Statistical Analysis Plan

A Double Masked, Placebo Controlled, Single Center, Randomized Clinical Trial to Assess the Safety and Efficacy of ADX-629 in Subjects with Mild Asthma Induced by the Bronchial Allergen Challenge (BAC)

Protocol Number:	ADX-629-AA-001
Protocol Version and Date:	Version 5.0, 19May2021 Version 4.0, 14Apr2021 Version 3.0, 24Feb2021 Version 2.0, 16Nov2020 Version 1.0, 20Aug2020
Sponsor:	Aldeyra Therapeutics, Inc
Indication:	Mild asthma
Analysis Plan Date:	14Feb2022
Analysis Plan Version:	Version 1.0

SPONSOR SIGNATURE PAGE

Protocol Title:	A Double Masked, Placebo Controlled, Single Center, Randomized Clinical Trial to Assess the Safety and Efficacy of ADX-629 in Subjects with Mild Asthma Induced by the Bronchial Allergen Challenge (BAC)
Protocol Number:	ADX-629-AA-001
Study Products:	Treatment A: ADX-629, 600 mg (2 x 300 mg tablets) Treatment B: Placebo, 600 mg (2 x 300 mg tablets)
Clinical Trial Phase:	Phase 2
Sponsor:	Aldeyra Therapeutics, Inc. 131 Hartwell Avenue, Suite 320 Lexington, MA 02421

By signing this document, I acknowledge that I have read the document and approve of the planned statistical analyses described herein. I agree that the planned statistical analyses are appropriate for this study, are in accordance with the study objectives, and are consistent with the statistical methodology described in the protocol and all applicable regulatory guidance and guidelines.

Author:

[redacted]

[redacted]

Approver:

[redacted]

Table of Contents

LIST OF TABLES

ABBREVIATIONS

Abbreviation	Explanation
ACQ	Asthma Control Questionnaire
AE	Adverse Event
AHR	Airway Hyper Responsiveness
AR [1]	autoregressive 1
ATC	Anatomical Therapeutic Chemical
AUC	Area Under the Curve
BAC	Bronchial Allergen Challenge
BMI	Body Mass Index
CBC	Complete Blood Count
CI	Confidence Interval
COVID-19	Corona Virus Disease 2019
CRF	Case Report Form
CV	Coefficient of Variation
ECG	Electrocardiogram
ETV	Early Termination Visit
FeNO	Fractional Exhaled Nitric Oxide
FEV1	forced expiratory volume in one second
ITT	Intent-to-Treat
Mch	Methacholine
MCT	Methacholine Challenge Test
MedDRA	Medical Dictionary for Regulatory Activities
MMRM	Mixed Model Repeated Measures
PP	Per-Protocol
PT	Preferred Term
RASP	Reactive Aldehyde Species
SAE	Serious Adverse Event
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SD	Standard Deviation
SOC	System Organ Class
TEAE	Treatment-Emergent Adverse Event
UN	Unstructured
WHO	World Health Organization

1. INTRODUCTION

This statistical analysis plan is designed to outline the statistical methods for protocol ADX-629-AA-001:

"A Double Masked, Placebo Controlled, Single Center, Randomized Clinical Trial to Assess the Safety and Efficacy of ADX-629 in Subjects with Mild Asthma Induced by the Bronchial Allergen Challenge (BAC)"

Biomarker and pharmacokinetic analysis will be addressed separately from this statistical analysis plan.

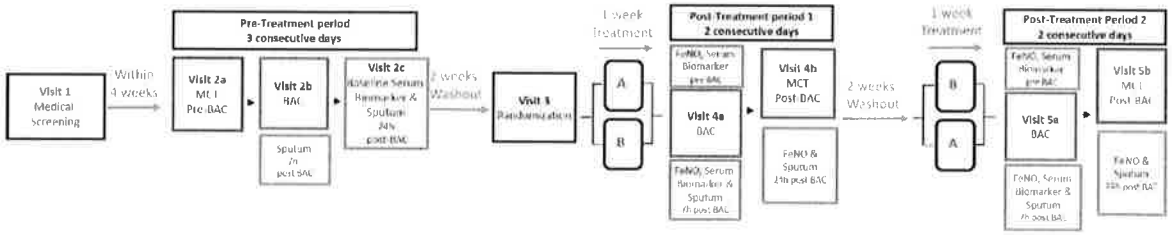
This document has been prepared based on protocol amendment version 5.0 dated 19 May 2021 and Case Report Form (CRF) dated 01 October 2020.

2. STUDY DESIGN

2.1 OVERVIEW OF STUDY DESIGN

ADX-629-AA-001 is a double-masked, cross-over, placebo-controlled, single center, randomized clinical trial to assess the clinical safety and efficacy of ADX-629 compared to placebo in mild cat or HDM-induced asthmatics using the bronchial allergen challenge (BAC) model. The clinical trial will consist of 9 visits to the clinic (Visits 1, 2a, 2b, 2c, 3, 4a, 4b, 5a, and 5b) over a period of approximately 75 days (Figure 1). During this period there will be 4 additional visits, 1 visit for safety lab and 3 visits for COVID-19 testing.

Figure 1: Study Design



MCT: Methacholine Challenge Test; BAC: Bronchial Allergen Challenge; FeNO: Fractional Exhaled Nitric Oxide
A: ADX-629 600 mg PO bid for 7 (+3) Days
B: Placebo 600 mg PO bid for 7 (+3) Days

A re-screening visit will be included to the subjects who failed under previous versions of the Protocol.

The end of study is defined as the time at which the last subject has completed all study procedures in the clinical trial. Upon completion of the clinical trial, the study product will no longer be available to the subject but the Investigator can, at their discretion, discuss alternative treatments with the subject.

2.2 Sample Size

Based on repeatability analyses in allergen-induced airway inflammation responses, a sample size

of 12 subjects yields more than 80% power to detect a difference of 0.1 with standard deviation of 0.1 in change from baseline FEV1 across treatment groups.

2.3 Randomization and Masking

2.3.1 Randomization

At Visit 3, eligible subjects will be randomized 1:1 to one of two treatment sequences of AB or BA where:

- Treatment A: ADX-629, 600 mg (2 x 300 mg tablets)
- Treatment B: Placebo, 600 mg (2 x 300 mg tablets)

12 subjects (6 per treatment sequence) will be enrolled.

2.3.2 Masking

This is a double-masked clinical trial. Investigators, qualified site personnel (except pharmacy staff), and subjects will be masked to the investigational product administered. The Sponsor will also be masked to the IP administered until database lock. Only the pharmacy staff and Scientific Affairs staff involved with preparing the randomization will have access to the treatment under evaluation.

3. STUDY OBJECTIVES AND ENDPOINTS

3.1 Study Objectives

- Primary Objective
  - To assess the safety of ADX-629 in subjects with allergen-induced mild asthma
- Secondary Objective
  - To assess the clinical efficacy of ADX-629 in subjects with allergen-induced mild asthma

3.2 Study Endpoints

- Safety Endpoint
  - Adverse events (AEs) and serious adverse events (SAEs)
- Efficacy Endpoints
  - Change from baseline (within visit) in forced expiratory volume in one second (FEV1) to post-BAC (during 0-3 h post-BAC [Key Efficacy Endpoint] and 3-7 h post BAC)
  - Absolute count and percentage differential count of sputum eosinophils and neutrophils at approximately 7 h and 24 h post-BAC
  - Allergen-induced shift in airway hyper responsiveness (AHR) as assessed by Methacholine PC20 (Mch PC20) post-BAC

  - Change from baseline in fractional exhaled Nitric Oxide (FeNO) at approximately 7 h and 24 h post-BAC

- Exploratory Endpoints
  - Biomarkers (RASP and endotoxin-induced cytokine release) pre-BAC (at approximately 1 hour post-dose) and 7 h post-BAC
  - Area under curve (AUC) of FEV1 during 0-3 h post-BAC and/or 3-7 h post BAC

4. DEFINITIONS AND CONVENTIONS FOR DATA HANDLING

4.1 Handling of Missing Data

Generally, imputation of missing data will not be performed.

4.2 Unscheduled Visits for Safety Analysis

All unscheduled visit values will be excluded from summary tables but will be included on data listing.

4.3 Handling of Partial Dates for Medications

When determining prior or concomitant medications, partial start dates of prior and concomitant medications will be assumed to be the earliest possible date consistent with the partial date.

For the purposes of analysis, incomplete medication start dates and stop dates will be imputed.

- If a medication start date is incomplete, January will be imputed for missing month and/or the first day of the month will be imputed for missing day.
- If a medication stop date is incomplete, December will be imputed for missing month and the last day of the month will be imputed for missing day.
- If the imputed medication stop date is after the date of study completion, the date of study completion will be used instead.

4.4 Handling Partial Dates for Adverse Events

When determining the treatment emergent AE, partial dates will be handled as follows.

- If the day of the month is missing, the onset day will be set to the first day of the month unless it is the same month and year as first dose date. In this case, the onset date will be assumed to be the first date of treatment.
- If the onset day and month are both missing, the day and month will be assumed to be January 1, unless the event occurred in the same year as the study treatment. In this case, the event onset will be coded to the day of treatment to conservatively report the event as treatment-emergent.
- A missing onset date will be coded as the day of treatment. If the resulting onset date is after a reported date of resolution, the onset date will be set equal to the date of resolution.

- Imputation of partial dates is used only to determine whether an event is treatment-emergent; data listings will present the partial date as recorded in the eCRF.

5. ANALYSIS POPULATIONS

5.1 Enrolled Population

Enrolled Population includes all subjects with a signed informed consent form.

5.2 Safety Population

The Safety Population includes all subjects who receive at least one dose of any of the study products regardless of randomization status.

The Safety Population will be used for safety analyses. Subjects will be evaluated based on the actual treatment received.

5.3 Intent-to-Treat Population

Intent-to-Treat (ITT) population includes who are randomized and received both treatments.

The ITT Population will be used for efficacy analyses. Subjects will be evaluated according to the investigational drug treatment of the visit as per the randomized treatment sequence

5.4 Per-Protocol Population

The Per-Protocol (PP) Population includes all subjects who are randomized and complete both treatments (receive treatment at visit 5b and 80% $\leq$ overall treatment compliance (%) $\leq$ 125% in each treatment arm) and have no major protocol deviations.

The PP Population will be used for sensitivity analyses of the efficacy findings. Subjects will be evaluated according to the investigational drug treatment of the visit as per the randomized treatment sequence.

5.5 Pharmacokinetic Population

The Pharmacokinetic (PK) Population includes all subjects in Safety Population who have at least one pharmacokinetic assessment.

The pharmacokinetic analysis will be addressed separately from this statistical analysis plan.

5.6 Application of Analysis Populations

Unless otherwise noted, the analysis populations that will be used for creating the summary tables of each type is provided in Table 1.

Table 1. Application of Populations on Tables

Type	Enrolled	Safety	ITT	PP
Disposition	X			

Type	Enrolled	Safety	ITT	PP
Demographics		X	X	X
Medical and Social History		X		
Protocol Deviations		X		
Prior/Concomitant Medications		X		
Drug Exposure		X		
Safety Evaluations Endpoints		X		
Efficacy Endpoints			X	X

6. STATISTICAL CONSIDERATIONS

All analyses described in this plan are considered a priori analyses in that they have been defined prior to locking the database. All other analyses, if any, designed subsequently to locking the database, will be considered post hoc analyses and will be described as exploratory analyses in the Clinical Study Report.

All summaries and statistical analysis will be performed using SAS v9.4 or later.

6.1 General Statistical Procedures

Frequency distributions for categorical variables will be provided as number of patients in the category and the percentages of the total number of patients in the given population as noted. Percentages will be reported to one decimal place.

The descriptive statistics for continuous variables will be number of subjects (n), mean, standard deviation (SD), median, minimum, and maximum. Mean and median will be reported to 1 more decimal place than the raw data, while the SD will be reported to 2 more decimal places than the raw data. Minimum and maximum will be reported the same as the original data. In addition, geometric and coefficient of variation (CV%) will also be provided for computation of AUC.

All statistical tests will be two-sided with a significance level of 0.05 ($\alpha = 0.05$) unless otherwise specified. The Confidence interval (CI) for differences between treatment will be two-sided at 95%.

The p-value will be rounded to at most 3 decimal places and will be reported as < 0.001 if smaller than 0.001.

Unless otherwise specified, subjects' characteristics at baseline will be summarized by treatment sequences (ADX-629/Placebo, Placebo/ADX-629). Safety and efficacy will be summarized by treatment (ADX-629, Placebo) where appropriate.

In general, all listings will be ordered by subject number and visit for available data unless otherwise specified in the text.

6.2 Subject Disposition

Frequency and percentage of subject disposition will be summarized by treatment sequence and all subjects for the Enrolled Population and will include:

- Number of subjects with screen failure
- Number of subjects randomized
- Number of subjects in each analysis population
- Number of subjects who completed each treatment (ADX-629, placebo)
- Number of subjects only completed the first treatment session

Disposition will be listed for the Enrolled Population, and disposition percentages will be based on number of subjects in the Enrolled Population.

Study discontinuation will be summarized by treatment for the Safety Population, and will include:

- Number of subjects discontinued from study
- Reasons for study discontinuation
  - Withdrawal by subject
  - Adverse event
  - Non-compliance with study schedule
  - Pregnancy
  - Significant worsening of asthma
  - Protocol violations
  - Non-compliance with study drug
  - Positive COVID-19 test
  - Other

Study discontinuation will be listed for the Safety Population, and discontinuation percentages will be based on number of subjects in the Safety Population.

In addition, subjects excluded from study will be listed for Enrolled Population.

6.3 Protocol Deviations

Protocol deviations will be reviewed, assessed, and documented by sponsor personnel before database lock.

The number and percentage of subjects with one or more major protocol deviations will be tabulated by treatment for the Safety Population.

All protocol deviations will be listed for the Enrolled Population.

6.4 Demographic and Baseline Characteristics

Subjects' demographic and baseline characteristics will be summarized by treatment sequence, and will include:

- Age at screening (years; if not reported, age will be calculated as integer of [(year of informed consent signed – year of birth)/365.25]
- Sex
- Ethnicity
- Race
- Height (cm)
- Weight (kg)
- BMI (kg/m^2)

Demographics and baseline characteristics will be listed for the Safety Population.

6.5 Medical, Surgical, and Social History

Medical, surgical, and social history will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 21.1 or higher.

The frequency count and percentage of patients experiencing any medical conditions will be tabulated by system organ classifications (SOC) and preferred term (PT) for treatment sequence. If a preferred term or system organ class was reported more than once for a subject, the subject will be counted once in the incidence for that preferred term or system organ class.

Medical, surgical, and social history data will be listed for the Safety Population.

6.6 Prior and Concomitant Medications

All medications will be coded according to the WHO drug dictionary which includes the WHO Drug Preferred Name and the ATC Classification Level 2 and 4.

Prior medications are medications taken prior to first dose of the study drug.

Concomitant medications are medications being taken on or after first dose of study drug. Medications taken prior to first dose date and were ongoing on the date of the first dose will be considered concomitant medications. Medications with missing end dates are assumed to be concomitant medications.

Prior medications will be summarized by ATL Level 2 and preferred term (ATC Level 4) by treatment sequence. Concomitant medications will be summarized by treatment. If an ATC Level 2 or Level 4 term was reported more than once for a subject, the subject will be counted once in the incidence for that term.

Prior and concomitant medications will be listed for the Safety Population.

7. Safety Analysis

7.1 Extent of Study Drug Exposure

Exposure to study drug (ADX-629, placebo) in the treatment/post-treatment periods (Visit 3, Treatment period 1, Post-Treatment Period 1 (Visits 4a and 4b), Treatment Period 2, Post-Treatment Period 2 (Visits 5a and 5b)) will be summarized by treatment for the Safety Population, and will include:

- Total number of days on treatment
- Total number of tablets taken
- Compliance (%), defined as total number of doses taken/planned total number of doses taken x100
- Compliance category: $< 80\%$, $\geq 80\%$ - $\leq 125\%$, $> 125\%$

Exposure to study drug will be listed for the Safety Population.

7.2 Adverse Events

AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) coding dictionary (Version 20.0 or higher) preferred term (PT) and system organ classification (SOC).

An AE will be considered treatment emergent (TEAE) if it begins or worsens in severity after the first dose of the study drug in Treatment Period 1.

All TEAEs with start dates prior to first dose date of Treatment Period 2 will be classified as AEs that occurred in Treatment/Post-Treatment Period 1.

TEAEs are classified as related and not related to the study drug. Any TEAEs with missing relationship to study drug will be considered related to study drug.

An overall summary for number of subjects with events in each treatment will be provided, and will include:

- Any TEAE
- Any drug-related TEAE
- Any serious TEAE
- Any TEAE leading to study drug discontinuation

The following types of summaries for each treatment will be provided. Summaries will be sorted by decreasing frequency of PT within SOC, which will be sorted alphabetically, and will include:

- TEAEs by SOC and PT
- Drug-Related TEAEs by SOC and PT
- Serious TEAEs by SOC and PT
- TEAEs by SOC, PT, and maximum Severity

- TEAEs leading to study drug discontinuation by SOC and PT

If a PT or SOC was reported more than once for a subject, the subject will be counted once in the incidence for that PT or SOC.

In the tabulation of TEAE by severity, only the most severe PT or SOC for each subject will be included. Missing severity will be counted as Severe.

In the summary of drug-related TEAEs, only the strongest relationship will in included.

Listings will be provided including for Safety Population, and will include:

- All AEs
- Serious TEAEs
- TEAEs leading to study drug discontinuation

7.3 Clinical Laboratory Tests

Chemistry (complete blood count [CBC] with differential), hematology, and urinalysis laboratory tests are performed at the Medical Screening Visit and within 3 days prior to the first dose of study drug. Additional tests are also performed at Visit 4a and 5a. Laboratory tests will be classified as clinically significant or not clinically significant.

All laboratory tests including pregnancy testing will be listed for Safety Population.

7.4 Vital Signs

All vital signs throughout the clinical trial will be measured in a rested (5 minutes) and seated position. Blood pressure, heart rate, respiratory rate, and oral temperature will be measured at the Medical Screening Visit, the Pre-Treatment Period (Visits 2a, 2b, and 2c), the Randomization Visit (Visit 3), the Post-Treatment Periods 1 and 2 (Visits 4a, 4b, 5a, and 5b), and any ETV.

Observed vital signs and changes from baseline (the last non-missing assessment prior to the first dose of ADX-629 or placebo) for vital signs will be summarized over visit by treatment. Baseline is defined as the last non-missing measurement taken prior to the first dose.

Listings will be provided for the Safety Population.

7.5 12-Lead ECG

A 12 lead ECG will be recorded at the Screening Visit and 1 hour (±15 min) post dose at Visit 3, Visit 4a, and Visit 5a. Additional ECGs may be obtained at the discretion of the Investigator(s) or designee.

ECG will be listed for the Safety Population.

7.6 COVID-19 Test (SARS-CoV-2)

Within 5 days of study Visits 2a, 4a, and 5a, a nasal/nasopharyngeal/oropharyngeal swab will be

collected at the clinical site for SARS-CoV-2 testing. Subjects with a positive test result will be withdrawn from the study.

COVID-19 testing results will be listed for the Enrolled Population.

8. Efficacy Analyses

All efficacy endpoints will be summarized for the Intent-to-Treat Population. Sensitivity analysis will be performed on the Per-Protocol Population.

All efficacy data will be listed for the Safety Population.

8.1 Forced Expiratory Volume in One Second (FEV1) Following Allergen Challenge

FEV1 measurement is reported in litres. Normal values for FEV1 vary according to age, sex, height, weight, and ethnicity. To account for such variations, predicted FEV1% will be calculated. Predicted FEV1% is defined as subject's FEV1 value expressed as a percentage of the reference average FEV1 for a person of similar age, sex, and body composition.

Observed and changes from baseline (pre-saline within the visit) over time points as follows during post-BAC assessment (Visits 4a and 5a) for FEV1% will be summarized by treatment:

- Post-BAC (during 0-3 h) (the Key Efficacy Endpoint is change from baseline)
- Post-BAC (during 3-7 h)

[redacted]

[redacted]

[redacted]

The difference between treatments of mean predicted FEV1% change from baseline at each time point from 0 to 3 hours will be calculated along with the 2-sided 95% CI and the associated p-value from the MMRM model.

If 3 or more predicted FEV1% change from baseline over all time points from 30 to 180 minutes (in aggregate) of ADX-629 minus placebo is > 0, and corresponding p-value of the contrast is < 0.05, then the Key Efficacy Endpoint will be achieved.

If the model does not converge after fitting different covariance matrices, to compare treatment groups, a paired t-test based on averaged values across all timepoints (ignoring the correlation within subjects) will be provided.

The following plots will be provided for Intent-to-Treat Population:

- Spaghetti plots of the difference of predicted FEV1% (ADX-629 minus vehicle) over time
- Mean and mean changes of the difference of predicted FEV1% (ADX-629 minus vehicle) from baseline over time
- Difference of changes in least square means from the MMRM model over time

In addition, area under curve (AUC) over 0-3 hours post-BAC, 3-7 hours post-BAC, and 0-7 hours post-BAC will be summarized. To be included in the 0–3-hour AUC analysis, subjects must have non-missing data at baseline and non-missing data from at least three other timepoints. To be included in the 3–7-hour AUC analysis, subjects must have non-missing data at 3 hours and non-missing data from at least three other timepoints. To be included in the 0-7 hour AUC analysis, subjects must have non-missing data at baseline and satisfy all other conditions for the 0-3-hour and 3-7 hour AUC analyses.

8.2 Sputum Analysis

- Sputum is induced, collected, and processed at following timepoints: Approximately 7 hours post-BAC (Visit 2b, Visit 4a and Visit 5a), and
- Approximately 24 hours post-BAC (Visit 2c, Visit 4b and Visit 5b).

Observed and changes of absolute count and percentage (%) of leukocyte counts of sputum eosinophils and neutrophils from baseline (Visit 2b) at 7 hours post-BAC during the treatment periods (Visits 4a and 5a) will be summarized.

A similar analysis will also be performed at 24 hours post-BAC using the last non-missing assessment at Visit 2c as baseline.

P-values for comparison of changes from baseline between treatments at 7 hours and 24 hours, and comparison of change from baseline from 7 hours to 24 hours, will be provided using the paired t-test and the Wilcoxon signed-rank test.

8.3 Allergen-Induced Shift in Airway Hyper-Responsiveness

At Visits 2a, 4b, and 5b, subjects will undergo methacholine challenge testing (MCT). At Visit 2a, subjects will undergo bronchoprovocation challenge with progressively increasing concentrations of methacholine (Mch) to determine the baseline provocation concentration of inhaled methacholine required to reduce FEV1 by 20% (PC_{20}). The PC_{20} concentration will then be

administered to assess FEV1 and predicted FEV1 % at Visits 4b and 5b.

Following the Mch PC_{20} exposure, FEV1 (and predicted FEV1%) and change from within-visit baseline (post-saline/baseline timepoint at Visits 4b and 5b) will be summarized by treatment. In addition, time to return to 10% of baseline after Mch administration will be summarized by treatment.

P-values for comparison of changes from baseline between treatments will be provided using the paired t-test and the Wilcoxon signed-rank test.

8.4 Fractional Exhaled Nitric Oxide (FeNO)

FeNO will be measured at Visit 4a, 4b, 5a and 5b at following timepoints:

- Pre-BAC at Visit 4a and Visit 5a (baseline)
- Approximately 7 hours post BAC at Visit 4a and Visit 5a
- Approximately 24 hours post BAC at Visit 4b and Visit 5b.

Observed and changes from baseline (pre-BAC assessment at Visit 4a or 5a) at approximately 7 hours and 24 hours post BAC will be summarized.

P-values for the comparison of treatment groups at 7 hours and 24 hours, and comparison of change from baseline from 7 hours to 24 hours, will be provided using the paired t-test and the Wilcoxon signed-rank test.

8.5 Asthma Control Questionnaire (ACQ)

The ACQ has 7 questions. It includes the 5 most important symptoms, one question about rescue bronchodilator use and one about FEV1 % predicted. Patients recall their experiences during the previous 7 days and respond to each of the first 6 questions using a 7-point scale (0 = totally controlled to 6 = extremely poorly controlled). The seventh question, concerning FEV1 % predicted, is completed by clinic staff.

The overall ACQ score is calculated as the mean of the response to all 7 questions. An ACQ score of 1.50 or greater indicates that a subject has inadequate asthma control.

The ACQ score and change from baseline (the last non-missing assessment at Visit 2a) and adequate of asthma control will be summarized over time. Subjects who didn't complete all 7 questions will be excluded from analysis. The inadequate asthma rate between treatments will be tested using Fisher's exact test and the overall ACQ score and change from baseline between treatments at Visit 4a/5a will be tested by paired t-test and Wilcoxon signed-rank test.

Listings will be provided for the Safety Population.

9. Appendix

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