA1c	X								X							X							
Urinalysis	X	X[6]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Serum pregnancy test	X[5]	X[5,6]			X[5]		X[5]		X[5]			X[5]		X[5]		X[5]	X[5]	X[5]	X[5]	X[5]	X[5]	X[5]	X[5]
Serum B12 levels	X								X							X			X		X		X[7]
Serum tryptase levels	X	X[6]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Serum immunoglobulin levels	X								X							X			X		X		X
Serum IgE levels	X								X							X			X		X		X
Tetanus Toxoid IgG Antibody									X[2]								X[2]						
Research laboratory studies																							

Eosinophil and PBMC purification from whole blood	X								X							X			X		X		X
Serum, plasma and urine for eosinophil granule protein and mediator levels	X	X[6]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X[10]
Serum for PK studies and ADA measurement	X				X		X		X			X		X		X	X	X	X	X	X	X	X
Bone marrow flow cytometry	X								X														
T and B cell receptor rearrangement studies	X								X												X		X
IL-5 Receptor expression	X								X							X			X		X		
Whole Blood pre/post		X							X														
TBNK cell subsets									X							X			X		X		X
Study Assessments																							
Assessment of end organ involvement	X								X							X			X		X		X
Concomitant medication history	X	X[6]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Collection of adverse events		X[6]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Appendix C: Blood Volumes for Specimen Collection

Evaluations	**Study Schedule**																													
[1] Visit	**BL 01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
Week of Study	0	1	1	1	1	1	2	3	4	6	8	10	12	12	12	12	13	14	15	16	18	20	22	24	28	32	36	40	44	48
Day of Study	-14 to 0	[4]0	1	2	3	7	14	21	28	42	56	70	84	85	86	87	91	98	105	112	126	140	154	168	196	224	252	280	308	336
Clinical																														
Chemistry	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
CBC/diff	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
PT/PTT	4.5												4.5																	
ESR, CRP	5								5		5		5							5		5		5	5	5	5	5	5	5
LDH, CPK	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Troponin	4												4											4			4			4
HbA1c	3												3											3						
Pregnancy	4								4		4		4							4		4		4	4	4	4	4	4	4
Serum B12	4												4											4			4			4
Serum	4												4											4			4			4
Serum Ig	4												4											4			4			4
Serum IgE	4												4											4			4			4
Tetanus Ab																							4		4					
Research																														
Cell	50												50											60			60			60
Serum	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
Plasma Storage	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
PK, ADA	6								6		6		6							6		6		6	6	6	6	6	6	6
TCR, BCR	4.5												4.5																	4.5
Flow	9												9											9			9			9
WholeBlood		20											20																	
Daily Vol (mL)	133	47	27	27	27	27	27	27	42	27	42	27	153	27	27	27	27	27	27	42	27	42	31	134	44	42	131	42	42	136
Cum Vol (mL)	133	180	207	234	261	288	315	342	384	411	**453**	480	633	660	687	714	741	768	795	**837**	864	906	937	**1071**	1115	**1157**	1288	**1330**	1372	**1508**
Vol/8 wks											**453**									**357**				**399**		**86**		**173**		**178**

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 8week period. Exceptions to this policy shall be approved by the IRB.

Appendix D: Research Studies

Research blood and bone marrow will be collected at a variety of timepoints to 1) assess the effects of benralizumab on eosinophil activation and other biomarkers of disease activity in HES, 2) assess the effects of benralizumab on basophils, mast cells and eosinophil precursors, 3) identify predictors of response/resistance to benralizumab, 4) explore the immunologic consequences of eosinophil depletion, including the effects of plasma cells and vaccine responses, and 5) examine the role of eosinophil and/or NK activation in post-injection reactions.

1) Eosinophil activation and biomarkers of disease activity in HES

Eosinophil activation will be assessed in a variety of ways including:

- measurement of serum levels of eosinophil degranulation products (MBP, ECP, EDN, EPO) using a suspension array assay in multiplex
- assessment of eosinophil surface marker expression, including CD69 and CD25, in whole blood and bone marrow aspirates by flow cytometry
- immunohistochemical staining of tissue biopsies obtained for clinical indications for degranulation products

Although validated biomarkers of disease activity in HES have not been identified to date, a number of mediators, including cytokines (IL-5, chemokines (i.e., TARC, eotaxin), and soluble receptors (i.e., soluble IL-5 receptor alpha, soluble CD25), have been shown to correlate with disease activity in some settings. Since subjects will be maintained on a stable HES treatment regimen for the first 12 weeks of benralizumab therapy, blood samples obtained immediately prior to and after 12 weeks of therapy will be used to explore potential biomarkers. Eosinophils, peripheral blood mononuclear cells and plasma will be purified from peripheral blood (60 cc) using standard methods. Plasma will be stored at -80^{o}C. Depending on the total numbers of cells isolated, the purified eosinophils and PBMC may be processed for RNA, viably frozen in liquid nitrogen (PBMC only) and/or snap frozen for proteomic analysis.

2) Basophil, mast cell and eosinophil precursors

- 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.

- Mast cells: Mast cells will be quantified and assessed for spindle-shape (activation) in bone marrow biopsy specimens stained for tryptase and CD117 before and during benralizumab therapy. Flow cytometry of the aspirate specimens will be used to assess surface markers of activation (CD2 and CD25). Serum tryptase levels will also be assessed.

- Eosinophil precursors: Eosinophil precursors will be assessed morphologically and by flow cytometry using antibodies to IL-5R and CD34 before and after 12 weeks of benralizumab therapy.

3) Predictors of response/resistance to benralizumab

- Prior studies have shown a correlation between decreased IL-5R levels on the surface of eosinophils and increased soluble IL-5R levels in serum (7). Consequently, surface expression of IL-5R will be quantified by whole blood flow of eosinophils using Quantibrite beads and soluble IL-5R will be measured by ELISA before and at 12, 24, 36 and 48 weeks of benralizumab therapy.

- The presence of anti-drug antibodies will be assessed before and at specified time points during the study. Drug levels will also be assessed.

4) Immunologic consequences of benralizumab therapy

Activated eosinophils are a source of a wide variety of cytokines, chemokines, and other mediators. Thus, depletion of eosinophils is in patients with HES is likely to have secondary effects on other immune cells. Whole blood flow cytometry will be perfomed at baseline and every 12 weeks on benralizumab therapy to look at T/B/NK cell numbers, activation status and cytokine production. In addition, PBMC will be purified from whole blood at the same time points for generation of supernatants for cytokine analyses by suspension array in multiplex and isolation of RNA for microarray and/or RNAseq analysis.

- In view of murine studies demonstrating that eosinophil-deficient mice have decreased plasma cells and impaired repsonses to secondary immunization with T cell dependent antigens (12), plasma cells will be quantified in the bone marrow prior to and after 12 weeks of benralizumab therapy. In addition, anti-tetanus antibody responses will be assessed in all subjects at week 28 (6 weeks following immunization).

5) Mechanism of post-treatment reactions

- Post-injection reactions have been observed in some, but not all, subjects 4-6 hours following the first dose of benralizumab vs. placebo or the first dose of open-label benralizumab. These reactions have been characterized by fever, nausea, fatigue and mylagias and a rise in LDH. Reactions have not been observed following injections at other study time points and the severity of the reaction does not appear to be related to pre-treatment eosinophil count. Similar reactions have not been described in studies of benralizumab in subjects with asthma, but were seen in some subjects in phase i dose finding studies.

- Potential mechanisms for these reactions include 1) NK activation and cytokine release and/or 2) eosinophil lysis. In order to begin to examine these possibiltiies, whole blood flow will be performed to assess markers of activation and degranulation of NK cells (CD63 and CD107a) and eosinophils (CD69 and CD63) prior to and at 3-6 hours post the study drug injection at week 0 and week 12. Plasma levels of cytokines, granule proteins and other inflammatory markers will also be assessed.

Appendix E: HES Questionnaire

Symptom	Y	N	If yes, please specify:
Itching Rashes?			
Fatigue?			
Joint Pain?			
Muscle Aches?			
Chest Pain?			
Shortness of breath or wheezing?			
Abdominal pain or GI symptoms?			
Neuropathy?			
Hives?			
Angioedema?			
Allergies or sinus symptoms?			
Mouth sores?			
Fever?			
Weight loss?			
Lymphadenopathy?			

Appendix F: FASENRA PEN: Instructions for Use

Instructions for Use
FASENRA PEN™ (fas-en-rah)
(benralizumab)
for Subcutaneous Injection
Single-dose Autoinjector

Before using your FASENRA PEN, your healthcare provider should show you or your caregiver how to use it the right way.

Read this Instructions for Use before you start using your FASENRA PEN and each time you get a refill. There may be new information. This information does not take the place of talking to your healthcare provider about your medical condition or your treatment.

If you or your caregiver have any questions, talk to your healthcare provider.

Important information:

- **Store FASENRA in a refrigerator between** 36°F to 46°F (2°C to 8°C) **in its original carton until you are ready to use it**.
- FASENRA may be kept at room temperature between 68°F to 77°F (20°C to 25°C) for a maximum of 14 days.
- Once removed from the refrigerator and brought to room temperature, FASENRA must be used within 14 days or thrown away (disposed of).

Do not use your FASENRA PEN if:

- it has been frozen
- it has been dropped or damaged
- the security seal on the carton has been broken
- the expiration date (EXP) has passed

Do not:

- shake your FASENRA PEN
- share or reuse your FASENRA PEN
- expose your FASENRA PEN to heat

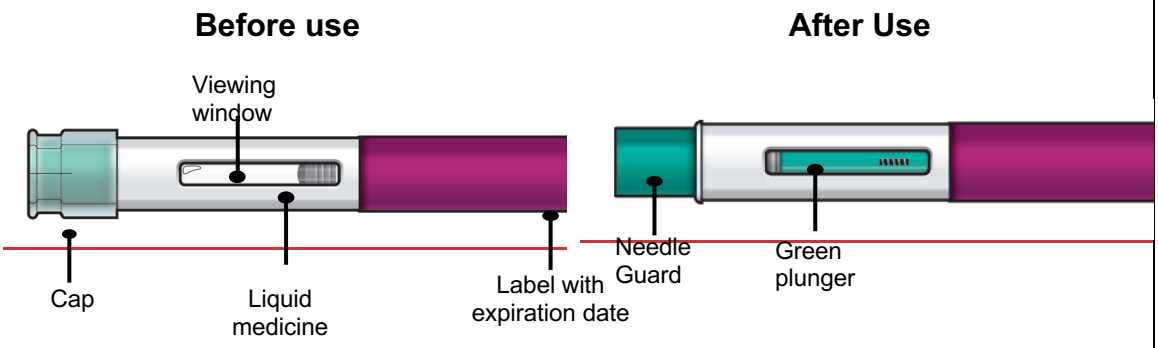
If any of these happen, throw away the FASENRA PEN in a puncture-resistant sharps disposal container and use a new FASENRA PEN.

Each FASENRA PEN contains 1 dose of FASENRA that is for one time use only.

Keep FASENRA and all medicines out of the sight and reach of children.

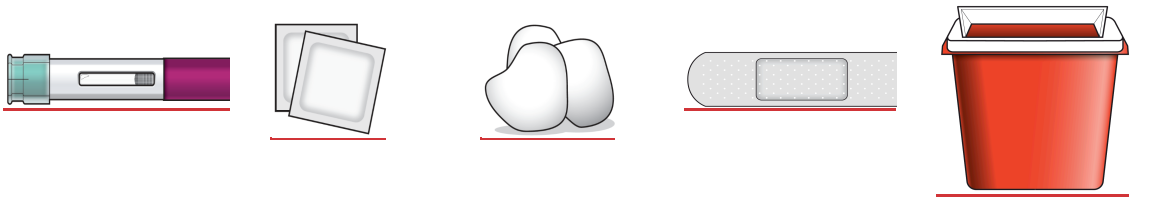
Your FASENRA PEN

Do not remove the cap until you have reached Step 6 of these instructions and are ready to inject FASENRA.



Step 1 – Gather supplies

- 1 FASENRA PEN from the refrigerator
- 1 alcohol wipe
- 1 cotton ball or gauze
- 1 bandage
- 1 puncture-resistant sharps disposal container. See Step 10 for instructions on how to throw away (dispose of) the used FASENRA PEN safely.



FASENRA PEN | Alcohol wipe | Cotton ball or gauze | Bandage | Sharps container

Step 2 – Prepare to use your FASENRA PEN

Check the expiration date (EXP). Do not use if the expiration date has passed.

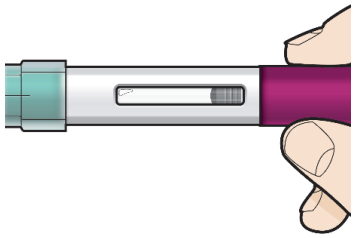
Let FASENRA warm up at room temperature between 68°F to 77°F (20°C to 25°C) **for about 30 minutes before giving the injection**.

Do not warm the FASENRA PEN in any other way. For example, do not warm it in a microwave or hot water, or put it near other heat sources.

Use FASENRA within 14 days of removing from the refrigerator. After 14 days, throw away the FASENRA PEN.

Do not remove the cap until you have reached Step 6.

Step 3 – Check the liquid

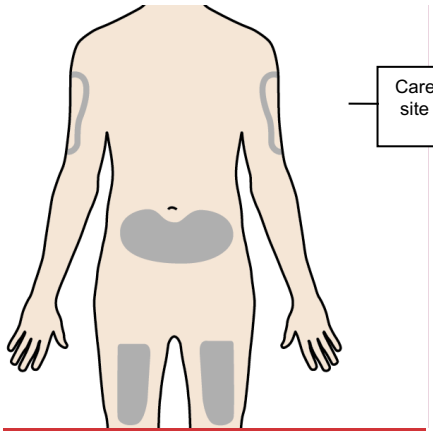


Look at the liquid in the FASENRA PEN through the viewing window. The liquid should be clear and colorless to slightly yellow. It may contain small white particles.

Do not inject FASENRA if the liquid is cloudy, discolored, or contains large particles.

You may see small air bubbles in the liquid. This is normal. You do not need to do anything about it.

Step 4 – Choose the injection site



If you are giving yourself the injection, the **recommended injection site** is the front of your thigh or the lower part of your stomach (abdomen).

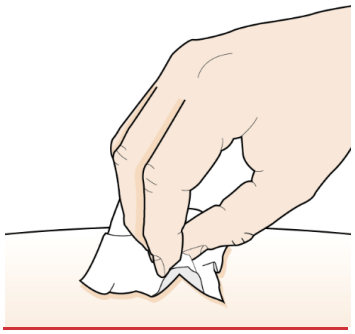
A caregiver may inject you in the upper-arm, thigh, or abdomen. **Do not** try to inject yourself in the arm.

For each injection, choose a different site that is at least 1-inch (3-cm) away from where you last injected.

Do not inject:

- into the 2-inch (5-cm) area around your belly-button
- where the skin is tender, bruised, scaly or hard
- into scars or damaged skin
- through clothing

Step 5 – Clean the injection site



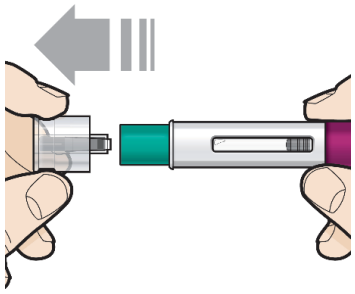
Wash your hands well with soap and water.

Clean the injection site with an alcohol wipe in a circular motion. Let it air dry.

Do not touch the cleaned area before injecting.

Do not fan or blow on the cleaned area.

Step 6 – Pull off the cap



Hold the FASENRA PEN with 1 hand. Carefully pull the cap straight off with your other hand.

Put the cap aside to throw away later.

The green needle guard is now exposed. It is there to prevent you from touching the needle.

Do not try to touch the needle or push on the needle guard with your finger.

Do not try to put the cap back on the FASENRA PEN. You could cause the injection to happen too soon or damage the needle.

Complete the following steps right away after removing the cap.

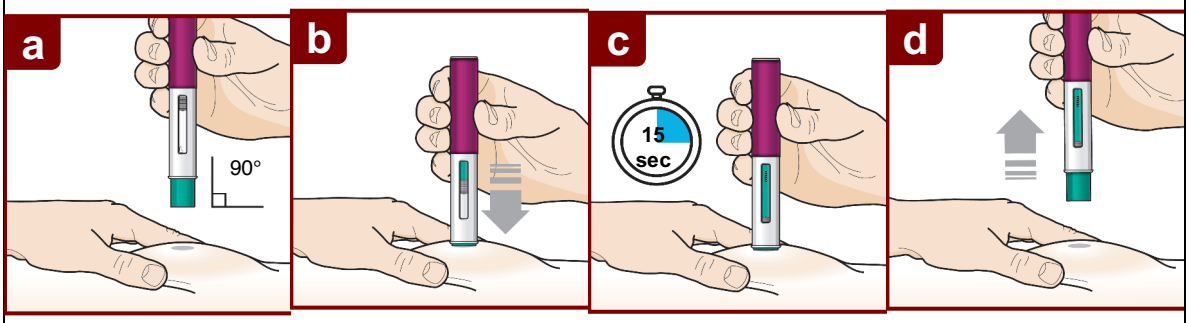
Step 7 – Inject FASENRA

Follow your healthcare providers instructions on how to inject. You can either gently pinch at the injection site or give the injection without pinching the skin.

Inject FASENRA by following the steps in figures **a, b, c,** and **d**.

Hold the FASENRA PEN in place for the entire injection.

Do not change the position of the FASENRA PEN after the injection has started.



Position the FASENRA PEN at the injection site.

Place the needle guard of the FASENRA PEN flat against your skin (90 degree angle).
Make sure you can see the viewing window.

Press down firmly.

You will hear a click. A **'click'** tells you the injection has started. The green plunger will move down in the viewing window during the injection.

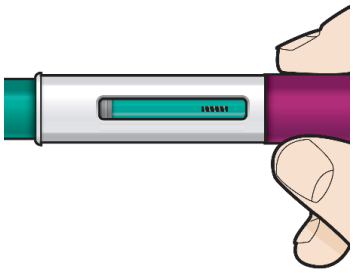
Hold down firmly for 15 seconds.

You will hear a second **'click'**. The second click tells you the injection has finished. The green plunger will fill the viewing window.

Lift the FASENRA PEN straight up.

The needle guard will slide down and lock into place over the needle.

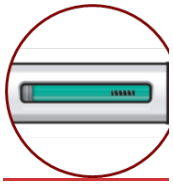
Step 8 – Check the viewing window



Check the viewing window to make sure all the liquid has been injected.

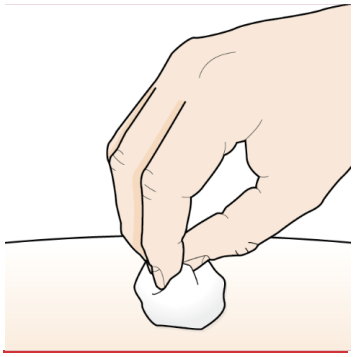
If the green plunger does not fill the viewing window, you may not have received the full dose. If this happens or if you have any other concerns, call your healthcare provider.

Before Injection



After Injection

Step 9 – Check the injection site



There may be a small amount of blood or liquid where you injected. This is normal.

Gently hold pressure over your skin with a cotton ball or gauze until the bleeding stops.

Do not rub the injection site.

If needed, cover the injection site with a small bandage.

Step 10 – Dispose of the used FASENRA PEN safely

- Each FASENRA PEN contains a single dose of FASENRA and **cannot be reused.**
- Put your used FASENRA PEN in a FDA-cleared **sharps disposal container** right away after use.

Do not throw away the FASENRA PEN in your household trash.

Throw away the cap and other used supplies in your household trash.

Disposal guidelines

If you do not have a FDA-cleared sharps disposal container, you may use a household container that is:

- made of a heavy-duty plastic,
- can be closed with a tight-fitting puncture-resistant lid, without sharps being able to come out,
- upright and stable during use,
- leak-resistant, and
- properly labeled to warn of hazardous waste inside the container.

When your sharps disposal container is almost full, you will need to follow your community guidelines for the right way to dispose of your sharps disposal container. There may be state or local laws about how you should throw away

used needles and syringes. For more information about safe sharps disposal, and for specific information about sharps disposal in the area that you live in, go to the FDA's website at: http://www.fda.gov/safesharpsdisposal.

Do not dispose of your used sharps disposal container in your household trash unless your community guidelines permit this.
Do not recycle your used sharps disposal container.

For more information go to www.FasenraPen.com or call 1-800-236-9933. If you still have questions, call your healthcare provider.

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