STATISTICAL ANALYSIS PLAN

STUDY TITLE:

COCKROACH IMMUNOTHERAPY IN CHILDREN AND ADOLESCENTS

PROTOCOL NUMBER:

ICAC-28

SHORT TITLE: CRITICAL

NCT#: 03541187

COMPOUND #: Non-standardized glycerinated German cockroach allergenic extract (U.S. License 467).

CLIENT: [redacted]

SPONSOR: [redacted]

REGULATORY AGENCY IDENTIFIER NUMBER(S): IND #: 17979

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VERSION HISTORY

SAP Version	Version Date	Change(s)	Rationale
1.0	2022-06-09	Initial Document	

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1. LIST OF ABBREVIATIONS

Table 1: List 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

2. PURPOSE OF THE ANALYSES

The purpose of this statistical analysis plan (SAP) is to describe the planned analyses and data displays to be included in the Clinical Study Report (CSR) for Protocol ICAC-28. This document provides details on study populations, how the variables will be derived, how missing data will be handled and details on statistical methods to be used to analyze the safety and efficacy data.

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

The document may evolve over time, for example, to reflect the requirements of protocol amendments or regulatory requests. However, the final SAP must be finalized, approved by the Client, and placed on file before database is locked. If differences occur between analyses described in the SAP and the current protocol, those found in this SAP will assume primacy. Deviations from the final approved plan will be noted in the clinical study report.

3. PROTOCOL SUMMARY

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 ≥ 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 (≥ 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.

4. GENERAL ANALYSIS AND REPORTING CONVENTIONS

The following analyses and reporting conventions will be used:

- Categorical variables will be summarized using counts (n) and percentages (%) and will be presented in the form "n (%)." Percentages will be rounded to one decimal place. If a count is 0, 0% will be shown for the percentage.
- Continuous variables will be summarized using sample size (n), mean, standard deviation (SD), median, first and third quartiles, minimum (min), and maximum (max), as appropriate. The mean will be reported at one more significant digit than the precision of the data, the standard deviation will be reported at two more significant digits than the precision of the data, and first and third quartiles, minimum, maximum, and median will be reported at the same precision as the data. The median will be reported as the average of the two middle numbers if the dataset contains an even number of observations.
- Test statistics including t, z, and $\chi 2$ test statistics will be reported to two decimal places.
- P-values will be reported to three decimal places if greater than or equal to 0.001. If less than 0.001, the value will be reported as "<0.001." A p-value can be reported as "1.000" only if it is exactly 1.000 without rounding. A p-value can be reported as "0.000" only if it is exactly 0.000 without rounding.
- Each data listing will be sorted by treatment arm and subject identifier (ID) unless otherwise noted.

If departures from these general conventions are present in the specific evaluations section of this SAP, then those conventions will take precedence over these general conventions.

5. ANALYSIS SAMPLES

ICH E3: 11.4.2.6; ICH E9: 5.2

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 at least 0.4 mL of the 1:10 maintenance dose, and receive at least 3 doses of maintenance SCIT/placebo.

6. STUDY SUBJECTS

6.1. Disposition of Subjects

ICH E3: 10.1

The disposition of all enrolled subjects will be summarized in tables and listed. The following disposition information will be summarized:

- The number of participants screened.
- Among screened participants:
  - The number and percentage of participants who failed Screening, including those that attended the Screening visit but did not meet the inclusion/exclusion criteria.
  - The number and percentage of participants in the mITT sample.
  - The number and percentage of participants in the safety sample.
  - The number and percentage of participants in the PP sample.
- Among participants in the mITT sample and grouped by treatment arm:
  - The number and percentage of participants in the safety sample.
  - The number and percentage of participants in the PP sample.

- The number and percentage of participants who completed the 12 month NAC.
- The number and percentage of participants who withdrew during the study due to:
    - Withdrawal by participant
    - Lost to follow-up
    - Death
    - Pregnancy
    - Physician decision
    - Participant experienced an anaphylactic reaction to study drug injection
    - Participant was diagnosed with an exclusionary malignancy
    - Participant acquired an opportunistic infection
    - Non-compliance with the study protocol
    - Participant refused protocol-mandated procedure at the Screening of Treatment Initiation Visit
    - Adverse Event
    - Other

A data listing of subject disposition will be provided for the mITT population.

6.2. Demographic and Other Baseline Characteristics

ICH E3: 11.2

Summary descriptive statistics for demographic and baseline characteristics will be summarized in the mITT population grouped by randomized treatment arm and overall.

Demographic data will include age at randomization, sex, race, ethnicity, and site.

Baseline will be defined as the last measurement prior to or on the day of randomization (beginning of Month 0). Baseline characteristics will include:

- Age
- Sex
- Race
- Caretaker completed high school
- Annual household income (<15k)
- CASI & CASI subscales
- Cockroach-specific IgE and IgG4
- NAC Responsive Dose

Demographic and baseline characteristics for continuous variables will be summarized using the number of participants, mean, standard deviation, median, and first and third quartiles (as applicable). Demographic and baseline characteristics for qualitative or categorical variables will be summarized by the number and percentage of participants within each category. Demographic and baseline characteristics in the mITT sample will also be compared between treatment arms using T-tests, Wilcoxon Sign-Rank Tests, or Chi-Square Tests, as applicable.

A data listing of demographics, baseline characteristics, and medical history will be provided for the mITT sample. A data listing of any inclusion/exclusion criteria not met will also be provided for all screened participants as well as all participants in the mITT sample separately.

6.3. Prior and Concomitant Medications

Medications will be coded according to the World Health Organization (WHO) Drug Dictionary (version 2017.01). Medications reported on the CRF will be categorized for analysis as prior, concomitant, or after study treatment by comparing the medication start and stop dates with the first and last dose of study medication dates. Prior medications will have both the medication start and stop dates prior to the first dose of study medication date. After medications will have both the medication start and stop dates after the last dose of study medication date. All other medications will be classified as concomitant, indicating that use of the medication overlapped with use of the study medication by at least one day.

The number and percentage of subjects receiving prior, concomitant, and after medications will be presented overall and by medication class. When reporting the number of subjects receiving the medication, a subject will only be counted once if they ever received the medication within the medication class. Percentages will be based on the number of subjects in the analysis population.

7. STUDY OPERATIONS

7.1. Protocol Deviations

ICH E3: 10.2

Protocol deviations will be recorded on the protocol deviation electronic case report form (eCRF). Major protocol deviations will be identified by the DAIT Medical Monitor and study team prior to unblinding of treatment assignment and database freeze.

A data listing of all protocol deviations sorted by site and subject ID will be provided to the DAIT Medical Monitor and study team for the manual identification of major protocol deviations.

Following database freeze, a data listing will be provided for protocol. The listing will be sorted by site, treatment arm, and subject ID and will also include type of deviation, severity of the deviation (major or non-major), date of occurrence, and the reason for the deviation.

Protocol deviations will be summarized in tabular format by site and by type of deviation (including the major deviations listed above).

7.2. Randomization

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

7.3. Measures of Treatment Compliance

ICH E3: 9.4.8

Subjects are intended to escalate to at least 0.4 mL of the 1:10 maintenance dose and continue the maintenance dose regimen every 4 weeks up to 12 months after the beginning of therapy per the protocol (section 8.6.2). Dose escalation takes a minimum of 26 weeks and can take up to a maximum of 40 weeks. Thus, the minimum number of maintenance doses remaining in the 12-month study would be 3 and the maximum would be 6.

Treatment compliance during the maintenance period will be summarized separately for each treatment arm and listed.

Treatment compliance during the maintenance period will be calculated as:

$$\%\ \text{Compliance} = \frac{\#\ \text{of actual maintenance doses}}{\#\ \text{of expected maintenance doses}} \times 100$$

8. ENDPOINT EVALUATION

8.1. Overview of Efficacy Analysis Methods

8.1.1. Multicenter Studies

ICH E3: 11.4.2.4; ICH E9: 3.2

8.1.2. Assessment Time Windows

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

At the onset of the COVID-19 pandemic, all in-person study visits were suspended including all SCIT/placebo injections. All participant follow-up was conducted via telephone during this time. As in-clinic injection visits resumed, the following procedures were restricted: Spirometry, Nasal Lavage, Peak Flow, Nasal Allergen Challenge. Resumption of all study procedures was conducted via study-specific approvals reviewed by site and network leadership. Each participant whose therapy was interrupted was re-escalated to maintenance therapy. The first dose a participant received upon resumption of immunotherapy was based on their dose at the time of suspension of therapy, the time elapsed, and their reaction history (see Appendix B of the ICAC-28 protocol.

8.1.3. Missing Data

The primary method of handling missing efficacy data will be the use of complete case analysis for the primary analyses. Indeed, participants with missing data for the 12 months NAC will not be included in the ANCOVA with factors for arm and site and the respective baseline TNSS as a covariate.

8.1.4. Multiple Comparisons/Multiplicity

ICH E3; 11.4.2.5; ICH E9; 5.6

As ICAC-28 is a pilot study, adjustment for multiplicity will not be performed for the primary or secondary endpoints.

8.1.5. Definitions of Outcome Measures

Many of the study endpoints derive from the nasal allergen challenge (NAC) procedure, where increasing doses of allergen are administered in defined time intervals (every 10-20 minutes) up to a specified maximum dose (up to 9 doses in ICAC-28) or a symptom threshold. After administration of each dose, staff count the number of sneezes for 10 minutes; five or more sneezes results in the maximum sneeze score of 3. After the 10 minutes have passed, participants rate their symptoms separately for runny nose, stuffy nose, and itchy nose on a scale of 0-3. The sum of the 4 symptoms is the Total Nasal Symptom Score or TNSS, which ranges from 0-12. In the ICAC-28 study, a NAC is considered positive when the TNSS ≥ 6 or the sneeze score = 3. The dose that elicits the positive response is called the NAC responsive dose. The baseline NAC is stopped at the responsive dose. The 12-month NAC is continued to its responsive dose or the baseline responsive dose, whichever is later.

8.2. Primary Endpoint

ICH E9: 2.2.2-2.2.7

Variable (Endpoint): Change in mean TNSS after 12 months of treatment with cockroach SCIT.

Population: Of all subjects defined by the study inclusion / exclusion criteria, the analysis population will include subjects who are in the modified ITT and per protocol populations.

Intercurrent events: All intercurrent events will be handled following hypothetical strategy (imputation). Missing data of this nature, as well as missing data due to withdrawal, will be multiple imputed as described above in section 7.1.3.

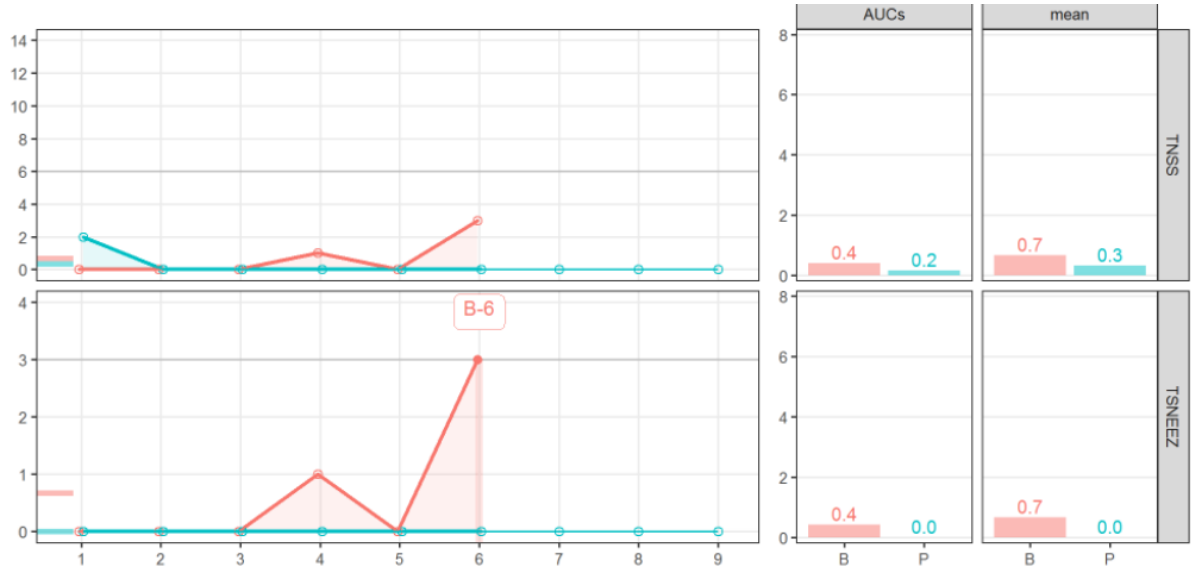
Population-level summary: Difference between treatment groups, Cockroach SCIT vs Placebo, in the change in mean TNSS after 12 months of treatment between the placebo and treatment arm.

8.2.1. Computation of the Primary Endpoint

The primary endpoint of the study is the change in the mean TNSS from baseline to 12 months, calculated as the difference between the mean TNSS at baseline up to their baseline responsive dose and the mean TNSS at 12 months up to their baseline responsive dose.

Below are a couple of examples describing the above calculations. The top row displays the participant's TNSS scores and bottom row displays the participants sneeze scores. The pink line is the measurement taken at baseline while the blue line is the measurement taken at 12 months.

1. Example



In this example, the participant reacted at baseline with sneeze at dose 6.

The baseline mean TNSS is calculated up to dose 6:

$$\frac{0+\ 0+\ 0+1+0+3}{6} \approx 0.7$$

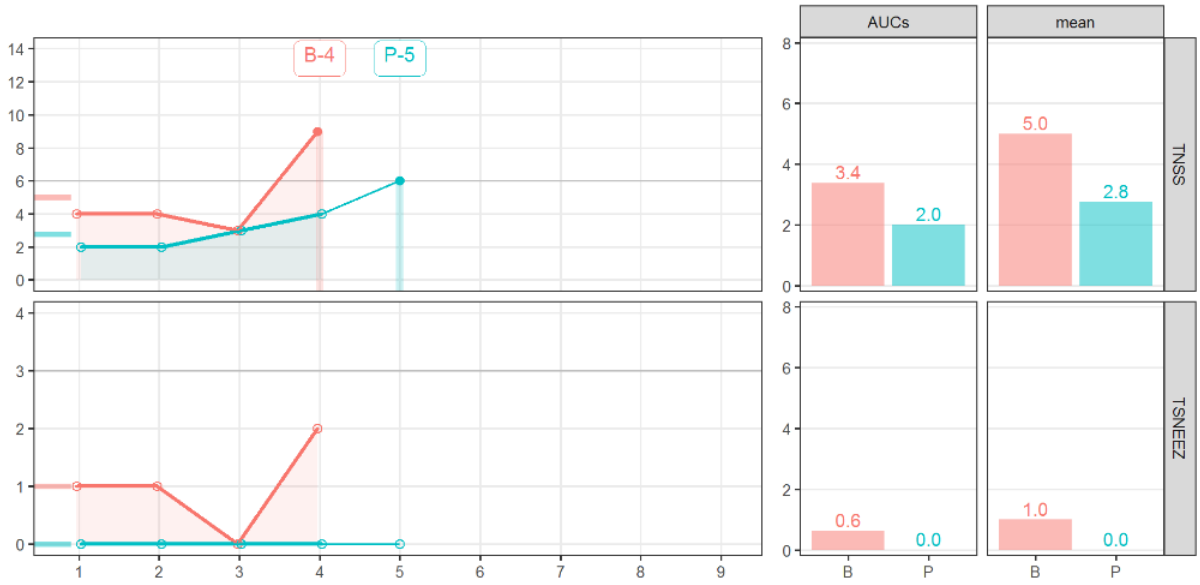
The 12-month mean TNSS is calculated the same as the baseline mean:

$$\frac{2+\ 0+0+0+0+0}{6} \approx 0.3$$

Then change in the mean TNSS from baseline to 12 months will be calculated as:

$$0.3 - 0.7 = -0.4$$

2. Example



Unlike the participant in the example above, this participant reacted at baseline with TNSS, dose 4. However, the calculation of the mean and change in mean are similar.

The baseline mean TNSS is calculated up to dose 4:

$$\frac{4 + 4 + 3 + 9}{4} \approx 5$$

The 12-month mean TNSS is calculated the same as the baseline mean:

$$\frac{2 + 2 + 3 + 4}{4} \approx 2.8$$

Then change in the mean TNSS from baseline to 12 months will be calculated as:

$$2.8 - 5 = -2.2$$

8.2.2. Primary Analysis of the Primary Endpoint

ICH E3: 11.4.1

The comparison between arms will be conducted using an analysis of covariance (ANCOVA) model, where the change from baseline to 12-month mean TNSS score is the outcome. The ANCOVA model will include fixed categorical effects for treatment and baseline mean TNSS score, with adjustment for site. Least square means (LSmeans) and associated SEs will be presented for each treatment group. The comparison between treatment groups will be presented using the LSmeans difference and associated 2-sided 95% confidence interval (CI) and p-value.

8.3. Secondary Endpoints

8.3.1. Endpoint 1

Variable: Number of immunotherapy related adverse events, overall and by participant, and number of immunotherapy related serious adverse events in the course of treatment.

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

8.3.2. Endpoint 2a

Variable: Change in TNSS AUC from baseline to 12 months, calculated as the difference between the AUC TNSS at baseline up to their baseline responsive dose and the AUC TNSS at 12 months up to their baseline responsive dose.

Analysis: For the mITT population, like the rules referenced in section 8.2.1, the TNSS AUC will be calculated separately at baseline and 12 months for each subject using the trapezoidal rule and divided by their baseline reactive dose. Change in TNSS AUC will be compared between treatment arms using an analysis of covariance (ANCOVA) model. The model will contain the change from baseline to 12-month TNSS AUC as the outcome, and fixed effects for baseline TNSS AUC, treatment group, and site. Least square means and associated SEs will be presented for each treatment group. Difference in least square means between treatment groups will be presented along with associated 2-sided 95% confidence interval (CI) and p-value.

8.3.3. Endpoint 2b

Variable: 12-Month responsive dose. 12-Month responsive dose will be calculated as the first dose for which a reaction (TNSS >= 6 or sneeze score of 3, whichever occurs first) occurs in the sequence of up to 9 doses.

Analysis: For the mITT population, the 12-month responsive dose will be analyzed using a Cox Regression model. 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. Participants who reach the last dose without reacting will be considered right censored. The baseline responsive dose and site will be controlled and there will be no imputation for missing 12-month NAC observations.

8.3.4. Endpoint 3

Variable: Change in log-transformed German cockroach-specific IgE and IgG4 from baseline to 12 months.

Analysis: For the mITT population, 12-month log-transformed IgE and IgG4 will be modeled as ANCOVA with factors for treatment arm, site, and the respective log-transformed baseline as covariates. Geometric least square means (LSmeans) and associated SEs will be presented for each treatment group. The geometric least square mean ratio comparing treatment vs placebo group, associated 95% confidence interval (CI) and p-value will be presented.

8.3.5. Sensitivity Analyses of the Secondary Analysis

For the mITT population, baseline and 12-month log transformed IgE and IgG4 will be modeled using a linear mixed model that will include a random intercept and fixed effects for each treatment arm, site, and an interaction term between treatment arm and time of measurement. Similarly to the ANCOVA, geometric least square means (LSmeans) and associated SEs will be presented for each treatment group. The geometric least square mean ratio comparing treatment vs placebo group, associated 95% confidence interval (CI) and p-value will be presented.

8.3 Other Endpoints

8.4.1 Exploratory Endpoint 1

Variable: CASI score over the 2 assessments occurring between 10 and 12 months of treatment

Analysis: 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.

8.4.2 Exploratory Endpoint 2

Variable: Change in mean sneeze score after 12 months of treatment with cockroach SCIT.

Analysis: The comparison between arms will be conducted using an analysis of covariance (ANCOVA) model, where the change from baseline to 12-month mean sneeze score is the outcome. The ANCOVA model will include fixed categorical effects for treatment and baseline mean sneeze score, with adjustment for site. Least square means (LSmeans) and associated SEs will be presented for each treatment group. The comparison between treatment groups will be presented using the LSmeans difference and associated 2-sided 95% confidence interval (CI) and p-value.

8.4.3 Exploratory Endpoint 2

Variable: Additional asthma severity outcome measures

Analysis: 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. FEV1

8.4.4 Exploratory Endpoint 3

Variable: Rhinitis severity outcome measures

Analysis: 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)

8.4.5 Exploratory Endpoints 4-9

Variable: Changes in component allergens, including Bla g 1, Bla g 2, Bla g 4, Bla g 5, and Per A 1, change in cockroach-specific blocking antibodies, Change in cockroach-specific blocking antibodies, Change in cockroach skin test reactivity, Changes in cockroach-specific T cell response, Changes in PBMC gene expression response to CR stimulation, Changes in nasal lavage gene expression

Analysis: Continuous variables with baseline value and one post-baseline measurement (12-months) will be analyzed and reported similarly to Secondary Endpoint 3 (Section 8.3.4, ANCOVA technique). Binary variables with baseline and post-baseline measures will be analyzed using logistic regression analyses. Models will contain12-month measures as the outcome and factors for treatment arm, site, and the respective baseline measure as covariates. Odds ratios and 95% CI for the odds ratio of treatment group comparisons will be given.

8.4.6 Exploratory Endpoint 10

Variable: Sneeze score AUC at NAC-12 and NAC baseline. AUC will be calculated from sneeze score across 9 doses or until reaction (TNSS >= 6 or sneeze score of 3, whichever occurs first).

Analysis: Sneeze score AUC will be calculated separately at baseline and 12 months for each subject using the trapezoidal rule. AUC will be calculated from sneeze score across 9 doses or until reaction (TNSS >= 6 or sneeze score of 3, whichever occurs first). Change in sneeze score AUC will be compared between treatment arms using an analysis of covariance (ANCOVA) model. The model will contain 12-month sneeze score AUC as the outcome, and fixed effects for baseline sneeze score AUC, treatment group, and site. Least square means and associated SEs will be presented for each treatment group. Difference in least square means between treatment groups will be presented along with associated 95% confidence interval and p-value.

8.5 Examination of Subgroups

ICH E3: 11.4.2.8; ICH E9: 5.7

Because of the temporary halt on ICAC-28/CRITICAL study procedures due to the influence of COVID-19, the study population can be divided into two subgroups for the analysis of the primary endpoint.

The first is the group of participants who were expected to follow all guidance per protocol. These participants were expected to reach maintenance-level SCIT/placebo injections within 40 weeks of dose initiation, for example. These participants completed the study prior to the onset of COVID-19 or were enrolled after the restart of study procedures.

The second is the group of participants who were expected to follow guidance in the protocol appendices designed to allow for modifications related to COVID-19. These participants could not be expected to reach maintenance-level SCIT/placebo injections within 40 weeks of dose initiation, for example, because of the period of time in which they could not attend clinic visits. These participants were those already enrolled, but not yet completed, at the onset of COVID-19.

As a sensitivity analysis, the primary endpoint will be calculated by treatment separately for each of the COVID-19 groups above.

9. SAFETY EVALUATION

9.1. Overview of Safety Analysis Methods

The safety analyses will be performed in the safety sample defined in Section 4. Safety assessment summaries will include:

- Adverse events (AEs), defined in Protocol Section 12.2.1.
- Suspected Adverse Reaction (SAR), defined in Protocol Section 12.2.1.1.
- AEs leading to discontinuation of study drug
- Serious adverse events (SAEs), defined in Protocol Section 12.2.3.
- Deaths
- Clinical laboratory results
- Vital signs
- Physical examinations

These analyses will not be stratified by site.

For grading an abnormal value or result of a clinical or laboratory evaluation (including, but not limited to, a radiograph, an ultrasound, an electrocardiogram etc.), a treatment-emergent AE is defined as an increase in grade from baseline or from the last post-baseline value that doesn't meet grading criteria. Changes in grade from screening to treatment initiation at Day 0 will also be recorded as AEs, but are not treatment-emergent.

9.2. Extent of Exposure

ICH E3: 12.1

9.3. Adverse Events

ICH E3: 12.2

All AEs will be classified by system organ class (SOC) and preferred term, according to a standardized thesaurus (Medical Dictionary for Regulatory Activities [MedDRA]). The latest edition of MedDRA will be used at the time of classification.

The severity of AEs will be classified, as applicable, using the criteria set forth in the NCI-CTCAE Version 4.03.

Each AE will be entered on the electronic case report form (eCRF) once at the highest severity.

The relationship, or attribution, of an AE to the study therapy regimen or study procedures is defined in Protocol Section 12.3.2.

Overall summary tables will be developed using the Safety Population to report the number of events and the number and percentage of participants having at least one event in the following categories:

- AEs
- AEs by maximum grade
- AEs by relationship to a study drug

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

- AEs
- AEs by maximum grade
- AEs by relationship to study drug

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

Similar summaries will be generated for SARs (defined in Protocol Section 12.2.1.1) and AEs leading to discontinuation of study drug separately.

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

9.4. Deaths, Serious Adverse Events, and Other Significant Adverse Events

ICH E3: 12.3

SAEs will be listed and summarized in the same manner described in Section 8.2. Separate displays listing and summarizing death, including time to death and cause of death, will also be created.

Data listing will be provided separately for SAEs and AEs resulting in death.

9.5. Clinical Laboratory Evaluation

ICH E3: 12.4

Clinical laboratory measurements will be measured at Screening and include IgE. Results will be converted to standardized units where possible. For numeric laboratory results, descriptive

statistics of laboratory values and the change from baseline of laboratory values will be presented for each treatment group and overall. For categorical laboratory results, the number and percentage of subjects reporting each result will be presented for each treatment group and overall.

9.6. Vital Signs, Physical Findings, and Other Observations Related to Safety

ICH E3: 12.5

9.6.1. Vital Signs

Vital sign measurements (including weight in kg, height, pulse rate, respiratory rate, blood pressure, and temperature) and growth parameters will be collected at all E&M visits unless otherwise noted. A data listing of all vital sign and growth parameter measurements will be provided.

9.6.2. Physical Examinations

A study clinician performed a physical examination at Screening visit. A chest auscultation and current asthma symptom assessment were performed at all injection visits. An abbreviated physical examination was performed at all E&M visits. Additional examinations may have been completed at the remainder of the visits at the study clinician's discretion. Significant findings that met the definition of an adverse event (AE) were recorded on the AE form.

9.6.3. Other Safety Measures

Prior to March 16, 2020, peak expiratory flow (PEF) was performed at all clinic visits with the exception of E&M visits. At Screening, Asthma Control, and Randomization visits, this was done to prove asthma control for study eligibility. At injection visits, this was done before and after SCIT injections as a safety measure to ensure no reaction in relation to the treatment. During NAC visits, this was done many times as a safety measure to ensure the challenge was stopped and rescue medications were administered if there were reductions in lung function.

As of June 26, 2020, PEF was replaced during clinic visits with an alternate safety measure, the targeted pulmonary exam. PEF conducted outside of the clinic after NAC visits was replaced with a questionnaire conducted by phone. This decision was made in light of COVID-19 to avoid unnecessary procedures involving forceful exhalation while maintaining assurance of lung function. The frequency of drops in PEF during and after a NAC procedure prior to the onset of COVID-19 were reviewed when considering these alterations.

10. OTHER ANALYSES

10.1. Use of Medications

Medications will be coded according to the World Health Organization (WHO) Drug Dictionary (version 2017.01). Medications reported on the CRF will be categorized for analysis as prior, concomitant, or after study treatment by comparing the medication start and stop dates with the first and last dose of study medication dates. Prior medications will have both the medication start and stop dates prior to the first dose of study medication date. After medications will have both the medication start and stop dates after the last dose of study medication date. All other medications will be classified as concomitant, indicating that use of the medication overlapped with use of the study medication by at least one day.

The number and percentage of subjects receiving prior, concomitant, and after medications will be presented overall and by medication class. When reporting the number of subjects receiving the medication, a subject will only be counted once if they ever received the medication within the medication class. Percentages will be based on the number of subjects in the analysis population.

11. INTERIM ANALYSES AND DATA MONITORING

ICH E3: 11.4.2.3; ICH E9: 4.5

The progress of the study will be monitored by the Data and Safety Monitoring Board (DSMB). The NIAD Allergy and Asthma DSMB will be chartered to review safety data and to make recommendations regarding continuation, termination, or modification of the study. The DSMB will formally review the safety data at least yearly. The discontinuation of study treatment will also be periodically reported to the DSMB.

In addition, safety data will be reviewed by the DSMB when an event occurs that is of sufficient concern to the National Institute of Allergy and Infectious Diseases (NIAID) medical monitor or protocol chair to warrant review, or when an event occurs that could contribute to a predefined stopping rule specified in the protocol.

Findings will be reported to Institutional Review Boards (IRBs) and health authorities.

12. CHANGES TO THE ANALYSES PLANNED IN THE PROTOCOL

ICH E3: 9.8

The protocol describes the Per Protocol analysis population to be "All participants who are randomized, are escalated to at least 0.4 mL of the 1:10 maintenance dose and receive at least 66% of expected doses of maintenance."

This analysis plan has changed the definition of the Per Protocol analysis population to be "All participants who are randomized, are escalated to at least 0.4 mL of the 1:10 maintenance dose and receive at least 3 doses of maintenance." This change was made to simplify the definition due to the pause in immunotherapy caused by the COVID-19 pandemic.

13. REFERENCES

1. Chinchilli VM, Fisher L, Craig TJ. Statistical issues in clinical trials that involve the double-blind, placebo-controlled food challenge. J Allergy Clin Immunol 2005;115:592-7.

2. Sampson HA, Gerth van Wijk R, Bindslev-Jensen C, Sicherer S, Teuber SS, Burks AW, et al. Standardizing double-blind, placebo-controlled oral food challenges: American Academy of Allergy, Asthma & Immunology-European Academy of Allergy and Clinical Immunology PRACTALL consensus report. J Allergy Clin Immunol 2012;130:1260-74.

14. APPENDIX

14.1. Table of Estimands

Objective Clinical Category	Statistical Category	Estimand/Variable
Primary Objective: To compare efficacy of cockroach subcutaneous immunotherapy (SCIT) superiority within placebo/active in participants with participants with asthma and german cockroach sensitization with respect to:		
Efficacy Category 1	Primary/MCP	• Variable: Change in mean TNSS after 12 months of treatment with cockroach SCIT. • Population: Of all subjects defined by the study inclusion / exclusion criteria, the analysis population will include subjects who are modified ITT and per protocol population. • Intercurrent Event Strategy (IES): All intercurrent events will be handled following hypothetical strategy (imputation). Missing data of this nature, as well as missing data due to withdrawal, will be multiple imputed as described above in section 7.1.3. • Population Level Summary (PLS): Difference between treatment groups, Cockroach SCIT vs Placebo, in the change in mean TNSS after 12 months of treatment between the placebo and treatment arm. • Analysis: The comparison between arms will be conducted using an analysis of covariance (ANCOVA) model, where the change from baseline to 12-month mean TNSS score is the outcome. The ANCOVA model will include fixed categorical effects for treatment and baseline mean TNSS score, with adjustment for site. Least square means (LSmeans) and associated SEs will be presented for each treatment group. The comparison between treatment groups will be presented using the LSmeans difference and associated 2-sided 95% confidence interval (CI) and p-value.

Efficacy Category 2	Secondary/MCP	• Variable: Change in TNSS AUC from baseline to 12 months. AUC at baseline will be calculated from the TNSS score across 9 doses or until reaction (TNSS >= 6 or sneeze score of 3, whichever occurs first). The TNSS AUC at 12-months will be calculated from the TNSS score across 9 doses or until TNSS = 12 or the baseline reacting dose has been reached, whichever occurs first. • For the mITT population, like the rules referenced in section 8.2.1, the TNSS AUC will be calculated separately at baseline and 12 months for each subject using the trapezoidal rule and divided by their baseline reactive dose. Change in TNSS AUC will be compared between treatment arms using an analysis of covariance (ANCOVA) model. The model will contain the change from baseline to 12-month TNSS AUC as the outcome, and fixed effects for baseline TNSS AUC, treatment group, and site. Least square means and associated SEs will be presented for each treatment group. Difference in least square means between treatment groups will be presented along with associated 2-sided 95% confidence interval (CI) and p-value.
	Secondary/MCP	• Variable: Change in Responsive dose from baseline. Responsive dose will be calculated as the first dose for which a reaction (TNSS >= 6 or sneeze score of 3, whichever occurs first) occurs in the sequence of up to 9 doses. • Analysis: For the mITT population, compare the change in responsive dose (the dose which elicits the threshold response of a TNSS ≥ 6 or a sneeze score ≥ 3) from baseline to the 12-month NAC between treatment groups, using discrete-time (discrete-dose) survival analyses[1],[2]. 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

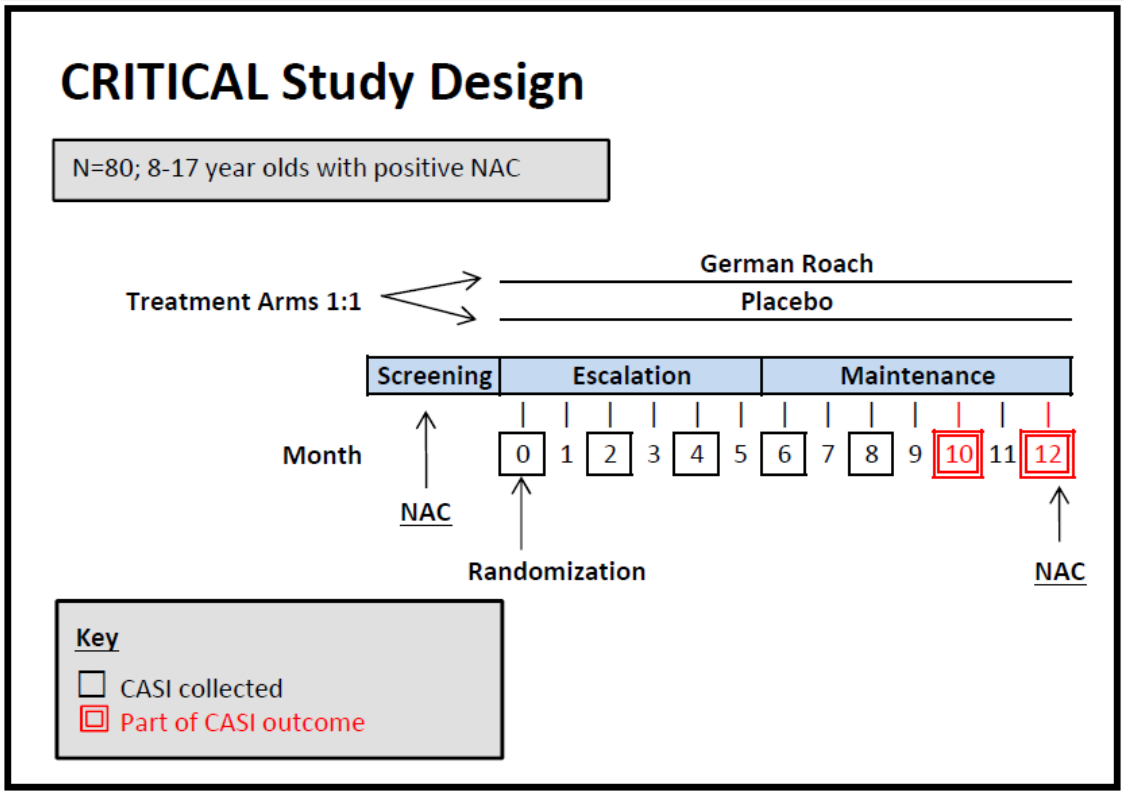
1
2

		reacted to previously administered doses. Participants who reach the last dose without reacting will be considered right censored. The baseline NAC responsive dose and site will be controlled and there are no imputations for missing 12-month NAC observations.
	Secondary/MCP	• Variable: Change in log-transformed German cockroach-specific IgE and IgG4 from baseline to 12 months. • Analysis: For the mITT population, 12-month log-transformed IgE and IgG4 will be modeled as ANCOVA with factors for treatment arm, site, and the respective log-transformed baseline as covariates. Geometric least square means (LSmeans) and associated SEs will be presented for each treatment group. The geometric least square mean ratio comparing treatment vs placebo group, associated 95% confidence interval (CI) and p-value will be presented.
	Sensitivity	• Analysis: For the mITT population, baseline and 12-month log transformed IgE and IgG4 will be modeled using a linear mixed model that will include a random intercept and fixed effects for each treatment arm, site, and an interaction term between treatment arm and time of measurement. Similarly to the ANCOVA, geometric least square means (LSmeans) and associated SEs will be presented for each treatment group. The geometric least square mean ratio comparing treatment vs placebo group, associated 95% confidence interval (CI) and p-value will be presented
Secondary Objective: To compare efficacy of cockroach subcutaneous immunotherapy (SCIT) superiority within placebo/active in participants with participants with asthma and german cockroach sensitization with respect to:		
Safety Category 1 (e.g., AEs)		• Variable: Rate of immunotherapy related adverse events and immunotherapy related serious adverse events in the course of treatment • Analysis: For the safety samples, the difference in the number and percentage of serious and non-serious adverse events between treatment groups. The analysis will be stratified by grade.

IES = Intercurrent event(s) strategy; PLS = Population-level summary; MCP=Multiple comparisons procedure

All estimand attributes explicitly identified for primary/secondary and select key estimands only.

14.2. Study Flow Chart



14.3. Schedule of Events

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.