INNER-CITY ASTHMA CONSORTIUM

PROTOCOL ICAC-28

COCKROACH IMMUNOTHERAPY IN CHILDREN AND ADOLESCENTS

5.0 / 11 JANUARY 2021

IND# 17979

Study Sponsor(s)	The Division of Allergy, Immunology, and Transplantation (DAIT), The National Institute of Allergy and Infectious Diseases (NIAID)
NIAID Funding	Grants 1 UM1 AI114271-01 and UM2AI117870
IND Sponsor	DAIT/NIAID, NIH
Study Drug Manufacturer	Stallergenes Greer

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Protocol: ICAC-28	Version/Date: 5.0 / 11 January 2021
Title: Cockroach Immunotherapy in Children and Adolescents	
Study Sponsor: The National Institute of Allergy and Infectious Diseases (NIAID)	
INSTRUCTIONS: *The original signature page must be kept for your records. Return an electronic PDF copy of the signed signature page (*as described below) to the DAIT Regulatory Management Center via the applicable DAIT RMC email address for the protocol/network:* **DAITRegulatory_ICAC (SM) <DAITRegulatory_ICAC@ppdi.com>**	

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 United States Code of Federal Regulations (CFR) – 45 CFR part 46 and 21 CFR parts 50, 56, and 312, and in the International Conference on Harmonisation (ICH) document *Integrated Addendum to ICH E6(R1): Guideline for Good Clinical Practice E6(R2)*. . Further, I will conduct the study in keeping with local legal and regulatory requirements.

As the site 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 the written permission of the IRB and NIAID.

*[*The site Principal Investigator should sign and date at the indicated location below. A written signature/date is acceptable (e.g., scanned and sent via email as a PDF version). An electronic signature is also acceptable (e.g., sent via email as a PDF version).]*

_____
Site Principal Investigator (Print)

_____ _____
Site Principal Investigator (Signature) **Date**

Protocol Synopsis

Title	Cockroach Immunotherapy in Children and Adolescents
Short Title	CRITICAL
Clinical Phase	II
Number of Sites	Multiple sites in the United States
IND Sponsor/IND Number	DAIT NIAID, NIH /IND #17979
Study Objectives	The primary objective of the study is to determine if the NAC response will be changed with treatment with cockroach subcutaneous immunotherapy (SCIT). Secondary objectives are: 1. To assess safety of cockroach SCIT in children and adolescents 2. To assess the effect of cockroach SCIT on German cockroach-specific IgE and IgG4
Study Design	This is a 1:1 randomized, double-blind, placebo-controlled, multicenter trial with 2 arms: • Cockroach SCIT • Placebo This study will enroll 80 children from 8-17 years of age who are sensitized to cockroach and have asthma and a positive cockroach Nasal Allergen Challenge (NAC) before randomization.
Primary Endpoint(s)	Change in mean TNSS after 12 months of treatment with cockroach SCIT.
Secondary Endpoint(s)	1. Rate of immunotherapy related adverse events and immunotherapy related serious adverse events in the course of treatment 2. Change in additional NAC outcomes from baseline a. TNSS AUC b. Responsive dose 3. Changes in German cockroach-specific IgE and IgG4
Accrual Objective	~80
Study Duration	~48 months

Treatment Description	Non-standardized glycerinated German cockroach (*Blattella germanica*) allergenic extract or placebo used by subcutaneous administration in escalating doses up to 0.4 ml of 1:10 wt./vol.
Inclusion Criteria	Individuals who meet all of the following criteria are eligible for enrollment as study participants: 1. Subject and/or parent guardian must be able to understand and provide informed consent. 2. Are male or female children, 8-17 years of age at recruitment. 3. Have a primary place of residence in one of the pre-selected recruitment census tracts as outlined in the Protocol ICAC-28 Manual of Operations (MOP) a. Participants who do not live in the pre-selected census tracts but live within the Office of Management and Budget (OMB) defined Metropolitan Statistical Area and have publicly-funded health insurance will qualify for inclusion. 4. Have a history of persistent asthma for a minimum of 1 year before study entry. a. A diagnosis of asthma will be defined as a report by the caretaker that the participant had a clinical diagnosis of asthma made by a clinician $\geq$ 1 year ago, resulting in a prescription of preventative asthma medication. b. The participant must have persistent asthma defined by the current need for at least 88 mcg fluticasone (or the equivalent of another inhaled corticosteroid) to control asthma at the time of screening. 5. Before randomization, the participant's asthma must be well-controlled as defined by: a. A FEV_1 greater than or equal to 80% predicted, and b. An Asthma Control Test (ACT) or Childhood Asthma Control Test (CACT) score ≥ 20. 6. Are sensitive to German cockroach as documented by a positive (≥ 3 mm greater than negative control) skin prick test result and detectable German cockroach-specific IgE ($\geq$ 0.35 kU_A/L). 7. Have no known contraindications to therapy with glycerinated German cockroach allergenic extract or placebo. 8. Have a positive cockroach Nasal Allergen Challenge (NAC), as defined by reaching a Total Nasal Symptom Score (TNSS) of ≥ 6 or a sneezing score of 3 at dose 2 or above during the challenge before randomization. 9. Have documentation of current medical insurance with prescription coverage at randomization.
Exclusion Criteria	Individuals who meet any of these criteria are not eligible for enrollment as study participants but may be reassessed while enrollment is ongoing. Participants are ineligible if they:

	1. Are unable or unwilling to give written informed consent or comply with study protocol. 2. Are pregnant or lactating. Post-menarcheal females must be abstinent or use a medically acceptable birth control method throughout the study (e.g. oral, subcutaneous, mechanical, or surgical contraception). 3. Cannot perform acceptable spirometry or peak flow before randomization. 4. Have an asthma severity classification of severe or unstable at the time of randomization, as evidenced by at least one of the following: a. Require a dose of greater than 500 mcg of fluticasone per day or the equivalent of another inhaled corticosteroid. b. Have received more than 2 courses of oral or parenteral corticosteroids within the 12 months or one course within the last 3 months prior to study entry. c. Have been treated with depot steroids within the 3 months prior to study entry. d. Have been hospitalized for asthma within the 6 months prior to study entry. e. Have had a life-threatening asthma exacerbation that required intubation, mechanical ventilation, or that resulted in a hypoxic seizure within 2 years prior to study entry. 5. Do not have access to a phone (needed for scheduling appointments). 6. Have received allergen immunotherapy (SLIT or SCIT) in the last 12 months prior to study entry or who plan to initiate or resume allergen immunotherapy during the study. 7. Have received biologic therapy (e.g., anti-IgE, anti-IL-4, anti-IL-5) within 6 months of the NAC procedure, if applicable, or Randomization, if no NAC is performed. 8. Have received an investigational drug in the 30 days prior to study entry or who plan to use an investigational drug during the study. 9. Have past or current medical problems or findings from physical examination or laboratory testing that are not listed above, which, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the participant's ability to comply with study requirements or that may impact the quality or interpretation of the data obtained from the study. 10. Have nasal polyps or other major structural abnormalities in their nasal cavities as assessed by anterior rhinoscopy. Participants who meet any of the following criteria are not eligible for enrollment and may not be reassessed. Participants are ineligible if they: 1. Plan to move from the area during the study period 2. Have a history of anaphylaxis grade 3 or higher as defined in section 12.3.1.1c, World Allergy Organization Subcutaneous Immunotherapy Systemic Reaction Grading System.

	3. Have unstable angina, significant arrhythmia, uncontrolled hypertension, history of autoimmune disease, or other chronic or immunological diseases that in the opinion of the investigator might interfere with the evaluation of the investigational product or pose additional risk to the participant. 4. Past or current medical problems or findings from physical examination or laboratory testing that are not listed above, which, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the participant's ability to comply with study requirements or that may impact the quality or interpretation of the data obtained from the study. 5. Are using tricyclic antidepressants or beta-adrenergic blocker drugs (both oral and topical).
Study Stopping Rules	Study enrollment and treatment will be suspended pending expedited review of all pertinent data after the occurrence of: 1) One death if at least possibly related to the investigational agent 2) Two Grade 4 systemic reactions (see Table 12.3.1.1c) possibly related to the injection of the immunotherapy agent 3) Grade 4 local reaction (see Table 12.3.1.1a) or Grade 4 systemic reaction (see Table 12.3.1.1c) possibly related to the Nasal Allergen Challenge (NAC) a. The study will be halted for this occurrence pending review of pertinent data by DAIT MM and the DSMB.

Table of Contents

Glossary of Abbreviations

ACT	Asthma Control Test
AE	Adverse Event
ATS	American Thoracic Society
AUC	Area under the curve
BID	Twice a day
CASI	Composite Asthma Severity Index
CFR	Code of Federal Regulations
CR	Cockroach
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
DAIT	Division of Allergy, Immunology, and Transplantation
DSMB	Data Safety Monitoring Board
eCRF	Electronic Case Report Form
E&M	Evaluation and Management
FDA	Food and Drug Administration
FENO	Fractional Exhaled Nitric Oxide
FEV_1	Forced Expiratory Volume in 1 second
GCP	Good Clinical Practice
HIPPA	Health Insurance Portability and Accountability Act
IB	Investigator Brochure
ICAC	Inner-City Asthma Consortium
ICH	International Conference on Harmonization
ICS	Inhaled Corticosteroid
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IND	Investigational New Drug
IRB	Institutional Review Board
ITT	Intent-to-treat
LABA	Long-acting Beta Agonist

MCG	Microgram
MCL	Microliter
MM	Medical Monitor
MOP	Manual of Procedures
NAC	Nasal Allergen Challenge
NAEPP	The National Asthma Education and Prevention Program
NCI	National Cancer Institute
NIAID	National Institute of Allergy and Infectious Diseases
OMB	Office of Management and Budget
PCP	Primary Care Provider
PD	Protocol Deviation
PEF	Peak Expiratory Flow
PFT	Pulmonary Function Tests
PI	[Site] Principal Investigator
SACCC	Statistical and Clinical Coordinating Center
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Suspected Adverse Reaction
SCIT	Subcutaneous Immunotherapy
SLIT	Sublingual Immunotherapy
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TLC	Total Lung Capacity
TNSS	Total Nasal Symptom Score
WAO	World Allergy Organization

1 Background and Rationale

1.1 Background and Scientific Rationale

Cockroach allergen has been established as one of the leading causative agents for morbidity in inner-city children with asthma.[1] Although the negative effects of cockroach exposure have been known for almost 20 years, eradication and avoidance, which can be impossible to achieve in the housing available to lower socioeconomic urban populations, is still the mainstay of treatment.[2] Given these data, a major goal of the NIAID-funded Inner-City Asthma Consortium (ICAC) is to conduct a large multi-center efficacy trial of cockroach immunotherapy in inner-city asthma. While we have pursued the possibility of using sublingual immunotherapy (SLIT) given its excellent safety profile, data from the completed DAIT NIAID-sponsored Protocol ICAC-12 BioCSI and Protocol ICAC-17 BioCSI2 studies of cockroach SLIT have suggested that SLIT for cockroach may not be effective due to limitations in the maximum doses that can be provided with currently available cockroach extracts.[3,4] These studies have also generated concerns about the likelihood of compliance with daily SLIT therapy, an issue that may both reduce efficacy and potentially increase risk. A pilot study of cockroach SCIT was conducted in 10 adults (Protocol ICAC-18 SCITCO) with a primary focus on safety to lay the groundwork for a possible efficacy study using SCIT given the distinct possibility that cockroach SLIT will not prove effective and / or feasible. The SCITCO study demonstrated an excellent safety profile, as well as robust markers of immunologic response, and with the current study our goal is to extend that approach into a pediatric population.[3]

There is a vast clinical and research experience with the use of SCIT for the treatment of asthma and allergic rhinitis, as well as stinging insect hypersensitivity.[5-12] While there is a higher risk of systemic reactions with SCIT compared to SLIT, the overall safety profile of SCIT is very favorable, as is the overall SCIT risk benefit ratio given the markedly salutary effects demonstrated in most studies. As with SLIT, the efficacy of SCIT depends on the use of adequate dosages, but we are confident that the available extracts will prove adequate in this regard.[9,13]

The rationale for this study is that SCIT may be the only option for effective cockroach immunotherapy. In fact, the only studies that demonstrated efficacy of cockroach immunotherapy used SCIT.[14,15] We intend in this study to test the effects of cockroach SCIT on asthma control, as well as safety and immunologic biomarkers, in a pediatric population.

Unlike pollen allergy, where symptoms characteristically peak during a particular season, or allergy to animals (e.g., cat, dog), where symptoms are induced upon exposure, there is no specific period during the year at which assessment of the efficacy of SCIT on natural cockroach allergen exposure can be undertaken. This only leaves the option of year-round clinical evaluations of symptoms, medication use, lung function and asthma exacerbations as clinical outcomes of a cockroach allergen immunotherapy study. Although these are very relevant outcomes, our ability to attribute them directly to the effect of single allergen immunotherapy is uncertain. A negative outcome could be due either to the failure of SCIT to reduce allergic responses to cockroach, or because ameliorating cockroach allergy is insufficient to change the clinical outcome in polysensitized individuals with asthma. In this context, provocation testing with cockroach allergen could be used to objectively document the response to SCIT. Nasal Allergen Challenge (NAC) is an established type of provocation testing commonly used in the research setting to assess early stage efficacy of nasal allergy treatments, including allergen immunotherapy, and to study the pathophysiology of allergic reactions in an accessible part of the respiratory tract.[16,17] The

outcomes of Nasal Allergen Challenge (NAC) are not lower airway outcomes, but the purpose of using this form of assessment in the context of this trial is to establish that treatment is effective against mucosal reactions to the allergen in question and not to substitute for asthma outcomes. As the majority of children with allergic asthma also have allergic rhinitis and improvement in upper respiratory control is beneficial in asthma, the nasal challenge may have a direct clinical relevance in a study of allergen immunotherapy for asthma. An alternative approach would have been to establish a bronchial cockroach allergen provocation[18], but this procedure raises more safety concerns and is more complicated and time consuming than Nasal Allergen Challenge (NAC).

Very few studies examining nasal challenge with cockroach allergen have been reported, and no study provides adequate information on safety and dosing in adults or children.[19-21] For further details, please refer to DAIT NIAID's Investigator Brochure (IB) for German Cockroach Allergenic Extract for Nasal Allergen Challenge (NAC).

1.2 Rationale for Selection of Investigational Product or Intervention

The investigational agent for Protocol ICAC-28 is 1:10 w/v non-standardized glycerinated German cockroach (*Blattella germanica*) allergenic extract obtained from Stallergenes Greer (Lenoir, NC) administered by the subcutaneous route. Placebo for immunotherapy is (glycerin 50% v/v, Sodium Bicarbonate 0.091%, Sodium Chloride 0.166%, water for injection q.s.) administered by the subcutaneous route obtained from Allergy Laboratories, Inc. (Oklahoma City, OK).

The selection of this particular extract was based on the potency of German cockroach extracts available for skin testing and allergen immunotherapy on the market. The maintenance dose for ICAC-28 will be approximately 6 micrograms (mcg). No data exists as to the clinically effective dose allergen for SCIT. Therfore the maintenance dose was selected based on information from other allergens where maintenance doses range from 5 to 15 micrograms have proven effective,[9,13] and in the SCITCO trial (Protocol ICAC-18) a maintenance containing 6 mcg of Bla g 2 induced consistent immunologic responses.[3]

1.3 Preclinical Experience

Not applicable.

1.4 Clinical Studies

1.4.1 Immunotherapy

While only two published studies have assessed the potential efficacy of cockroach SCIT,[14,15] there is a vast body of literature and clinical experience with the use of SCIT for other allergens. A recent practice parameter detailed the use of SCIT for inhalant allergens as well as stinging insects.[5] In addition, meta-analyses have confirmed the effectiveness of SCIT for the treatment of asthma, allergic rhinitis, and stinging insect hypersensitivity.[7,8,11,12] A 2003 Cochrane review of 75 trials including 3188 patients with asthma found that overall SCIT led to a significant reduction in asthma symptoms, medication use, and bronchial hyperreactivity.[8] For example, in that review, it was determined that it would have been necessary to treat 4 patients with immunotherapy to avoid 1 deterioration in asthma symptoms and 5 patients to avoid 1 requiring increased asthma medication.

With regard to the published studies on allergy to cockroach, Kang et al. studied 28 adults with asthma and positive cockroach allergen skin tests; all had positive skin tests to other inhalant allergens as well.[14]

Subjects were randomized to immunotherapy with cockroach allergen extract (active group, n = 15) or immunotherapy with allergens other than cockroach (control, n = 13), and treated for 5 years. Upon study completion, 5 of the control subjects had switched to cockroach immunotherapy and 6 subjects (4 active, 2 control) were dropped from the study. In the active group, average symptom scores decreased from 7.2 at baseline to 1.2, while the control group showed no change. IgG antibodies against cockroach increased in the treated group. Adverse reactions were not mentioned. We do not know how the extract used in this trial compares in terms of antigen content and stability with the one that will be used in Protocol ICAC-28.

The second study on cockroach SCIT actually focused on American cockroach, an allergen far less important in inner cities, and the U.S. in general, than German cockroach.[15] In this double-blind, placebo-controlled trial, 50 patients with asthma, rhinitis or both were treated with cockroach SCIT or placebo for one year. They found that active SCIT led to significant improvement in clinical parameters compared with baseline values and the placebo group. They also demonstrated modest reductions in cockroach-specific IgE levels and significant increases in specific IgG4 levels.

1.4.2 Nasal Allergen Challenge (NAC)

There have been three reports of Nasal Allergen Challenge (NAC) with cockroach extract in the literature.[19-21]

In the Hosen study, 174 patients of unreported ages underwent nasal provocation testing to insects common to Texas. An unclear number of these patients underwent nasal provocation testing specifically to cockroach. The extract used as the diagnostic agent was a powdered concentration of cockroach, which was directly inhaled by the patients in the setting of Nasal Allergen Challenge (NAC) with multiple allergen extracts in powdered form. Participants would be treated with phenylephrine hydrochloride and, if needed, a bronchodilator in between allergen challenges. Sixty-nine patients were reported to have had a systemic reaction to 1 of the allergen extracts. For cockroach in particular, 16 total reactions were reported, of which 11 were nasal, 2 were listed as bronchial asthma, and 3 were listed as post-nasal and upper bronchial.[21]

In the Okuda study, 560 patients from the ages of 4 to 80 years old (mean 31.3 years; 112 participants were 19 years old or younger) with allergic rhinitis were recruited to undergo an evaluation for insect allergy. Sixty-five participants of unknown ages were selected by unspecified means from the pool of 560 patients to undergo Nasal Allergen Challenge (NAC) with a mixture of German cockroach, *Chironomus yoshimatsui*, and silkworm moth. The publication did not mention whether there were any adverse reactions from the Nasal Allergen Challenge (NAC).[19]

The most recent and informative study evaluated the concept of localized allergy in patients with nonallergic rhinitis. In that study, 16 adult volunteers underwent sequential nasal allergen testing with a variety of aeroallergens, including a cockroach extract mix. The cockroach mix nasal allergen testing was done with an arbitrarily chosen single dose of 100 µL of 1:5000 (w/v), and no testing was conducted prior to the challenge to establish a dose. No adverse events were reported in this published report.[20]

For further details, please refer to the Investigator Brochure for German Cockroach Allergenic Extract for Nasal Allergen Challenge (NAC).

2 Study Hypotheses/Objectives

2.1 Hypotheses

The primary hypothesis to be tested by this study is that subcutaneous treatment with German cockroach (*Blattella germanica*) extract will induce changes in nasal allergen challenge outcomes. A major secondary hypothesis is that subcutaneous treatment with German cockroach (*Blattella germanica*) extract will induce significant changes in immunologic biomarkers indicative of desensitization in children with persistent, well-controlled asthma.

2.2 Primary Objective

The primary objective of this study is to determine if the response to nasal allergen challenge (NAC) will be changed with treatment with cockroach SCIT.

2.3 Secondary Objectives

1. To assess safety of cockroach SCIT in adolescents and children
2. To assess the effect of cockroach SCIT on German cockroach-specific IgE and IgG4

2.4 Exploratory Objectives

1. To assess the effect of cockroach SCIT on asthma severity as measured by the CASI score
2. To assess improvements in components of the CASI asthma severity score
3. To assess the effect of cockroach SCIT on rhinitis symptoms and medication requirements
4. To assess the effect of cockroach SCIT on component allergens, including, but not limited to, Bla g 1, Bla g 2, Bla g 4, Bla g 5, and Per A 1
5. To assess the effect of cockroach SCIT on cockroach-specific blocking antibodies
6. To assess the effect of cockroach SCIT on skin test reactivity
7. To assess the effect of cockroach SCIT on other key mechanistic parameters
8. To assess the role of environmental cockroach exposure on the relationship between treatment with cockroach SCIT and asthma and rhinitis outcomes
9. To assess the relationship of change in NAC to change in CASI

3 Study Design

3.1 Description of Study Design

This study is a randomized, double-blind, placebo-controlled, multicenter trial of non-standardized glycerinated German cockroach (*Blattella germanica*) allergenic extract or placebo treatment administered by subcutaneous injection.

Participants will be randomized to 1 of 2 treatment groups in a ratio of 1:1:

1. German cockroach allergenic extract administered by subcutaneous injection.
2. Placebo administered by subcutaneous injection.

Protocol ICAC-28 will enroll eighty 8 to 17-year-old cockroach sensitive children with asthma with a positive cockroach Nasal Allergen Challenge (NAC) before randomization.

The intended maintenance dose of German cockroach allergenic extract is ≈6 mcg of Bla g 2. The study will be based on a treatment schedule with German cockroach allergenic extract or placebo for 12 months.

There will be a Screening phase to determine eligibility, including a positive IgE and skin test to German cockroach. If asthma control parameters are not met, participants will begin a run-in period, during which participants' asthma medications will be adjusted for up to 4 months to achieve symptom control before completing an initial NAC procedure. Eligible participants with a positive NAC will then be randomized to one of the two treatment arms and receive their first injection. Up to 2 doses of SCIT will be given weekly during dose escalation, separated by a minimum of 2 days. Dose escalation can be delayed at any point for systemic or local reactions, although to continue to maintenance each participant will need to reach the minimum maintenance dose in a maximum of 40 weeks (see Appendix B: Study Restrictions Due to COVID-19). Once the maintenance dose is achieved, participants will receive two additional maintenance level-doses (for a total of three maintenance level-doses) spaced approximately 2 weeks apart, followed by maintenance injections every 4 weeks for the remainder of the treatment period.

At Randomization and every 2 months during treatment, an asthma Evaluation and Management (E&M) process will occur where participants' asthma medication regimen will be adjusted based on symptoms, albuterol/levalbuterol use, FEV_1, recent exacerbations, and their current level of therapy.

All participants must have a positive cockroach NAC, as defined by reaching a Total Nasal Symptom Score (TNSS) of ≥ 6 or a sneezing score of 3 at Dose 2 or higher during the challenge before randomization and will undergo follow-up NAC procedures at 12 months.

Blood will be collected at Screening, prior to Randomization, and after 12 months of treatment for assessment of biomarkers of allergen immunotherapy, such as cockroach-specific IgE, IgG4, and component allergens, including Bla g 1, Bla g 2, Bla g 4, Bla g 5, and Per A 1, and inhibition of cockroach antigen binding to B-cells. Skin testing (German cockroach) will be performed during the Screening phase and at 12 months. CASI will be collected every 2 months (See Visit Activities Summary, Appendix A).

Adverse events (AEs) will be assessed at each study visit. Pulmonary function and rhinitis and asthma symptom questionnaires will also be administered at Screening and every two months thereafter.

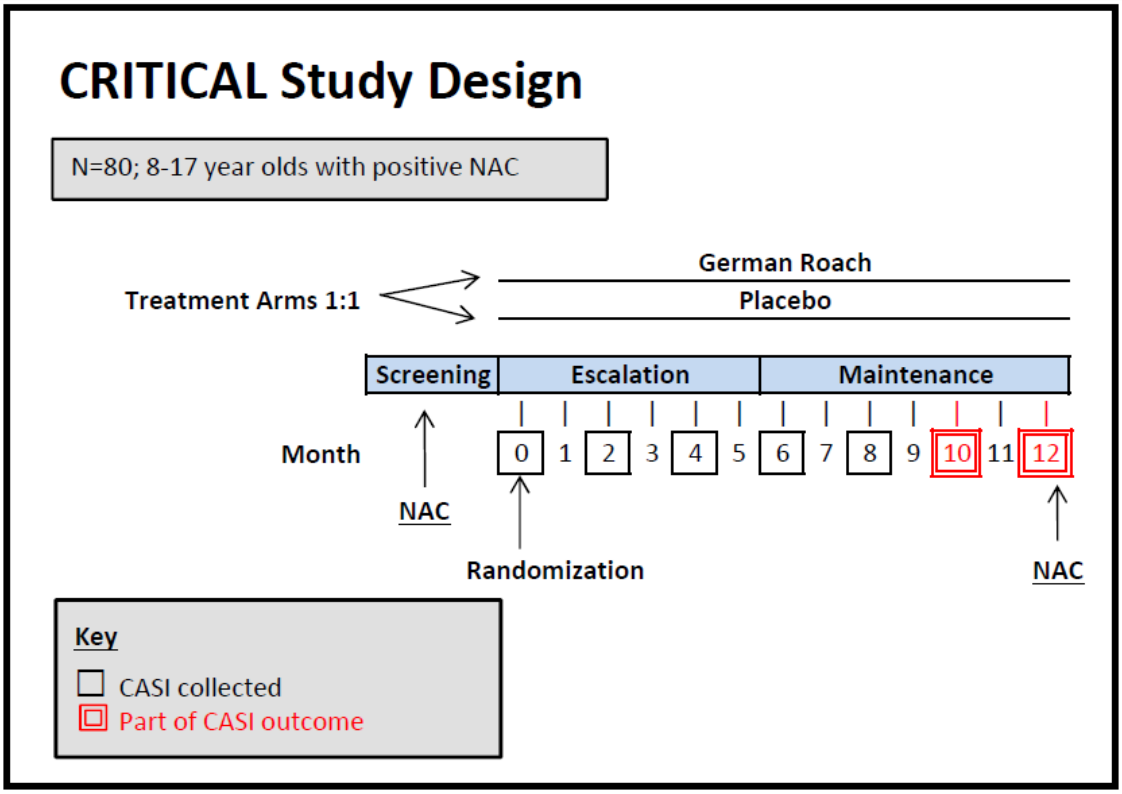

Figure 1. Protocol ICAC-28 CRITICAL Study Design Schematic

3.2 Primary Endpoint

The primary study endpoint is the change in mean TNSS after 12 months of treatment with cockroach SCIT.

3.3 Secondary Endpoints

1. Rate of immunotherapy related adverse events and immunotherapy related serious adverse events in the course of treatment
2. Change in additional NAC outcomes from baseline
    a. TNSS AUC
    b. Responsive dose
3. Changes in German cockroach-specific IgE and IgG4

3.4 Exploratory Endpoints

1. CASI score over the 2 assessments occurring between 10 and 12 months of treatment
2. Additional asthma severity outcome measures
    a. Number of days with asthma symptoms (wheezing or tightness in the chest or cough)
    b. Number of nights with asthma symptoms (waking up because of wheezing or tightness in the chest or cough)
    c. Number of days with albuterol use
    d. Number of nights with albuterol use
    e. Asthma treatment step (medication requirements)
    f. Asthma exacerbations
    g. FEV_1

3. Rhinitis severity outcome measures
    a. Modified Rhinitis Symptom Utility Index
    b. Rhinitis treatment step (medication requirements)
4. Changes in component allergens, including Bla g 1, Bla g 2, Bla g 4, Bla g 5, and Per A 1
5. Change in cockroach-specific blocking antibodies
6. Change in cockroach skin test reactivity
7. Changes in cockroach-specific T cell response
8. Changes in PBMC gene expression response to CR stimulation
9. Changes in nasal lavage gene expression

3.5 Stratification, Randomization, and Blinding/Masking

The randomization schedule will be generated by the DAIT NIAID Statistical and Clinical Coordinating Center (SACCC) and implemented in a validated system that will be used by site personnel to automate the random assignment of treatment groups to study participants. The randomization scheme will be reviewed and approved by a statistician at the DAIT SACCC and will not be modified thereafter except to accommodate any changes required by amendments to the protocol . Participants will be randomized using a 1:1 ratio of active (German cockroach extract) and control (placebo) participants. Randomization will be stratified by site.

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

The clinical site's research pharmacy will dispense to the clinical research site unblinded nurse the study drug based on the unique vial IDs communicated by the randomization system at randomization and any subsequent dosing visits. The SCIT injections will be administered by trained clinical research staff. Prior to injection, an unblinded staff member will confirm the expiration date, the dose, and randomization/investigational product assignment kit number.

3.5.1 Procedure for Unblinding/Unmasking

Unblinding must be approved by the DAIT/NIAID Medical Monitor (MM) unless an immediate life-threatening condition has developed and the Medical Monitor is not accessible. Automatic notifications of any unblinding event will be sent to study personnel as specified in the Protocol ICAC-28 MOP. Unblinding events will also be reported to the NIAID Allergy and Asthma Data and Safety Monitoring Board (DSMB).

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

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

4 Selection of Participants and Clinical Sites/Laboratories

4.1 Rationale for Study Population

Because the combination of cockroach allergy and cockroach exposure is one of the most important factors contributing to the increased asthma morbidity seen in inner-city children with asthma, a major goal of the NIAID-funded ICAC is to conduct a large multi-center efficacy trial of cockroach immunotherapy in this population. DAIT NIAID-sponsored studies of SLIT – Protocols ICAC-10, ICAC-12, and ICAC-17 (IND # 13163) in both adults and children had limited success and pointed to challenges with adherence to the daily dosing regimen. Daily adherence may be particularly difficult for children and adolescents. DAIT NIAID-sponsored Protocol ICAC-18 SCITCO (IND exempt, BB-IND # 14330) study in adults, on the other hand, demonstrated an excellent safety profile, as well as robust biomarkers of immunologic response, and with the current study our goal is to extend that approach into a pediatric population using CASI as the primary measure of efficacy.

4.2 Inclusion Criteria

Individuals who meet all of the following criteria are eligible for enrollment as study participants:

1. Subject and/or parent guardian must be able to understand and provide informed consent.
2. Are male or female children, 8 through 17 years of age at recruitment.
3. Have a primary place of residence in one of the pre-selected recruitment census tracts as outlined in the Protocol ICAC-28 Manual of Operations (MOP).
    a. Participants who do not live in the pre-selected census tracts but live within the Office of Management and Budget (OMB) defined Metropolitan Statistical Area and have publicly-funded health insurance will qualify for inclusion.
4. Have a history of persistent asthma, for a minimum of 1 year before study entry.
    a. A diagnosis of asthma will be defined as a report by the caretaker that the participant had a clinical diagnosis of asthma made by a clinician $\geq$ 1 year ago, resulting in a prescription of preventative asthma medication.
    b. The participant must have persistent asthma defined by the current need for at least 88 mcg fluticasone (or the equivalent of another inhaled corticosteroid) to control asthma at the time of screening.
5. Before randomization, the participant's asthma must be well-controlled as defined by:
    a. A FEV_1 greater than or equal to 80% predicted
    b. An Asthma Control Test (ACT) or Childhood Asthma Control Test (CACT) score ≥ 20.
6. Are sensitive to German cockroach as documented by a positive (≥ 3 mm greater than negative control) skin prick test result and detectable German cockroach-specific IgE (≥ 0.35 kU_A/L).
7. Have no known contraindications to therapy with glycerinated German cockroach allergenic extract or placebo.
8. Have a positive cockroach Nasal Allergen Challenge (NAC), as defined by reaching a Total Nasal Symptom Score (TNSS) of ≥ 6 or a sneezing score of 3 at dose 2 or above during the challenge before randomization.
9. Have documentation of current medical insurance with prescription coverage at randomization.

4.3 Exclusion Criteria

Individuals who meet any of these criteria are not eligible for enrollment as study participants but may be reassessed while enrollment is ongoing. Participants are ineligible if they:

1. Are unable or unwilling to give written informed consent or comply with study protocol.
2. Are pregnant or lactating. Post-menarcheal females must be abstinent or use a medically acceptable birth control method throughout the study (e.g. oral, subcutaneous, mechanical, or surgical contraception).
3. Cannot perform spirometry or peak flow before randomization.
4. Have an asthma severity classification at the time of randomization of severe persistent, using the NAEPP classification, as evidenced by at least one of the following:
   a. Require a dose of greater than 500 mcg of fluticasone per day or the equivalent of another inhaled corticosteroid.
   b. Have received more than 2 courses of oral or parenteral corticosteroids within the 12 months or one course within the last 3 months prior to study entry.
   c. Have been treated with depot steroids within the 3 months prior to study entry.
   d. Have been hospitalized for asthma within the 6 months prior to study entry.
   e. Have had a life-threatening asthma exacerbation that required intubation, mechanical ventilation, or that resulted in a hypoxic seizure within 2 years prior to study entry.
5. Do not have access to a phone (needed for scheduling appointments).
6. Have received allergen immunotherapy (SLIT or SCIT) in the last 12 months or who plan to initiate or resume allergen immunotherapy during the study.
7. Have received biologic therapy (e.g., anti-IgE, anti-IL-4, anti-IL-5) within 6 months of the NAC procedure, if applicable, or Randomization, if no NAC is performed.
8. Have received an investigational drug in the 30 days prior to recruitment or who plan to use an investigational drug during the study.
9. Have past or current medical problems or findings from physical examination or laboratory testing that are not listed above, which, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the participant's ability to comply with study requirements or that may impact the quality or interpretation of the data obtained from the study.
10. Have nasal polyps or other major structural abnormalities in their nasal cavities as assessed by anterior rhinoscopy.

Participants who meet any of the following criteria are not eligible for enrollment and may not be reassessed. Participants are ineligible if they:

1. Plan to move from the area during the study period.
2. Have a history of anaphylaxis grade 3 or higher as defined in section 12.3.1.1c, World Allergy Organization Subcutaneous Immunotherapy Systemic Reaction Grading System.
3. Have unstable angina, significant arrhythmia, uncontrolled hypertension, history of autoimmune disease, or other chronic or immunological diseases that in the opinion of the investigator might interfere with the evaluation of the investigational product or pose additional risk to the participant.
4. Past or current medical problems or findings from physical examination or laboratory testing that are not listed above, which, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the participant's ability to comply with study requirements or that may impact the quality or interpretation of the data obtained from the study.

5. Are using tricyclic antidepressants or beta-adrenergic blocker drugs (both oral and topical).

5 Known and Potential Risks and Benefits to Participants

5.1 Risks of Investigational Product or Intervention as cited in Investigator Brochure or Package Insert

Per "Allergenic Extract: Instructions for Use and Dosing Schedule" (Allermed Laboratories, Inc., San Diego, CA), "Allergenic Extracts Pollens, Mold, Intradermal, Insects, Dust, Food and Miscellaneous Inhalants: Suggested Dose Schedule and Instructions" (Greer, Lenoir, NC) and the current version of DAIT NIAID's Investigator Brochure German Cockroach Allergenic for Nasal Allergen Challenge (NAC) , risks of exposing participants to allergens via immunotherapy include, but are not limited to: itching of the mouth, ears, and throat, itching or swelling of the eyes, nasal congestion, sneezing, rhinorrhea, oral or throat angioedema, cough, chest tightness, wheezing, gastrointestinal symptoms (vomiting, diarrhea, abdominal pain), skin symptoms (rash, hives, swelling, itching), and anaphylaxis. We expect that the main sign and/or symptom experienced by participants will be localized swelling at the injection site occurring within 30 minutes after administration of the immunotherapy injection. Large local reactions associated with allergen immunotherapy are common, with a frequency ranging from 26% to 86% of injections.[22]

Additionally, clinical research staff may apply a topical anesthetic to the skin before giving the injection to reduce the discomfort of the stick. Side effects from topical anesthetics include erythema, burning, paleness at the skin site, edema, and alterations in temperature. Reactions are mild and transient. There is a potential for cutaneous allergic reactions to topical anesthetics.

The risks to a fetus are unknown and SCIT is generally not initiated during pregnancy. Pregnant females will be excluded from the Protocol ICAC-28. All females who have reached menarche will be required to have a urine pregnancy test before completing study activities at Screening, Randomization, 12-month visit, visits where the NAC is performed, monthly during the escalation phase, and at each Evaluation and Management Visit (E&M) during maintenance. Urine pregnancy testing will ensure no pregnant female will be entered into the study, and any female becoming pregnant during the study will be discontinued. Safety follow-up of pregnant participants is described in section 12.6.

5.2 Risks of Investigational Product Cited in Medical Literature

In published studies of SCIT, systemic anaphylactic reactions with other inhalant allergens have been reported to occur in from 1% of patients to as many as 36% of patients receiving SCIT using a rush schedule.[23,24] Protocol ICAC-28 does not involve a rush immunotherapy schedule. Fatalities are very rare but have been reported, as have specific risk factors for fatal reactions, which include unstable asthma, beta-blocker therapy, rush immunotherapy, and use of high doses of potent standardized extracts.[23] In our SCITCO study (Protocol ICAC-18), in 10 adults undergoing six months of SCIT, there were 2 serious adverse neither of which were considered related to the therapy.[3]

5.3 Risks of Other Protocol Specified Medications

The risks from inhaled corticosteroids (ICS) are minimal but may include upper respiratory tract infection, throat irritation, sinusitis/sinus infection, upper respiratory inflammation, rhinitis, oral candidiasis, nausea and vomiting, gastrointestinal discomfort, viral gastrointestinal infection, non-specific fever, viral infection, viral respiratory infection, cough, bronchitis, headache, muscle injury,

musculoskeletal pain, and injury. ICS have also been associated with modest growth effects. Height will be monitored throughout the study, by calibrated stadiometers.

Oral corticosteroids can cause hoarseness, sore throat, and yeast infection of the mouth or throat if taken in high doses for lengthy periods of time. In addition, they can cause effects on the body such as weight gain, growth delay, bruising of the skin, cataracts, and diabetes. These effects are more likely if the medicine is taken at very high doses for extended periods of time. These side effects are not anticipated in this study because of the length of time (4 days on any occasion) that oral corticosteroids will be taken.

The risks of long acting beta agonists (LABAs) include diarrhea, nausea, asthma exacerbation, bronchitis, respiratory infection, anxiety, fever, dizziness, insomnia, chest pain. There is also a small, but statistically significant risk of asthma-related death. This study will follow black box guidelines by using LABAs as an adjuvant therapy in patients not adequately controlled on inhaled steroids.

The risks from inhaled albuterol include increased heart rate and blood pressure, nausea, headache, and a jittery or nervous feeling. These symptoms usually resolve within one hour.

5.4 Risks of Study Procedures

5.4.1 Skin Prick Tests

Participants may experience mild to moderate pruritus or local discomfort at the sites of skin pricks with allergen and the positive control (histamine dihydrochloride 10 mg/mL). Usually, the allergen-induced wheal and flare responses resolve within 1–2 hours, but rarely a participant may have local swelling that takes 2-3 days to clear entirely. Rarely skin testing will cause the participant being tested to have systemic allergic symptoms. These symptoms may include sneezing, ocular pruritus and tearing, rhinorrhea and/or generalized pruritus or urticaria. Treatment with topical corticosteroids and oral antihistamines is available and is effective. A study clinician is always present and drugs and equipment for treatment of anaphylactic reactions are available. Stopping antihistamines before skin testing may make allergy (but not asthma) symptoms worse. Participants will be told they can take their medications if they need them, but the test will need to be rescheduled.

There is also a very rare chance that the participant may experience a systemic allergic reaction or fainting. A study clinician will be available to provide immediate treatment, if needed. Further, we believe that performance of such allergen evaluation is consistent with national asthma guidelines, and provides direct benefits to participants. The results of the skin tests are provided to participants.

5.4.2 Venipuncture

The risks associated with a blood draw include possible pain from the stick, as well as bleeding, bruising, and infection of the skin. Lightheadedness and fainting rarely occur. To minimize these risks, a clinical research staff member who is trained to draw blood from children will collect the samples. Additionally, clinical research staff may apply a topical anesthetic to the skin before the blood draw to reduce the discomfort of the stick. Side effects from topical anesthetics include erythema, burning, paleness at the skin site, edema, and alterations in temperature. Reactions are mild and transient. There is a potential for cutaneous allergic reactions to topical anesthetics. Topical anesthetics are routinely used for usual care venipunctures to minimize discomfort. The amount of blood to be collected is within the NIH pediatric guidelines.

5.4.3 Spirometry and Peak Flow Measurement

Spirometry and peak flow measurements will be performed by trained and certified clinical research staff according to American Thoracic Society (ATS) standards as performed routinely in usual care as part of subspecialist management of asthma.

Spirometry and peak expiratory flow measurement can cause coughing or lightheadedness, which will go away shortly after the test is finished.

5.4.4 Nasal Allergen Challenge

Nasal Allergen Challenge (NAC) involves the direct application by trained clinical research staff of one or more defined doses of allergen onto the participant's nasal mucosa using a nasal drug delivery system (LMA® MAD Nasal delivery system LMA/Teleflex, San Diego, CA). Nasal Allergen Challenge is expected to cause allergic symptoms such as nasal congestion, sneezing, nasal discharge, and itchy, watery eyes which may last up to several hours after the last dose is administered. If nasal and/or ocular symptoms persist after the period of observation in the clinical unit, participants will be offered treatment with oral antihistamines. There is a very small risk of provoking asthma symptoms. If PEF is reduced by more than 20% from baseline or if asthma symptoms develop at any step during the NAC, the procedure will be suspended, the participant will be treated, and FEV_1 will be measured. To minimize this risk, individuals with a pre-bronchodilator FEV_1 of less than 80% of predicted are not allowed to perform this procedure. In addition, a targeted pulmonary exam will be conducted at each NAC visit. The allergen will be delivered to the nose while subjects are breath holding at Total Lung Capacity (TLC) and will be asked to exhale through their nose thereafter thus minimizing the potential for allergen to reach the lower airways. As for any intervention with allergen to which the patient is sensitive, there is the slight risk of developing an anaphylactic reaction. Trained personnel, including a study clinician, as well as medications and equipment, will be immediately available to treat any reaction.

A recent DAIT NIAID-sponsored clinical study evaluated the safety of cockroach nasal allergen challenge in adults and children with asthma. Protocol ICAC-27 CoNAC conducted under DAIT NIAID-sponsored IND #16512 performed cockroach nasal allergen challenges on twenty-five children, and no reportable adverse events were linked to the study drug. Nasal allergen challenge has been performed with other allergens (perennial and seasonal) in children with asthma and found to be low risk. Mild cough or wheezing developed shortly after the nasal challenge in 2 out of 25 children,[25] while in another study a slight drop (10%) in FEV_1 was noted in 1 out of 50 children immediately following nasal allergen challenge.[26] Twenty-four hours after the NAC, 1 out of 50 children had an asthma exacerbation with a febrile viral infection.[26] Pedroletti et al. demonstrated that a single nasal allergen challenge with cat allergen was not associated with increased bronchial inflammation, as assessed by F_ENO, among cat allergic children with asthma.[27] For further details, please refer to the Investigator Brochure for German Cockroach Allergenic Extract for Nasal Allergen Challenge.

5.4.5 Nasal Lavage

Nasal lavage may be associated with some transient discomfort due to the <10 second breath hold and the feeling of fluid in the nares. A very rare potential complication of nasal lavage is acute bacterial sinusitis (sinus infection). The nasal lavage procedure is considered minimal risk, and is often referred to as a nasal wash or nasal irrigation. A variety of over-the-counter products (such as the neti-pot filled with a saline solution) have been widely available for home use to safely flush out nasal passages and alleviate both nasal congestion and even sinusitis symptoms.

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

5.4.6 Influenza Vaccine

As part of standard of care, the study clinician will recommend the participant obtain the current influenza vaccine. Minor side effects are soreness, redness or swelling at the injection site, low grade fever and aches. If these problems occur, they begin soon after vaccination and are mild and short-lived. Almost all people who receive influenza vaccine have no serious problems from it. On rare occasions, flu vaccination can cause serious problems, such as severe allergic reactions.

5.4.7 Questionnaires

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

5.5 Potential Benefits

The results of the skin testing will be provided to participants. Participants will receive relevant asthma education consistent with Guidelines for the Diagnosis and Management of Asthma (EPR 3) for patients with asthma, including handouts listing ways to avoid common allergy and asthma triggers. The national asthma guidelines have emphasized that exposure of patients who have asthma to allergens to which they are sensitive has been shown to increase asthma symptoms and precipitate asthma exacerbations. Guidelines for the Diagnosis and Management of Asthma (EPR 3) state that the clinician should evaluate the potential role of allergens, particularly indoor inhalant allergens via medical history and skin testing, and then assess the significance of positive tests in the context of the patient's medical history. Patients who have asthma at any level of severity should: (1) reduce, if possible, exposure to allergens to which the patient is sensitized and exposed; (2) know that effective allergen avoidance requires a comprehensive approach; and (3) be provided with relevant educational materials for control of environmental factors.

While the participant's asthma may or may not improve while in this study, participants may directly benefit from study participation by receiving study-based asthma assessments and care from an asthma specialist. The participants will receive close monitoring and direct management of asthma exacerbations on a 24/7 basis throughout the study. Participants with uncontrolled asthma at Screening will benefit by having additional visits to bring their asthma under control before Randomization. Participant families will receive contact numbers for the study site and a tailored asthma action plan and education. A supply of prednisone will be provided in concert with this management.

In the longer term, this study may promote the further development of cockroach SCIT for asthma and allergic rhinitis, which could be a major advance in the care of individuals with these conditions who are allergic to cockroach antigens.

6 Investigational Agent

6.1 Investigational Agent

Non-standardized glycerinated German cockroach (*Blattella germanica*) allergenic extract (50% glycerin) will be used as the investigational agent in Protocol ICAC-28. Commercially-labeled licensed product was

purchased by the IND Sponsor from the manufacturer - Stallergenes Greer (Lenoir, NC) and distributed to the clinical research sites by DAIT NIAID drug distributor (Eminent Services Corporation, Frederick, MD).

6.1.1 Formulation, Packaging, and Labeling

The active ingredient of the investigational product is a non-standardized allergen derived from the extraction and purification of proteins from German cockroach (*Blattella germanica*). The allergenic extract contains the aqueous extractable from allergenic source material in extracting solution containing 0.25% sodium chloride, 0.125% sodium bicarbonate, and 50% glycerol. Phenol (0.4%) is added after extraction as a preservative.

Non-standardized glycerinated German cockroach allergenic extract is approved in the United States for diagnostic skin testing and immunotherapy by subcutaneous injection (U.S. License 467). It has been used in humans for at least 35 years. The recommended storage conditions for the investigational agent are 2°C to 8°C. For this protocol, German cockroach extract will be stored at minus 20°C. Storage at minus 20°C is to decrease the rate of degradation of allergen (Bla g5) in the extract.

6.2 Placebo

The placebo product for SCIT in Protocol ICAC-28 is a commercially-labeled licensed product, sterile diluent for allergenic extract [glycerin (50%), sodium bicarbonate (0.091%), sodium chloride (0.166%) with water for injection (q.s.)]. The commercially-labeled licensed product was purchased by the IND Sponsor from the manufacturer – Allergy Laboratories (Oklahoma, OK) and distributed to the clinical research sites by DAIT NIAID drug distributor (Eminent Services Corporation, Frederick, MD).

6.2.1 Formulation, Packaging, and Labeling

The placebo for immunotherapy will consist of the same inactive ingredients as the non-standardized glycerinated German cockroach allergen extract. It is not colored and is in 8mL multi-dose vials.

6.3 Dosage, Preparation, and Administration

6.3.1 Subcutaneous Immunotherapy

Cockroach allergenic extract or placebo is prepared by the clinical site research pharmacist. Complete details of the dilutions can be found in the Pharmacy Manual: Investigational Product Preparation and Dispensing for Subcutaneous Immunotherapy (SCIT) for Protocol ICAC-28.

Participants will receive escalating doses of glycerinated German cockroach allergenic extract or placebo administered subcutaneously according to the dose escalation schedule (Section 8.7.1).

6.4 Drug Accountability

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

Records for receipt, storage, use, and disposition will be maintained by the study site. The drug-dispensing log will be kept current for each participant. These logs 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 IND sponsor (DAIT, NIAID) or sponsor's representative after study termination, or destroyed with the permission of the sponsor in accordance with applicable law and study site procedures.

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

6.5 Assessment of Participant Compliance with Investigational Agent

As participants receive their injections in the clinic, compliance will be monitored by assessing the number of expected and completed injection visits.

6.6 Toxicity Prevention and Management

In the case of a reaction to the subcutaneous investigational agent, dosing will be altered according to Section 8.6.1 Dose Escalation and 8.6.2. Maintenance Visits.

6.7 Premature Discontinuation of Investigational Agent

Participants who are unable to reach the minimum maintenance dose by 40 weeks following the start of dose escalation will be unable to proceed to the maintenance period and will have their study therapy discontinued (see Section 11.2.1 Discontinuation of Study Therapy; see Appendix B Study Restrictions Due to COVID-19).

Study therapy may be prematurely discontinued for any participant for any of the reasons identified in Section 11.2.1 Discontinuation of Study Therapy. Study therapy may also be prematurely discontinued for any participant if the study clinician believes that the study treatment is no longer in the best interest of the participant. Participants whose therapy is prematurely discontinued will be followed when possible for the duration of the study and seen for all remaining visits except Injection Visits.

7 Other Medications

7.1 Concomitant Medications

7.1.1 Protocol-mandated

All participants will receive asthma step care according to the Guidelines for the Diagnosis and Management of Asthma.[28] Treatment will be supervised by a study clinician. The study clinician will prescribe initial appropriate concomitant asthma therapies (see Section 7.1.1.1) and will provide a written Asthma Action Plan for the participant at Screening, Randomization, and every two months thereafter (see Section 7.1.1.4). The regimen will then be adjusted as necessary at those visits throughout the duration of the trial based on the principles of the EPR-3 guidelines (see Table 7.1.1.2a, below).

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

All participants are required to have medical insurance that will cover, at least in part, the costs of their asthma medications. It is likely, however, that some participants may lose this coverage during the time that they are in the study. To maintain participant safety and avoid a loss of these participants to the study, Protocol ICAC-28 CRITICAL will fund the cost of these participants' asthma medications during the remainder of the study or until the participant regains insurance coverage during the study. Medications will be prescribed in the same manner using the same algorithms as all insured participants. Each research site will develop plans to use local pharmacies in a cost efficient and timely manner. This design is in place to mimic the support typical insured participants receive, i.e. they receive a prescription and have to fill it at a pharmacy. To limit the time that Protocol ICAC-28 CRITICAL is required to fund the cost of asthma medications for a participant who has lost coverage, each research site will develop a plan for evaluation of insurance options and referral to appropriate support systems to obtain coverage for medication costs. In addition to addressing potential insurance from employers and governmental sources, this may include free clinics and support programs available through pharmaceutical firms.

The study clinician is allowed to prescribe treatment for rhinitis symptoms as clinically indicated. No specific rhinitis medications are recommended or required to be used. The choice is study clinician discretion.

7.1.1.1 Initial Regimen

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

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

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

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

4	14 days	5-14 nights	< 70	>2

FEV_1 = forced expiratory volume in 1 second; pred = predicted
* Determined from participant recall, based on the 2-week interval directly preceding the study visit.
**Predicted references are provided in the Protocol ICAC-28 MOP.
*** Defined as a prescription of a course of systemic steroids by a clinician or initiation of a course of systemic steroids by a participant to prevent a serious asthma outcome. If a participant initiates and completes a course of systemic steroids without clinician involvement, this course will be counted only if it meets the following dosage: the course for prednisone, prednisolone, or methylprednisolone will be 2 mg/kg/day as a single daily dose for 4 days with a maximum dose of 60 mg per day. The course for dexamethasone will be 0.3 to 0.6 mg/kg/day as a single daily dose for 2 days with a maximum dose of 16 mg per day. If a corticosteroid burst for the treatment of an asthma exacerbation is prescribed by a non-ICAC clinician, it will be counted regardless of dose.

Next, the study clinician will determine the current level of controller therapy that the participant is using. Table 7.1.1.1b shows the asthma regimens that will be used during the treatment phase of Protocol ICAC-28 CRITICAL. If the participant is currently on one of the therapies below, then the step level of that therapy will be noted. Otherwise, the study clinician must refer to the Protocol ICAC-28 MOP for equivalency tables for other asthma therapies. From the information in the Protocol ICAC-28 MOP, the study clinician will determine the corresponding step level of therapy that the participant is currently using. For example, if the participant is currently taking Flovent® Diskus® 250 mcg twice per day (bid), then he/she is receiving Step 3 therapy.

Table 7.1.1.1b Asthma Regimen by Step Level of Therapy

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

bid = twice per day; LABA = long-acting beta agonist
*Equivalent medication covered by participant's insurance formulary – see Protocol ICAC-28 MOP.

Based on the participant's current control level (from Table 7.1.1.1a) and the participant's current step level of therapy (from Table 7.1.1.1b and the Protocol ICAC-28 MOP), the study clinician will find the corresponding row in Table 7.1.1.1c below that includes both the current regimen and the current control level. The last column of that row gives the corresponding regimen as a step level, which refers back to Table 7.1.1.1b. The study clinician will prescribe this regimen.

Continuing the example from above, since the participant is taking Step 3 therapy and is at control level 3, the regimen assigned would be Step 4 (fluticasone 250 mcg bid with LABA).

Table 7.1.1.1c Algorithm for Prescribing Treatment Regimen at Screening

Current Regimen	Current Control Level	Regimen Assigned
Regimen equivalent to Step 0 (or 1-3 days/week of a regimen equivalent to Step 1)	Control level 1 Control level 2 Control level 3 Control level 4	Step 0 Step 1 Step 1 Step 2 (and may also need prednisone burst)
Regimen equivalent to Step 1 (or 1-3 days/week of a regimen equivalent to Step 2)	Control level 1 Control level 2 Control level 3 Control level 4	Step 1 Step 2 Step 2 Step 3 (and may also need prednisone burst)
Regimen equivalent to Step 2 (or 1-3 days/week of a regimen equivalent to Step 3)	Control level 1 Control level 2 Control level 3 Control level 4	Step 2 Step 3 Step 3 Step 4 (and may also need prednisone burst)
Regimen equivalent to Step 3 (or 1-3 days/week of a regimen equivalent to Step 4)	Control level 1 Control level 2 Control level 3 Control level 4	Step 3 Step 4 Step 4 Step 5 (and may also need prednisone burst)
Regimen equivalent to Step 4 (or 1-3 days/week of a regimen equivalent to Step 5)	Control level 1 Control level 2 Control level 3 Control level 4	Step 4 Step 5 Step 5 Step 5 (and may also need prednisone burst)
Regimen equivalent to Step 5	Control level 1 Control level 2 Control level 3 Control level 4	Step 5 Step 5 Step 5 (and may also need prednisone burst) Step 5 (and may also need prednisone burst)

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

In addition to the controller regimen assigned above, all participants will be prescribed albuterol by metered-dose inhaler beta-agonist or nebulizer (per clinician discretion) to be used as needed and will be provided oral prednisone to be used as per protocol – see Section 7.4.1 and the Protocol ICAC-28 MOP. The study clinician will prescribe enough medication to last until the next E&M visit. If a participant is not eligible to continue, the study clinician may prescribe an appropriate treatment regimen based on clinical judgment.

7.1.1.2 Study Period Treatment Regimen

At Randomization and every 2 months during treatment, an asthma Evaluation and Management (E&M) process will occur where participants' asthma medication regimen will be adjusted based on symptoms, albuterol/levalbuterol use, FEV_1, recent exacerbations, and their current level of therapy.

Participants may be stepped down from controller medications if their control level is equal to 1 at Screening, Asthma Control Visits, and at some E&M visits while the participant is in maintenance (see Appendix B: Study Restrictions Due to COVID-19). Participants may not be stepped down from controller

medications at Randomization or during Dose Escalation. Participants may be stepped up as per the protocol algorithm at any visit where indicated.

Participants will undergo pre-bronchodilator spirometry and an assessment of symptoms, rescue bronchodilator usage, recent exacerbations and medication adherence. Bronchodilator use as a preventive measure prior to exercise will not count toward the assessment of rescue bronchodilator use. Based on these data, a study clinician will determine the participant's current control level from Table 7.1.1.2a below. The control levels originated from the published national guidelines, Guidelines for the Diagnosis and Management of Asthma.[28]

For all participants, the highest control level for days with symptoms or albuterol/levalbuterol use, nights with symptoms or albuterol/levalbuterol use, percent personal best FEV_1, and exacerbation history will be determined by the study clinician. The day and night symptoms, albuterol/levalbuterol use, adherence to prescribed asthma controller medications by self-report, and exacerbation history will be determined from questionnaires. The FEV_1 personal best is defined in the Protocol ICAC-28 MOP. If the quality of the spirometry maneuver at the current visit is unacceptable according to the technician's judgment, FEV_1 in Table 7.1.1.2a will not be used, and the control level will be based on day and night symptoms, albuterol/levalbuterol use, and exacerbation history. For example, if a participant has 0 days with symptoms (control level 1), 4 nights with symptoms (control level 3), FEV_1 at 85% of personal best (control level 1), and 1 exacerbation (control level 2), then the participant's overall control level is 3 because that is the maximum value of control levels 1, 3, 1, and 2.

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

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

FEV_1 = forced expiratory volume in 1 second

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

**Defined as a prescription of a course of systemic steroids by a clinician or initiation of a course of systemic steroids by a participant to prevent a serious asthma outcome. If a participant initiates and completes a course of systemic steroids without clinician involvement, this course will be counted only if it meets the following dosage: the course for prednisone, prednisolone, or methylprednisolone will be 2 mg/kg/day as a single daily dose for 4 days with a maximum dose of 60 mg per day. The course for dexamethasone will be 0.3 to 0.6 mg/kg/day as a single daily dose for 2 days with a maximum dose of 16 mg per day. If a corticosteroid burst for the treatment of an asthma exacerbation is prescribed by a non-ICAC clinician, it will be counted regardless of dose.

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

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

Table 7.1.1.2b Treatment Adjustment Based on Control Levels and Adherence

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

*Defined as a prescription of a course of systemic steroids by a clinician or initiation of a course of systemic steroids by a participant to prevent a serious asthma outcome. If a participant initiates and completes a course of systemic steroids without clinician involvement, this course will be counted only if it meets the following dosage: the course for prednisone, prednisolone, or methylprednisolone will be 2 mg/kg/day as a single daily dose for 4 days with a maximum dose of 60 mg per day. The course for dexamethasone will be 0.3 to 0.6 mg/kg/day as a single daily dose for 2 days with a maximum dose of 16 mg per day.
Note: Shading in the table corresponds to the example described in the text above.

Based on the participant's step level assigned at the last E&M visit and the change in steps determined above, the new regimen will be prescribed according to Table 7.1.1.2b above. In the example above, if the participant were currently on Step 2 and having no problems with adherence, then the recommended new treatment step based on symptoms, albuterol/levalbuterol use, FEV_1, and exacerbations is Step 4.

7.1.1.3 Final Study Visit

At the Final Study Visit (12-month Visit), the final treatment regimen prescribed will be based on the protocol-based medications management algorithm or the discretion of the study clinician who examines the participant. The study clinician will base the treatment on factors such as symptoms, albuterol use, spirometry, adherence, and the participant's current level of therapy. The participant's care will then be transferred back to their primary care provider (PCP) and/or asthma specialist.

7.1.1.4 Asthma Action Plan

Clinical research staff will provide a written Asthma Action Plan consistent with NAEPP guidelines for the participant at all E&M visits. This plan describes a Green, Yellow, and Red Zone for the participant based on his/her symptoms. In the Green Zone, the participant has no symptoms of an asthma episode and is directed to use his/her usual asthma medications. In the Yellow Zone, the participant is experiencing asthma, allergy, or cold symptoms, and is directed to increase rescue medication use until the symptoms have been resolved. In the Red Zone, the participant is having persistent symptoms that cannot be controlled. The participant is instructed to contact a study clinician or go to the emergency department (ED) if unable to contact a study clinician.

7.1.1.5 Asthma Education

As part of the meeting at E&M visits with a clinical research staff member for asthma education, participants will be educated on topics related to asthma. These topics include background on asthma as a disease, use of the Asthma Action Plan (see Section 7.1.1.4), adherence to medications, using medications at school (when applicable), and allergen and irritant avoidance. Handouts will be provided and the education content will be tailored to each participant. For example, the allergen avoidance education will be based on the participant's aeroallergen skin test/serum IgE results. The participant will receive education about the allergens to which he/she is sensitive. The information contained in the handouts will be based on the EPR-3: Guidelines for the Diagnosis and Management of Asthma and on experience from previous ICAC clinical trials.

7.1.2 Other permitted concomitant medications

During the study, after the initial skin testing, rhinitis and asthma medications will be permitted along with other maintenance medications, aside from those excluded in Section 4.3, Exclusion Criteria. Short courses of oral corticosteroids or doses of greater than 500 mcg of fluticasone per day or the equivalent of another inhaled corticosteroid may be used short-term (not to exceed 6 months) to control an asthma exacerbation.

7.2 Prophylactic Medications

Pre-treatment with antihistamines can be used before immunotherapy injection at the study clinician's discretion.

7.3 Prohibited Medications

1. A regular dose of greater than 500 mcg of fluticasone per day or the equivalent of another inhaled corticosteroid for a duration of ≥ 6 months
2. Allergen immunotherapy (SLIT or SCIT)
3. Biologic therapies (e.g., anti-IgE, anti-IL-4, anti-IL-5)
4. Tricyclic antidepressants
5. Beta-adrenergic blocker drugs (both oral and topical)
6. Any investigational drug

7.4 Rescue Medications

Participants may use antihistamines as needed for large local reactions to the injections.

Participants may use antihistamines and/or a nasal steroid as needed for persistent nasal and/or ocular symptoms following the NAC. If at any time during the NAC a participant experiences asthma symptoms or anaphylaxis, treatment with inhaled short-acting bronchodilators, corticosteroids and epinephrine will be immediately available to treat the reaction. Participants will be instructed to bring an albuterol inhaler to any visit in which a NAC will be performed and carry it with them for the first 24 hours after the NAC. Participants who do not bring an albuterol inhaler to a NAC visit will be provided one.

7.4.1 Definition and Management of Acute Exacerbations

Consistent with NAEPP 2007 guidelines, study clinicians caring for participants in the Protocol ICAC-28 CRITICAL study will start a course of corticosteroids for severe and/or unresponsive exacerbations of asthma. For the purposes of the proposed analyses, an asthma exacerbation is defined as a prescription of a course of systemic steroids by a clinician or initiation of a course of systemic steroids by a participant or a hospitalization to prevent a serious asthma outcome. If a participant initiates and completes a course of systemic steroids without clinician involvement, this course will be counted only if the study clinician agrees the treatment was warranted and it meets the following dosage: the course for prednisone, prednisolone, or methylprednisolone will be at least 20 mg daily for 3 of 5 consecutive days. The course for dexamethasone will be a single daily dose. Study clinicians will strongly consider initiation of corticosteroids if:

- Albuterol is needed by inhaler (with or without a spacer) or by nebulization for more than 6 individual treatments in 24 hours; or
- Moderate-severe wheeze, cough, shortness of breath, and/or chest tightness or pain occurs for at least 5 of the preceding 7 days; or
- Symptoms of wheeze, cough, shortness of breath, and/or chest tightness or pain severe enough to place a participant in his or her “Red Zone” on their Asthma Action Plan (see Section 7.1.1.4) do not significantly improve after 3 doses of albuterol. (In such situations, in addition to the initiation of oral corticosteroids, the study clinician should consider directing the participant to the nearest ED.); or
- There is an unscheduled visit for acute asthma care requiring repeated doses of albuterol (clinician office, urgent care, ED); or
- Hospitalization is needed for asthma.

Permissible drugs for systemic corticosteroid treatment of asthma exacerbations include: prednisone, prednisolone, methylprednisolone, and dexamethasone. The course for prednisone, prednisolone, or

methylprednisolone will be 2 mg/kg/day as a single daily dose for 4 days with a maximum dose of 60 mg per day. The course for dexamethasone will be 0.3 to 0.6 mg/kg/day as a single daily dose for 2 days with a maximum dose of 16 mg per day. Failure to respond to initial treatment will result in additional evaluation and possible hospitalization for further management. This management approach has been applied to previous ICAC clinical trials and is consistent with asthma specialist usual care practices.

8 Study Procedures

Detailed instructions for all procedures are provided in the Protocol ICAC-28 MOP. Please see Appendix B for information regarding institutional or ICAC-wide restrictions on study activities due to COVID-19.

8.1 Study Assessments

8.1.1 Vital Signs and Growth Parameters

The following vital signs and growth parameters will be collected at all E&M visits unless otherwise noted:

- Weight in kilograms
- Height (using a calibrated stadiometer) at all visits when spirometry is performed
- Pulse rate
- Respiratory rate
- Blood pressure
- Chest auscultation,
- Influenza vaccine - All participants will be recommended to receive an annual flu vaccine.

8.1.2 Medical History and Physical Examination

The participant's medical history will be obtained and a study clinician will perform a physical examination at the Screening Visit. A chest auscultation and current asthma symptom assessment will be performed at all injection visits. An abbreviated physical examination will be performed at all E&M visits. Additional examinations may be completed at the study clinician's discretion.

Significant findings that are present prior to the start of the study must be included on the appropriate case report form (CRF). Significant findings that meet the definition of an adverse event (AE) must also be recorded on the AE form.

If the participant is currently having an asthma exacerbation or other symptoms that the study nurse or study clinician feel may compromise the participant's ability to complete the study procedures safely, a physical exam may be performed, and the study visit may be rescheduled. If the study clinician observes any AEs during the study visit, a targeted physical exam may be performed. Additional details regarding AE assessment are outlined in Section 12, Safety Monitoring and Reporting.

8.1.3 Aeroallergen Skin Testing

The participant's atopic status will be assessed with skin prick tests to assess allergy to dust mites, cockroaches and other aeroallergens relevant to an urban environment and the local area. Participants will be asked to stop taking antihistamines for 5 days prior to the visit at which the skin test is performed to limit interference with the results of the skin test.

Skin testing will be done by the prick technique using the GreerPick system (Stallergenes Greer; Lenoir, NC). Testing will be performed by certified clinical research staff according to the instructions in the Protocol ICAC-28 MOP. Tests will be read after 15 minutes by measuring the wheal for each antigen and for the controls.

Acceptable skin testing completed as part of a participant's participation in other ICAC protocols within 12 months will meet the requirement for this procedure. In this situation, the skin testing results documentation from the previous study will be added to the participant's study file, and the skin testing procedure will not be repeated, with the exception of the German cockroach test for study eligibility.

8.1.4 Measurement of Pulmonary Function

Spirometry will be conducted following the ATS and European Respiratory Society guidelines. For children under 8 years of age, ATS Preschool Guidelines will be used for spirometry acceptance evaluation. This procedure has been successfully implemented in previous DAIT NIAID-sponsored ICAC trials. Details regarding pulmonary function testing (PFT) are provided in the Protocol ICAC-28 MOP. All PFTs will be performed by certified clinical research staff.

Peak flow will be measured with the participant sitting upright in a chair as described in the Protocol ICAC-28 MOP.

8.1.5 Nasal Allergen Challenge

The NAC must be rescheduled if the participant meets the following criteria:

- Has uncontrolled asthma at the time of the NAC, as defined by an FEV_1 < 80% predicted or an ACT score < 20.
- Has active rhinitis symptoms at the time of the NAC, defined as a Baseline TNSS > 3, or any individual symptom score > 1.
- Is currently using oral or nasal antihistamines, nasal corticosteroids, nasal decongestants, nasal anticholinergics, biologic therapies, prednisone, or cromolyn, which cannot be suspended for the required washout periods.

Baseline peak flow (PEF) and TNSS will be measured before the start of the challenge. Nasal lavage will be performed as described in Section 8.1.6.

Increasing doses of allergen will be administered in defined time intervals (every 10-20 minutes) up to a specified maximum dose or symptom level, will be given as described in an accompanying MOP for Protocol ICAC-28. Briefly, one spray (100mcL) of diluent or diluted allergen solution is applied to each of the participant's nostrils using a nasal drug delivery system (LMA/Teleflex, San Diego, CA) while the participant breath-holds at TLC. After the dose is delivered, the participant is asked to exhale through the nose and then to avoid sniffing or swallowing in the immediate 1 minute after application. For the baseline NAC, the challenge will begin with a 0 mcg/ml of Bla g 1 [negative control – 50% glycerin, 50% COCAS with 0.2% phenol (Stallergenes Greer, Lenoir, NC)] dose followed by up to 8 doses of increasing concentration, until the participant reaches a TNSS of 6 or more, has a sneezing score of 3, or their symptoms become intolerable. Collection of TNSS and PEF will be done 10 minutes after each diluent or allergen dose is administered.

The follow-up challenge at 12 months will be performed as described above, except the challenge will continue until the participant reaches at least the dose that elicited a positive response at the baseline NAC or the participant reaches a TNSS of 12 or their symptoms become intolerable. If a participant reaches the dose that elicited a positive response at the baseline NAC without having a TNSS of 6 or more or a sneezing score of 3, dosing will continue until the participant reaches a TNSS of 6 or more, has a sneezing score of 3, or symptoms become intolerable.

If a participant begins exhibiting asthma symptoms during the NAC, or the PEF is reduced by more than 20% from baseline, the challenge will be suspended, and the participant will be evaluated by the study clinician. If it is determined treatment is needed based on clinical judgment, then the challenge will not be continued. In the absence of an immediate need for treatment, spirometry will be performed to confirm a change in lung function. If the FEV_1 is < 85% of baseline value, the challenge will be stopped. If spirometry is performed because of a low PEF and the FEV_1 is ≥ 85% of baseline value, the challenge can continue at the clinician's discretion.

The day after the NAC is performed, the participant will receive a follow-up phone call to assess if he or she is experiencing any late-onset respiratory symptoms. Participants will be given the peak flow meter they used during the challenge and will be asked to record two PEF measurements, one 4-8 hours after the completion of the challenge and one 24 hours after the completion of the challenge (or as soon as possible after the school day, if applicable). AEs and concomitant medications will also be assessed. Participants who report significant asthma symptoms will receive additional follow-up to assess safety.

For further details, please refer to the Investigator Brochure for German Cockroach Allergenic Extract for Nasal Allergen Challenge.

8.1.6 Nasal Lavage

Each NAC will accompanied by collection of nasal lavage samples. Samples will be collected prior to the challenge and approximately 3 hours from initiation of the challenge as specified in the Protocol ICAC-28 MOP. Nasal lavage samples will be collected by instilling preservative-free buffered saline into the nostrils and allowing fluid to return passively into collection vessels. If the nostrils are occluded, a nasal decongestant may be administered at the discretion of the study clinician. Samples will be split and processed for both cell differentials and RNA extraction. Site clinical research staff who are trained, study-authorized, and certified will perform the nasal lavage and processing procedure as specified in the Protocol ICAC-28 MOP.

8.1.7 Dust Sample Collection

The caretaker or participant will be given a dust collection kit, which includes instructions on how to collect a dust sample (Refer to the MOP for Protocol ICAC-28 for detailed instructions). A combined dust sample from the participant's bed and the participant's bedroom floor will be collected. The room where the participant sleeps most nights will be considered the participant's bedroom. Measuring templates will be used to delineate the areas to be vacuumed. Dust will be collected using a vacuum cleaner with a special dust collection filter attached. The dust collector will be placed into a sealable plastic bag and returned to the study center for temporary storage (frozen). Crude samples will be batched and shipped to a central laboratory by express mail for sieving, extraction, and analysis. The dust specimens will be assayed to measure the concentration of cockroach allergens such as: Bla g 1, Bla g 2, and Bla g 5. Additional allergens of interest and markers of fungal and microbial exposure may be measured. In

addition, the caretaker/participant will complete a dust collection questionnaire, which will be returned with the dust sample.

The caretaker or participant will be given the dust collection kit at the Randomization Visit and will be asked to return the dust sample and questionnaire either by mail or by bringing it to the next visit.

8.1.8 Questionnaire Assessments

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

8.2 Clinical and Research Laboratory Evaluations and Specimen Collection

8.2.1 Blood Sample Collection

Whole blood will be collected by venipuncture at Screening to determine eligibility. Blood collected prior to Randomization and at 12 months of treatment will be tested for assessment of biomarkers of allergen immunotherapy, such as cockroach-specific IgE and IgG4 antibodies. Mechanistic studies are described in Section 9.

8.2.2 Urine Pregnancy Testing

All females who have reached menarche will be required to have a urine pregnancy test before completing study activities at Screening, Randomization, 12-month visit, visits where the NAC is performed, monthly during the escalation phase, and at each Evaluation and Management Visit (E&M) during maintenance. Results of all pregnancy tests will be given to the participant or caretaker as per site-specific guidelines and relevant state laws.

8.3 Recruitment

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

Retention methods involve a number of different approaches. We will use an appointment reminder system that consists of phone calls several days and one day prior to scheduled appointments for confirmation. To facilitate telephone contact with subjects whose phone service may change during the study, up to three telephone contact numbers (relatives, neighbors, friends) will be collected for each subject. This has proven to be an effective strategy in previous DAIT NIAID-sponsored ICAC studies. Those who have no obvious characteristics making them ineligible and who are interested will be invited to the clinic for a Screening Visit.

8.4 Screening Procedures

8.4.1 Screening Eligibility Visit

This research study will be explained in lay terms to the parent/legal guardian of each potential research participant. Written informed consent and Health Insurance Portability and Accountability Act (HIPAA)

authorization will be obtained from all parents/legal guardians at the beginning of this visit and before the child undergoes any screening study procedures. Children (age dependent on local IRB guidelines) will sign a written assent form. When the informed consent has been signed, the participant is considered enrolled in the study and will be assigned a unique participant number. Study procedures will be stopped and the participant will be deemed ineligible for the study at any point during the Screening Visit if and when they fail to meet eligibility criteria.

After the consent (and assent, if applicable) is signed, participants will undergo screening study procedures, including prick skin tests with German cockroach to ensure participants are sensitive to the test product, since those who do not react to German cockroach are excluded from the remainder of the study. In addition, participants will undergo prick skin tests to a panel of allergens detailed in the MOP for Protocol ICAC-28 to characterize the study population. Participants will not need additional skin testing if they have a positive skin test result using the same lot of non-standardized German cockroach extract used for Protocol ICAC-28 immunotherapy within the previous year. If a different German cockroach extract was used for skin testing, the participant will have a repeat skin test of German cockroach, histamine, and diluent control to confirm eligibility.

A urine pregnancy test will be performed on all female participants that have reached menarche. A medical history will also be taken and a physical examination will be performed to verify the participant's suitability for inclusion in the study. Participants will be encouraged to receive a flu vaccine during each flu season.

Spirometry will be performed by all participants to verify the participant's suitability for inclusion in the study.

For participants with a positive skin prick test to cockroach, blood will be collected by venipuncture for cockroach-specific IgE antibody testing to determine study eligibility. Participants who have valid IgE test results within the previous year under another ICAC protocol will not need to be re-tested.

The study clinician will assign the participant to the appropriate asthma treatment step based on the participant's current asthma medications and symptoms according to the treatment algorithm (see Section 7.1.1.1), write an appropriate prescription, and provide an asthma action plan (see Section 7.1.1.4). Participants will receive basic asthma education (see Section 7.1.1.5).

8.4.2 Screening Nasal Allergen Challenge

The NAC will be performed on all participants. The NAC can be performed as part of a separately scheduled visit, if necessary.

Each NAC will be accompanied by collection of nasal lavage samples. Samples will be collected prior to the challenge and approximately 3 hours from initiation of the challenge as described in Section 8.1.6 and the Protocol ICAC-28 MOP.

A urine pregnancy test will also be performed on all female participants that have reached menarche.

Blood samples will be collected prior to the NAC for mechanistic studies (see Section 9). Blood will also be evaluated for baseline assessment of biomarkers of allergen immunotherapy, such as cockroach-

specific IgE and IgG4 antibodies. Total IgE and specific IgE to a panel of additional allergens detailed in the Protocol ICAC-28 MOP will also be measured.

Participants who have:

- a positive German cockroach skin prick test,
- German cockroach-specific IgE ≥0.35 kU_A/L,
- reached the TNNS of ≥ 6 or a sneeze score of 3 at doses 2-9,
- FEV_1 is at least 80% of predicted normal, and
- an Asthma Control Test/Childhood Asthma Control Test score ≥ 20

are considered eligible for the study, and at the discretion of the study clinician, will be invited to proceed to Randomization.

Candidates whose FEV_1 is less than 80% of predicted normal or with an Asthma Control Test/Childhood Asthma Control Test score $<$ 20 will enter a run-in period, as described in Section 8.4.3 below.

Participants may also qualify for rescreening if they are ineligible due to reversible illness if their condition improves while recruitment is ongoing.

8.4.3 Asthma Control Optional Visits

Participants whose FEV_1 is less than 80% of predicted normal or whose Asthma Control Test/Childhood Asthma Control Test score is $<$ 20 may have their asthma managed by a study clinician for up to four months in an attempt to get their asthma under control. Participants will return to the clinic for visits approximately every two weeks to report on their asthma symptoms and medication use, and have their medication adjusted if indicated. When control has been established based on the Asthma Control Test/Childhood Asthma Control Test, the participant may proceed in the study. If control cannot be established, the participant's care will then be transferred back to their primary care provider (PCP) and/or asthma specialist. The participant will be provided with a summary of their treatment plan, which can be shared with their PCP.

8.5 Randomization

At randomization, the study clinician will review all inclusion and exclusion criteria to confirm the participant's eligibility. Blood will be drawn, if needed, for tests described in Section 8.4.2. A dust sample collection kit will be provided (see Section 8.1.7).

The study clinician will adjust the participant's asthma medications as necessary according to the treatment algorithm (see Section 7.1.1.2), write an appropriate prescription, and provide a new asthma action plan (see Section 7.1.1.4). Participants will receive basic asthma education (see Section 7.1.1.5).

Study participants will be randomized (see Section 13.3) to one of two groups: 1) cockroach SCIT or 2) placebo SCIT (1:1).

8.6 Follow-up

8.6.1 SCIT Dose Escalation

The dose escalation of glycerinated German cockroach allergenic extract or placebo is detailed in the Dose Escalation Table 8.6.1. Each injection will be administered by the trained research staff under the

supervision of the study clinician along with any rescue medications, as required. Refer to the ICAC-28 Reaction Orders on handling treatment-related reactions.

During the dose escalation phase, all females who have reached menarche will be required to have a urine pregnancy test monthly.

Prior to the administration of each dose of allergenic extract or placebo, pre-assessment will be completed to assess the eligibility of the participant to receive the allergenic extract injection.

Post-assessment of participants, 30 minutes after each injection, will be completed for symptoms or changes.

During all dosing visits, a study clinician and appropriate emergency supplies will be readily available to treat any adverse reactions.

Participants will receive weekly injections during escalation (Table 8.6.1). From Dose 1 through Dose 20, injections must be separated by a minimum of 2 days. From Dose 21 through Dose 26, injections must be separated by a minimum of 5 days. To maximize flexibility during dose escalation, injections can be given once weekly, and can be delayed at any point if there are systemic reactions, local reactions or other concerns. To continue on to maintenance, each subject will need to reach the minimum maintenance dose in a maximum of 40 weeks (see Appendix B: Study Restrictions Due to COVID-19).

Dose escalation will be conducted by the following schedule(s):

Table 8.6.1 SCIT Dose Escalation
All participants will start SCIT with Dose 1. Doses -1 and -2 are included for participants who experience a reaction at Doses 1 or 2 that require dosage reductions.

Dose #	Concentration	Volume
-2	1:10,000	0.03 ml
-1	1:10,000	0.04 ml
1	1:10,000	0.05 ml
2	1:10,000	0.1 ml
3	1:10,000	0.2 ml
4	1:10,000	0.3 ml
5	1:10,000	0.4 ml
6	1:1,000	0.05 ml
7	1:1,000	0.1 ml
8	1:1,000	0.2 ml
9	1:1,000	0.3 ml
10	1:1,000	0.4 ml
11	1:100	0.05 ml
12	1:100	0.1 ml
13	1:100	0.2 ml
14	1:100	0.3 ml
15	1:100	0.4 ml
16	1:10	0.05 ml
17	1:10	0.1 ml

18	1:10	0.2 ml
19	1:10	0.3 ml
20	1:10	0.4 ml
21	1:1	0.05 ml
22	1:1	0.1 ml
23	1:1	0.15 ml
24	1:1	0.2 ml
25	1:1	0.3 ml
26	1:1	0.4 ml

Dose escalation will continue according to the schedule unless there are lapses in dosing or reactions occur. Dosing modifications as a result of reaction or lapse in dosing during dose escalation are detailed in the MOP for Protocol ICAC-28.

Participants who are unable to reach the minimum maintenance dose (the highest volume of the 1:10 concentration; Dose 20 in Table 8.6.1) by 40 weeks following the start of dose escalation will be unable to proceed to the maintenance period and will have their study therapy stopped (see Section 11.2.1; see Appendix B Study Restrictions Due to COVID-19).

8.6.2 Maintenance Visits

Participants who have completed dose escalation will receive one injection of investigational agent every 2 weeks for 3 doses, followed by maintenance injections every 4 weeks to complete a maximum of 12 months of treatment.

During the maintenance phase, a urine pregnancy test will be performed at the E&M visits (every two months) on all female participants that have reached menarche.

Dosing modifications as a result of reaction or lapse in dosing for the three, initial, maintenance-level injections, planned at two week intervals, are detailed in the MOP for Protocol ICAC-28. Additionally, dosing modifications as a result of reaction or lapse in dosing for the subsequent maintenance level injections planned at four-week intervals are detailed in the MOP for Protocol ICAC-28.

Participants will be instructed to immediately report any severe sign or symptom possibly related to the subcutaneous administration of the investigational agent and to contact the study site when they need medical advice. At each visit, participants will be queried regarding any delayed reactions or side effects experienced. For those participants with asthma, exacerbations will be handled through an approach consistent with standard clinical practice as described in the Protocol ICAC-28 MOP.

8.7 Asthma Evaluation and Management (E&M) Visits

Every two months, an asthma E&M process will occur where participants' asthma medication regimen will be adjusted based on symptoms, albuterol/levalbuterol use, FEV_1, recent exacerbations, and their current level of therapy (see Section 7.1.1.2). At these visits, asthma and rhinitis endpoints, including the CASI (see Section 3) will also be measured. The E&M Visits will include:

- Pregnancy test (post-menarcheal females)
- Physical exam and AE assessment
- Vital signs, with height and weight

- Spirometry and peak flow
- CASI and other symptom measurements
- Assignment of asthma medication regimen by study clinician (see Section 7.1.1)
- Asthma education/counseling (see Section 7.1.1.5)

8.8 12-Month Visit

The 12-Month Visit is the final visit and will include:

- Pregnancy test (post-menarcheal females)
- Final physical exam and AE assessment
- Vital signs, with height and weight
- Blood draw
- Allergen skin test for German cockroach
- Spirometry and peak flow
- CASI and other symptom measurements
- Targeted pulmonary exam
- NAC
- Nasal lavage, before and after NAC

8.9 Early Termination Visit

Participants who withdraw from the study or are dropped from the study will be invited to the study center for an Early Termination Visit as specified in the Protocol ICAC-28 MOP. The Early Termination Visit can include:

- Pregnancy test (post-menarcheal females)
- Final physical exam and AE assessment
- Vital signs, with height and weight
- Blood draw
- Allergen skin test for German cockroach
- Spirometry and peak flow
- CASI and other symptom measurements

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

8.10 Unscheduled Visits

Participants who experience asthma-related problems (not requiring emergency room care) or concerns between scheduled study visits will be seen by a study clinician as warranted by the situation. If the site staff are notified by the participant, his/her family, an ED, a hospital, or clinic that the participant was seen for an unscheduled visit elsewhere, the site staff will contact the participant within 2 weeks of discharge to obtain an update on the participant's clinical status. A follow-up visit at the study center

will be scheduled if needed. For details on the management plan, please refer to Section 7.1.1 and the Protocol ICAC-28 MOP.

Participants who are at Control Level 4 at a study visit will either be seen in the clinic or will receive a follow-up phone call within 1-2 weeks of the visit to check the status of their symptoms.

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

8.11 Visit Windows

During dose escalation, 1-2 injections will be given each week separated by at least 2 days. Dose escalation must be completed over a maximum of 40 weeks (See Appendix B: Study Restrictions Due to COVID-19). Bi-weekly and monthly maintenance injections should be scheduled as close as possible to the every 2-week or every-4-week target dates.

See the Protocol ICAC-28 MOP for details regarding visit scheduling and Appendix A for the Table of Events.

9 Mechanistic Assays

Exploratory objectives will be addressed using mechanistic assays to evaluate biological responses to CR immunotherapy. Detailed descriptions of the methodology for biological sample collection and processing and for assay implementation are described in the ICAC-28 MOP and in separate mechanistic study proposals.

Whole blood will be collected by venipuncture according to the visit activity schedule (see Appendix A) prior to Randomization and at the 12-Month Visit or the Early Termination Visit. Nasal lavage samples will be collected prior to each NAC and at approximately 3 hours after the initiation of each NAC.

9.1 Antibody Assays

Serum processed from study blood draws will be tested for biomarkers of allergen immunotherapy, such as cockroach-specific IgE and IgG4 antibodies to cockroach component allergens. These include, but may not be limited to, Bla g 1, Bla g 2, Bla g 4, Bla g 5 and Per a 7.

Serum from these blood draws will also be used to measure in-vitro cockroach antigen binding to B-cells.

9.2 Cellular Assays

Peripheral blood mononuclear cells (PBMCs) will be separated from study blood draws and used to measure the magnitude and phenotype of the CR-specific T cell response to CR immunotherapy.

9.3 Gene Expression

Blood drawn at each study blood draw will be used to extract RNA samples for whole transcriptome RNA sequencing to identify the changes in PBMC gene expression response to CR stimulation over time and compare those changes between the treated group and the untreated group. Significant differences will allow the identification of mechanisms of immune tolerance in CR SCIT.

Nasal lavage samples collected before and after each NAC will be used to compare gene expression by RNA-sequencing in nasal lavage samples pre-CR NAC with samples post-CR NAC. Samples collected at 12 months will be used to identify the changes in nasal lavage gene expression in response to CR immunotherapy between the treated group and the untreated group.

10 Biospecimen Storage

Unused samples of biological specimens collected during the course of the study will be stored (described in the Protocol ICAC-28 MOP) for future use for tests that may or may not be planned. These tests may or may not be related to the study of asthma and allergy and may include genetic studies such as DNA. Participants will be asked to give permission for long-term storage and future use during the consent process. With the participant's approval and as approved by the central IRB, de-identified biological samples will be stored indefinitely at the DAIT NIAID central repository (Eminent Services Corporation, Frederick, MD).

11 Criteria for Participant and Study Completion and Premature Study Termination

11.1 Participant Completion

Participants are considered to have completed the study once they have completed the final visit (12-month Visit).

11.2 Participant Stopping Rules and Withdrawal Criteria

11.2.1 Discontinuation of Study Therapy

Study therapy may be prematurely discontinued for any participant for any of the following reasons:

1. SAE related to investigational agent.
2. Systemic reaction grade 3 or 4 (see Table 12.3.1.1c, WAO Systemic Reaction Grading System).
3. Failure to reach the minimum maintenance dose in a forty-week period (See Appendix B: Study Restrictions Due to COVID-19).
4. Development of any serious medical illness whose natural history, sequela, or treatment would be worsened or impaired by continuation in the protocol.
5. The participant has a need to start immunotherapy or any chronic immunosuppressive medications.
6. The participant has a need for a dose of greater than 500 mcg of fluticasone per day, or the equivalent of another inhaled corticosteroid, to maintain asthma control for greater than 6 months.

11.2.2 Discontinuation of Study Participation

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

1. Pregnancy
2. The participant elects to withdraw consent from all future study activities, including follow-up.
3. The participant is "lost to follow-up" as defined in the Protocol ICAC-28 MOP.
4. The participant dies.
5. The Investigator no longer believes participation is in the best interest of the participant.
6. The participant has a need for > 3 bursts of systemic corticosteroids for asthma exacerbations during the treatment period OR has a need for > 2 bursts of systemic corticosteroids for asthma

exacerbations within 6 months during the treatment period. If prescribed by an ICAC study clinician, a burst is defined as a course for prednisone, prednisolone, or methylprednisolone will be 2 mg/kg/day as a single daily dose for 4 days with a maximum dose of 60 mg per day. The course for dexamethasone will be 0.3 to 0.6 mg/kg/day as a single daily dose for 2 days with a maximum dose of 16 mg per day. If a corticosteroid burst for the treatment of an asthma exacerbation is prescribed by a non-ICAC clinician, it will be counted regardless of dose.

11.3 Participant Replacement

Participants who are discontinued prior to completing the study may be replaced as long as randomization for the study is still ongoing. All reasons for withdrawal will be captured on the appropriate electronic case report form (eCRF).

11.4 Follow-up after Study Therapy Discontinuation

Participants who discontinue study therapy after receiving one dose of investigational product will be asked to continue study follow-up for the remainder of the treatment period, using an altered visit schedule.

11.5 Study Stopping Rules

Study enrollment and treatment will be suspended pending expedited review of all pertinent data for the following reasons:

1) One death if at least possibly related to the investigational agent
2) Two Grade 4 systemic reactions possibly related to the injection of the investigational agent (see Table 12.3.1.1c)
3) One local Grade 4 reaction (see Table 12.3.1.1a) or Grade 4 systemic reaction (see Table 12.3.1.1c) possibly related to the NAC
    a) The study will be halted for this occurrence pending review of pertinent data by DAIT/NIAID Medical Monitor (MM) and the NIAID Asthma and Allergy DSMB

If the study enrollment is stopped due to meeting the above criteria, it may not be resumed until all pertinent information is discussed with DAIT/NIAID, NIAID Asthma and Allergy DSMB, and the Central IRB, and all parties concur with the resumption of the study. The FDA will be notified if stopping rules are met and also on resumption of the study.

The study may be terminated by the IND sponsor (DAIT/NIAID) or the NIAID Allergy and Asthma DSMB upon review of any observations, events, or new information that merits such action.

12 Safety Monitoring and Reporting

12.1 Overview

This section defines the types of safety data that will be collected under this protocol and outlines the procedures for appropriately collecting, grading, recording, and reporting those data. Adverse events that are classified as serious according to the definition of health authorities must be reported promptly (per Section 12.5, Reporting of Serious Adverse Events and Adverse Events) to the IND sponsor (DAIT NIAID). Appropriate notifications will also be made to site Principal Investigators, Institutional Review Boards (IRBs), and appropriate Health Authorities.

Information in this section complies with ICH Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, ICH Guideline E-6: Guideline for Good Clinical Practice, 21CFR Parts 312 and 320, and applies the standards set forth in the National Cancer Institute (NCI), Common Terminology Criteria for Adverse Events (CTCAE), Version [enter 4.0 or correct version applicable to trial] : http://ctep.cancer.gov/reporting/ctc.html.

12.2 Definitions

12.2.1 Adverse Event (AE)

Any untoward or unfavorable medical occurrence associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research (modified from the definition of adverse events in the 1996 International Conference on Harmonisation E-6 Guidelines for Good Clinical Practice) (from OHRP "Guidance on Reviewing and Reporting Unanticipated Problems Involving Risks to Subjects or Others and Adverse Events (1/15/07)" http://www.hhs.gov/ohrp/policy/advevntguid.html#Q2)

The following are adverse events associated with study therapy and study procedures:

- **Study therapy regimen**: German cockroach allergenic extract administered by the subcutaneous route
    - Local Injection site reaction which requires a change in SCIT dose
    - Systemic reactions such as, but not limited to, those listed below:
    - Eye symptoms (itchy, runny, swelling)
    - Nose symptoms (sneezing, itching, runny, stuffy)
    - Mouth/ears/throat symptoms (itchy mouth, throat irritation, oral or throat angioedema, cough, itchy ears)
    - Skin symptoms other than local injection site reactions (angioedema, urticaria, generalized itching, rash)
    - Gastrointestinal symptoms (vomiting, diarrhea, cramps, nausea)
    - Chest (cough, tightness, wheezing)
    - Anaphylaxis (see Table 12.3.1.1b)
- **Study procedures**: AEs that occur due to a study procedure will be collected and recorded. The following clinical situations, when associated with study procedures, are defined as adverse events and will be recorded on the AE eCRF.

Blood Draws

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

Pulmonary Function Testing

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

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

Nasal Allergen Challenge

- Any clinical response requiring medication (see table 12.3.1.1a)

Allergen Skin Testing

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

12.2.2 Suspected Adverse Reaction (SAR)

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

12.2.3 Unexpected Adverse Event

An adverse event or suspected adverse reaction (SAR) is considered "unexpected" when its nature (specificity), or severity, or rate of occurrence is not consistent with the risk information described in the general investigational plan or elsewhere in the IND.

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

12.2.4 Serious Adverse Event (SAE)

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

1. Death.
2. A life-threatening event: An AE or SAR is considered "life-threatening" if, in the view of either the Principal Investigator or IND Sponsor, its occurrence places the 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.
3. Inpatient hospitalization or prolongation of existing hospitalization.
4. Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
5. Congenital anomaly or birth defect.
6. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment,

they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

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

12.3 Grading and Attribution of Adverse Events

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

- *Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (published September 2007)* for allergic local reactions to study procedures.
- The definition of anaphylaxis is described in Table 12.3.1.1b (Clinical Criteria for Diagnosing Anaphylaxis)[29]
- World Allergy Organization (WAO) Subcutaneous Immunotherapy Systemic Reaction Grading System.[9] National Cancer Institute (NCI) *Common Terminology Criteria for Adverse Events Version 4.03* (published June 14, 2010) for all other reactions. (This document is referred to herein as the "NCI-CTCAE manual.")

12.3.1 Grading Criteria

12.3.1.1 Adverse Events Related to Nasal Allergen Challenge, Skin Testing and Immunotherapy

Adverse events related to nasal allergen challenge, skin testing, and immunotherapy not associated with systemic signs or symptoms will be graded according to Table 12.3.1.1a below. All AEs should be recorded on the eCRF.

Table 12.3.1.1a Grading of Local Reactions to Study Procedures

Grade	1	2	3	4
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 (e.g. upper airway obstruction)
Skin testing	Meets the minimum criteria listed in section 12.2.1, but requiring no medication other than topical corticosteroids or antihistamines.	Interfering with usual daily activities or sleep and requiring oral steroids.	Requiring a visit to a health care provider for treatment	Not applicable
Immunotherapy	Requires a change in IT dosing, but requiring no medication other than topical corticosteroids or antihistamines.	Interfering with usual daily activities or sleep and requiring oral steroids.	Requiring a visit to a health care provider for treatment	Not applicable

Criteria from Sampson, et al.[29] (Table 12.3.1.1b), will be used to determine whether systemic allergic reactions constitute anaphylaxis. To be considered anaphylaxis, the event must involve at least two organ systems (e.g. not be simply a skin reaction).

Table 12.3.1.1b Clinical Criteria for Diagnosing Anaphylaxis

Anaphylaxis is highly likely when any <u>one</u> of the following 3 criteria are fulfilled:
1) Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (e.g., generalized hives, pruritus or flushing, swollen lips-tongue-uvula) *AND AT LEAST ONE OF THE FOLLOWING:* a) Respiratory compromise (e.g., dyspnea, wheeze-bronchospasm, stridor, reducted PEF, hypoxemia b) Reduced BP or associated symptoms of end-organ dysfunction (e.g., hypotonia [collapse], syncope, incontinence) 2) Two or more of the following that occur rapidly after exposure *to a <u>likely</u> allergen for that patient* (minutes to several hours): a) Involvement of the skin-mucosal tissue (e.g., generalized hives, itch-flush, swollen lips-tongue-uvula) b) Respiratory compromise (e.g., dyspnea, wheeze-bronchospasm, stridor, reducted PEF, hypoxemia) c) Reduced BP or associated symptoms (e.g., hypotonia [collapse], syncope, incontinence) d) Persistent gastrointestinal symptoms (e.g., crampy abdominal pain, vomiting) 3) Reduced BP after exposure to *<u>known</u> allergen for that patient* (minutes or several hours): a) Infants and children: low systolic BP (age specific) or greater than 30% decrease in systolic BP* b) Adults: systolic BP of less than 90 mm Hg or greater than 30% decrease from that person's baseline
PEF, Peak expiratory flow; *BP,* blood pressure. *Low systolic blood pressure for children is defined as less than 70 mm Hg from 1 month to 1 year, less than (70 mm Hg + [2 x age]) from 1 to 10 years, and less than 90 mm Hg from 11 to 17 years.

All anaphylaxis events, regardless of severity grade, and other systemic reactions will be recorded as Adverse Events and will be graded according to Table 12.3.1.1c World Allergy Organization Subcutaneous Immunotherapy Systemic Reaction Grading System.[9]

Table 12.3.1.1c World Allergy Organization Subcutaneous Immunotherapy Systemic Reaction Grading System

World Allergy Organization Subcutaneous Immunotherapy Systemic Reaction Grading System (see text)				
Grade 1	**Grade 2**	**Grade 3**	**Grade 4**	**Grade 5**
Symptom(s)/ sign(s) of one organ system present[i] **Cutaneous** Generalized pruritus, urticaria, flushing or sensation of heat or warmth[ii] **or** Angioedema (not laryngeal, tongue or uvular) **or** **Upper respiratory** Rhinitis (e.g., sneezing, rhinorrhea, nasal pruritus and/or nasal congestion) **or** Throat-clearing (itchy throat) **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	***Symptom(s)/ sign(s) of more than one organ system present*** **or** **Lower respiratory** Asthma: cough, wheezing, shortness of breath (e.g., less than 40% PEF or FEV1 drop, responding to an inhaled bronchodilator) **or** **Gastrointestinal** Abdominal cramps, vomiting, or diarrhea or **Other** Uterine cramps	**Lower respiratory** Asthma (e.g., 40% PEF or FEV1 drop, NOT responding to an inhaled bronchodilator) **or** **Upper respiratory** Laryngeal, uvula or tongue edema with or without stridor	**Lower or Upper respiratory** Respiratory failure with or without loss of consciousness **or** **Cardiovascular** Hypotension with or without loss of consciousness	Death
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.				

12.3.1.2 Grading and Attribution of All Other Adverse Events

The study clinician will grade the severity of other adverse events experienced by the study participants according to the criteria set forth in the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. This document (referred to herein as the NCI-CTCAE manual) provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all adverse events. The NCI-CTCAE has been reviewed by the Principal Investigators and has been deemed appropriate for the participant population to be studied in this protocol.

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

Events Grade 1 or higher will be recorded on the appropriate AE electronic case report form for this study.

During the course of the study, to determine if an abnormal value or a clinical or laboratory evaluation (including, but not limited to, a radiograph, an ultrasound, an electrocardiogram etc.), is an adverse event will rely on clinical judgment of the study clinician.

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

12.3.2 Attribution Definitions

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

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

Table 12.3.2 Attribution of Adverse Events

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

12.4 Collection and Recording of Adverse Events

12.4.1 Collection Period

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

12.4.2 Collecting Adverse Events

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

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

12.4.3 Recording Adverse Events

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

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

12.5 Reporting of Serious Adverse Events and Adverse Events

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

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

Principal Investigators must report all serious adverse events (see Section 04, Serious Adverse Event), regardless of relationship or expectedness within 24 hours of becoming aware of the event.

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

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

However, unavailable details of the event will not delay submission of the known information. As additional details become available, the AE/SAE eCRF will be updated and submitted. Every time the SAE eCRF is submitted, it should be electronically signed by the investigator or sub-investigator.

12.5.2 Reporting to FDA

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

12.5.2.1 Standard Reporting (Report in the IND Annual Report)

This option applies if the AE is classified as one of the following:

- Serious, expected, suspected adverse reactions (see Section 12.2.2, Suspected Adverse Reaction, and Section 12.2.3, Unexpected Adverse Event).
- Serious and not a suspected adverse reaction (see Section 12.2.2, Suspected Adverse Reaction).
- Pregnancies.

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

12.5.2.2 Expedited Safety Reporting

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

Category 1: **Serious and unexpected suspected adverse reaction [SUSAR]** (see Section 12.2.2, Suspected Adverse Reaction (SAR), and Section 12.2.3, Unexpected Adverse Event and 21 CFR 312.32(c)(1)i).

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

1. 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);
2. An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group.

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

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

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

12.5.3 Reporting of Adverse Events to IRBs

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

12.6 Pregnancy Reporting

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

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

Pregnancy eCRF shall be updated and submitted to the DAIT SACCC when details about the outcome are available.

Information requested about the delivery shall include:

- Gestational age at delivery
- Birth weight, length, and head circumference
- Gender
- Appearance, pulse, grimace, activity, and respiration (APGAR) score at 1 minute, 5 minutes, and 24 hours after birth, if available
- Any abnormalities

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

12.7 Reporting of Other Safety Information

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

12.8 Review of Safety Information

12.8.1 Medical Monitor Review

The DAIT NIAID Medical Monitor shall receive monthly reports from the DAIT SACCC compiling new and accumulating information on major protocol deviations, AEs, SAEs, and pregnancies recorded by the study sites on appropriate eCRFs.

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

12.8.2 DSMB Review

12.8.2.1 Planned DSMB Reviews

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

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

12.8.2.2 Ad hoc DSMB Reviews

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

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

12.8.2.2.1 Temporary Suspension of enrollment and drug dosing for ad hoc DSMB Safety Review

A temporary halt in both enrollment and drug dosing will be implemented if an ad hoc DSMB safety review is required.

13 Statistical Considerations and Analytical Plan

13.1 Overview

The primary hypothesis to be tested by this study is whether nasal allergen challenge outcomes, in children with persistent, well-controlled asthma, will be affected by subcutaneous treatment with non-standardized glycerinated German cockroach (*B. germanica*) allergenic extract. Major secondary hypotheses are that subcutaneous treatment with German cockroach (*B. germanica*) extract can affect asthma severity measures as well as induce significant changes in immunologic biomarkers indicative of desensitization in children with persistent, well-controlled asthma.

13.2 Endpoints

The primary endpoint of the study is the average TNSS (total nasal symptom score) over the 9 protocol-defined doses at the 12-month NAC. Secondary and exploratory endpoints include other NAC outcomes, safety, in vivo and in vitro biomarkers of immunotherapy, other asthma and rhinitis severity endpoints, and key mechanistic parameters.

13.3 Measures to Minimize Bias

To minimize bias, a randomization schedule will be generated by the DAIT SACCC and implemented in a validated system that will be used by site personnel to automate the random assignment of treatment groups to study participants. The randomization scheme will be reviewed and approved by a statistician at the DAIT SACCC (Rho Federal) and will not be modified thereafter except to accommodate any changes required by amendments to the protocol. Participants will be randomized using a 1:1 ratio of active (German cockroach (*B. germanica*) extract) and control (placebo) participants. Randomization will be stratified by site.

All laboratory assays for serum IgE and IgG antibodies and other parameters will be performed in a central laboratory by technicians who do not know the participants' group assignments.

13.4 Analysis Plan

13.4.1 Analysis Populations

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

- Modified Intent-to-treat (ITT) sample: All participants who are randomized, received at least one dose of study treatment, and receive at least one dose during the 12-month NAC. Participants will be analyzed according to the treatment arm to which they were randomized, regardless of the medication they actually received.
- Safety sample: All participants who sign consent and undergo study procedures at Screening. Participants will be analyzed according to the medication they actually received, regardless of the

treatment arm to which they were randomized. Non-treatment-emergent adverse events (e.g., any adverse event that occurs before the first injection) will be summarized in all participants who are enrolled, while treatment-emergent adverse events (e.g., any adverse event that occurs on or after the first injection) will be summarized in the safety sample.

- Per-protocol (PP) sample: All participants who are randomized, are escalated to 0.4 mL of the 1:10 maintenance dose, and receive at least 66% of expected doses of maintenance.

13.4.2 Primary Analysis of Primary Endpoint

The primary outcome of the study is the average TNSS, calculated as the average TNSS over the observed doses. The comparison between arms will be conducted using an analysis of covariance (ANCOVA) model. The ANCOVA model will include fixed categorical effects for treatment and baseline TNSS score, with adjustment for site. Least square means (LSmeans), SE, LSmeans difference, 95% confidence interval (CI) and p-value will be presented. Further details regarding the analyses of the 12-month TNSS will be provided in the Statistical Analysis Plan.

13.4.3 Supportive Analyses of the Primary Endpoint

13.4.3.1 Effects of Cockroach SCIT on TNSS

Additionally, it will be possible to compare the responsive dose at the 12-month NAC (the dose which elicits the threshold response of a TNSS ≥ 6 or a sneeze score ≥ 3) at the 12-month NAC by treatment group, using discrete-time (discrete-dose) survival analyses as described by Chinchilli et al.[30] and Sampson et al.[31] This analysis, which will estimate a hazard ratio, is based on a model for the conditional probability of a subject reacting to a given dose, given that the subject has not reacted to previously administered doses. Details of right censoring, adjustment for the baseline NAC, imputation for missing 12-month NAC observations, and evaluation of the proportional hazards assumption (and analytical options when the assumption is not met) will be provided in the Statistical Analysis Plan.

13.4.3.2 Approaches for Missing Data

The primary method of handling missing efficacy data will be the method of MCMC multiple imputation which does not rely on the assumption of data missing at random. After imputing with MCMC, each complete data set will be analyzed with an ANCOVA with factors for arm and site and the respective baseline TNSS as a covariate. Lastly, we will combine the sets of estimates (and their standard errors) generated from different ANCOVA multiply imputed datasets into one set of results.

13.4.4 Analyses of Secondary and Other Endpoints

13.4.4.1 Safety

Safety will be summarized by arm in the safety sample by the number and percentage of serious and non-serious adverse events stratified by grade and relationship to treatment as well as the number and percentage of participants who have a serious and non-serious adverse event stratified by grade and relationship to treatment.

13.4.4.2 German Cockroach-specific IgE and IgG4

12-months log-transformed German cockroach-specific IgE and IgG4 will also be modeled as ANCOVA with factors for treatment arm, site and the respective log-transformed baseline as covariate. Similarly, Least square means (LSmeans), SE, LSmeans difference, 95% confidence interval (CI) and p-value will be

presented, modeled using a linear mixed model that will include a random intercept and fixed effects for each treatment arm, and an interaction term between treatment arm and time of measurement.

13.4.5 Analyses of Exploratory Endpoints

13.4.5.1 CASI outcome measures

To account for the correlation among repeated measures within subject, the effect of cockroach SCIT on asthma severity will be analyzed using a mixed effect repeated measures model looking at the CASI assessments at 10 and 12 months of treatment.

The mixed model will include fixed effects for treatment group, baseline CASI score at randomization, site as well as a within-subject random effects. Treatment group differences will be tested using the F statistics from the mixed model with Kenward-Roger degrees of freedom. Least squares estimates of the CASI for both of the study arms will be obtained from the model and presented. Ninety-five percent confidence intervals for the estimated treatment effect (LS Mean for the German Roach arm – LS Mean for the Placebo arm) will also be obtained using the fitted-regression model. The validity of model assumptions will be explored using regression diagnostics and may include separate models to assess possible modification of the treatment effect by CASI or other fixed effects.

In addition, for each subject, the average CASI overall assessments will be computed and summarized by treatment group (mean, standard deviation, median, minimum and maximum). For each visit where CASI is collected following the first injection of study drug, summary statistics will be presented by treatment arm.

13.4.5.2 Rhinitis severity outcome measures

The same mixed effect repeated measures model as used for the CASI outcomes, with the same covariates, will be used to examine treatment differences in the secondary rhinitis severity endpoints:

1. Modified Rhinitis Symptom Utility Index
2. Rhinitis treatment step (medication requirements)

13.4.5.3 Additional asthma severity outcome measures

The same mixed effect repeated measures model as used for the CASI, with the same covariates, will be used to examine treatment differences in the additional asthma severity endpoints:

1. Number of days with asthma symptoms (wheezing or tightness in the chest or cough)
2. Number of nights with asthma symptoms (waking up because of wheezing or tightness in the chest or cough)
3. Number of days with albuterol use
4. Number of nights with albuterol use
5. Asthma treatment step (medication requirements)
6. Asthma exacerbations
7. FEV_1

13.4.5.4 Mechanistic Endpoints

For continuous variables with baseline value and one post-baseline measurement (12-months), ANCOVA will be used, unless specified otherwise. Least square means (LSmeans), SE, LSmeans difference, 95%

confidence interval (CI) and *p*-value will be presented. Logistic regression analyses adjusted for baseline will be performed for the binary variables, and the odds ratio and 95% CI for the odds ratio of treatment group comparisons will be given.

Analysis plans for other mechanistic endpoints, as described in Section 9, will be detailed in their respective mechanistic proposals.

13.4.6 Descriptive Analyses

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

13.5 Interim Analyses

13.5.1 Interim Analysis of Efficacy Data

Not applicable.

13.5.2 Interim Analysis of Safety Data

Not applicable.

13.5.3 Futility Analysis

Not applicable.

13.6 Sample Size Considerations

Sample size calculations are based on the primary endpoint of the study, mean TNSS over the 9 protocol-defined doses at the 12-month NAC following the primary analysis, modeled as ANCOVA with factors for treatment arm, site and TNSS baseline as covariate. For sample size calculations we assumed a mean TNSS score of 2.94 for the placebo group with varying effect sizes. A risk reduction of 0.30 is obtained as (2.94 – 2.94*(1-0.3)) / 2.94 = 0.30 and translates into an absolute difference of 2.94*0.3 = 0.88. A mean squared error (pooled SD) of 1.09 was assumed for these sample sizes calculations.

Assuming a risk reduction of 30%, absolute difference of 0.88 an ANCOVA with a total sample size of 54 participants will provide 83% power for a 2-sided, 0.05 significance level test.

Table 13.6: Power Calculations under different scenarios of total sample size.

Risk Reduction	Absolute Diff	n = 44	n = 54	n = 64
0.20	0.59	0.42	0.50	0.57
0.25	0.74	0.59	0.68	0.76
0.30	0.89	0.75	0.83	0.89
0.35	1.03	0.87	0.93	0.96
0.40	1.18	0.94	0.97	0.99
0.45	1.33	0.98	0.99	1.00
0.50	1.48	0.99	1.00	1.00

Code for table:

```
pacman::p_load(tidyverse, rio)
pacman::p_load(arsenal, gt)
nac_per <- import("S:/RhoFED/NIAID/DAIT/Allergy_Asthma/ICAC/CRITICAL/Statistics/Manuscripts/Baseline/Data/nac_per.rds")
t1 <- tableby(nac_pos ~ tnss_mean, data = nac_per, digits = 2) %>%
 summary()
t1
m1 <- lm(tnss_mean ~ 1, data = nac_per, subset = nac_pos == 1)
summary(t1)
anova(t1)
# rr and abs diff
(2.94 - 2.94*(1-0.3))/2.94
(2.94 - 2.94*(1-0.3))
2.94*0.3
#table
crossing(  n = c(44, 54, 64)/2,
           sd = 1.09,
           m1 = 2.95,
           rr = seq(0.20, 0.50, 0.05)) %>%
 mutate( m2 = m1*(1-rr),
       d = m1-m2,
       N = n*2) %>%
 rowwise() %>%
 mutate(pw = power.t.test(n = n, delta = d, sd = sd)$power %>%
        round(2)) %>%
 pivot_wider(id_cols = c(rr,d),
          names_from = N,
          values_from = pw,
          names_prefix = "n_") %>%
 gt()
```

14 Identification and Access to Source Data

14.1 Source Data

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

14.2 Access to Source Data

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

15 Protocol Deviations

15.1 Protocol Deviation Definitions

15.1.1 Protocol Deviation

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

15.1.2 Major Protocol Deviation (Protocol Violation)

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

15.1.3 Non-Major Protocol Deviation

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

15.2 Reporting and Managing Protocol Deviations

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

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

16 Ethical Considerations and Compliance with Good Clinical Practice

16.1 Quality Control and Quality Assurance

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

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

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

16.2 Statement of Compliance

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

16.3 Informed Consent Process

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

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

16.4 Privacy and Confidentiality

A participant's privacy and confidentiality will be respected throughout the study. Each participant will be assigned a unique identification number and these numbers rather than names will be used to

collect, store, and report participant information. Site personnel will not transmit documents containing personal health identifiers (PHI) to the study sponsor or their representatives.

17 Publication Policy

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

18 References

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Appendix A: Schedule of Procedures/Evaluations

Visit	Screening Eligibility Visit	Screening NAC Visit	Asthma Control Visits[6]	Random-ization Visit	Dose Escalation Injection Visits[5]	Maintenance Injection Visits	Injection Visits with Evaluation and Management (E&M)	12-Month Visit	Early Termination Visit[7]	Unscheduled Asthma Control Visits/ Phone Calls[8]
Informed consent	X									
Medical history	X									
Physical exam[1]	X		X	X	X	X	X	X	X	X
Targeted Pulmonary Exam		X						X		
Vital signs and growth parameters[2]	X	X	X	X			X	X	X	X
Asthma Evaluation & Management	X		X	X			X	X	X	X
Asthma Counseling	X		X	X			X	X	X	X
Spirometry	X	X	X	X			X	X	X	X
Peak flow	X	X	X	X	X	X	X	X	X	X
Adverse event assessment	X	X	X	X	X	X	X	X	X	X
Concomitant medication assessment	X	X	X	X	X	X	X	X	X	X
Symptom questionnaires	X	X	X	X	X	X	X	X	X	X
CASI	X			X			X	X	X	
Allergen skin test[3]	X							X	X	
Blood collection	X	X		X				X	X	
Pregnancy test	X	X		X	X[4]		X[4]	X	X	
Injection					X	X	X			
Nasal allergen challenge		X						X		
Nasal lavage		X						X		
Dust sample collection				X						

[1]A detailed physical exam will occur at Screening and 12 months. A more limited physical exam will be completed at other visits.

[2] Height will be assessed at Screening and at visits where spirometry is performed. Weight will be assessed at screening and every 2 months at E&M Visits.

[3] Full panel of allergens at Screening, German cockroach only at 12 months and Early Termination.

[4] Pregnancy testing will be performed monthly during escalation and at E&M Visits during maintenance.

[5]Dose Escalation will normally take 16-26 weeks, but can take place over up to 40 weeks, if needed. See Appendix B Study Restrictions Due to COVID-19.

[6] Visits occur approximately every two weeks until participant's asthma is under control as defined by the ACT.

[7] An Early Termination Visit will be conducted for participants who withdraw from the study or are dropped from the study.

[8] Procedures, e.g. spirometry, can be performed at the discretion of the clinician.

Appendix B: Study Restrictions Due to COVID-19

In the event of institutional or ICAC-wide restrictions on certain types of procedures or certain classes of research, some study activities will be suspended. This includes certain visits and specific inhalation and nasal procedures, such as:

- In-person study visits, including injection visits
- Spirometry
- Nasal Allergen Challenges
- Nasal sampling, including nasal lavages
- Peak Expiratory Flow

When all in-person study activities are suspended, study staff will continue to make contact with study participants over the phone and clinicians will continue to manage the participants' asthma remotely. Prescriptions for asthma management medications can be sent to local pharmacies and additional prednisone, if needed, can be mailed to the participant. Should reconsenting be necessary, the consent process will occur remotely.

Participants whose immunotherapy regimen was halted as a result of study activity restrictions will be asked to resume therapy once the site is able to administer injections. The initial restart dose for these participants will be dictated by the dose modification rules in the Protocol ICAC-28 MOP. Re-escalation of immunotherapy will follow the rules referenced in Section 8.6 and in the Protocol ICAC-28 MOP, taking into account each participant's total time off therapy and history of reactions. The 40-week requirement for reaching maintenance will be reset for participants whose immunotherapy was interrupted by the COVID-19 pandemic.

While participants may typically be stepped down from controller medications during the maintenance phase, participants in this phase who had their therapy level modified as a result of the COVID-19 pandemic will not be stepped down in MEDS treatment level until they have successfully re-escalated to their original maintenance dose.