Immune
Tolerance
Network

Anti-TSLP (AMG 157) plus antigen-specific immunotherapy for induction of tolerance in individuals with cat allergy

Protocol ITN057AD

Version 6.0 (August 14, 2017)

IND # 117,529

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Protocol Approval

Trial ID: ITN057AD	**Protocol Version: 6.0**
	Date: August 14, 2017
IND # 117,529	**Protocol Chair:** Jonathan Corren, MD
Title: Anti-TSLP (AMG 157) plus antigen-specific immunotherapy for induction of tolerance in individuals with cat allergy	

I confirm that I have read the above protocol in the latest version. I understand it, and I will work according to the principles of good clinical practice (GCP) as described in the US Code of Federal Regulations (CFR)—45 CFR part 46 and 21 CFR parts 50, 56, and 312, and in the International Conference on Harmonization (ICH) document *Guidance for Industry: E6 Good Clinical Practice: Consolidated Guidance* dated April 1996. Further, I will conduct the study in keeping with local legal and regulatory requirements.

As the principal investigator, I agree to carry out the study by the criteria written in the protocol and understand that no changes can be made to this protocol without written permission of the NIAID.

By participating in this protocol, investigators and their designees also agree to:

i) Use AMG 157 and placebo for AMG 157 only in accordance with the Protocol and for no other Purpose
ii) Not transfer AMG 157 or Placebo for AMG 157 to any parties other than the Distributor identified by the NIAID
iii) Not chemically modify, replicate, make derivatives of, or reverse engineer AMG 157 or Placebo for AMG 157

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Principal Investigator **(*Print*)**

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Principal Investigator **(*Sign*)** **Date**

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Synopsis

Title	Anti-TSLP plus antigen-specific immunotherapy for induction of tolerance in individuals with cat allergy
IND Sponsor	NIAID
Conducted by	Immune Tolerance Network
Protocol Chair(s)	Jonathan Corren, MD
Accrual Objective	121 participants total enrollment
Study Design and Treatment	This is a randomized, placebo-controlled trial in which four groups of cat-allergic subjects are treated until week 48 and then are followed without additional treatment until week 104. Allergy-related endpoints and exploratory endpoints related to mechanisms of immune modulation and tolerance will be assessed at baseline and at 26, 52, 78 and 104 weeks. Starting 1-3 days prior to immunotherapy or placebo for immunotherapy, AMG 157 or placebo will be administered intravenously using investigational product supplied by the manufacturer. AMG 157 will then be administered once every 4 weeks at a dose of 700 mg IV. Each AMG 157 dose will be administered at least 1 day before immunotherapy through week 24, then on the same day as immunotherapy thereafter. The treatment period for AMG 157 or placebo will be 48 weeks. Concurrently, immunotherapy using a standard cat allergen extract or placebo will be administered subcutaneously with a cluster dose and schedule currently in widespread use in the clinical practice of allergy in the United States. There will be an up-dosing period (approximately 12 weeks) during which small doses are gradually increased to a maintenance dose. Dosing levels may be individually tailored based on subject response. The combined up-dosing and maintenance periods will total 48 weeks.
Study Duration	Total study duration will be 154 weeks: • Study enrollment to occur over 46 weeks. • Individual subject study participation will be 108 weeks.
Primary Objective	To determine if anti-TSLP in conjunction with antigen-specific immunotherapy can induce tolerance to cat allergen.

Primary Endpoint

Total nasal symptom score (TNSS) AUC from 0 to 1 hour after cat allergen challenge at 104 weeks. TNSS is calculated with a scale of 0 to 3 on the 4 parameters of sneezing, rhinorrhea, nasal congestion and blockade, and pruritus. Maximum score is 12

Secondary Endpoints

Other measures of allergic response including:

- Skin prick test endpoint titration at baseline, 1, 4, 12, 26, 52, 78, 104 weeks.
- Skin early phase response (EPR) to intradermal testing at baseline, 26, 52, 104 weeks. EPR is the skin response at 15 minutes.
- Skin late phase response (LPR) to intradermal testing at baseline, 26, 52, 104 weeks. LPR is the skin response at 6 hours.
- Peak TNSS EPR at baseline, 26, 52, 78, 104 weeks. Peak is the highest value recorded between 0 and 1 hour inclusive
- TNSS EPR at baseline, 26, 52, 78, 104 weeks. EPR is the TNSS AUC from 0 to 1 hour.
- TNSS LPR at baseline, 26, 52, and 104 weeks. LPR is the TNSS AUC from 5 to 6 hours.
- Peak nasal inspiratory flow (PNIF) LPR AUC at baseline, 26, 52, 104 weeks. LPR is the PNIF AUC from5 to 6 hours.
- PNIF EPR AUC at baseline, 26, 52, 78, 104 weeks. EPR is the PNIF AUC from 0 to 1 hour.

Inclusion Criteria

Patients must meet *all* of the following criteria to be eligible for this study:

1. Age 18-65 years.
2. Removed in protocol version 4.0.
3. History of moderate-severe allergic rhinitis (AR) caused by cat exposure for at least 2 years.
4. Skin prick test wheal greater than or equal to 5 mm to standardized cat extract.
5. Removed in protocol version 3.0.
6. Screening nasal allergen challenge in which:
    - TNSS is less than or equal to 3 after the 0 concentration (vehicle control only) dose,

- TNSS increase is less than or equal to 1 from the TNSS prior to allergen administration to the TNSS after the 0 concentration (vehicle control only) dose,

- TNSS is greater than or equal to 8 after the highest dose,

- Between the first non-zero dose and 10 minutes after the highest dose, either:

  - 3 or more sneezes are counted or

  - Greater than 20% drop in PNIF is recorded.

7. Body mass index (BMI) is greater or equal to 18.0 and less than or equal to 35.0 kg/m^2, at screening.

8. Clinically acceptable physical examination and electrocardiogram (ECG) results (12-lead reporting RR, PR, QRS, QT and QTcF) prior to Day 0 based on the opinion of the investigator.

9. Adequate renal function defined as CrCl greater than 80 mL/min using the Cockcroft Gault equation.

10. For women of childbearing age, a willingness to use a highly effective form of contraception for four months after last dose of study medication. Highly effective methods of birth control include abstinence, vasectomy by the male partner, or a condom with spermicide in combination with either hormonal birth control, IUD or barrier methods used by the woman.

11. For men with female partners of childbearing potential, agreement not to donate sperm and to inform their female partner of their participation in this clinical study and use highly effective methods of birth control for four months after last dose of study medication. Highly effective methods of birth control include abstinence, vasectomy, or a condom with spermicide in combination with either hormonal birth control, IUD or barrier methods used by the woman.

12. The ability to give informed consent and comply with study procedures.

13. Not currently taking and, for the duration of the study,

agreement not to take any form of immunotherapy, including sublingual, subcutaneous or investigational immunotherapy.

Exclusion Criteria

Patients who meet any of the following criteria will *not* be eligible for this study:

1. Removed in protocol version 4.0.
2. Prebronchodilator FEV1 less than 80% of predicted value at screening visit.
3. Removed in protocol version 3.0.
4. History of asthma meeting the NAEPP EPR3 classification of mild-persistent or worse in the past year, other than with cat exposure, requiring regular inhaled corticosteroids for greater than 4 weeks per year.
5. History of serious chronic medical conditions which might interfere with treatment or assessments.
6. History of emergency visit or hospital admission for asthma in the previous 12 months.
7. History of chronic obstructive pulmonary disease (COPD).
8. History of significant recurrent acute sinusitis, defined as 2 episodes per year for the last 2 years, all of which required antibiotic treatment.
9. History of chronic sinusitis, defined as a sinus symptoms lasting greater than 12 weeks that includes 2 or more major factors or 1 major factor and 2 minor factors. Major factors are defined as facial pain or pressure, nasal obstruction or blockage, purulent or discolored postnasal discharge, purulence in nasal cavity, or impaired or loss of smell. Minor factors are defined as headache, fever, halitosis, fatigue, dental pain, cough, and ear pain, pressure, or fullness.
10. History of systemic disease affecting the immune system such as autoimmune diseases, immune complex disease, or immunodeficiency, where, in the opinion of the study physician, participation in the trial would pose a risk or significant effect on the immune system.
11. Type I or type II diabetes.
12. Evidence of any clinically significant active or suspected

bacterial, viral, fungal or parasitic infections within 14 days prior to screening nasal allergen challenge. Participants may be re-evaluated for eligibility after an appropriate course of treatment has been completed and symptoms have resolved.

13. High risk of parasitic disease as judged by the investigator.
14. Positive QuantiFERON® tuberculin test unless the potential subject has been treated with appropriate chemoprophylaxis.
15. Exposure to an individual with active tuberculosis within six months from randomization.
16. Subjects tested positive for HIV antibody, Hep B surface antigen, or Hep C antibody.
17. Removed in protocol version 3.0.
18. History of malignancy of any type, including basal cell and squamous cell cancers of the skin, within 5 years of enrollment
19. Any smoking within the last year or a history of greater than or equal to 10 pack years.
20. Previous immunotherapy treatment with cat allergen within the previous 10 years.
21. Any history of grade 4 anaphylaxis due to any cause as defined by the CTCAE grading criteria for immunotherapy.
22. History of bleeding disorders or treatment with anticoagulation therapy.
23. Treatment with omalizumab within 6 months prior to randomization.
24. Currently taking any of the following medications: beta blockers; tricyclic antidepressants; monoamine oxidase inhibitors; or anti-IgE monoclonal antibody treatment.
25. Ongoing systemic immunosuppressive treatment.
26. History of intolerance to the study therapy, rescue medications, or their excipients.
27. For women of childbearing age a positive serum or urine pregnancy test with sensitivity of less than 50 mIU/mL within 72 hours before the start of study therapy.
28. The use of any investigational drug or currently using an

investigational device within 30 days or five half-lives (whichever is longer) prior to randomization.

29. The presence of any medical condition that the investigator deems incompatible with participation in the trial.

Abbreviations

AE	adverse event
AR	Adverse reaction
ARIA	Allergic Rhinitis and its Impact on Asthma
AUC	area under the curve
CBC	complete blood count
CFR	Code of Federal Regulations
CFSE	fluoroscein succinimidyl ester
CRF	case report form
COPD	chronic obstructive pulmonary disease
CRO	contract research organization
DSMB	Data and Safety Monitoring Board
EDC	electronic data capture
EPR	early phase reaction
FDA	US Food and Drug Administration
GCP	good clinical practice
IB	Investigator Brochure
ICH	International Conference on Harmonisation
IRB	institutional review board
ITN	Immune Tolerance Network
LPR	late phase reaction
MedDRA	Medical Dictionary for Regulatory Activities
NAC	nasal allergen challenge
NCI-CTCAE	National Cancer Institute *Common Terminology Criteria for Adverse Events* (version 4.03, June 14, 2010)
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
PD	pharmacodynamics
PK	pharmacokinetics

PNIF	peak nasal inspiratory flow
SAE	serious adverse event
SAP	statistical analysis plan
SAR	serious adverse reaction
SUSAR	suspected unexpected serious adverse reaction
SIT	allergen-specific immunotherapy
SOE	schedule of events
SPT	skin prick test
TNSS	total nasal symptom score
WAO	World Allergy Organization
WHO	World Health Organization

1 BACKGROUND AND RATIONALE

1.1 CAT ALLERGY AS A CLINICAL PROBLEM

1.1.1 Overview

Sensitization to cats is a common contributor to nasal allergy symptoms in the United States. The 2005-2006 NHANES survey demonstrated that approximately 28% of current hay fever sufferers had IgE to cat extract.[1] Cat allergen exposure is also a widespread issue, with as many as 33% of homes in the United States having a pet cat (Humane Society of United States, 2011). Cat dander allergens have also been shown to exist in significant quantities in public buildings [2] and even in homes that do not have cats. [3]

1.1.2 Current Approaches to Cat Allergy

Treatment of cat allergy usually includes a recommendation to remove the cat from the home environment. Often, however, even highly sensitive patients will not comply with this. Once a cat has been removed from inside the home, concentrations of cat allergen do not decrease for periods of a year or longer unless very aggressive allergen abatement also takes place, including removal of the carpet and thorough cleaning.[4] An alternative strategy of allergen avoidance consists of leaving the cat in the home and removal of carpet, exclusive use of non-fabric furniture, and high-flow ventilation.[4] Unfortunately, despite these efforts patients may continue to have significant cat exposure outside of the home. Medications for rhinoconjunctivitis and asthma including oral, intranasal, and ocular antihistamines, oral leukotriene receptor antagonists, and intranasal and inhaled corticosteroids are variably effective in patients who are allergic to cats and have ongoing exposure.[5,6] Prevention of cat-induced asthma may be particularly challenging, with most available therapies (including omalizumab) inhibiting cat-induced decreases in FEV1 by approximately 50%.[6]

1.1.3 Clinical Outcomes with Subcutaneous Cat Dander Immunotherapy

Subcutaneous immunotherapy with high doses of cat dander extract has been shown to effectively reduce symptoms in 3 studies. Alvarez-Cuesta et al. performed a double-blind trial of cat dander immunotherapy (containing 13.2 mcg of Fel d 1) for 1 year in 28 patients with cat-induced rhinitis and asthma.[7] In patients who received active therapy, the medication-symptoms score, skin prick tests, conjunctival provocation test, and allergen bronchoprovocation test were all significantly improved compared with placebo. Varney et al. compared cat dander extract (containing 15 mcg of Fel d 1) in a controlled trial of 28 patients for 3 months. The actively treated group showed significant reductions in symptoms induced by cat room exposure (mean score 61.6 vs. 17.1) with no change in the placebo group (64.7 vs. 62.1).[8] The active group also showed reductions in conjunctival provocation sensitivity and skin sensitivity to cat extract. In the third study, patients with cat allergy received either cat dander immunotherapy (at a dose of 17.3 mcg of Fel d 1) or placebo for 3 years.[9] Bronchial sensitivity to inhaled cat allergen decreased

significantly after 1 year and continued to improve during the next 2 years compared with placebo.

Two more recent studies attempted to identify whether high doses of cat dander immunotherapy has greater immunologic and clinical effects than lower doses. Ewbank et al. examined the efficacy of cat immunotherapy in a placebo-controlled trial.[10] Escalating doses were given in 8 visits over a 4-week period to a target maintenance dose of 0 (placebo), 0.6, 3.0 or 15 microgram. Titrated skin prick tests, nasal allergen challenges and immunologic measurements were performed one week after the first maintenance dose. The concentration of antigen needed to generate an immediate 5 mm wheal was increased in the dose groups with 3.0 or 15 micrograms. Nasal allergen challenge thresholds were not increased. These data indicate that detectable responses to subcutaneous immunotherapy can be detected as early as five weeks after starting therapy.

The same group performed a similar small trial (N = 26 evaluable) with longer follow-up to examine the impact of extended treatment.[11] This study yielded inconsistent results, with some effects seen at 5 weeks being less apparent at one year. Nasal challenges were reported as symptom scores rather than as a threshold dose. With this endpoint both placebo and high dose treatment groups showed a decline in nasal symptom scores. These data demonstrate that the surrogate measures of response in skin and nasal mucosa may be detectable early but should be interpreted with caution in small groups of patients.

1.1.4 Mechanisms of Allergen-specific Immunotherapy

Mechanisms of immunotherapy have been described for grass immunotherapy. These may include stimulation of Th 1-type helper lymphocytes, decrease in Th 2 cells and induction of T regulatory cells that produce IL-10 and TGF-beta.[12,13] Immunotherapy has an effect on early allergic responses which occur in minutes and are due to IgE-mediated effects on mast cells, and on late phase responses which occur in hours and are due to effects mediated by T cells, eosinophils, and basophils that infiltrate skin or other tissues. There is experimental evidence, however, that immunotherapy preferentially suppresses the late phase response.[14]

Immunotherapy consistently results in long-term increase in antigen-specific IgG4. These antibodies may inhibit allergic responses through inhibitory effects on Fc-gamma-RII receptors on basophils or via direct interactions with antigen. Serum activity associated with IgG4 has been shown to block IgE-facilitated antigen presentation after effective immunotherapy.[15]

Immunotherapy can also result in long-lasting immune deviation from an allergic Th 2-based response to a protective Th 1-type response. This likely occurs as a local event in nasal mucosa in patients with rhinitis. In addition, allergen immunotherapy may influence both native and induced T regulatory cells. Local production of IL-10 may play a central role in suppression of mucosal responses in successfully treated allergic rhinitis.

The time course of effects of immunotherapy varies according to the immune measure: late phase skin responses are suppressed after as little as two weeks of therapy whereas clinical symptoms require 2-3 months for a reduction in severity.

Less is known about the corresponding mechanisms after cat immunotherapy. Ewbank et al. and Nanda et al. both reported approximately two to three-fold increases in cat-specific IgG4 after as little as 5 weeks of subcutaneous immunotherapy.[10,11] In one report there was a decrease in CD4/IL-4-positive (Th 2-like) cells[10] but this has not been a consistent finding.[3]

1.1.5 Why New Approaches are Needed

Prior studies of cat dander immunotherapy have demonstrated a significant but variable effect upon cat-induced nasal and pulmonary symptoms. While cat immunotherapy has been administered for up to 3 years in past studies, it is unknown whether this therapy is capable of inducing tolerance. Immunotherapy studies employing other allergens have demonstrated that at least 2 years of maintenance treatment is necessary in order to achieve tolerance. Therefore, it would be highly beneficial to determine whether achievement of tolerance with cat immunotherapy could be achieved in a shorter time than with other allergens using adjunct agents such as specific cytokine inhibitors.

1.2 THYMIC STROMAL LYMPHOPOIETIN (TSLP)

1.2.1 Role in Immune Responses

Thymic stromal lymphopoetin is an IL-7-like cytokine discovered in thymic stromal cell supernatents originally found to support B cell development.[16] It is largely produced by epithelial cells in nasal and bronchial mucosa and is increased in states of allergic and non-allergic inflammation. The TSLP receptor complex comprises the TSLP-binding chain (TSLP-R) and the IL-7R-alpha chain. The receptor is expressed on dendritic cells, T cells, B cells, mast cells, and NKT cells. Although originally recognized as a B cell factor, it acts on a variety of cell types to promote inflammation.[16,17]

TSLP produced by epithelium can activate dendritic cells leading to OX40L expression and resulting in differentiation of naïve CD4 cells into Th 2 cells. These secrete high levels of inflammatory cytokines IL-4, IL-5 and IL-13. Thus TSLP is associated with a Th 2-skewed immune response consistent with a role in allergy.

TSLP also acts directly on T cells to promote Th 2 differentiation at least in murine model systems. This effect is enhanced in the presence of IL-4. Kitajima et al. demonstrated that the TSLP receptor was expressed at high levels on mouse effector Th 2 cells compared to Th 1 and Th 17 cells and showed that TSLP preferentially induced proliferation of effector Th 2 cells. These results indicate that TSLP is involved in exacerbation of mouse Th 2-mediated allergic inflammation.[18]

TSLP also has direct effects on mast cells and enhances mast cell IL-13 production. In a study of in vitro-generated mast cells derived from peripheral or cord blood,

TSLP in the presence of IL-1 was found to act directly on mast cells to stimulate production of inflammatory cytokines IL-5, IL-13 and IL-6.[19]

1.2.2 Role in Asthma

Several studies indicate that TSLP may have a role in allergic asthma. In a study in two cohorts of 36 and 16 subjects with mild to severe asthma, Shikotra et al. reported an increase in expression of TSLP protein primarily in epithelial cells from bronchial biopsies; increased TSLP levels were associated with expression of other Th 2-related genes.[20] IL-13 expression was increased in non-epithelial cells. These findings suggest a role for TSLP in generating or maintaining the inflammatory state associated with allergic asthma and are supported by mouse models.[21]

1.2.3 Role in Allergic Rhinitis

Miyata et al. described a role for TSLP in a mouse model of allergic rhinitis.[22] In this study neutralizing antibody to TSLP also diminished response in an OVA-induced model of allergic rhinitis. TSLP protein and mRNA are overexpressed in nasal mucosa in turbinate biopsies of patients with allergic rhinitis.[19] Epithelial cells expressing TSLP were found in higher numbers and TSLP protein was expressed at higher levels assessed by immunohistochemistry in patients with atopic as compared to non-allergic rhinitis.[23] Genetic studies indicated that a polymorphism at the TSLP gene locus was associated with reduced risk of allergic rhinitis in children with asthma in three distinct populations, supporting a role for TSLP in the pathogenesis of allergic rhinitis.[24]

1.3 TSLP INHIBITION

1.3.1 Principle

Preclinical data indicate inhibition of TSLP may block immune-mediated responses in an animal model of allergic skin inflammation.[25] Zhang et al. examined TSLP inhibition in a murine model of allergic asthma. They found an inhibitor comprising a soluble TSLP receptor-immunoglobulin molecule reduced inflammatory cell infiltrates, and levels of Th 2 cytokines, IL-4 and IL-5, were also reduced in bronchoalveloar lavage fluid.[26] TSLP is involved in disease pathogenesis in a murine model of allergic rhinitis induced by OVA. In this model neutralizing TSLP antibody administered during the mucosal challenge phase after intraperitoneal sensitization inhibited allergic inflammation in the nasal mucosa.[22] These studies support the examination of TSLP inhibition in human allergic disease.

1.3.2 AMG 157

1.3.2.1Name of Investigational Product

In this protocol the investigational anti-TSLP agent is referred to as AMG 157 with Amgen, Inc. as the manufacturer. In 2012, MedImmune partnered with Amgen on AMG 157 and as a result the agent can also be identified as MEDI9929/AMG 157.

http://www.amgen.com/media/media_pr_detail.jsp?year=2012&releaseID=1679295
http://www.medimmune.com/research/pipeline

1.3.2.2Description and Previous Experience

The following information has been obtained from the Amgen Investigator's Brochure for MEDI19929/AMG 157.

AMG 157 is a fully human monoclonal IgG2λ directed against TSLP expressed in a Chinese hamster ovary cell line (Investigator's Brochure for MEDI9929/AMG 157). The molecule is a heterotetramer consisting of two heavy chains of the IgG2 subclass and two light chains of the lambda subclass, which are covalently linked through disulfide bonds. The antibody binds with high affinity to human and cynomolgus TSLP. It inhibits TSLP-induced STAT5 phosphorylation in human dendritic cells. It does not bind to mouse TSLP.

AMG 157 has been assessed in four completed phase I pharmacokinetic trials in healthy subjects and in individuals with atopic dermatitis, and mild atopic asthma. It is currently (September 2015) being assessed by the Manufacturer in three phase I/IIa trials evaluating pharmacokinetics, pharmacodynamics, safety/efficacy in adults with moderate to severe atpic dermatitis, adults with inadequately controlled severe asthma and adolescents with mild to moderate asthma.

In summary, information is available for four of the completed and two of the ongoing trials. Based on data collected in these trials, AMG 157 has demonstrated an acceptable safety profile, predictable and consistent pharmacokinetics, and high bioavailability after subcutaneous and intravenous dosing. It has a prolonged half-life which makes intermittent dosing feasible.To date, limited pharmacodynamics and clinical efficacy information is available. Based on the most recent version of MedImmune's Investigator's Brochure for AMG 157 (Version 3.0), a multiple dose study in subjects with mild atopic asthma (Study 20101183) demonstrated a decrease in blood and sputum eosinophils and a decrease in the Th2/Th1 balance based upon a new exploratory biomarker assay. Study 20101183 also showed that AMG 157 attenuates both the early and late asthmatic responses after allergen challenge comparing to pre-allergen challenge days. AMG 157 has not shown any effect on several lymphocyte subsets (CD3+, CD4+, CD8+ or CD19+), IgE scores, or plasma levels of CCL17, CCL22, or CCL27.

1.4 COMBINATION OF IMMUNOTHERAPY WITH TSLP INHIBITION

1.4.1 Combining Antigen with an Immunomodulatory Agent

The pathways affected by allergen-specific immunotherapy may also be effectively modified by adjuvant anti-inflammatory strategies. Based on the emerging role of TSLP in allergic responses, TSLP inhibition has the potential to combine with allergen-specific immunotherapy with beneficial effects on both the antigen-specific T-cell mediated elements and the inflammatory elements mediated by basophils and mast cells.

If TSLP stimulates Th 2 responses and allergic rhinitis related to cat allergy is a Th 2-mediated condition, then TSLP inhibition may be combined with immunotherapy to tip the balance in favor of an inhibitory, rather than an allergic, response.

The current trial plans for administration of anti-TSLP during immunotherapy. Several experiments in animal models suggest that TSLP may be most important during the sensitization or priming phase of allergy. Immunotherapy presents a strong antigenic challenge to individuals who are already allergic with effects that may to some extent mimic those of sensitization. The concurrent administration of anti-TSLP and antigen may thus provide clear advantages over single interventions.

1.4.2 Why the Combination Might Lead to Long-lasting Effects or Tolerance

As discussed in Section 1.2, there are at least two key effects of TSLP which contribute significantly to the persistence of allergic responses.

First, TSLP-activated dendritic cells support the maintenance and further polarization of CRTH2+ Th 2 effector memory cells.[27] Only dendritic cells (DCs) activated by TSLP can induce a robust expansion of CRTH2+CD4+ Th 2 memory cells, while maintaining their central memory phenotype and Th 2 commitments.

Second, some studies have indicated that TSLP might hinder the production and/or maintenance of Foxp3 + T-regulatory (Treg) cells in allergy. Specifically, in both mice and humans, low doses of TSLP have been shown to be capable of inhibiting Treg cell induction without significantly promoting Th 2 development, indicating that there is some degree of separation of these functions.[28] These effects may be complex, however, as data from patients with Crohn's disease have suggested that intestinal epithelial cells are also capable of promoting the differentiation of tolerogenic DCs which drive the development of Treg cells.[29] Clinical trials of TSLP inhibition will help resolve which effect is predominant in humans.

Given the generally pro-inflammatory and T regulatory-inhibitory effects of TSLP, inhibition of this cytokine as proposed in the current trial may lead to a reduction in Th 2 memory cells and increase in Treg cells and, when co-administered with allergen to allergic patients, may enhance or expedite the achievement of tolerance.

1.5 TRIAL DESIGN

1.5.1 Overview

The primary goal of this study is to determine whether the combination of anti-TSLP (AMG 157) plus antigen-specific immunotherapy will result in tolerance to the therapeutic antigen, as demonstrated by the persistence of a therapeutic effect for a substantial period after stopping immunotherapy. To address this we propose a randomized, placebo-controlled trial in which four groups of cat-allergic subjects are treated until week 48 and then are followed without additional treatment until week 104. Allergy-related endpoints and exploratory endpoints related to mechanisms of immune modulation and tolerance will be assessed at baseline and at 26, 52, 78, and 104 weeks during participation.

1.5.2 Why Total Nasal Symptom Score (TNSS) was Chosen as an Endpoint

The use of total nasal symptom score (TNSS) relies on its relation to clinical symptoms and to experience gained in mechanistic analysis of nasal and other samples after in vivo challenge in the target organ for allergic rhinitis. TNSS is calculated with a scale of 0 to 3 on the 4 parameters of sneezing, rhinorrhea, nasal congestion and blockade, and pruritus. The maximum score is 12.

TNSS is a reflection of disease activity in response to allergen exposure.[30] Prior studies have demonstrated that both early and late allergen-induced symptoms are reproducible and amenable to change with a variety of clinically effective therapies.

Nasal allergen challenge has been studied in detail after grass allergen challenge. Scadding and colleagues have reported on a standardized approach to nasal challenge in which grass pollen-allergic individuals received increasing doses of grass pollen to determine the threshold dose that elicited a response. In subsequent challenges they received the same threshold dose that elicited a response and symptoms; tryptase and nasal cytokine levels were assessed over 6 hours.[31] Consistent findings among 18 subjects included an early peak of tryptase levels in nasal fluid coincident with a peak in symptom scores and increasing levels of Th 2 cytokines IL-4, IL-5 and IL-13. This response was first detected at 2 hours, and was still increasing at 6 hours. This procedure provides a robust platform to assess the effects of distinct treatment arms on in vivo allergen challenge which can be considered a surrogate for clinical efficacy while at the same time probing mechanism such as Th 2 deviation.

Nasal allergen challenges were used in a study of IL-13 blockade. In this study systemic treatment with anti-IL-13 resulted in a significant decrease in IL-13 isolated from nasal secretions 5 days after antibody administration.[32] This demonstrates the potential for nasal cytokine measurement to serve as a pharmacodynamic marker for anti-allergic treatment strategies.

1.5.3 Why a Tolerance Trial

The current trial proposes a treatment period followed by a period of observation off the therapy. The demonstration of a persistent treatment effect in the absence of ongoing therapy, is a characteristic of immune tolerance. We hypothesize that a major effect of anti-TSLP will be to shorten substantially the duration of allergen immunotherapy that is needed to induce tolerance.

There are substantial unknowns in this trial of previously tested and untested interventions. The consistency and magnitude of the effect of cat allergen immunotherapy on nasal challenge and other endpoints is incompletely defined in the published literature. Analysis at the conclusion of the treatment phase will allow confirmation of treatment effect for immunotherapy in the selected patient group and investigation of whether there is a detectable effect of TSLP blockade alone. The role of anti-TSLP alone or in combination in providing immunotherapeutic benefit for patients with allergic rhinitis during and after treatment has not been tested. We do know that a tolerance effect cannot be demonstrated without a foundation of a treatment effect. The interim analysis aims to control the risk that a treatment effect is

not observed. It does not address the likelihood of a tolerance outcome but provides some protection against proceeding in a trial highly unlikely to reach its aims.

1.5.4 Why Cat Dander as an Allergen for Study

There are several important clinical reasons justifying the choice to study cat allergic patients in this study: 1) Cat-induced rhinitis and asthma occur commonly in the general population and represent a significant source of morbidity[4]; 2) Clinical responses to cat allergen can be studied using reproducible methods, including nasal allergen challenge (NAC) and cat room challenge[11]; 3) Commercial cat dander extracts are well-standardized with respect to the allergenic potency.[33]

In addition to its relevance as a clinical problem, there are good technical and scientific reasons to study cat allergy and the response to the immunodominant protein Fel d1.[34] There are excellent reagents to study the immune response in cat allergy. Many of the epitopes of the major immunogenic protein Fel d1 are well-characterized. A variety of assays exist to measure T cell responses. Tetramers may be produced with principal epitopes for a variety of HLA alleles, which can be used to study the evolution of antigen-specific reactions in individuals.

1.5.5 Mechanistic Questions: Overview

The current trial allows for the exploration of mechanistic questions in several areas. The mechanisms of immunotherapy previously identified for other allergens including Th 1 deviation, production of blocking antibodies, and the generation of regulatory cells can be examined for cat immunotherapy. The study arm in which subjects receive both immunotherapy and anti-TSLP inhibition allows for the exploration of whether these same effects are magnified in extent or duration when TSLP signaling is impaired. It will be important to assess whether anti-TSLP alone has measurable mechanistic effects. These issues will be addressed in peripheral blood, skin, and nasal compartments. The mechanistic hypotheses and proposed techniques are laid out in more detail in Section 7.

1.6 SUMMARY OF KNOWN AND POTENTIAL RISKS AND BENEFITS FOR HUMAN PARTICIPANTS

1.6.1 Study Medication Risks

1.6.1.1 Allergenic Extract SQ (Standardized Cat Hair)

Injection immunotherapy is commonly associated with local swelling at the allergen injection site, sometimes occurring within minutes but more typically several hours after injection. Large local cutaneous reactions may have associated pain and pruritus and occur in up to 10% of recipients. Occasionally systemic allergic symptoms can occur, such as rhinitis, ocular symptoms, or urticaria. These symptoms can generally be managed with oral antihistamines. Rarely, anaphylaxis with wheezing and hypotension may occur. In this trial immunotherapy will be carried out in well-equipped facilities by personnel trained and experienced in immunotherapy and its potential complications. Both local and systemic reactions are most common during the build-up phase of immunotherapy.

1.6.1.2 AMG 157

As discussed in Section 1.3.2, the investigational drug AMG 157 or placebo has been administered to a total of 652 subjects in phase I and Phase II clinical trials either as single or multiple SC or IV doses as of September 2015. No specific risks have been identified with AMG 157 administration.

Important potential risks include Immune Complext Disease (Type III Hypersensitivity Reactions), Serious Infections, Parasitic Infestation/Infections , Hypersensitivity/Allergic Reactions and Infusion Reactions.

Immune Complex Disease results from the formation of ADA, which can be a result of the administration of a mAb. No subjects received AMG 157 in the previous studies developed ADA.

Due to the blockade of TSLP, AMG 157 has the potential to modulate systemic immune responses by reducing number or activity of Th 2-type T-cells. Therefore,there is a theoretical increased risk of serious infection,includingparasitic infections.In this trial participants will be screened and excluded for history of known immunosuppressive disorder, clinically significant infection and untreated systemic helminthic parasitic infection.

As with any large molecule therapeutics, administration of AMG 157 may result in systemic and/or local reactions, as well as infusion-hypersensitivity reactions. To mitigate the risk of severe hypersnsitivy and infusion reactions, subjects will be monitored for AEs and vital sign before, during and for 1 hour after the AMG 157 administration. In addition, medical equipment and study personnel trained to treat acute anaphylactic reactions will be immediately available.

1.6.2 Study Procedure Risks

1.6.2.1Intradermal and Skin Prick Tests

Participants may experience mild to moderate itchiness or local discomfort at the sites of intradermal injections and skin pricks with allergen and the positive control (histamine dihydrochloride 10 mg/mL). The symptoms are not bothersome and treatment with oral antihistamines is always available and is effective although almost never required. Systemic reactions including anaphylaxis after skin tests with standardized aeroallergen extracts are a theoretical risk but exceedingly rare. However, a physician is always present and drugs and equipment for treatment of anaphylactic reactions available.

1.6.2.2Nasal Allergen Challenge (NAC)

This trial will use nasal allergen challenge as one of several outcome measures. Challenge is expected to cause early (0-1 hr) and late (1-6 hr) allergic symptoms such as nasal congestion, sneezing, nasal discharge, and itchy, watery eyes. If nasal and/or ocular symptoms persist after the period of 6 hours observation in the clinical unit participants will be offered treatment with oral antihistamines. There is a small risk of provoking asthmatic symptoms. Participants will perform peak flow recordings

before and after nasal challenge and treatment with inhaled bronchodilators and corticosteroids will be immediately available. To minimize this risk, individuals with a pre-bronchodilator FEV1 of less than 80% of predicted are excluded from this trial. As for any intervention with allergen to which the patient is sensitive there is the theoretical risk of developing an anaphylactic reaction. Trained personnel, including a physician, as well as medications and equipment, will be immediately available to treat any reaction.

1.6.2.3Nasal Lavage

Prior to nasal challenge a saline nasal lavage with be performed with a commercially available preparation (SinuRinse®, NeilMed Pharmaceuticals) diluted in 240 mL (8 oz) of warm distilled water. This is a non-irritant, preservative free, pH neutral solution that contains sodium chloride, sodium bicarbonate and iodine. SinuRinse® is widely available and no adverse effects are expected.

1.6.2.4 Nasal Brushings

Cells from the nasal epithelium may be collected by nasal brushing after the nasal allergen challenges. Risks include local burning with eye tearing, and rarely local bleeding. The symptoms are generally mild and resolve within minutes.

1.6.2.5 Venipuncture and Intravenous Access

Participation in the trial requires blood drawing for laboratory assessment and intravenous access for drug administration. Potential risks are those associated with phlebotomy and include ecchymosis and infections at the site of needle puncture.

1.6.3 Potential Benefits

Based on previous studies[8] it is possible that participants who receive cat immunotherapy may have an improvement in symptoms. The impact of anti-TSLP, however, is unknown. Some participants will not receive either cat immunotherapy or anti-TSLP. There is thus no assurance of therapeutic benefit from participation.

2 OBJECTIVES

2.1 PRIMARY OBJECTIVE

To determine if anti-TSLP in conjunction with antigen-specific immunotherapy can induce tolerance to cat allergen.

2.2 SECONDARY OBJECTIVES

To determine if anti-TSLP alone modifies the nasal allergic response to cat allergen.

2.3 EXPLORATORY OBJECTIVES

To explore immunologic mechanisms of tolerance induction by the combination of anti-TSLP and antigen-specific immunotherapy.

3 STUDY DESIGN

3.1 DESCRIPTION

3.1.1 Overview

This is a randomized, double-blind, multi-center, placebo-controlled, four-arm study in 121 cat-allergic subjects.

Potentially eligible subjects will be screened for eligibility with assessments starting with a skin prick test.

It is estimated that:

- of those, 80% will meet the skin test eligibility criterion and undergo the nasal allergen challenge,
- of those, 90% will meet the screening nasal allergen challenge eligibility criteria and undergo the remaining screening assessments;
- of those, 90% will meet the remaining eligibility criteria and be enrolled; and
- of those enrolled, 90% will successfully complete the baseline nasal allergen challenge and be randomly assigned.

Thus it is estimated that about 205 individuals will need to be screened to meet the target randomization.

Random assignment will be 1:1:1:1 to four groups:

A) AMG 157 plus immunotherapy,

B) placebo for AMG 157 plus immunotherapy,

C) AMG 157 plus placebo for immunotherapy, or

D) placebo for AMG 157 plus placebo for immunotherapy.

Participants are treated in their assigned group for 48 weeks. At that point study therapy is stopped. Participants undergo observation until week 104.

3.1.2 Dose and Schedule

Starting 1-3 days prior to immunotherapy or placebo for immunotherapy, AMG 157 or placebo will be administered intravenously using investigational product supplied by the manufacturer. AMG 157 will then be administered once every 4 weeks at dose of 700 mg IV. Each AMG 157 dose will be administered at least 1 day before immunotherapy through week 24. After week 24, AMG 157 may be given before or on the same day as immunotherapy. The treatment period for AMG 157 or placebo will be 48 weeks.

Concurrently, immunotherapy using a standard cat allergen extract or placebo will be administered subcutaneously with a cluster dose and schedule currently in

widespread use in the clinical practice of allergy in the United States. There will be an up-dosing period (approximately 12 weeks) during which small doses are gradually increased to a maintenance dose. Dosing levels may be individually tailored based on subject response. The combined up-dosing and maintenance periods will total 48 weeks.

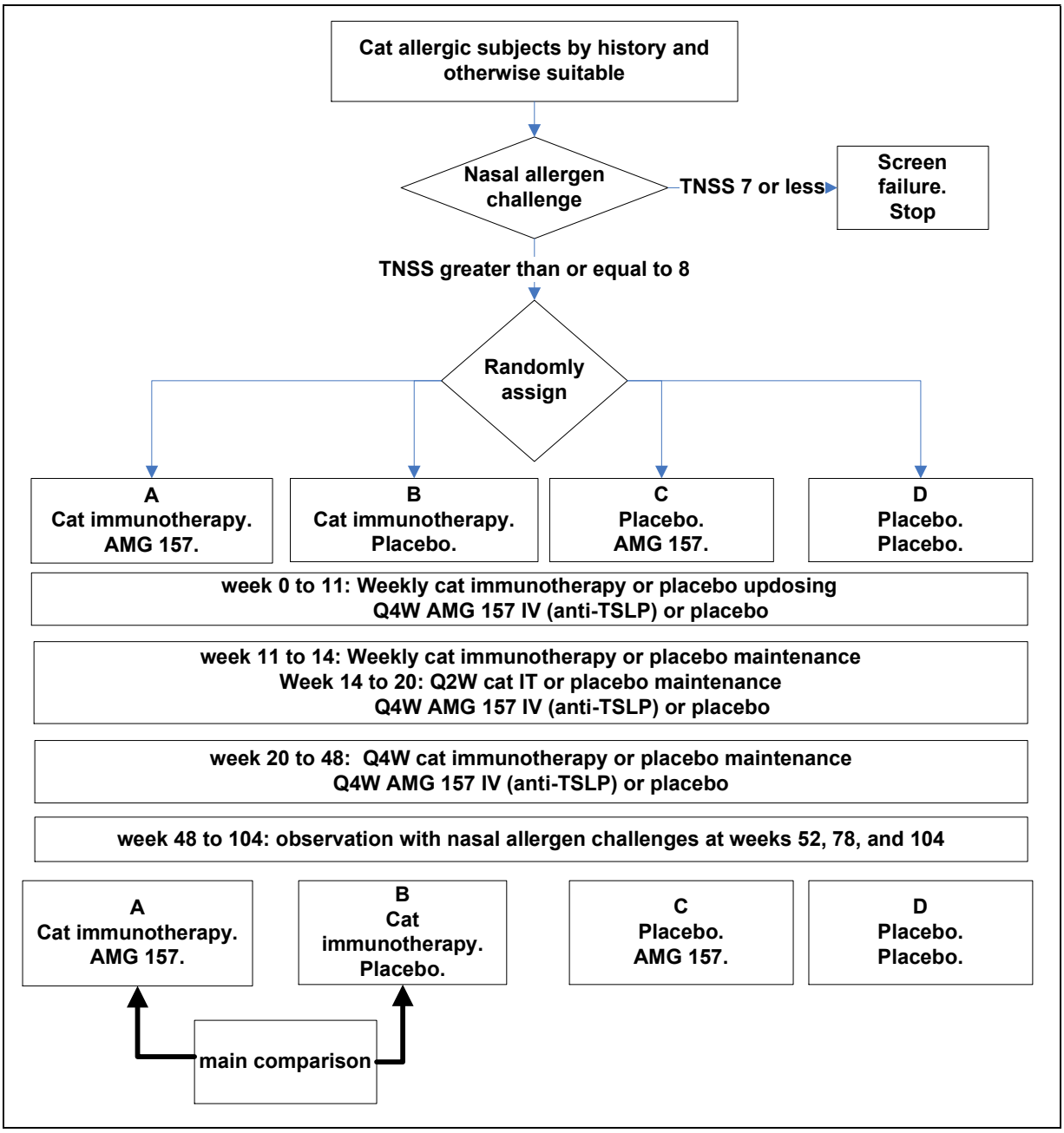


Figure 1: Screening, allergen challenge, random assignment, treatment, and observation

3.1.3 Interim Analysis

The first 10 participants enrolled into each treatment group will form initial cohorts for a planned interim analysis based on week 52 assessment. When the last participant in this cohort completes the week 52 assessment, an interim analysis of TNSS response to challenge with cat allergen will compare the initial cohorts of the combined groups A and B to the initial cohort of group D. During the interim analysis, enrollment in the rest of the trial will continue. Treatment of this cohort and of other enrolled participants will also continue.

If there is strong evidence that the fully enrolled groups A and B will not show a significant decrease from baseline in TNSS compared to fully enrolled group D, the trial will be stopped. Otherwise, the trial will continue. This evaluation will be made based on an analysis described in Section 9.2.5.

3.2 STUDY DURATION

Total study duration will be 154 weeks:

- Study enrollment to occur over 46 weeks.
- Individual subject study participation will be 108 weeks..

3.3 STUDY ENDPOINTS

3.3.1 Primary Endpoint

Total nasal symptom score (TNSS) AUC from 0 to 1 hour after cat allergen challenge at 104 weeks. TNSS is calculated with a scale of 0 to 3 on the 4 parameters of sneezing, rhinorrhea, nasal congestion and blockade, and pruritus. Maximum score is 12.

3.3.2 Secondary Endpoints

Other measures of allergic response including:

1. Skin prick test endpoint titration at baseline, 1, 4, 12, 26, 52, 78, 104 weeks.
2. Skin early phase response (EPR) to intradermal testing at baseline, 26, 52,104 weeks. EPR is the skin response at 15 minutes.
3. Skin late phase response (LPR) to intradermal testing at baseline, 26, 52, 104 weeks. LPR is the skin response at 6 hours.
4. Peak TNSS EPR at baseline, 26, 52, 78, 104 weeks. Peak is the highest value recorded between 0 and 1 hour inclusive.
5. TNSS EPR at baseline, 26, 52, 78, 104 weeks. EPR is the TNSS AUC from 0 to 1 hour.
6. TNSS LPR at baseline, 26, 52, 104 weeks. LPR is the TNSS AUC from 5 to 6 hours.

7. Peak nasal inspiratory flow (PNIF) LPR AUC at baseline, 26, 52, 104 weeks. LPR is the PNIF AUC from5 to 6 hours.
8. PNIF EPR AUC at baseline, 26, 52, 78, 104 weeks. EPR is the PNIF AUC from 0 to 1 hour.

3.3.3 Exploratory Endpoints

Exploratory endpoints related to mechanisms of immune modulation and tolerance assessed prior to, during, and after therapy, including those related to:

1. Local immune response in nasal fluid and/or cells for inflammatory cytokines and inflammatory mediators.
2. Cells in peripheral blood will be collected for basophil activation at various time points during and after immunotherapy to measure basophil activation, cat antigen tetramers and cytokine profiles in response to in vitro cat antigen stimulation; cat antigen tetramers for changes during and after immunotherapy; and cytokine profiles after in vitro cat antigen stimulation.
3. Serum components of cat-specific IgE; IgG and subclasses; and IgA inhibition of facilitated antigen presentation.

3.4 STOPPING RULES

3.4.1 Ongoing Review

The progress of the study will be monitored by the NIAID Allergy-Asthma Data and Safety Monitoring Board (NIAID DSMB), which will review safety data and make recommendations regarding continuation, termination, or modification of the study. The DSMB will formally review the safety data six months after the first participant is randomized and then annually thereafter. The number of subjects who discontinue study treatment will be included in the reports prepared for the DSMB.

In addition, safety data will be reviewed by the DSMB when an event occurs that is of sufficient concern to the NIAID medical monitor, ITN clinical trial physician, or protocol chair to warrant review, or when events occur that contribute to a stopping rule listed in Section 3.4.2.

3.4.2 Stopping Rules Guidance

Enrollment in the study will be stopped and administration of the investigational medication AMG 157 will be halted pending review by DSMB, DAIT and the ITN if any of the following criteria are met:

1. Death of any participant at least possibly related to study participation.
2. Grade 4 anaphylaxis at least possibly related to study participation.
3. Two participants have a grade 3 or higher adverse event at least possibly related to AMG 157.
4. Five participants meet the first individual stopping rule in Section 5.3.

5. Two participants have a systemic infection which in the judgment of the NIAID medical monitor is unusual or unusually severe.
6. Grade 3 serum sickness at least possibly related to study participation.

3.5 DEFINITION OF HIGH AND LOW EXPOSURE TO CAT ALLERGEN

Exposure to cats is assessed at screening. This assessment will determine whether a participant is stratified as described in section 6.2.1 to the high exposure or low exposure stratum. Exposure to cats is also assessed at intervals during participation. These assessments will allow tabulation of a participant's exposure to cats during the trial.

High exposure is defined as exposure to cats at least three times per week during each of the four weeks prior to the time of assessment.

Low exposure is defined as not meeting the high exposure definition. In cases of uncertainty the clinical site investigator will determine high versus low exposure.

4 ELIGIBILITY

4.1 INCLUSION CRITERIA

Patients must meet *all* of the following criteria to be eligible for this study:

1. Age 18-65 years.
2. Removed in protocol version 4.0.
3. History of moderate-severe allergic rhinitis (AR) caused by cat exposure for at least 2 years.
4. Skin prick test wheal greater than or equal to 5 mm to standardized cat extract.
5. Removed in protocol version 3.0.
6. Screening nasal allergen challenge in which:
    - TNSS is less than or equal to 3 after the 0 concentration (vehicle control only) dose,
        - TNSS increase is less than or equal to 1 from the TNSS prior to allergen administration to the TNSS after the 0 concentration (vehicle control only) dose,
    - TNSS is greater than or equal to 8 after the highest dose, and
    - Between the first non-zero dose and 10 minutes after the highest dose, either:
        - 3 or more sneezes are counted or
        - Greater than 20% drop in PNIF is recorded.
7. Body mass index (BMI) is greater or equal to 18.0 and less than or equal to 35.0 kg/m2, at screening.

8. Clinically acceptable physical examination and electrocardiogram (ECG) results (12-lead reporting RR, PR, QRS, QT and QTcF) prior to Day 0 based on the opinion of the investigator.

9. Adequate renal function defined as CrCl greater than 80 mL/min using the Cockcroft Gault equation.

10. For women of childbearing age, a willingness to use a highly effective form of contraception for four months after last dose of study medication. Highly effective methods of birth control include abstinence, vasectomy by the male partner, or a condom with spermicide in combination with either hormonal birth control, IUD or barrier methods used by the woman.

11. For men with female partners of childbearing potential, agreement not to donate sperm and to inform their female partner of their participation in this clinical study and use highly effective methods of birth control for four months after last dose of study medication. Highly effective methods of birth control include abstinence, vasectomy, or a condom with spermicide in combination with either hormonal birth control, IUD or barrier methods used by the woman.

12. The ability to give informed consent and comply with study procedures.

13. Not currently taking and, for the duration of the study, agreement not to take any form of immunotherapy, including sublingual, subcutaneous or investigational immunotherapy.

4.2 EXCLUSION CRITERIA

Patients who meet any of the following criteria will *not* be eligible for this study:

1. Removed in protocol version 4.0.

2. Prebronchodilator FEV1 less than 80% of predicted value at screening visit.

3. Removed in protocol version 3.0.

4. History of asthma meeting the NAEPP EPR3 classification of mild-persistent or worse in the past year, other than with cat exposure, requiring regular inhaled corticosteroids for greater than 4 weeks per year.

5. History of serious chronic medical conditions which might interfere with treatment or assessments.

6. History of emergency visit or hospital admission for asthma in the previous 12 months.

7. History of chronic obstructive pulmonary disease (COPD).

8. History of significant recurrent acute sinusitis, defined as 2 episodes per year for the last 2 years, all of which required antibiotic treatment.

9. History of chronic sinusitis, defined as a sinus symptoms lasting greater than 12 weeks that includes 2 or more major factors or 1 major factor and 2 minor factors. Major factors are defined as facial pain or pressure, nasal obstruction or blockage, purulent or discolored postnasal discharge, purulence in nasal cavity, or impaired or loss of smell. Minor factors are defined as headache, fever, halitosis, fatigue, dental pain, cough, and ear pain, pressure, or fullness.

10. History of systemic disease affecting the immune system such as autoimmune diseases, immune complex disease, or immunodeficiency, where, in the opinion of the study physician, participation in the trial would pose a risk or significant effect on the immune system.

11. Type I or type II diabetes.

12. Evidence of any clinically significant active or suspected bacterial, viral, fungal or parasitic infections within 14 days prior to screening nasal allergen challenge. Participants may be re-evaluated for eligibility after an appropriate course of treatment has been completed and symptoms have resolved.

13. High risk of parasitic disease as judged by the investigator.

14. Positive QuantiFERON® tuberculin test unless the potential subject has been treated with appropriate chemoprophylaxis.

15. Exposure to an individual with active tuberculosis within six months from randomization.

16. Subjects tested positive for HIV antibody, Hep B surface antigen, or Hep C antibody.

17. Removed in protocol version 3.0.

18. History of malignancy of any type, including basal cell and squamous cell cancers of the skin, within 5 years of enrollment.

19. Any smoking within the last year or a history of greater than or equal to 10 pack years.

20. Previous immunotherapy treatment with cat allergen within the previous 10 years.

21. Any history of grade 4 anaphylaxis due to any cause as defined by the CTCAE grading criteria for immunotherapy.

22. History of bleeding disorders or treatment with anticoagulation therapy.

23. Treatment with omalizumab within 6 months prior to randomization.

24. Currently taking any of the following medications: beta blockers; tricyclic antidepressants; monoamine oxidase inhibitors; or anti-IgE monoclonal antibody treatment.

25. Ongoing systemic immunosuppressive treatment.

26. History of intolerance to the study therapy, rescue medications, or their excipients.

27. For women of childbearing age a positive serum or urine pregnancy test with sensitivity of less than 50 mIU/mL within 72 hours before the start of study therapy.

28. The use of any investigational drug or currently using an investigational device within 30 days or five half-lives (whichever is longer) prior to randomization.

29. The presence of any medical condition that the investigator deems incompatible with participation in the trial.

4.3 PREMATURE TERMINATION OF A PARTICIPANT FROM THE STUDY

Withdrawal of consent. Participants who withdraw consent for further treatment or study procedures will be asked if they would be willing to complete an early termination visit (Appendix 3).

Investigator decision. The principal investigator may choose to withdraw a participant from the study for any reason.

Failure to return. Participants who do not return for visits and who do not respond to repeated attempts by the site staff to have them return will be considered *lost to follow-up*.

Participants who terminate after randomization from the study will not be replaced.

Participants who prematurely terminate due to withdrawal of consent for further treatment or investigator decision will be invited to complete study assessments at week 104 and any other preceding clinical allergy assessment visits including visits at weeks 26, 52, and 78 if applicable. However if they decline they will be asked to complete an early termination visit (Appendix 3) and will be discharged from the study.

5 STUDY MEDICATIONS

5.1 INVESTIGATIONAL MEDICATION - AMG 157 AND PLACEBO

5.1.1 Formulation and Packaging

AMG 157 is not approved for any use in the US or elsewhere.

AMG 157 is a fully human monoclonal IgG2λ directed against TSLP, expressed in a Chinese hamster ovary cell line. The molecule is a heterotetramer consisting of 2 heavy chains of the IgG2 subclass and 2 light chains of the lambda subclass, which are covalently linked through disulfide bonds.

AMG 157 (manufactured by Amgen, Thousand Oaks, CA) is presented as a sterile, clear, and colorless to slightly yellow liquid for subcutaneous or intravenous administration. Each sterile vial is filled with 1 mL deliverable volume of 70 mg/mL

AMG 157 formulated with 10 mM sodium acetate, 9% (w/v) sucrose, 0.004% (w/v) polysorbate 20, pH 5.2.

Placebo for AMG 157, maufactured by Amgen, has the same formulation as ther drug product except there is no active AMG157.

AMG 157 and placebo will be packaged and distributed by a Drug Distributor under contract to DAIT, NIAID.

5.1.2 Dosage, Preparation, and Administration

AMG 157 at 700 mg or placebo of similar appearance will be administered by intravenous injection every four weeks as specified in the schedule of events. Every four-week dosing has been previously tested and is based on information obtained in pharmacokinetic studies which demonstrate a prolonged (approximately 21 day) half-life which supports dosing at this interval. The duration of AMG 157 specified in the current protocol has not been previously assessed.

Both the drug and the placebo will be delivered by an infusion in 100 ml of sterile diluent (5% dextrose) over a 1-hour period using a peristaltic pump. Participants will be observed in the clinic for 2 hours prior to discharge or receiving cat immunotherapy or placebo.

Infusion of AMG 157 or placebo should not be given if the participant has significant illness. The infusion may be rescheduled when the symptoms have resolved and treatment has been completed. Persistent of serous otitis is not a reason to continue to hold the infusion. If the illness will require a hold of greater than 14 days, then the infusion should be skipped and the next dose given per the original schedule.

5.1.3 Recommended Storage Conditions

AMG 157 should be stored frozen, protected from light and according to the storage and expiration information provided on the label that is affixed to the package containing the investigational product.

AMG 157 should be thawed per the instructions provided in the Pharmacy Guide or Investigational Product Information Manual. Vials should be checked for cracks or damage that may occur during the thawing process if not performed properly. Damaged product should not be administered and if damage is observed, the study sponsor (DAIT NIAID) should be notified to obtain further instructions.

The placebo for AMG 157 should be handled in the same way as AMG 157.

5.2 INVESTIGATIONAL MEDICATION – CAT IMMUNOTHERAPY AND PLACEBO

5.2.1 Formulation and Packaging

Cat immunotherapy will comprise standardized cat allergen extract and is licensed in the US for allergen immunotherapy, and it is formulated as a long-acting suspension for subcutaneous injection.

The standardized allergen extract will be obtained from ALK-Abelló, Inc. Port Washington, NY.

The cat immunotherapy is 10,000 BAU/ml with inactive ingredients sodium chloride (0.5%) sodium bicarbonate (0.25%) and glycerin 50% (v/v).

The placebo for immunotherapy will consist of the same inactive ingredients as the standardized cat allergen extract. The placebo will be obtained from Allergy Laboratories, Inc., Oklahoma City, OK.

Allergen and placebo are diluted in normal saline with phenol (0.4%) and 0.03% human serum albumin also obtained from Allergy Laboratories, Inc., Oklahoma City, OK.

Cat immunotherapy/placebo will be packaged and distributed by a vendor under contract to DAIT, NIAID

5.2.2 Dosage, Preparation, and Administration

Cat immunotherapy or placebo will be administered weekly during the updosing phase. The dosing schedule is shown in the table 1 in this section and in the schedule of events. Allergen extract for immunotherapy is supplied as a custom kit for each individual participant. These kits were designed to contain sufficient cat allergen extract or placebo and diluent to see the participant through the entire course of immunotherapy. **In the event that this is not possible, the site will contact the NIAID Project Manager** who will work with the pharmacy distribution center to identify a new kit that was made from the same lot of cat allergen extract or placebo that was used in the kit to be replaced. In the case where a kit with a different lot of cat hair allergen or placebo must be used as a replacement, the first dose from the new kit will be reduced to half of the most recent dose from the kit that is being replaced, unless the investigator feels further dose reduction is needed based on their assessment of the situation. The participant will re-escalate per schedule to the maintenance dose.

Participants will be pre-medicated with a fast acting antihistamine (loratadine, or similar) at least 60 minutes but not more than 6 hours prior to each dose. Each dose should be administered as a subcutaneous injection with monitoring of peak expiratory flow and monitoring for clinical signs of anaphylaxis. Participants will be observed in the clinic for 30 minutes prior to discharge. When two doses are administered in one visit, there should be a 30 minute interval between each injection.

Additional details on formulation, dosage, preparation and administration can be found in the package insert available at:

http://www.alk-abello.com/US/products/productinformation/Lists/Package%20Inserts/Standardized%20Cat%20Hair.pdf

Table 1: Schedule of cluster immunotherapy injections

Day	Visit	Volume (ml)	Vial #	Fel d 1 (BAU/ml)	Feld1 (BAU)	Fel d 1 (approx mcg)
1	1	0.10	4	10	1	0.003
		0.40	4	10	4	0.012
8	2	0.10	3	100	10	0.03
		0.20	3	100	20	0.06
15	3	0.40	3	100	40	0.12
		0.07	2	1000	70	0.21
22	4	0.10	2	1000	100	0.3
		0.15	2	1000	150	0.45
29	6	0.25	2	1000	250	0.75
		0.35	2	1000	350	1.05
36	7	0.50	2	1000	500	1.5
		0.07	1	10,000	700	2.1
43	8	0.10	1	10,000	1000	3
50	9	0.15	1	10,000	1500	5
57	11	0.20	1	10,000	2000	6
64	12	0.30	1	10,000	3000	9
71	13	0.40	1	10,000	4000	12
78	14	0.50 (end of updosing)	1	10,000	5000	15

Additional visits at the discretion of the investigator may be required for adjustment of allergen doses for individual participants during the updosing or maintenance phases of immunotherapy. These extra visits for dose adjustments could result in the requirement for more than the 4.5- 5.0 mls in vials 2, 3 or 4 that are created in the original dilution series. The clinical coordinator should inform the NIAID Project Manager of this need, and a new kit of diluent will be supplied.

If participants develop side effects during the updosing phase of subcutaneous immunotherapy, such as repeated large immediate local reactions or grade 1-3 systemic reactions requiring dosage adjustment, the site principle investigator, in consultation with the protocol chair and NIAID medical monitor, may decide to limit the planned maintenance dose of immunotherapy injections to less than 5,000 BAU., .Efforts should be made to reach a dose of at least 3000 BAU to maximize potential for effectiveness.

In the case of a failure to reach a dose of 3000 BAU by week 26, and if the participant cannot, for whatever reason, continue weekly dosing attempts to increase to 3000 BAU, a panel comprised of the protocol chair, site investigator and NIAID medical monitor, will convene to determine the best course of action for the particular participant to determine an appropriate maintenance dose.

Similar visits may be required for dosage adjustments due to intercurrent illness, concomitant allergen exposure, or a prolonged interval between doses for personal or other reasons.

5.2.3 Recommended Storage Conditions

The extract concentrate and all dilutions should be kept refrigerated at 2-8°C. Do not freeze, and do not open extract concentrate or placebo vials after the expiration date indicated on the vial label. The extract concentrate or placebo vial can be used for 13 weeks after first puncture. Sites will write on the vial the 13 week expiration date upon initial puncture of the vial.

5.3 DISCONTINUATION OF STUDY TREATMENT

Study treatment, defined as the dosing and administration of study medication according to study specification, will be discontinued for an individual participant if *any* of the following criteria are met:

- Two occurrences of grade 3 systemic reaction after administration of study therapy or nasal challenge.
- An adverse event that, in the judgment of the principal investigator or the medical monitor, presents an unacceptable consequence or risk to the participant.
- An illness or infection that is not associated with the condition under study and that requires treatment not consistent with protocol requirements; or, if a participant develops an intercurrent illness that in the judgment of the principal investigator in any way justifies discontinuation.
- Grade 3 serum sickness at least possibly related to study participation.
- An inability or unwillingness to comply with the study protocol, and the protocol deviations are sufficient to jeopardize the participant's well-being or the integrity of the study.
- Pregnancy occurs during study participation.

Participants who prematurely discontinue study treatment will be invited to complete study assessments at week 104 and any other proceeding clinical allergy assessment visits including visits at weeks 26, 52, and 78 if applicable. However if they decline they will be asked to complete the early termination visit (Appendix 3).

If study treatment is discontinued, the NIAID medical monitor should be notified.

5.4 CONCOMITANT MEDICATIONS

5.4.1 Prophylactic and Emergency Rescue Medications

Antihistamine (loratadine) will be provided and should be taken prior to each immunotherapy injection.

EpiPen® (epinephrine) will be provided and participants will be instructed to bring the medication to each immunotherapy visit. A research staff member will verify that

the participant has the EpiPen® with them prior to each study treatment administration.

5.4.2 Contraception

Low-dose estrogen containing oral contraception is permitted.

5.4.3 Prohibited Medications

Use of the medications listed in the exclusion criteria in Section 4.2 is prohibited during study participation.

5.5 DRUG ACCOUNTABILITY

Under federal regulations (21CFR 312.62) an investigator is required to maintain adequate records of the disposition of the investigational product, including the date and quantity of drug that was received, the participants to whom drug was dispensed (participant by participant accounting), and an account of any drug accidentally or deliberately destroyed. The investigator will ensure that the investigational product supplies are stored as specified in the protocol and pharmacy manual in a secured area, with access limited to authorized study personnel as described in the clinical study agreement.

Records for receipt, storage, use, and disposition of the study drug will be maintained by the study sites. A drug-dispensing log will be kept current for each participant and will contain the identification of each participant and the date and quantity of drug dispensed. All remaining unused investigational product will be returned to the sponsor or sponsor's representative after study termination, or destroyed with the permission of the sponsor in accordance with applicable law and study site procedures. If investigational product is to be destroyed locally, the investigator will provide documentation in accordance with sponsor's specifications.

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

5.6 ASSESSMENT OF COMPLIANCE WITH STUDY MEDICATION

All study medications will be administered at sites by trained medical staff. Compliance, therefore, will be monitored by the site and documented on the eCRF.

6 STUDY PROCEDURES

6.1 VISIT WINDOWS

6.1.1 Scheduled Visits

Appendices 1, 2, and 3 present the schedule of events for this trial. Windows for visits -1 and visit 0 are defined with respect to visit -2. Windows for the remainder of the visits are defined with respect to visit 0. All scheduled study visits must occur within the time limits specified below:

Visit -2: no window

Visit -1: +14 days
Visit 0: +7 days
Visits 1 through 25: ±3days
Visits 26 – 31: AMG 157/placebo and cat immunotherapy/placebo may be given on separate days, ±3 days
Visit 32: ±7 days
Visits 33 through 39: ±30 days

6.1.2 Unscheduled Visits

Unscheduled visits may be performed to investigate poorly controlled allergic symptoms or symptoms that may be related to study therapy. Assessments for an unscheduled visit are listed in Appendices 1, 2 and 3.

6.2 RANDOMIZATION, BLINDING, AND UNBLINDING

6.2.1 Randomization and Stratification

Participants who sign the informed consent, meet the eligibility criteria, and successfully complete the baseline nasal allergen challenge will be randomly assigned to one of the four study groups. Randomization will be accomplished through a password-protected, web-based, randomization system (RhoRAND™) maintained by the Statistical and Data Coordinating Center (SDCC).

Random assignment will be stratified by high versus low exposure to cat allergen assessed at screening and as defined in Section 3.5, and by clinical investigative site.

6.2.2 Blinding

Blinding will be maintained for all study participants and trial personnel after randomization, with the exception of the site's pharmacy staff and unblinded qualified individual to administer cat immunotherapy or placebo. This unblinded qualified individual may also infuse the AMG 157 or placebo but will not be involved in performing any study assessments .

6.2.3 Unblinding

Unblinding before the study is completed will occur only if a participant's well-being is threatened and the investigator believes unblinding is necessary to protect the participant.

Before treatment assignment for an individual participant is unblinded, the principal/site investigator must confer with the NIAID medical monitor. The site investigator will notify the protocol chair of the unblinding event, and the medical monitor will notify the study management team (SMT).

The emergency unblinding will be recorded and reported to the DSMB. A full account of the event will be recorded, including the date and time of the emergency, the reason for the decision to unblind, and the names of the medical monitor and others who were notified of the emergency. During site visits, the site monitor must verify that the medical monitor was notified and that a written account was

completed. The reasons for unblinding of a participant's treatment will be included in the final study report. ITN and NIAID approval is required for unblinding the treatment of an individual participant or subgroups of participants for unplanned interim analyses to support DSMB reviews and final analysis.

An exception to the above rule is that IND Safety Reports that will be reported to the FDA, DSMB, and IRBs in an unblinded fashion as requested by current FDA guidance.

6.3 GENERAL ASSESSMENTS

- Medical history
- Allergy history
- Comprehensive physical examination (includes height and weight)
- Limited physical examination
- Vital signs: blood pressure, temperature, pulse, and respiratory rate
- Pulmonary function testing (spirometry)
- Pre and post peak flow testing
- Adverse events
- Concomitant medications

6.4 CLINICAL ALLERGY ASSESSMENTS

6.4.1 Overview and Washout Periods

Clinical allergy assessments comprise nasal allergen challenges and several forms of skin testing and provide eligibility information, clinical characterization and assessments of allergy status. It is important that participants avoid medications in the period prior to all such assessments that might unduly modify allergic responses independent of the study interventions. Washout periods for specific medications are shown in the accompanying table.

Table 2: Medication washout periods

Medication	Time
Inhaled β-agonists	
Short acting (e.g., Albuterol)	6 hours
Long acting (e.g., Salmeterol, Serevent, Formoterol, Foradil, Oxis)	2 days
Oral β-agonists	
Conventional release (e.g., Albuterol, Ventolin)	12 hours
Extended release (e.g., albuterol-ER)	2 days
Romolyn drugs (e.g., Intal nebulizer solution)	7 days
Leukotriene modifiers, short-acting (e.g., zafirlukast, Accolate)	3 days
Leukotriene modifiers, long-acting (e.g., montelukast, Singulair)	7 days
Inhaled corticosteroids (e.g., beclomethasone, Qvar, Budesonide, Pulmicort, Symbicort, mometasone, Asmanex, Dulera, fluticasone, Flovent, Advair)	14 days
Oral steroid (e.g., prednisone, prenisolone)	14 days
Theophylline product	14 days
Short-acting preparation (e.g., theophylline, Aminophylline)	3 days
Long-acting preparation (e.g., Slo-bid, Theodur)	7 days
Rhinitis Medications	**Time**
Cromolyn sodium (e.g., Nasalcrom)	7 days
Oral Antihistamines (e.g., Cetirizine, Desloratadine, Claritin, Fexofenadine, Allegra, Levocetirizine, Xyzal. Loratadine, Chlorphenamine, Bendryl, Atarax)	5 days
Topical Antihistamines (e.g. azelastine, Astelin)	5 days
Decongestants (e.g., pseudoephedrine, phenylephrine)	3 days
Antihistamine-decongestant tablets/liquids (e.g., Zyrtec D, Claritin-D)	5 days
Nasal corticosteroids (e.g., Flonase, Nasonex, Nasacort, Fluticasone Propionate, Qnasl, Beclomethasone, flunisolide, Budesonide, Rhinocort Aqua, Dymista)	14 days
Topical anticholinergics (e.g. Atrovent, ipratropium)	3 days
Anticoagulants and Anti-platelet agents	**Time**
Low-dose aspirin, NSAIDs, other anti-platelet agents	2 days

6.4.2 Nasal Allergen Challenges

The screening nasal allergen challenge is carried out as a graded, up-dosing allergen exposure starting with the vehicle control only (16.7% glycerin and normal saline) dose and then with the same, commercially available, standardized cat allergen extract used for immunotherapy as described in an accompanying standard operating procedure. A procedure similar to that reported by Scadding et al. (2012) will be used. Subjects who meet protocol inclusion criteria with respect skin testing may undergo the nasal allergen challenge at screening visit, baseline visit and subsequent challenge visits(See Section 4.). Assessments performed during the challenges include peak expiratory flow, forced expiratory volume in 1 second, total nasal symptom score, and peak nasal inspiratory flow rate.

When possible, all nasal allergen challenges in an individual participant will be completed using the same lot of cat allergen extract manufactured by ALK-Abello. Investigators should contact NIAID Project Manager if the lot of cat allergen extract used for a NAC must be changed for any reason. The NIAID Project Manager will be responsible for communicating replacement lot information to the sites.

If the same lot of cat allergen is not available or expires before the week 104 assessment, the participants will be challenged with a new lot of cat allergen extract with a similar Fel d 1 microgram/mL levels. The choice of a replacement lot will be

based on a side-by-side ELISA assay performed at ALK-Abello. The new lot must be within +/- 26% of the lot being substituted.

When a lot change is anticipated due to the expiration of the original lot prior to the week 104 assessment, the lot change will take place at the week 52 nasal allergen challenge.

The lots of cat allergen extract used for the nasal allergen challenges may be the same as, or different than, the lot used for immunotherapy in any one participant.

6.4.3 Skin Testing

6.4.3.1 Overview

All skin test procedures are consistent with current clinical practice and product labeling. All cat allergen extracts will be purchased from ALK-Abelló and packaging and delivery of these materials will be done by a designated drug distributor under contract to NIAID. Skin testing should be done with the same lot of cat allergen extract that is used for nasal allergen assessments in an individual participant.

Washout periods for specific medications prior to skin testing are listed in the table.

6.4.3.2 Skin Prick Test for Cat Allergen

Skin prick test (SPT) for cat allergen will be done as indicated in the SOE.

Controls include: negative control (50% glycerin), and positive control (10 mg/mL histamine dihydrochloride). Subjects will be assessed 15 (+2) minutes after application.

6.4.3.3 Intradermal Skin Test for Early and Late Phase Responses: Cat Allergen Extract

Intradermal skin testing with cat allergen extract will be done at times indicated in the SOE.

For this test, concentrations of standardized cat hair extract specified in an SOP will be applied intradermally to the forearm. Subjects will be assessed 15 (+2) minutes and 6 hours (± 15 minutes) after application.

6.4.3.4 Skin Prick Test (SPT) Serial Titration: Cat Allergen Extract

Skin prick test titration with cat allergen extract will be done at times indicated in the SOE.

A dilution series of standardized cat allergen extracts will be prepared. Each dilution will be applied and punctured in duplicate. Subjects will be assessed 15 (+2) minutes after application.

6.5 CLINICAL LABORATORY ASSESSMENTS

- Serum pregnancy test
- Urine pregnancy test

- Hematology: Complete blood count with differential
- Comprehensive chemistry: Albumin, bilirubin total, creatinine, glucose, potassium, protein total, sodium, alamine amino transferase (ALT) (SPGT), aspartate amine transferase (AST) (SGOT), and urea nitrogen (BUN)
- QuantiFERON® TB test
- HIV Antibody
- Hepatitis B surface antigen test
- Hepatitis C antibody test
- Creatinine Clearance

6.6 PHARMACOKINETIC AND AMG 157 NEUTRALIZING ANTIBODY TESTING

Serum samples to assess pharmacokinetic and AMG 157 serum antibody levels will be collected at drug trough from all participants at the times indicated in the schedule of events.

7 TOLERANCE ASSAYS

7.1 RATIONALE FOR IMMUNE STUDIES

Thymic stromal lymphopoietin (TSLP), a member of the IL-2 cytokine family and an IL-7 like cytokine, was initially identified as being involved in lymphocyte development.[35] TSLP is now known to impact many different cell types including: dendritic cells (DCs), basophils, eosinophils, mast cells, T cells, B cells, and epithelial cells.[16] Multiple murine studies in 2005, two by Steven Ziegler and colleagues at the Benaroya Institute, demonstrated that TSLP is a key initiator of allergic diseases, that is, asthma and atopic dermatitis.[21,36] _ENREF_37 More recent studies have implicated TSLP in the pathogenesis of allergic diseases in humans.[16,20] _ENREF_21

Mechanisms of immunotherapy have been described for grass immunotherapy. These may include stimulation of Th 1-type helper lymphocytes, decrease in Th 2 cells and induction of T regulatory (Treg) cells that produce IL-10 and TGF-β.[12,13] They also include the production of blocking antibodies as well as effects on other inflammatory cells including mast cells, eosinophils and basophils. As noted in the introduction, less is known about the corresponding mechanisms after cat immunotherapy. Ewbank et al. and Nanda et al. both reported approximately two to three-fold increases in cat-specific IgG4 after as little as 5 weeks of subcutaneous immunotherapy.[10,11] In one report there was a reported decrease in CD4/IL-4-positive (Th 2-like) cells[10] but this was not a consistent finding.[11]

Recent data elucidating the role of TSLP in allergic responses suggests that TSLP inhibition has the potential to combine with allergen-specific immunotherapy to give beneficial effects on both the antigen-specific T-cell mediated elements and

inflammatory elements mediated by basophils and mast cells. We hypothesize that TSLP blockade by anti-TSLP will inhibit Th 2 responses and thus combine with immunotherapy to lead to a long-lasting desensitization to cat allergens and potentially to tolerance of these allergens.

Mechanistically tolerance could occur through multiple routes and the experiments summarized below are designed to address these questions. Note that the primary goal of this study is to determine whether the combination of anti-TSLP (AMG 157) plus antigen-specific immunotherapy will result in tolerance to the therapeutic antigen that persists for a substantial period after stopping immunotherapy.

Previous studies of the mechanism of subcutaneous immunotherapy, including those performed by the ITN, have highlighted the value of studying immune and inflammatory responses in the target organ rather than just in the peripheral blood. Therefore, this study will compare changes in the nasal mucosa/secretions with systemic changes in T-cell and humoral responses and the clinical response to nasal allergen provocation before, during and at intervals after immunotherapy and TSLP blockade.

7.2 HYPOTHESES TO BE TESTED

Subcutaneous immunotherapy with cat allergens induces desensitization to cat allergens of unknown durability. We hypothesize that adding anti-TSLP to cat immunotherapy will increase the duration of desensitization and potentially induce tolerance to cat antigens by all or some of the mechanisms proposed below:

1. Long-lived suppression of allergen-induced early and late phase responses for at least 52 weeks after discontinuation of immunotherapy.
2. Change in the T effector/T regulatory cell balance. We predict that either antigen-specific T effector cells will decrease and/or T regulatory cells will increase.
3. Immune deviation in favor of Th 1 responses and relative suppression of local Th 2-mediated allergic inflammation in the nasal mucosa.
4. “Protective” inhibitory antibody responses that persist for at least 56 weeks after discontinuation and are surrogate and/or predictive of the clinical response to immunotherapy.

In order to test these hypotheses, we shall perform intradermal skin provocation testing with measurement of immediate and late skin responses before and at intervals after immunotherapy. Early skin responses occur in minutes and represent IgE-driven mast cell activation and degranulation. Late responses occur several hours later and are mediated by T cells, eosinophils, and basophils. At corresponding time points we will perform nasal allergen challenge with measurement of early and late-phase nasal symptoms, along with nasal fluid, nasal epithelial cell brushings, and peripheral blood sampling. The hypotheses described above will be tested using a variety of assays, examples of which are described in the sections below.

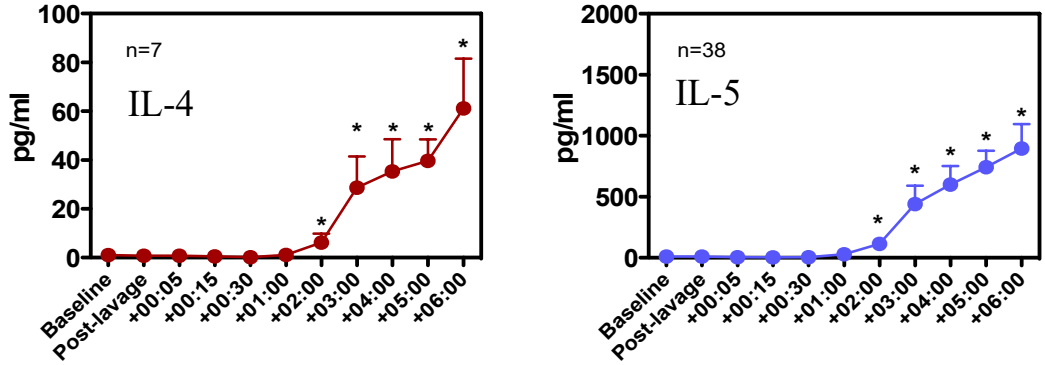
7.3 RETENTION OF SAMPLES

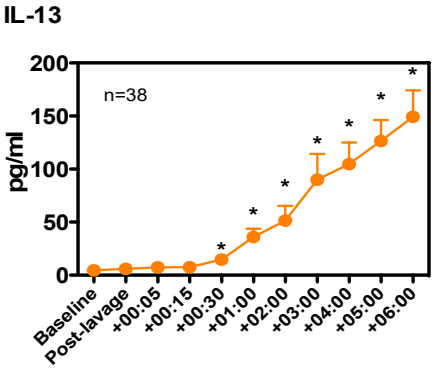
A major priority of the Immune Tolerance Network, in partnership with the National Institute of Allergy and Infectious Diseases of the NIH, USA, is the development of novel immunoassays in order to better understand mechanisms of tolerance and to develop biomarkers to predict the development and maintenance of clinical tolerance. As in all Immune Tolerance Network-funded clinical trials, informed consent will be obtained from all participants for their samples to be stored for use in future studies. Biological specimens collected in this trial will be stored long-term in order to re-evaluate biologic responses as new research tools to study tolerance become available. The specimens will therefore be stored at the ITN sample repository for a minimum of 10 years. Residual specimens may be used by the investigators for development of new immunologic assays or for cross-trial comparisons. Although specimens in this protocol are described in the context of assays to be performed, it should be noted that not necessarily all assays will be performed for all participants at each time point. Decisions to perform assays will be made based on statistical and scientific planning, hypotheses to be tested, and technologies available. Finally, clinical outcomes will be taken into account to determine the potential value of the assays. For example, if a clinical effect fails to occur, it may be decided that there is minimal value in performing certain mechanistic assays.

7.4 LOCAL IMMUNE RESPONSES IN NASAL MUCOSA

Nasal allergen challenge will be performed at baseline and at 26, 52, and 104 weeks. Along with clinical signs and symptoms score, nasal secretions will be collected at multiple time points at baseline, and at 26, 52, and 104 weeks. These analyses are based on pilot work performed by Drs. Scadding, Durham et al. in collaboration with the ITN, to support the GRASS study (ClinicalTrials.gov Identifier: NCT0335139). Note, that as shown in Figure 2 Th 2 cytokine levels continue to rise to 6 hours post challenge.[31] Therefore to ensure that we capture the entire late phase responses, samples in this trial will be collected several hours post-challenge.

Figure 2: Th 2 cytokine levels





Epithelial cells harvested by nasal brushing/scraping may also be collected at each nasal allergen challenge visit. However, brushings will only be taken 6 hours after the challenge to avoid inflammatory sampling effects on nasal secretions taken at earlier time-points.

Nasal secretions will be assayed for inflammatory mediators, including TSLP, and local antibodies. RNA will be extracted from the epithelial cells harvested by nasal brushing and the expression of various cytokines and chemokines assessed by quantitative gene expression technology, such as RT-PCR or RNAseq, for comparison between the four treatment groups.

- Nasal fluid will be obtained before and at intervals up to 6 hours after nasal allergen challenge for measurement of cytokines, such as IL-5, IL-13, IF -γ, IL-10, TGF-β , antibodies (IgE, IgG4 and IgA2 using CAPRAST) and inflammatory mediators such as ECP (eosinophilic cationic protein), tryptase, basogranulin, and TSLP.
- Nasal epithelial cells may be collected from the nasal epithelium by gently brushing the inferior turbinates using an interdental brush (2 or 3 mm). The brush will be introduced into a microcentrifuge tube, covered with 1.5 mL extraction buffer RLT (Qiagen), and stored in this buffer at −80 °C for up to 6 months until batched extraction of the RNA.

Depending on the results of pilot studies and future publications, RNA extracted from the epithelial cells may be analyzed for genes of interest by RT-PCR. These may include genes for:

- epithelial cell-derived chemokines such as TSLP, MDC, TARC, Eotaxin, RANTES
- adhesion molecules such as ICAM-1
- cytokines such as IL-8, GM-CSF and those that may possibly be detected in trans-epithelial migratory effector cells, including IL-4, IL-5, IL-9, IL-13, Interferon-gamma, IL-10 and TGF-beta

- newer cytokines of potential interest e.g., IL-25, IL-22, IL-33 and IL-35
- transcription factors of interest e.g., GATA-3, STAT-6, T Bet and FOXP3

7.5 WHOLE BLOOD ASSAYS

7.5.1 DNA-HLA Genotypes and Single Nucleotide Polymorphisms

MHC tetramers bind to the T-cell receptor in an HLA-specific context. Therefore, DNA will be collected from participants to perform sequence-based HLA typing, such that appropriate candidates can be identified for tetramer analysis.

In 2008, Dr. Ziegler conducted analyses of single nucleotide polymorphisms (SNPs) of the *TSLP* gene in human bronchial epithelial cells, to determine if there are functional genetic variants of TSLP that contribute to disease.[37] He surveyed SNPs on the *TSLP* gene by sequencing genomic DNA from 36 subjects, and characterized the linkage disequilibrium of the gene. Dr. Ziegler identified a total of 23 polymorphisms in the *TSLP* gene. Two splice variants of TSLP have been identified: the long form, which is associated with allergic inflammation, was highly induced by poly (I:C) (double-stranded RNA) stimulation in normal human bronchial epithelial cells (NHBE) ($P = 0.0060$). The SNP rs3806933 (−847C > T) in the promoter region of long-form *TSLP* was found to create a binding site for the transcription factor activating protein (AP)-1, and *in vitro* functional analyses demonstrated that the SNP enhanced AP-1 binding to the regulatory element. The functional variant increased promoter-reporter activity of long-form *TSLP* in response to poly (I:C) stimulation in NHBE. Functional genetic polymorphism of the *TSLP* gene thus appears to have the potential to contribute to Th 2-polarized immunity through higher TSLP production by bronchial epithelial cells in response to viral respiratory infections.[37]

Although there are relatively few patients in this study and all are cat allergic, so much is known about TSLP SNPs that we may wish to look for variants and correlate them with response to therapy and to the expected frequency of TSLP alleles in the general non-allergic, appropriately race-matched population.

7.5.2 Gene Expression Profiling

To further elucidate possible changes in cytokines, signaling pathways and cellular profiles, gene expression profiling analysis may be performed on RNA isolated from peripheral blood using RNAseq, nanostring, or high-throughput real-time PCR methods. The goal of these assays is to identify differences in transcriptional profiles between treatment arms and clinical outcome groups, and to monitor the durability of these potential changes after discontinuation of immunotherapy. These types of analyses may also explain why some individuals respond to treatment or elucidate mechanisms resulting in adverse responses to treatment.

7.6 CELL ASSAYS

7.6.1 PBMC Preparation

All blood samples collected for PBMC isolation will be shipped from the clinical sites to the ITN core facility for processing using ITN standard operating procedures

(SOPs) for PBMC separation, and freezing. This will ensure that standardized procedures are used and that high quality material is obtained for testing. PBMCs will be stored in the vapor phase of liquid nitrogen until use.

7.6.2 Cellular Assays

To address the functional status of T cell responses, various cell-based assays using previously frozen PBMCs can be used to estimate the frequency, phenotype, cytokine and transcriptional profiles of allergen-specific T cells.

Allergen specific CD4 T cells are present at very low frequencies in peripheral blood and differ in function/frequency in allergic vs. non-allergic individuals. MHC (major histocompatibility complex) class II "tetramers", a fluorescently tagged multivalent synthetic mimic of the peptide binding proteins found on the surface of antigen presenting cells can be used to directly identify and enumerate cat allergen Fel d1 epitope specific CD4 T cells using flow cytometry. Additional steps may be performed to determine the effector function of antigen-specific CD4 T cells. For example, intracellular cytokine staining for IL-4, IL-5, IL-9, IL-13, IFNg, IL-17, IL10, Foxp3 and TGFβ could be performed to assess frequencies and ratios of Th1, Th2, Th9, Th17, Th17.1, and Treg cells. Alternatively, antigen-specific CD4 T cells could be FACS sorted for transcriptional analysis by RNAseq or related platform. The ability to use these reagents will depend on the overlap between HLA alleles among study participants and available reagents.

Since tetramers are not currently available for all haplotypes other T-cell assays may also be performed. We plan to pilot a CD154 up-regulation assay for monitoring cat allergen-reactive CD4 T cells with Bill Kwok at BRI using PBMC isolated from ~70 mL fresh blood collected from 15 study participants at the Asmthma Inc Site. This assay requires overnight in vitro stimulation of ~40 million PBMCs with Fel d 1 and Feld 4 peptides/cat allergen extract in the presence of anti-human CD40 blocking mAb. ½ of the cells will be collected and analyzed by flow cytometry, and the other half will be used for sorting CD154+ and CD154- CD4 T cells for RNAseq analysis. Since additional PBMCs from these same study participants/visits will be processed and cryopreserved by the ITN Core PBMC Processing Lab, we will be able to directly compare the performance and utility of the CD154 up-regulation assay for cryopreserved PBMCs tested in a single batch compared to fresh PBMC tested in real-time. If the CD154 upregulation assay can be validated on cryopreserved PBMCs, this assay would be an attractive option for determining the impact and durability of treatment on frequencies and phenotypes of cat allergen specific CD4 T cells in all study participants.

For optimum use, these assays require 40 million viable cells per assay, so it is important that every effort is made to collect the full planned blood volumes at the time points specified the SOE.

We may also use surface flow cytometry to determine the frequency of T_H2A cells as this subset of Th2 cells may be a biomarker for allergy. Work by Wambre suggests that this subset includes the vast majority of allergen-specific CD4 T cells as

determined by tetramer analysis. This assay has the advantage that it can be reliably performed with only two million previously frozen, viable PBMCs.

Treatment with anti-TSLP may lead to decreased STAT 5/6 signaling and potentially increased STAT 1 or STAT 3 signaling in multiple immune cell types. Activation status of these signaling pathways may be measured by flow cytometry using antibodies that discriminate between the phosphorylation states of these molecules in specific cell populations upon appropriate stimulation in vitro.

This protocol has been designed to allow us to store multiple aliquots of PBMCs taken at major time points in the study. This will allow us to look at additional markers and to address new reports of relevant markers. For example, recent publications[38] suggest that changes in the surface expression of FcεRI may enhance local inflammation after cell recruitment to the site of allergen challenge. Up-regulation of FcεRI is known to amplify the capacity for uptake and presentation of allergen to Th 2 effector cells via IgE mediated allergen capture and secondarily programming of IL-4/IL-13 dependent activated macrophages. Therefore, we may measure surface expression of FcεRI on plasmacytoid and myeloid dendritic cells as well as monocytes by flow cytometry using frozen PBMCs.

7.7 SERUM ASSAYS

Serum antibody responses will be measured to determine levels of total and allergen-specific antibodies. These measurements will include testing for total IgE and IgG and allergen-specific IgE, IgG1, IgG4, and IgA2, and will be performed in the ITN core. A role for IgG1, IgG4, and IgA2 in blocking allergen-specific reactivity is postulated; however, previous studies showed various results in allergen-specific antibody induction after immunotherapy.

In addition to antibody testing, serum samples may be used in functional assays including measurement of IgG-associated serum inhibitory activity of IgE-facilitated binding of allergen-IgE complexes to B cells (IgE-FAB (facilitated antigen binding), a surrogate measure of IgE-FAP (facilitated antigen presentation) and serum inhibitory activity for basophil histamine release (BHR). These two assays address the degree of blocking of binding of allergen-IgE complexes to low- and high affinity IgE receptors (respectively). Serum from treatment and placebo groups will be assessed for its inhibitory activity for IgE-FAB. The time course and magnitude of changes in inhibitory activity for IgE-FAB will be compared with clinical symptoms, clinical scores, and allergen-specific IgE, IgG1, IgG4, and IgA2 levels. Similarly, serum will be screened for its inhibitory activity in BHR assays.

8 ADVERSE EVENTS

8.1 OVERVIEW

The investigator is responsible for the detection and documentation of events meeting the criteria and definition of an AE (adverse event) or SAE (serious adverse event) as described in Section 8.2.2 and Section 8.2.4 in this protocol. All AEs and SAEs will

be recorded in the source documents and on the appropriate electronic CRF(s). All data will be reviewed periodically by the DSMB, which may provide recommendations to NIAID about withdrawing any participant and/or terminating the study because of safety concerns.

Adverse events that are classified as serious according to the definition of health authorities must be reported promptly and appropriately to the NIAID, ITN, principal investigators in the trial, IRBs, and health authorities. This section defines the types of AEs and outlines the procedures for appropriately collecting, grading, recording, and reporting them. Information in this section complies with 21CFR 312; ICH Guideline E2A*: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting*; and ICH Guideline E-6: *Guidelines for Good Clinical Practice*; and applies the following standards:

- *Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (published September 2007)* for local reactions to immunotherapy and study procedures.
- WAO Subcutaneous Immunotherapy Systemic Reaction Grading System for systemic reactions to immunotherapy and study procedures.
- National Cancer Institute (NCI) *Common Terminology Criteria for Adverse Events Version 4.03* (published June 14, 2010) for all other reactions to immunotherapy and study procedures. (This document is referred to herein as the "NCI-CTCAE manual.")

8.2 DEFINITIONS

8.2.1 Adverse Event

An adverse event (AE) is any occurrence or worsening of an undesirable or unintended sign, symptom, laboratory finding, or disease that occurs during participation in the trial. An AE will be followed until it resolves or until 30 days after a participant terminates from the study, whichever comes first. All AEs will be reported as specified in Section 8 whether they are or are not related to disease progression or study participation.

8.2.2 Study-Specific Adverse Events

Allergic rhinitis

During the study it is anticipated that participants will experience allergic rhinitis symptoms related to cat exposure including itching, sneezing, watery discharge, nasal congestion, and eye symptoms. These symptoms are consistent with moderate-severe allergic rhinitis caused by cat exposure which is an entry criterion for the study. These symptoms therefore will not be reported as adverse events.

Local reactions to immunotherapy and study procedures

Common local symptoms due to immunotherapy, skin testing and nasal allergen challenge will be graded according to table 3 below. Only those reactions assessed as grade 2 or greater will be reported as adverse events. Please refer to Section 8.4.1 for specifications of local reactions related to nasal allergen challenge.

Table 3: Grading of local reactions to cat immunotherapy and to study procedures

(Adapted from Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (published September 2007).

Grade	1	2	3	4
Cat immunotherapy and skin testing				
Pruritis	Interfering with the usual daily activities or sleep, but requiring no medication other than topical corticosteroids, or antihistamines.	Interfering with the usual daily activities or sleep and requiring oral steroids.	Requiring a visit to a health care provider for treatment	Not applicable
Pain	Does not interfere with activity	Repeated use of non-narcotic pain reliever > 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	Emergency room (ER) visit or hospitalization
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	ER visit or hospitalization
Erythema/ Redness*	2.5 – 5 cm	> 5 cm and interferes with activity	>10 cm or prevents daily activity	Necrosis or exfoliative dermatitis
Induration/ Swelling**	2.5 – 5 cm and does not interfere with activity	> 5 cm and interferes with activity	>10 cm or prevents daily activity	Necrosis
Nasal allergen challenge				
	Requiring antihistamines, decongestants or nasal steroids as rescue medication	Requiring oral steroids as rescue medication	Requiring a visit to a health care provider for treatment.	Life threatening and/or requiring hospitalization (local upper airway obstruction)

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

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

8.2.3 Suspected Adverse Reaction and Adverse Reaction

Suspected adverse reaction (SAR) means any adverse event for which there is a reasonable possibility that the study drug caused the adverse event. For the purposes

of safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse reaction. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug (21 CFR 312.32(a)).

An adverse reaction (AR) means any adverse event caused by a study drug. Adverse reactions are a subset of all suspected adverse reaction for which there is reason to conclude that the drug caused the event.

8.2.4 Serious Adverse Event

An AE or SAR is considered "serious" if, in the view of either the investigator or DAIT/NIAID it results in any of the following outcomes (21 CFR 312.32(a)):

- Death: A death that occurs during the study or that comes to the attention of the investigator during the protocol-defined follow-up period must be reported whether it is considered treatment related or not.
- A life-threatening event: An AE or SAR is considered "life-threatening" if, in the view of either the investigator or the medical monitor, its occurrence places the subject at immediate risk of death. It does not include an AE or SAR that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization.
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- An event that requires intervention to prevent permanent impairment or damage. An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based on appropriate medical judgment, it may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.
- Congenital anomaly or birth defect.

8.2.5 'Expected' versus 'Unexpected' Suspected Adverse Reaction

A suspected adverse reaction is considered "expected" when it is listed in the investigator brochure, the package insert or the protocol. A suspected adverse reaction is considered "unexpected" when its nature (specificity), severity, or rate of occurrence is not consistent with applicable product information as described in the safety information provided in the investigator brochure, the package insert or the protocol (21 CFR 312.32(a)). A serious unexpected suspected adverse reaction is referred to as a SUSAR.

For this study, expectedness will be determined by product information provided in the package insert for cat immunotherapy and in the investigator's brochure for AMG 157. Expectedness for study procedures (nasal allergen challenge, intradermal skin test and skin prick test) will be determined by information in the protocol.

8.3 COLLECTING AND RECORDING ADVERSE EVENTS

8.3.1 Methods of Collection

Adverse events may be collected as follows:

- Observing the participant.
- Questioning the participant in an objective manner.
- Receiving an unsolicited complaint from the participant.

An abnormal value or result from a clinical or laboratory evaluation (e.g., a radiograph, an ultrasound, or an electrocardiogram) can also indicate an AE if it is either determined by the investigator to be clinically significant or meets the criteria for a grade 2 or greater AE per CTCAE criteria. If this is the case, it must be recorded in the source document and as an AE on the appropriate AE form(s). The evaluation that produced the value or result should be repeated until that value or result returns to normal or can be explained and the participant's safety is not at risk.

8.3.2 Collection Period for Adverse Events and Serious Adverse Events

8.3.2.1Adverse Events

All systemic reactions and all grade 2 or greater local reactions occurring within 72 hours after cat immunotherapy and/or study procedures (see Section 8.2.2) will be collected from visit -2 (week -4) until the participant completes the study (visit 40/week 104) or prematurely withdraws from the study. All other AEs will be collected from visit 0 (week 0) until the participant completes the study (visit 40/week 104) or prematurely withdraws from the study.

8.3.2.2Serious Adverse Events

Serious AEs will be collected from visit -2 (week -4) until 30 days after the participant completes the study (visit 40/week 104) or prematurely withdraws from the study.

8.3.3 Methods of Recording

8.3.3.1Recording AEs

Throughout the study, the investigator will record all AEs on the appropriate eCRF. The investigator will treat participants experiencing AEs appropriately and observe them at suitable intervals until their symptoms resolve or their status stabilizes.

8.3.3.2Recording SAEs

Serious AEs will be recorded on the SAE eCRF and health authorities will be notified as outlined in Section 8.5.2.

8.4 GRADING AND ATTRIBUTION OF ADVERSE EVENTS

8.4.1 Grading Criteria

8.4.1.1Adverse Events Related to Cat Immunotherapy or to AMG 157

Immunotherapy

Local reactions to cat immunotherapy not associated with systemic signs or symptoms will be graded according to the table in Section 8.2.2.

Systemic reactions to cat immunotherapy will be graded according to the WAO Subcutaneous Immunotherapy Systemic Reaction Grading System (see appendix 4).

All other adverse events related to cat immunotherapy will be graded according to the criteria set forth in the NCI-CTCAE (v4.03 published June 14, 2010).

AMG 157

All adverse reactions related to AMG 157 will be graded according to the NCI-CTCAE v4.03, published June 14, 2010.

8.4.1.2Adverse Events Related to Nasal Allergen Challenge and Skin Testing

Local reactions related to nasal allergen challenge and skin testing not associated with systemic signs or symptoms will be graded according to the table in Section 8.2.2. Only grade 2 or greater local reactions will be recorded as AEs. Systemic reactions related to skin testing or to nasal allergen challenge procedure will be graded according to the WAO Subcutaneous Immunotherapy Systemic Reaction Grading System (see Appendix 4). All grades of system reactions should be recorded as AEs and as SAEs if serious criteria are met.

The following specifications considered local, not systemic reactions will apply for nasal allergen challenges:

- rhinitis (e.g., sneezing, rhinorrhea, nasal pruritus and/or nasal congestion) and
- cough perceived to originate in the upper airway, not the lung, larynx, or trachea

All other adverse events related to study procedures will be graded according to the criteria set forth in the National Cancer Institute's *Common Terminology Criteria for Adverse Events, Version 4.03* (published June 14, 2010).

8.4.1.3All Other Adverse Events

All other adverse events will be graded on a scale from 1 to 5 according to the following standards in the NCI-CTCAE manual:

- Grade 1 = mild adverse event
- Grade 2 = moderate adverse event
- Grade 3 = severe and undesirable adverse event

- Grade 4 = life-threatening or disabling adverse event
- Grade 5 = death

For additional information and a printable version of the NCI-CTCAE manual, go to http://ctep.cancer.gov/reporting/ctc.html

8.4.2 Attribution Definitions

Adverse events will be categorized for their relation to one or more of the following:

- Study medications:
  - AMG157/AMG 157 placebo
  - Cat immunotherapy/Cat immunotherapy placebo
- Study procedures:
  - Nasal allergen challenge
  - Intradermal skin test
  - Skin prick test

The investigator will make the initial determination of the relation, or attribution, of an AE to participation and will record the initial determination on the appropriate eCRF and/or SAE reporting form. The relation of an AE to study drug will be determined using definitions in the Table 4. Final determination of attribution for safety reporting will be decided by DAIT/NIAID.

Table 4: Attribution of adverse events

Code	Descriptor	Definition
Unrelated Category		
1	Unrelated	The adverse event is clearly not related.
2	Unlikely	The adverse event is unlikely related.
Related Categories		
3	Possible	The adverse event has a reasonable possibility of being related; there is evidence to suggest a causal relationship.
4	Probable	The adverse event is likely related.
5	Definite	The adverse event is clearly related.

8.5 REPORTING SERIOUS ADVERSE EVENTS

8.5.1 Reporting SAEs to the IND Sponsor

The following process for reporting an SAE ensures compliance with 21CFR 312 and ICH guidelines. After learning that a participant has experienced an SAE, the principal investigator or designee will report the SAE via the electronic SAE report form (SAE eCRF) within 24 hours of becoming aware of the event. The initial SAE eCRF should include as much information as possible, but at a minimum must include:

- AE term
- Study drug treatment
- Relationship to study medications
- Reason why the event is serious
- Supplementary CRF pages must be current at the time of SAE reporting: medical history, concomitant medications, demographics, study drug administration, death.

As additional details become available, the SAE eCRF should be updated and submitted. Every time the SAE eCRF is submitted, it should be electronically signed by the investigator or subinvestigator.

For additional information regarding SAE reporting, contact Rho Product Safety:

Rho Product Safety
6330 Quadrangle Drive, Suite 500
Chapel Hill, NC 27517
Toll-free - (888) 746-7231
SAE Fax Line: 1-888-746-3293
Email: rho_productsafety@rhoworld.com

8.5.2 Reporting SAEs to Health Authorities

After the SAE has been reported by the principal investigator and assessed by the IND sponsor, the IND sponsor must report the event to the appropriate health authorities using one of these two options:

- **Standard reporting (IND annual report).** This option applies if the AE is classified as:
  - Serious, expected, suspected adverse reactions described in Section 8.2.2, Section 8.2.3 and Section 8.2.4.
  - Serious and not a suspected adverse reaction described in Section 8.2.2 and Section 8.2.3.
- **Expedited reporting (IND safety report).** This option applies if the AE is classified as one of the following:

1. Serious and unexpected suspected adverse reaction (SUSAR) per Section 8.2.2, Section 8.2.3 and Section 8.2.4.

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

   - A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure (e.g., angioedema, hepatic injury, or Stevens-Johnson Syndrome);
   - One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the drug (e.g., tendon rupture);
   - An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group.

2. Any findings from other studies: The sponsor must report any findings from other epidemiological studies, pooled analysis of multiple studies, or clinical studies that suggest a significant risk in humans exposed to the drug that would result in a safety-related change in the protocol, informed consent, investigator brochure, or other aspects of the overall conduct of the study.

Safety reports must be reported by DAIT/NIAID to the appropriate health authorities within 15 calendar days; fatal or immediately life-threatening, serious, unexpected, suspected adverse reactions must be reported within 7 calendar days.

All principal investigators must report SAEs to their respective IRBs as mandated by them.

8.5.3 Reporting SAEs to the DSMB

The NIAID and ITN will provide the DSMB with data of all SAEs on an ongoing basis, including quarterly reports of all SAEs and as indicated in the stopping rules.

8.5.4 Reporting Pregnancy

The principal investigator should be informed immediately of any pregnancy and all available pregnancy information should be entered into the electronic data capture (EDC) system within 24 hours of becoming aware of the event. The investigator should counsel the participant and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the participant should continue until the conclusion of the pregnancy. Follow-up information detailing the outcome of the pregnancy should be entered into the EDC system as it becomes available. Any premature termination of the pregnancy will be reported.

Any pregnancy complication or elective termination of a pregnancy for medical reasons will be recorded as an AE or SAE as described in Section 8.3.1 and Section 8.3.2.

9 STATISTICAL CONSIDERATIONS AND ANALYTICAL PLAN

9.1 ANALYSIS SAMPLES

Intent to treat (ITT) sample: All subjects who are randomly assigned to treatment or placebo.

Per protocol (PP) samples: All ITT sample participants who remain in the study for at least 2 years and in whom the primary endpoint is assessed. Participants in the PP sample must be compliant with study medication and study assessments, defined as 1) The primary TNSS AUC endpoint at 104 weeks is collected, and 2) Taking 75% of their study medication for the duration of the study. Compliance with study medication will be as assessed by meeting one or both of these conditions:

-48 weeks of both SIT and AMG157 treatment are taken regardless of dosage.

-At least 12 of 16 (≥75%) prescribed SIT doses of 3000+ and 10 of 13 (75%) AMG 157 infusions are taken.

Safety sample (SS): All randomized participants who receive at least one dose of study medication. Participants in the safety sample will be analyzed with the group according to the medication they actually received, regardless of their randomized assignment.

9.2 ANALYSIS OF ENDPOINTS

9.2.1 Overview

Analysis of study data will be conducted to address all objectives of the trial and other interrelationships among all data elements of interest to the investigators and of relevance to the objectives of the study.

9.2.2 Primary Endpoint

The primary endpoint is the nasal allergen challenge TNSS AUC from 0 to 1 hour at 104 weeks and will be analyzed as described below.

The primary analysis will compare the mean TNSS AUC from 0 to 1 hour after cat allergen challenge at 104 weeks, using a longitudinal repeated measures model in the ITT sample. The model will include fixed effects for treatment, time, and treatment by time interaction and will include covariates for site and baseline TNSS AUC. Since a nonlinear relationship between the TNSS AUC outcome and time is expected, time will be treated as a categorical variable. An unstructured covariance structure will be used to model the correlation among time points within a subject. If the model assuming an unstructured covariance matrix does not converge, a spatial power covariance structure will be used instead. The primary endpoint will be assessed at week 104 using a contrast in least squares means between the following

groups: cat immunotherapy plus AMG 157 (group A in Figure 1) and cat immunotherapy plus placebo (group B in Figure 1).

The 0 to 1 hour TNSS AUC will be calculated using the trapezoidal rule.

A supplementary analysis of the primary endpoint will also be performed using the PP sample.

9.2.3 Secondary Endpoints

This study is not designed or powered to perform hypothesis testing on secondary endpoints. All secondary analyses will be treated as supportive. P-values will be presented for the secondary endpoints but will not be adjusted for multiplicity and should be interpreted with caution. The secondary endpoints will be analyzed using the ITT sample and will include all pairwise comparisons among the four study groups.

Secondary endpoints include measures of allergic response assessed at the timepoints listed in Section 3.3.2 including:

- Skin prick test endpoint titration
- Skin early phase response (EPR) to intradermal testing
- Skin late phase response (LPR) to intradermal testing
- Peak TNSS EPR
- TNSS EPR
- TNSS LPR
- PNIF LPR AUC
- PNIF EPR AUC

9.2.4 Exploratory Endpoints

Exploratory endpoints related to mechanisms of immune modulation and tolerance assessed prior to, during and after therapy, including those related to:

- Local immune response in nasal fluid for inflammatory cytokines. Nasal brushing for cells for RNA extraction.
- Cells in peripheral blood basophil activation at various timepoints during and after immunotherapy, cat antigen tetramers for changes during and after immunotherapy, cytokine profiles after in vitro cat antigen stimulation.
- Serum components of cat-specific IgE, IgG and subclasses, IgA inhibition of facilitated antigen presentation.

9.2.5 Interim Analysis

An interim analysis for futility based on conditional power for the comparison between immunotherapy and placebo groups as described below will be performed

when the first 10 participants in each treatment group (30% of the total planned sample size) have all reached their week 52 TNSS assessment

The interim analysis will be performed by a statistician not associated with the study team. The data will only be reviewed by the DSMB and will not be made available to the study team.

The rationale for the interim analysis is that if there is good evidence for absence of a difference between immunotherapy groups and placebo group, the study hypotheses as outlined in Section 9.3 is unlikely to be provable.

Conditional power will be calculated assuming that there will be an observed difference in TNSS AUC 0-1 hr at week 52 of 32% comparing:

- cat immunotherapy plus AMG 157 (Figure 1, group A) to placebo-placebo (Figure 1, group D)
- cat immunotherapy plus placebo (Figure 1, group B) to placebo-placebo (Figure 1, group D)
- the combination of cat immunotherapy plus AMG 157 and cat immunotherapy plus placebo (Figure 1, groups A and B) to placebo-placebo (Figure 1, group D)

First, conditional power will be calculated using the originally planned sample size, the assumed treatment effect, and the currently observed distribution for the endpoint.

Second, conditional power will be calculated using the originally planned sample size, the currently observed treatment effect, and the currently observed distribution for the endpoint.

If the conditional power is ≥33% for any of the comparisons, that will be taken as evidence against futility and as an indication that the trial should proceed.

If the conditional power is <33% for all the comparisons, that will be taken as evidence of futility and consideration will be given to terminating the study based upon the recommendation of the DSMB and at the discretion of the ITN and NIH.

Since the main outcome of the study, tolerance, cannot be measured at week 52, and the main comparison for the primary endpoint, which is between groups A and B, is not used in the interim analysis, no alpha adjustment will be made for the final analysis.

9.2.6 Safety Analysis

Safety analyses will include AEs, SAEs, laboratory abnormalities, and physical examination abnormalities. All participants in the safety sample will be included in all safety analyses.

Frequency of AEs will be tabulated by system organ class and preferred term, as well as by seriousness, severity, and treatment relatedness. Frequency of SAEs will be tabulated by system organ class and preferred term. For pertinent laboratory

measurements, mean and mean change from baseline values will be presented by treatment group and visit. Frequency of physical examination abnormalities will be tabulated by treatment group, visit, and organ class. Safety data will also be listed by treatment group and subject.

9.2.7 Medical History

Medical history within the past 12 months—including the existence of current signs and symptoms—will be collected for each body system.

9.2.8 Use of Medications

All medications taken by or administered to study participants beginning 30 days before enrollment and continuing throughout the study will be collected. All medications used will be coded according to the WHO drug dictionary. The number and percentage of participants receiving prior and concomitant medications/therapies will be presented overall and by medication class.

9.3 SAMPLE SIZE

The sample size is based on detecting a difference in TNSS AUC 0-1hr between the immunotherapy plus AMG 157 combination group and the immunotherapy plus placebo group at 104 weeks.

We assume that both active cat immunotherapy groups will demonstrate a reduction from baseline in mean TNSS AUC 0-1 hr by the conclusion of therapy at 52 weeks. In contrast, the placebo arm (group D) will not show any improvement in TNSS over time. At week 52, we assume that the difference in mean TNSS AUC 0-1 hr between the active cat immunotherapy groups and placebo (group D) will be approximately 40%. These assumptions have been observed in previous cat immunotherapy trials.

We further assume that TNSS AUC will return close to pre-therapy levels in the group that receives immunotherapy alone. This is based on the assumption that the immunomodulatory effect of approximately 52 weeks of cat allergen immunotherapy alone is too short to induce tolerance, with tolerance defined as the persistent reduction of TNSS even after immunotherapy is discontinued.

In contrast, and consistent with the overall study hypothesis, we assume that the group receiving combination treatment will lead to tolerance and remain relatively stable in terms of TNSS from week 52 through week 104. This would demonstrate that AMG 157 contributes to the induction of tolerance.

These assumptions imply a 40% difference in mean TNSS AUC 0-1 hr for the primary comparison, which is between cat immunotherapy plus AMG 157 (group A) and cat immunotherapy plus placebo (group B).

Pilot studies were conducted to estimate the mean and standard deviation for the TNSS in response to cat nasal allergen challenge in untreated cat-allergic subjects (Stephen Durham Imperial College London, personal communication). Table 5 below illustrates the means and standard deviations for several durations of AUC.

The highest ratio for the mean to standard deviation is for the 0-1 hr period. For this period, the mean is estimated at 4.56 with standard deviation of 1.5, and an effect size for a 40% difference between groups of 1.82. This higher effect size in large measure drove the decision to use this AUC period for the primary endpoint.

Table 5: Sample size estimates based on pilot studies of TNSS

Total Nasal Symptom Score					Number of Subjects per Group			
					No dropout		15% dropout	
Parameter	Mean	SD	Diff between groups	Approximate Effect Size (mean/SD)	80% power	90% power	80% power	90% power
AUC 0-8h	14.93	7.1	40% 35% 30% 25%	1.26 1.37 1.47 1.58	24 30 41 58	31 40 54 78	29 36 49 69	37 48 64 92
AUC 0-6h	13.27	6.2	40% 35% 30% 25%	1.28 1.39 1.50 1.61	23 29 39 56	30 39 52 74	28 35 46 66	36 46 62 88
AUC 0-1h	4.56	1.5	**40%** 35% 30% 25%	**1.82** 1.98 2.13 2.28	13 16 21 30	16 21 28 39	16 19 25 36	**19** 25 33 46

Allowing for approximately 15% dropouts, for 90% power to determine this treatment effect at a two-sided 0.05 level of significance, a sample size of approximately 19 per group would be required. However, in order to account for differences compared to previous trials in immunotherapy dosing and uncertainties regarding the size and duration of the treatment effect, a sample size of approximately 30 per group will be enrolled in the treatment phase. This will allow the study to detect a treatment effect as small as 32%.

Repeated measures analysis commonly provides more statistical power than cross-sectional studies, since the greater total number of observations over all participants and time points increases the degrees of freedom available for hypothesis tests. Hence, with the current sample size, the power to detect the expected treatment effect is anticipated to be greater than 90%.

9.4 MISSING DATA

The dropout rate in this study is anticipated to be less than or equal to 15% and to be equally distributed among the randomized groups. The primary analysis does not require that missing data be imputed. However, any supportive cross-sectional analyses of the primary or secondary endpoints for the ITT population will require missing data to be imputed.

If missing data are not equally distributed between groups, biases can be created.

Optimistic and pessimistic imputation methods and sensitivity analyses will be used to provide upper and lower bounds for potential bias. These will provide a measure of robustness of the treatment effect as it relates to the causes and consequences of missing data. More specific details of the imputation methods and sensitivity analyses will be specified in the SAP. Generally, a combination of multiple imputation methods for missing data will be used, including for example: regression, propensity scoring, and/or Markov chain Monte Carlo methods.

9.5 REPORTING DEVIATIONS FROM THE ORIGINAL STATISTICAL PLAN

The principal features of both the study design and the plan for statistical data analysis are outlined in this protocol and in the statistical analysis plan (SAP). Any change in these features requires either a protocol or an SAP amendment, which is subject to review by the DSMB, the study sponsor(s), and the health authorities. These changes will be described in the final study report as appropriate.

10 ACCESS TO SOURCE DATA/DOCUMENTS

The investigational sites participating in this study will maintain the highest degree of confidentiality permitted for the clinical and research information obtained from participants in this clinical trial. Medical and research records should be maintained at each site in the strictest confidence. However, as a part of the quality assurance and legal responsibilities of an investigation, the investigational sites must permit authorized representatives of the ITN, sponsor, and health authorities to examine (and to copy when required by applicable law) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress. Unless required by the laws permitting copying of records, only the coded identity associated with documents or other participant data may be copied (and any personally identifying information must be obscured). Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that may be linked to identified individuals. The investigational sites will normally be notified in advance of auditing visits.

11 QUALITY CONTROL AND QUALITY ASSURANCE

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

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

The eCRFs will be completed online via a web-based electronic data capture (EDC) system that has been validated and is compliant with Part 11 Title 21 of the Code of Federal Regulations. Some data requirements will be addressed outside the EDC

using SAS® software. Data queries will be issued and resolved within the EDC system or SAS®.

Study staff at the site will enter information into the electronic CRFs, and the data will be stored remotely at a central database. Data quality will be ensured through the EDC system's continuous monitoring of data and real-time detection and correction of errors. All elements of data entry (i.e., time, date, verbatim text, and the name of the person performing the data entry) will be recorded in an electronic audit trail to allow all changes in the database to be monitored and maintained in accordance with federal regulations.

Study staff will enter data from a study visit on the relevant eCRFs within 3 days following the visit or the time when data become available.

12 ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE

12.1 STATEMENT OF COMPLIANCE

This trial will be conducted in compliance with the protocol, current Good Clinical Practice (GCP) guidelines—adopting the principles of the Declaration of Helsinki—and all applicable regulatory requirements.

Prior to study initiation, the protocol and the informed consent documents will be reviewed and approved by the sponsor and an appropriate ethics review committee or institutional review board (IRB). Any amendments to the protocol or consent materials must also be approved by the Sponsor, the IRB and submitted to FDA before they are implemented.

12.2 INFORMED CONSENT

The informed consent form is a means of providing information about the trial to a prospective participant and allows for an informed decision about participation in the study. All participants (or their legally acceptable representative) must read, sign, and date a consent form before participating in the study, taking the study drug, and/or undergoing any study-specific procedures. If a participant does not speak and read English, the consent materials must be translated into the appropriate language.

The informed consent form must be updated or revised whenever important new safety information is available, whenever the protocol is amended, and/or whenever any new information becomes available that may affect participation in the trial.

A copy of the informed consent will be given to a prospective participant for review. The study investigator, in the presence of a witness, will review the consent and answer questions. The participant will be informed that participation is voluntary and that he/she may withdraw from the study at any time, for any reason.

12.3 PRIVACY AND CONFIDENTIALITY

A participant's privacy and confidentiality will be respected throughout the study. Each participant will be assigned a sequential identification number. This number, rather than the participant's name, will be used to collect, store, and report participant information.

13 PUBLICATION POLICY

The ITN policy on publication of study results will apply to this study. Authorized participants may find details regarding the policy statement on the ITN internet website at http://www.immunetolerance.org.

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APPENDIX 1 SCHEDULE OF EVENTS: SCREENING AND UPDOSING

	Screening	Baseline																
Day	**-28**	**-14**	**0**	**1**	**8**	**15**	**22**	**28**	**29**	**36**	**43**	**50**	**56**	**57**	**64**	**71**	**78**	
Week	**-4**	**-2**	**0**	**0**	**1**	**2**	**3**	**4**	**4**	**5**	**6**	**7**	**8**	**8**	**9**	**10**	**11**	
Visit	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**UN**
General Assessments																		
Informed consent	X																	
Medical history	X																	
Allergy history	X																	
Comprehensive physical exam	X																	
Limited physical exam			X															X
Vital signs	X		X					X					X					X
Pulmonary function testing (spirometry)	X	X																
Pre and post peak flow testing	X	X		X	X	X	X		X	X	X	X		X	X	X	X	
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Cat exposure level	X		X					X					X					
Clinical Assessments																		
Screening nasal allergen challenge	X																	
Nasal allergen challenge		X																
Total nasal symptom score	X	X																
Peak nasal inspiratory flow	X	X																
Intradermal skin test		X																
Skin prick test cat allergen	X																	
Skin prick test serial titration		X			X			X										
ECG	X																	
Study Medication																		
Randomization			X															
AMG 157 or matched placebo			X					X					X					
SIT or matched placebo				X	X	X	X		X	X	X	X		X	X	X	X	
Local Clinical Laboratory Assessments																		
Serum pregnancy test	X																	
Urine pregnancy test	X		X					X					X					
Hematology	X		X															
Comprehensive chemistry	X		X															
QuantiFERON® TB testing	X																	
HIV antibody	X																	
Hepatitis B surface antigen	X																	
Hepatitis C antibody	X																	
Creatinine clearance	X		X															

	Screening	Baseline																
Day	**-28**	**-14**	**0**	**1**	**8**	**15**	**22**	**28**	**29**	**36**	**43**	**50**	**56**	**57**	**64**	**71**	**78**	
Week	**-4**	**-2**	**0**	**0**	**1**	**2**	**3**	**4**	**4**	**5**	**6**	**7**	**8**	**8**	**9**	**10**	**11**	
Visit	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**UN**
Pharmacokinetics and Antibody Assessments																		
Anti-AMG 157 antibody			X					X										
AMG 157 pharmacokinetics			X					X										
Mechanistic Laboratory Assessments																		
Nasal fluid cytokines		X																
Nasal brushing		X																
PBMC		X																
ImmunoCAP for multiple allergens	X																	
IgG		X																
IgA		X																
Total IgE		X																
Cat-specific IgE		X																
Serum (FAB and other assays)		X																
Whole-blood DNA-HLA genotypes	X																	
CD154 Assays		X[1]																
Whole blood RNA expression		X																

[1] Only to be collected for participants at ASTHMA Inc.

APPENDIX 2 SCHEDULE OF EVENTS: MAINTENANCE

Day	84	85	92	99	112	113	127	140	141	168	169	182	196	224	252	280	308	336	
Week	**12**	**12**	**13**	**14**	**16**	**16**	**18**	**20**	**20**	**24**	**24**	**26**	**28**	**32**	**36**	**40**	**44**	**48**	
Visit	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**UN**
General Assessments																			
Informed consent																			
Medical history																			
Allergy history																			
Comprehensive physical exam																			
Limited physical exam																			X
Vital signs	X				X			X		X			X	X	X	X	X	X	X
Pulmonary function testing (spirometry)												X							
Pre and post peak flow testing		X	X	X		X	X		X		X	X	X	X	X	X	X	X	
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Cat exposure level	X				X			X		X			X	X	X	X	X	X	
Clinical Assessments																			
Screening nasal allergen challenge																			
Nasal allergen challenge												X							
Total nasal symptom score												X							
Peak nasal inspiratory flow												X							
Intradermal skin test												X							
Skin prick test cat allergen																			
Skin prick test serial titration	X											X							
Study Medication																			
Randomization																			
AMG 157 or matched placebo	X				X			X		X			X	X	X	X	X	X	
SIT or matched placebo		X	X	X		X	X		X		X		X	X	X	X	X	X	
Local Clinical Laboratory Assessments																			
Serum pregnancy test																			
Urine pregnancy test	X				X			X		X			X	X	X	X	X	X	
Hematology	X									X					X			X	
Comprehensive chemistry	X									X					X			X	
Creatinine clearance	X									X					X			X	

Day	84	85	92	99	112	113	127	140	141	168	169	182	196	224	252	280	308	336	
Week	**12**	**12**	**13**	**14**	**16**	**16**	**18**	**20**	**20**	**24**	**24**	**26**	**28**	**32**	**36**	**40**	**44**	**48**	
Visit	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**UN**
Pharmacokinetics and Antibody Assessments																			
Anti-AMG 157 antibody													X						
AMG 157 pharmacokinetics													X						
Mechanistic Laboratory Assessments																			
Nasal fluid cytokines												X							
Nasal brushing																			
PBMC	X											X							
IgG	X											X							
IgA	X											X							
Total IgE	X											X							
Cat-specific IgE	X											X							
Serum (FAB and other assays)	X											X							
Whole-blood DNA-HLA genotypes																			
CD154 Assays	X[2]											X[2]							
Whole blood RNA expression	X											X							

[2] Only to be collected for participants at ASTHMA Inc.

APPENDIX 3 SCHEDULE OF EVENTS: OBSERVATION

Day	365	421	477	547	617	673	729		
Week	**52**	**60**	**68**	**78**	**88**	**96**	**104**		
Visit	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**UN**	**Early Termination**
General Assessments									
Informed consent									
Medical history									
Allergy history									
Comprehensive physical exam									
Limited physical exam	X			X			X	X	X
Vital signs	X			X			X	X	
Pulmonary function testing (spirometry)	X			X			X		
Pre and post peak flow testing	X			X			X		
Adverse events	X	X[3]	X[3]	X	X[3]	X[3]	X	X	X
Concomitant medications	X	X[3]	X[3]	X	X[3]	X[3]	X	X	X
Cat exposure level	X	X[3]	X[3]	X	X[3]	X[3]	X		X
Clinical Assessments									
Screening nasal allergen challenge									
Limited Nasal Allergen Challenge				X					
Nasal allergen challenge	X						X		
Total nasal symptom score	X			X			X		
Peak nasal inspiratory flow	X			X			X		
Intradermal skin test	X						X		
Skin prick test cat allergen									
Skin prick test serial titration	X			X			X		
Study Medication									
Randomization									
AMG 157 or matched placebo									
SIT or matched placebo									
Local Clinical Laboratory Assessments									
Serum pregnancy test									
Urine pregnancy test	X			X			X		
Hematology	X								
Comprehensive chemistry	X								
Creatinine clearance	X								

[3] For Visits 34, 35, 37, and 38, participants will be followed up via telephone.

Day	365	421	477	547	617	673	729		
Week	**52**	**60**	**68**	**78**	**88**	**96**	**104**		
Visit	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**UN**	**Early Termination**
Pharmacokinetics and Antibody Assessments									
Anti-AMG 157 antibody	X						X		X
AMG 157 pharmacokinetics	X						X		X
Mechanistic Laboratory Assessments									
Nasal fluid cytokines	X						X		
Nasal brushing	X						X		
PBMC	X			X			X		
IgG	X			X			X		
IgA	X			X			X		
Total IgE	X			X			X		
Cat-specific IgE	X			X			X		
Serum (FAB and other assays)	X			X			X		
Whole-blood DNA-HLA genotypes									
CD154 Assays	X[4]			X[4]			X[4]		
Whole blood RNA expression	X			X			X		

[4] Only to be collected for participants at ASTHMA Inc.

APPENDIX 4: WORLD ALLERGY ORGANIZATION SUBCUTANEOUS IMMUNOTHERAPY SYSTEMIC REACTION GRADING SYSTEM

Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Symptom(s)/ sign(s) of one organ system present[i]	Symptom(s)/ sign(s) of more than one organ system present	Lower respiratory Asthma (e.g., 40% PEF or FEV1 drop, NOT responding to an inhaled bronchodilator)	Lower or Upper respiratory Respiratory failure with or without loss of consciousness	Death
	or	or	or	
Cutaneous Generalized pruritus, urticaria, flushing or sensation of heat or warmth[ii]	Lower respiratory Asthma: cough, wheezing, shortness of breath (e.g., less than 40% PEF or FEV1 drop, responding to an inhaled bronchodilator)	Upper respiratory Laryngeal, uvula or tongue edema with or without stridor	Cardiovascular Hypotension with or without loss of consciousness	
or	or			
Upper respiratory Rhinitis (e.g., sneezing, rhinorrhea, nasal pruritus and/or nasal congestion)	Gastrointestinal Abdominal cramps, vomiting, or diarrhea			
	or			
Throat-clearing (itchy throat)	Other Uterine cramps			
or				
Cough perceived to come from the upper airway, not the lung, larynx, or trachea				
or				
Conjunctival Conjunctival erythema, pruritus or tearing				
Other Nausea, metallic taste, or headache				

Patients may also have a feeling of impending doom, especially in grades 2, 3, or 4.
Note: children with anaphylaxis seldom convey a sense of impending doom and their behavior changes may be a sign of anaphylaxis, e.g., becoming very quiet or irritable and cranky.
Scoring includes a suffix that denotes if and when epinephrine is or is not administered in relationship to symptom(s)/sign(s) of the SR: a, ≤ 5 minutes; b, >5 minutes to ≤10 minutes; c, >10 to ≤ 20 minutes; d, >20 minutes; z, epinephrine not administered.

The final grade of the reaction will not be determined until the event is over, regardless of the medication administered.

The final report should include the first symptom(s)/sign(s) and the time of onset after the subcutaneous allergen immunotherapy injection[iii] and a suffix reflecting if and when epinephrine was or was not administered, e.g., Grade 2a; rhinitis:10 minutes. Comments[iv] may be added.

i. Each Grade is based on organ system involved and severity. Organ systems are defined as: cutaneous, conjunctival, upper respiratory, lower respiratory, gastrointestinal, cardiovascular and other. A reaction from a single organ system such as cutaneous, conjunctival, or upper respiratory, but not asthma, gastrointestinal, or cardiovascular is classified as a Grade 1. Symptom(s)/sign(s) from more than one organ system or asthma, gastrointestinal, or cardiovascular are classified as Grades 2 or 3. Respiratory failure or hypotension, with or without loss of consciousness, defines Grade 4 and death Grade 5.The Grade is determined by the physician's clinical judgment.
ii. This constellation of symptoms may rapidly progress to a more severe reaction.
iii. Symptoms occurring within the first minutes after the injection may be a sign of severe anaphylaxis. Mild symptoms may progress rapidly to severe anaphylaxis and death.
iv. If signs or symptoms are not included in the Table or the differentiation between an SR and vasovagal (vasodepressor) reaction, which may occur with any medical intervention, is difficult, please include comment, as appropriate.

From: Cox L, Larenas-Linnemann D, Lockey RF, Passalacqua G. Speaking the same language: The World Allergy Organization Subcutaneous Immunotherapy Systemic Reaction Grading System. J Allergy Clin Immunol 2010;125:569-74, 74 e1-74 e7