AOBIOME

CLINICAL TRIAL PROTOCOL

Phase 1b/2a

COMPOUND:

B244

A Prospective, Controlled, Double Blind, Multi-Center, Randomized, 3 arm, Phase 1b/2a Study to Assess the Safety, Tolerability, and Preliminary Efficacy of B244 Delivered as an Intranasal Spray in Healthy Volunteers and Subjects with Seasonal Allergic Rhinitis

STUDY NUMBER: ARB244-001

VERSION DATE: August 29, 2018

Sponsor:

AOBiome LLC

One Broadway

14th Floor

Cambridge, MA 02142-1100

Revision Chronology

Original	August 15, 2016
Amendment 1	June 5, 2017
Amendment 2	July 31, 2017
Amendment 3	September 21, 2017
Amendment 4	November 27, 2017
Amendment 5	April 9, 2018
Amendment 6	August 29, 2018

SPONSOR APPROVAL

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Date: September 5, 2018

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Date: Sept 6th, 2018

INVESTIGATOR AGREEMENT

I have read this protocol and agree:

- To conduct the study as outlined herein, in accordance with Good Clinical Practices (GCPs), the Declaration of Helsinki and complying with the obligations and requirements of Clinical Investigators and all other requirements listed in 21 CFR part 312 and according to the study procedures provided by AOBiome LLC and local regulations.
- Not to implement any changes to the protocol without prior agreement from the Sponsor and prior review and written approval from the IRB or IEC, except as would be necessary to eliminate an immediate hazard to study participant (s), or for administrative aspects of the study.
- To ensure that all persons assisting me with the study are adequately informed about the investigational product(s) and of their study-related duties as described in the protocol.
- To completely inform all participants in this study concerning the pertinent details and purpose of the study prior to their agreement to participate in the study in accordance with GCP and regulatory authority requirements.
- To be responsible for maintaining each participant's consent form in a secure study file and providing each participant with a signed copy of the consent form.
- That I am thoroughly familiar with the appropriate use of the investigational product(s), as described in the protocol, and any additional information provided to me by, or on behalf of AOBiome LLC.

Investigator Printed Name:_____

Signature:_____ **Date:** _____

Statement of Compliance

The study will be carried out in accordance with Good Clinical Practice (GCP) as required by the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46; 21 CFR Part 50, 21 CFR Part 56, and 21 CFR Part 312)
- ICH E6; 62 Federal Register 25691 (1997)

All key personnel (all individuals responsible for the design and conduct of this study) have completed Good Clinical Practice Training.

CLINICAL TRIAL SYNOPSIS

COMPOUND: B244 STUDY NUMBER: **ARB244-001**

TITLE:	A Prospective, Controlled, Double Blind, Multi-Center, Randomized, 3 arm, Phase 1b/2a Study to Assess the Safety, Tolerability, and Preliminary Efficacy of B244 Delivered as an Intranasal Spray in Healthy Volunteers and Subjects with Seasonal Allergic Rhinitis
INVESTIGATIONAL PRODUCT	B244
STUDY ARMS	B244 (140ul per nostril; OD 0.5; $1x10^9$ cells/ml) twice-a-day for 14 days B244 (140ul per nostril; OD 2.0; $4x10^9$ cells/ml) twice-a-day for 14 days Vehicle (140ul per nostril) twice-a-day for 14 days
PURPOSE:	To assess the safety and tolerability of B244 relative to Vehicle in healthy volunteers and to evaluate the preliminary efficacy of B244 relative to Vehicle in subjects with seasonal allergic rhinitis.
OBJECTIVES:	**Primary Objective** To assess the safety and tolerability of B244 relative to vehicle in healthy volunteers during 14 days of treatment and up to 28 days of follow-up. **Secondary Objective(s)** ☒ To evaluate the efficacy of B244 relative to vehicle in treating Total Nasal Symptom Score (TNSS) in subjects with seasonal allergic rhinitis evaluated in a nasal allergen challenge model as a prophylaxis therapy after 14 days of treatment. ☒ To evaluate the safety and tolerability

	of B244 relative to vehicle in subjects with allergic rhinitis after 14 days of treatment. ☒ To evaluate the efficacy of B244 relative to vehicle in treating individual nasal symptom scores in subjects with allergic rhinitis evaluated in a nasal allergen challenge model as a prophylaxis therapy after 14 days of treatment. **Exploratory Objective** ☒ To test the hypothesis that B244 inhibits airway inflammation driven by nasal allergen challenge. The outcome measures will be changes in Peak Nasal Inspiratory Flow, nasal inflammation score using otoscope, intranasal nitric oxide levels and cytokine concentration from nasal cavity pre- and post- nasal allergen challenge in a prophylaxis treatment setting.
ENDPOINTS:	**Primary** Safety and tolerability will be assessed by reporting of AEs, physical examination and vital signs (blood pressure, heart rate, respiratory rate) during 14 days of treatment and up to 28 days of follow-up (Part 1). **Secondary** ☒ Change in TNSS from baseline to 14 days of prophylaxis treatment. ☒ Change in subjective nasal symptom scores of nasal congestion, rhinorrhea, nasal itching, and sneezing from baseline to 14 days of prophylaxis

	treatment. Nasal symptom-free response rate within each individual symptom scores and percentage of subjects with a 25% or 50% reduction in TNSS after 14 days of prophylaxis treatment. ☒ Safety and tolerability will be assessed in subjects with allergic rhinitis by reporting of AEs, physical examination and vital signs (blood pressure, heart rate, respiratory rate) during 14 days of treatment (Part 2). **Exploratory** ☒ Change in Peak Nasal Inspiratory Flow (PNIF), nasal inflammation score using otoscope, intranasal nitric oxide levels, and cytokine concentration in nasal cavity and blood from baseline to 14 days of B244 application and during prophylaxis treatment setting.
STUDY DESIGN:	This is a Prospective, Controlled, Double Blinded, Multi-Center, Randomized, 3 Arm, Parallel Assignment, Phase 1b/2a Study to assess the safety, tolerability, and preliminary efficacy of B244 delivered as an intranasal spray in healthy volunteers and subjects with seasonal allergic rhinitis. This will be a 2-part study. In Part 1, safety and tolerability will be evaluated during 14 days of study treatment twice-a-day followed by 4 weeks of follow-up in healthy volunteers. In Part 2, preliminary efficacy will be evaluated in subjects with a history of seasonal allergic rhinitis outside of the local pollen season. Part 1 (safety and tolerability evaluation in healthy volunteers) will enroll 24 subjects. Up

	to 12 subjects per dose cohort (n=8 active, n=4 vehicle) will be enrolled, with a target of 20 subjects completed (10 subjects per dose cohort; n=7 active, n=3 vehicle) assuming a 16.7% drop out rate. Safety and tolerability will be assessed by reporting of AEs, physical examination and vital signs (blood pressure, heart rate, respiratory rate) during 14 days of treatment and up to 28 days of follow-up. Subjects will be asked to report for a screening visit and if all inclusion/exclusion criteria are met, subjects will be asked to report for a baseline visit and randomization. Subjects will then be trained on the use of the nasal spray, and have their first dose of treatment on site. Subjects will remain onsite for observation for 1 hour post dosing. Subjects will then be dispensed study medication for 14 days of at home dosing. While at home, subjects will dose twice daily (am and pm), and record their dosing, any occurrence of an AE, and Total Nasal Symptom Score (TNSS) using a study diary. Subjects will be asked to visit the site at Day 14 (Week 2) and at follow-up visits at Day 21 (Week 3) and Day 42 (Week 6) for safety assessments and will additionally obtain Peak Nasal Inspiratory Flow (PNIF), intranasal fluid, intranasal nitric oxide (nNO) and nasal inflammation score. During the 2 week safety phase (Part 1), a continuous Ambulatory Blood Pressure Monitoring (ABPM) device will monitor blood pressure of each subject for 24 hours at baseline and Day 14. ABPM would be worn for 24 hours prior to the baseline visit and

again for 24 hours prior to the Day 14 visit.

For Part 1, study will be paused for safety review LQWKHHYHQWWKDWVXEMHFWUHSRUWVD□ serious DGYHUVHHYHQWJUDGHVHYHUH□ DGYHUVHHYHQWVRUJUDGHPRGHUDWH□ adverse events considered possibly, probably, or definitely related to the investigational product. In addition, a 180 day follow up contact for collecting serious adverse events and new onset medical conditions will be provided.

An internal safety committee meeting will review the 2 week Part 1 safety data and, if there are no safety concerns, the study will proceed to Part 2 to evaluate preliminary efficacy in subjects with a history of seasonal allergic rhinitis (SAR)

Part 2 (preliminary efficacy evaluation in subjects with a history of SAR to ragweed pollen) will enroll 42 subjects. Up to 14 subjects per arm ($1x10^9$ cells/ml; $4x10^9$ cells/ml or vehicle) will be enrolled, with a target of 12 subjects per arm for a total of 36 subjects completed, assuming a 10-15% drop-out rate.

Safety and tolerability will be assessed in subjects with allergic rhinitis by reporting of AEs, physical examination and vital signs (blood pressure, heart rate, respiratory rate) during 14 days of treatment.

A preliminary efficacy assessment will be performed in response to nasal allergen challenge (NAC) in a prophylaxis treatment setting.

	Subjects will be asked to report for a screening visit (Visit 1) and if all inclusion/exclusion criteria are met, subjects will be asked to report for a NAC titration (ragweed pollen nasal allergen titration) visit to administer a single provocative challenge dose for each subject (Visit 2). Subjects will then return 14 days after the washout period for basic health checks and baseline assessments (intranasal fluid, TNSS, PNIF, intranasal nitric oxide, and have a nasal inflammation score) followed by confirmatory NAC (NAC#1) (Visit 3). During this time subjects will use a study diary to complete TNSS at -30 (30 minutes prior to NAC), 0 (baseline prior to NAC), 15, 30, 45, 60, 90, and 120 minutes. PNIF at -30, 0, 15, 30, 60, and 120 minutes will also be obtained. TNSS is the sum of four individual symptom scores (running nose, congestion, itchy nose, and sneezing) rated on a four point scale (0-3). Subjects are required to meet the minimum qualifying TNSS score of a change of 6/12 from their baseline score, on at least 2 diary cards. Subjects will also provide samples for intranasal fluid at 0, 20, and 60 minutes, measure intranasal nitric oxide (nNO) and have a nasal inflammation score assessed by the investigator using a nasal otoscope at 0 and 120 minutes, in addition to blood collection at 120 min for biomarkers. Subjects meeting the requirement will be randomized to one of the three treatment arms. Randomization will be 1:1:1 so that equal number of subjects will be treated in each arm of the study. Subjects will then be trained on the use of the nasal spray, and have their first dose of treatment on site (self-dosing). Subjects will remain onsite for observation for 1 hour post dosing. Subjects will then be dispensed study medication for 14

	days of at home dosing. While at home, subjects will dose twice daily (am and pm), and record their dosing, and any occurrence of an AE and TNSS using a study diary. Subjects will return to the clinic for the last dose following the 14 days of at home dosing for their final visit (Visit 4). During this time subjects will again have their baseline assessments and be exposed to ragweed pollen challenge (NAC#2), and will use a study diary to complete TNSS at -30, 0, 15, 30, 45, 60, 90, and 120 minutes and PNIF at -30, 0, 15, 30, 60, and 120 minutes. Prior to and 20 and 60 minutes after NAC #2, subjects will additionally provide samples for intranasal fluid, and have nNO measured and nasal inflammation score assessed by the investigator using a nasal otoscope at 0 and 120 min, in addition to blood collection at 120 min for biomarkers. For Part 2, study will be paused for safety review using the same stopping criteria as in Part 1. Rescue medications will not be allowed during the study.
STUDY POPULATION:	Normal healthy volunteers and subjects with a history of seasonal allergic rhinitis to ragweed pollen are eligible for enrollment. Patients must be willing to refrain from using any treatments, investigational products, including herbal medicines while participating in this study.
MAIN INCLUSION/EXCLUSION CRITERIA:	**Inclusion Criteria** Males and Females, 18 to 65 years of age (committed to consistent use of an acceptable method of birth control). ☒ In good general health as determined by

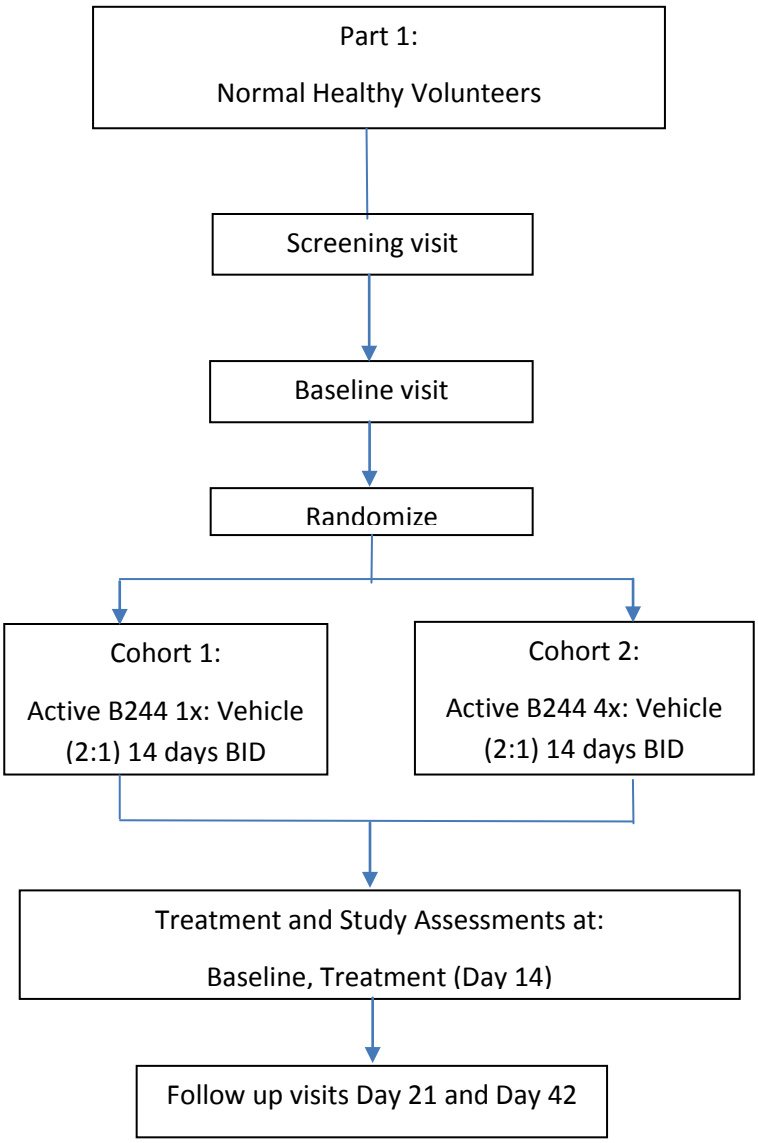
	a thorough medical history and physical examination, and vital signs. ☒ Nonsmoker or ex-smoker (stopped >1 year prior to study entry). ☒ Subjects willing and able to provide written informed consent. ☒ Is willing and able to comply with the requirements of the protocol and must be available for the full duration of the study. ☒ For Part 1, subjects asymptomatic from any seasonal or perennial allergens. ☒ For Part 1, elevated systolic blood pressure greater than 120 but less than 160 and never been on antihypertensive medications or have been off any hypertensive treatment for a period of 12 weeks or longer. ☒ For Part 2, subjects with a well-documented history of seasonal allergic rhinitis (specifically ragweed pollen) with documentation of sensitivity by positive skin testing and/or IgE testing to the relevant allergens 12 months prior to enrollment that correlate with clinical history. ☒ For Part 2, a ragweed positive skin prick test with a wheal diameter at least 5 mm larger than the negative control and/or a ragweed specific IgE greater or equal to 0.7 kU/L. ☒ For Part 2, subjects with confounding allergies, or sensitization to cat epithelia, dog epithelia, *Dermatophagoides farinae,* or *Dermatophagoides pteronyssinus* or prevalent and relevant seasonal allergens as per the skin prick test may be included if sensitization is not clinically relevant (ie. subject is non-

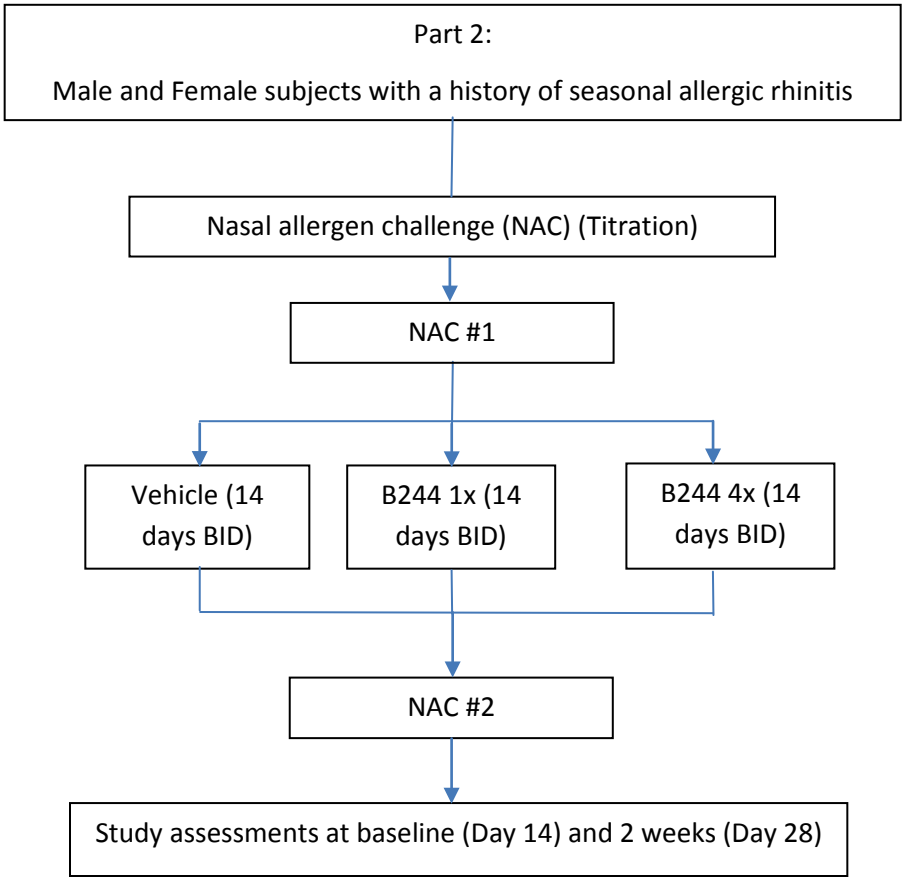
	symptomatic and/or can avoid the allergen during the study) at the discretion of the Investigator. x For Part 2, subjects with a baseline TNSS □ at Visit 3 and a minimum qualifying TNSS score of a change of 6/12 from their baseline score after NAC #1 on at least 2 diary cards. x For Part 2, subjects' average post diluents nasal congestion score must be □1 at admission for each study visit. **Exclusion Criteria** Patients who meet any of these criteria are not eligible for enrollment as study participants: x Pregnancy or breast-feeding x Female of childbearing potential not using adequate contraceptive measures x Smoking within the past year or during the protocol. x Systemic corticosteroid or other immunosuppressive medications use in the previous three months or during the protocol. x Intranasal corticosteroid use in the previous month or during the protocol. x Intranasal antihistamine or cromolyn use in the previous week or during the study. x Allergen immunotherapy during previous 12 months or during the protocol. x Omalizumab use in previous 12 months or during the protocol. x Systemic antihistamine or leukotriene modifying medication use in the previous week or during the protocol. x Use of antibiotics, NSAIDS, antihypertensives, beta-blockers,

	photosensitizing medications, or vitamin D supplements during study. Antihypertensive and beta-blocker exclusion applies to Part 1 only. ☒ Use of any intranasally administered over-the-counter product or nasal irrigation (e.g., neti pot) during study. ☒ Inability to give informed consent. ☒ Persistent asthma or any medical condition that in the opinion of the investigator may compromise the subject's ability to safely participate in the study. ☒ Subjects with any significant clinical abnormalities which may interfere with study participation. ☒ Prior use of AO+ Mist ☒ Subjects with immunodeficiencies, nasal lesions, nasal polyps, or sinus infections. ☒ Use of an investigational drug within 30 days before screening Visit 1
DOSE REGIMEN:	In Part 1, after screening and recruitment, participants will be randomized to one of the following arms of the study for 2 weeks of treatment and 4 weeks of follow-up: 1. B244 (140ul per nostril; OD 0.5; $1x10^9$ cells/ml) twice-a-day for 14 days, 2. B244 (140ul per nostril; OD 2.0; $4x10^9$ cells/ml) twice-a-day for 14 days 3. Vehicle (140ul per nostril) twice-a-day for 14 days In Part 2, subjects with a history of SAR to ragweed pollen will be enrolled to one of the following arms: 1. B244 (140ul per nostril; OD 0.5; $1x10^9$

	cells/ml) twice-a-day for 14 days, 2. B244 (140ul per nostril; OD 2.0; $4x10^9$ cells/ml) twice-a-day for 14 days, 3. Vehicle (140ul per nostril) twice-a-day for 14 days, Subjects will undergo nasal allergen challenge, followed by one-time treatment in clinic on Day 0, followed by twice-a-day in-home treatment for 14 days, followed by one additional treatment in clinic on Day 14 prior to second nasal allergen challenge.
ASSESSMENT SCHEDULE:	In Part 1, study assessments will occur at Baseline and Week 2 (Day 14) visit. Follow-up visit will be at Week 3 (Day 21) and Week 6 (Day 42) after treatment to perform safety assessments and clinical examination of the nasal mucosa, sinuses, and upper airway. In Part 2, study assessments will occur at NAC titration visit (Day 0), NAC#1 visit (Day 14) and NAC#2 visit (Day 28). Preliminary efficacy assessments will be after nasal allergen challenge in a prophylaxis treatment setting.
STATISTICAL CONSIDERATIONS:	Intent to treat analysis
INVESTIGATIONAL DRUG AND PLACEBO:	Investigational drug refers to B244. Investigational Product/treatment refers to either B244 or Vehicle.
PLANNED DURATION PER SUBJECT:	Up to 9 weeks
DURATION OF STUDY:	First subject first visit: Sep 2017 Study readout: Mar 2018

1 STUDY SCHEMA




Part 2:
Male and Female subjects with a history of seasonal allergic rhinitis
Nasal allergen challenge (NAC) (Titration)
NAC #1
Vehicle (14 days BID)
B244 1x (14 days BID)
B244 4x (14 days BID)
NAC #2
Study assessments at baseline (Day 14) and 2 weeks (Day 28)

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

ABPM	Ambulatory Blood Pressure Monitoring
AE	Adverse Event
AMO	Ammonia Monooxygenase
AOB	Ammonia Oxidizing Bacteria
BID	Twice-Daily
CBC	Complete Blood Count
CRF	Case Report Form
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
ECG	Electrocardiogram
E/T	Early Termination
FDA	Food and Drug Administration
HAO	NH_2OH oxidoreductase
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IP	Investigational Product
IRB	Institutional Review Board
IWRS	Interactive Web Response System
MedDRA	Medical Dictionary for Regulatory Activities
NAC	Nasal Allergen Challenge
NH_2OH	Hydroxylamine
NH_3	Ammonia
NO	Nitric Oxide
NO_2-	Nitrite
PNIF	Peak Nasal Inspiratory Flow
PT	Preferred Term
SAE	Serious Adverse Event
SPM	Study Procedures Manual
SOC	System Organ Class
TEAE	Treatment Emergent Adverse Event
TNSS	Total Nasal Symptom Score
WOCBP	Women of Child Bearing Potential

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2 BACKGROUND AND RATIONALE

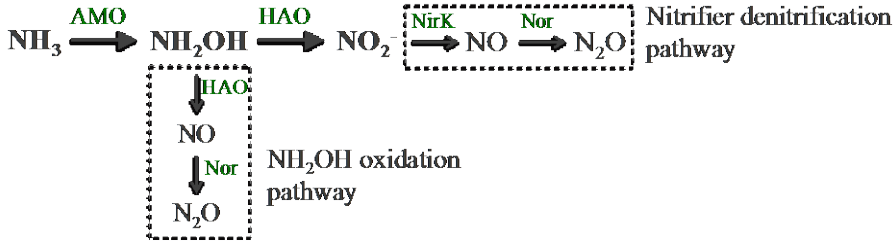
2.1 Background

Allergic rhinitis (AR) is an upper airway inflammatory disease, characterized by symptoms of rhinorrhea, sneezing, and nasal congestion. Although AR is often considered a benign condition, it greatly impacts the quality of life of affected individuals, with economic costs estimated at $5.3 billion per year in the USA (1,2). World-wide prevalence of AR is estimated at 9% to 42% (3,4). A variety of treatment approaches exist, including anti-histamines, corticosteroids, and immunotherapies, but have associated side effects and variable responses that warrant alternative options that exhibit improved safety and efficacy profiles. None of the current anti-allergic and anti-inflammatory interventions is designed to address a microbiome based approach to restore colonization of a human commensal bacteria to the nasal microbiome to regulate its anti-inflammatory and anti-infective properties. Intranasal administration of ammonia oxidizing bacteria maybe beneficial for the treatment of a variety of local and systemic indications through its anti-inflammatory and anti-infective properties. The objective of this study is to evaluate the safety of intranasally delivered ammonia oxidizing bacteria (AOB) in healthy volunteers and subsequently to evaluate preliminary efficacy of AOB as a prophylaxis treatment in response to a ragweed pollen nasal allergen challenge (NAC) in subjects with a history of seasonal allergic rhinitis (SAR).

Ammonia oxidizing bacteria are essential for the initial step in environmental nitrification processes, specifically the oxidation of ammonia (NH_3) to nitrite (NO_2-). *Nitrosomonas* are Gram-negative chemolithoautotrophic betaproteobacteria that obtain energy solely from NH_3 oxidation, while fixing CO_2 for their carbon needs (5). Oxidation of NH_3 proceeds in two steps (

Figure 1) leading to sequential generation of hydroxylamine (NH_2OH) and NO_2- that require two enzyme complexes: the membrane-bound ammonia monooxygenase (AMO) comprised of subunits AmoA, AmoB and AmoC; and the periplasmic NH_2OH oxidoreductase (HAO). In addition to high NO_2- levels, NH_3 oxidation leads to nitric oxide (NO) and N_2O production through two independent pathways downstream of NH_2OH production: nitrifier denitrification and NH_2OH oxidation (6).

Figure 1 Nitrifier Denitrification Pathway



B244 is a purified a strain of *Nitrosomonas eutropha* originally isolated from soil samples. Sequencing of the B244 genome revealed a distinct genetic profile from that of other published *Nitrosomonas* strains and AOB genomes. Based on *in vitro* co-culture studies, B244 was able to reduce survival of pathogenic bacteria. Nitrite generation from ammonia concurrently with medium acidification by B244 led to strong antibacterial effects and a marked reduction (~100-fold) in viable counts of methicillin-resistant *Staphylcoccus aureus* and *Pseudomonas aeruginosa*, two pathogens frequently isolated from infected skin and wound sites. In contrast, control cultures with B244 in the absence of ammonium or with heat-killed B244 supplemented with ammonium, had no antibacterial effects. The unique metabolic and antimicrobial activity of *Nitrosomonas*, in combination with their lack of virulence render these bacteria as attractive candidates for topical delivery of nitrite and nitric oxide on human skin with potential to improve health in both normal and abnormal skin conditions or wound sites. NO-releasing drugs or NO donors have also shown activity against *Propionibacterium acnes* and other pathogenic bacteria, anti-inflammatory activity, and inhibition of lipogenesis by insulin-stimulated immortal sebocytes (7-9).

To date, there have been no reported infections or health risks associated with topical application or ingestion of *Nitrosomonas* species. The absence of any illnesses attributed to these bacteria despite our widespread exposure indicates that they pose a minimal health risk, if any at all. Infection or tissue damage by *Nitrosomonas* is unlikely, because the sequenced genomes of several *Nitrosomonas* and other AOB lack genes encoding cytotoxins, or other known bacterial virulence factors. Further, AOB are slow growing, as compared to most heterotrophic bacteria, with optimum doubling times of 8 hours or higher. In particular, *Nitrosomonas* growth is rate limited by the availability of ammonia requiring the oxidation of 27 moles NH_3/mole CO_2 fixed. Due to their dependence on ammonia for their growth, the numbers of *Nitrosomonas* on the skin will be necessarily limited and naturally regulated by the amount of ammonia produced in sweat. This would ensure that the amount of nitrite and NO generated would be relatively low, without any adverse effects.

Currently, *Nitrosomonas eutropha* D23 in buffer at a concentration of $1x10^9$ cells/mL is available as a cosmetic product for topical application of a natural source of AOB and NO/NO_2 to improve the appearance of human skin. AOBiome plans to study *Nitrosomonas eutropha* D23 diluted in storage solution (50 mM Na_2HPO_4 and 2 mM $MgCl_2$, pH 7.6) at defined doses to be provided as an intranasal spray (B244) for therapeutic applications.

B244 is being developed under IND #16487 as a 'live topical' to provide a natural source of AOB and NO/NO_2 to the human skin. Under IND #16487, a phase 1b/2a clinical trial was completed in 2016 where 36 participants with clinical diagnosis of facial acne vulgaris were randomized to receive ascending doses of investigational product (IP) over 14 days. Safety analyses have been completed and there have been no attributable drug related SAEs reported. In addition, a phase 2b/3 clinical trial in 372 patients with clinical diagnosis of facial acne is

currently ongoing. To date, there have been no attributable drug related SAEs reported in this trial.

The proposed clinical study is designed to evaluate the safety of intranasally delivered AOB in healthy volunteers and subsequently to evaluate preliminary efficacy of AOB as a prophylaxis treatment in response to a ragweed pollen nasal allergen challenge in subjects with a history of seasonal allergic rhinitis.

3 OBJECTIVES

3.1 Primary Objective

- x To assess the safety and tolerability of B244 relative to vehicle in healthy volunteers during 14 days of treatment and up to 28 days of follow-up. Safety and tolerability will be assessed by reporting of AEs, physical examination and vital signs (blood pressure, heart rate, respiratory rate).

3.2 Secondary Objective(s)

- x To evaluate the efficacy of B244 relative to vehicle in treating Total Nasal Symptom Score (TNSS) in subjects with seasonal allergic rhinitis evaluated in a nasal allergen challenge model as a prophylaxis therapy after 14 days of treatment.
- x To evaluate the safety and tolerability of B244 relative to vehicle in subjects with allergic rhinitis after 14 days of treatment. Safety and tolerability will be assessed by reporting of AEs, physical examination and vital signs (blood pressure, heart rate, respiratory rate).
- x To evaluate the efficacy of B244 relative to vehicle in treating individual nasal symptom scores in subjects with allergic rhinitis evaluated in a nasal allergen challenge model as a prophylaxis therapy after 14 days of treatment.

3.3 Exploratory

- x To test the hypothesis that B244 inhibits airway inflammation driven by nasal allergen challenge. The outcome measures will be changes in Peak Nasal Inspiratory Flow, nasal inflammation score using otoscope, intranasal nitric oxide levels and cytokine concentration from nasal cavity pre- and post- nasal allergen challenge in a prophylaxis treatment setting.

4 ENDPOINTS

4.1 Primary

- Safety and tolerability will be assessed by reporting of AEs, physical examination and vital signs (blood pressure, heart rate, respiratory rate) during 14 days of treatment and up to 28 days of follow-up (Part 1).

4.2 Secondary

- Change in TNSS from baseline to 14 days of prophylaxis treatment.
- Change in subjective nasal symptom scores of nasal congestion, rhinorrhea, nasal itching, and sneezing from baseline to 14 days of prophylaxis treatment.
- Nasal symptom-free response rate within each individual symptom scores and percentage of subjects with a 25% or 50% reduction in TNSS after 14 days of prophylaxis treatment.
- Safety and tolerability will be assessed in subjects with allergic rhinitis by reporting of AEs, physical examination and vital signs (blood pressure, heart rate, respiratory rate) during 14 days of treatment (Part 2).

4.3 Exploratory

- Change in Peak Nasal Inspiratory Flow (PNIF), nasal inflammation score using otoscope, intranasal nitric oxide levels, and cytokine concentration in nasal cavity and blood from baseline to 14 days of B244 application and during prophylaxis treatment setting.

5 STUDY DESIGN

- The purpose of this study is to assess the safety and tolerability of B244 relative to Vehicle in healthy volunteers and to evaluate the preliminary efficacy of B244 relative to Vehicle in subjects with seasonal allergic rhinitis.
- This is a Prospective, Controlled, Double Blinded, Multi-Center, Randomized, 3 Arm, Parallel Assignment, Phase 1b/2a Study to assess the safety, tolerability, and preliminary efficacy of B244 delivered as an intranasal spray in healthy volunteers and subjects with seasonal allergic rhinitis. This will be a 2-part study.
    - In Part 1, safety and tolerability will be evaluated during 14 days of study treatment twice-a-day followed by 4 weeks of follow-up in healthy volunteers.
    - In Part 2, preliminary efficacy will be evaluated in subjects with a history of seasonal allergic rhinitis outside of the local pollen season.
- Subjects will be recruited to participate and randomized 1:1:1 to the following interventions:
    1. B244 (140ul per nostril; OD 0.5; $1x10^9$ cells/ml) twice-a-day for 14 days
    2. B244 (140ul per nostril; OD 2.0; $4x10^9$ cells/ml) twice-a-day for 14 days
    3. Vehicle (140ul per nostril) twice-a-day for 14 days

- Part 1 (safety and tolerability evaluation in healthy volunteers) will enroll 24 subjects. Up to 12 subjects per dose cohort (n=8 active, n=4 vehicle) will be enrolled, with a target of 20 subjects completed (10 subjects per dose cohort; n=7 active, n=3 vehicle) assuming a 16.7% drop out rate.
- Safety and tolerability will be assessed by reporting of AEs, physical examination and vital signs (blood pressure, heart rate, respiratory rate) during 14 days of treatment and up to 28 days of follow-up.
- Subjects will be asked to report for a screening visit and if all inclusion/exclusion criteria are met, subjects will be asked to report for a baseline visit and randomization. Subjects will then be trained on the use of the nasal spray, and have their first dose of treatment on site. Subjects will remain onsite for observation for 1 hour post dosing. Subjects will then be dispensed study medication for 14 days of at home dosing. While at home, subjects will dose twice daily (am and pm), and record their dosing, any occurrence of an AE, and Total Nasal Symptom Score (TNSS) using a study diary. Subjects will be asked to visit the site at Day 14 (Week 2) and at follow-up visits at Day 21 (Week 3) and Day 42 (Week 6) for safety assessments and will additionally record Peak Nasal Inspiratory Flow (PNIF), intranasal fluid, intranasal nitric oxide (nNO) and nasal inflammation score.
- During the 2 week safety phase (Part 1), a continuous Ambulatory Blood Pressure Monitoring (ABPM) device will monitor blood pressure of each subject for 24 hours at baseline and Day 14. ABPM would be worn for 24 hours prior to the baseline visit and again for 24 hours prior to the Day 14 visit.
- Safety monitoring will include infectious complications related to B244 (by symptoms and/or testing; pulmonary, nasal, neurological), local and systemic AEs related to intranasal route during treatment and at follow-up. Local AEs include runny nose, nasal congestion, sneezing, nasal itching, palate itching, anosmia, nasal ulceration, nasal bleeding, sore throat, cough, Bell's palsy, other neurologic complications, fevers, chills, headache, muscle aches, decreased appetite, nausea, vomiting, and rash.
- Sponsor will distinguish between immediate (onset of reaction is during the first 30 minutes after administration) and delayed (onset of action is after the first 30 minutes of administration) effects for safety reporting of adverse events.
- For Part 1, study will be paused for safety review [illegible] adverse events considered possibly, probably, or definitely related to the investigational product. In addition, a 180 day follow up contact for collecting serious adverse events and new onset medical conditions will be provided.
- An internal safety committee meeting will review the 2 week Part 1 safety data and, if there are no safety concerns, the study will proceed to Part 2 to evaluate preliminary efficacy in subjects with a history of seasonal allergic rhinitis (SAR).

- Part 2 (preliminary efficacy evaluation in subjects with a history of SAR to ragweed pollen) will enroll 42 subjects. Up to 14 subjects per arm ($1x10^9$ cells/ml; $4x10^9$ cells/ml or vehicle) will be enrolled, with a target of 12 subjects per arm for a total of 36 subjects completed, assuming a 10-15% drop-out rate.
- Safety and tolerability will be assessed in subjects with allergic rhinitis by reporting of AEs, physical examination and vital signs (blood pressure, heart rate, respiratory rate) during 14 days of treatment.
- A preliminary efficacy assessment will be performed in response to nasal allergen challenge (NAC) in a prophylaxis treatment setting.
- Subjects will be asked to report for a screening visit (Visit 1) and if all inclusion/exclusion criteria are met, subjects will be asked to report for a NAC titration (ragweed pollen nasal allergen titration) visit to administer a single provocative challenge dose for each subject (Visit 2). Subjects will then return 14 days after the washout period for basic health checks and baseline assessments (intranasal fluid, TNSS, PNIF, intranasal nitric oxide, and have a nasal inflammation score) followed by confirmatory NAC (NAC#1) (Visit 3). During this time subjects will use a study diary to complete TNSS at -30 (30 minutes prior to NAC), 0 (baseline prior to NAC), 15, 30, 45, 60, 90, and 120 minutes. PNIF at -30, 0, 15, 30, 60, and 120 minutes will also be obtained. TNSS is the sum of four individual symptom scores (running nose, congestion, itchy nose, and sneezing) rated on a four point scale (0-3). Subjects are required to meet the minimum qualifying TNSS score of a change of 6/12 from their baseline score, on at least 2 diary cards. Subjects will also provide samples for intranasal fluid at 0, 20, and 60 minutes, measure intranasal nitric oxide (nNO) and have a nasal inflammation score assessed by the investigator using a nasal otoscope at 0 and 120 minutes, in addition to blood collection at 120 min for biomarkers. Subjects meeting the requirement will be randomized to one of the three treatment arms. Randomization will be 1:1:1 so that equal number of subjects will be treated in each arm of the study. Subjects will then be trained on the use of the nasal spray, and have their first dose of treatment on site (self-dosing). Subjects will remain onsite for observation for 1 hour post dosing. Subjects will then be dispensed study medication for 14 days of at home dosing. While at home, subjects will dose twice daily (am and pm), and record their dosing, and any occurrence of an AE and TNSS using a study diary.
- Subjects will return to the clinic for the last dose following the 14 days of at home dosing for their final visit (Visit 4). During this time subjects will again have their baseline assessments and be exposed to ragweed pollen challenge (NAC#2), and will use a study diary to complete TNSS at -30, 0, 15, 30, 45, 60, 90, and 120 minutes and PNIF at -30, 0, 15, 30, 60, and 120 minutes. Prior to and 20 and 120 minutes after NAC #2, subjects will additionally provide samples for intranasal fluid, and have nNO measured and have a nasal inflammation score assessed by the investigator using a nasal otoscope at 0 and 120 min, in addition to blood collection at 120 min for biomarkers.

- For Part 2, study will be paused for safety review using the same stopping criteria as in Part 1.
- Rescue medications will not be allowed during the study.

6 SELECTION OF STUDY PARTICIPANTS

6.1 Study Population

Normal healthy volunteers and subjects with a history of seasonal allergic rhinitis to ragweed pollen are eligible for enrollment. Patients must be willing to refrain from using any treatments, investigational products, including herbal medicines while participating in this study.

6.2 Number of Participants Planned

Part 1 (safety and tolerability evaluation in healthy volunteers) will enroll 24 subjects. Up to 12 subjects per dose cohort (n=8 active, n=4 vehicle) will be enrolled, with a target of 20 subjects completed (10 subjects per dose cohort; n=7 active, n=3 vehicle) assuming a 16.7% drop out rate.

Part 2 (preliminary efficacy evaluation in subjects with a history of SAR to ragweed pollen) will enroll 42 subjects. Up to 14 subjects per arm ($1x10^9$ cells/ml; $4x10^9$ cells/ml or vehicle) will be enrolled, with a target of 12 subjects per arm for a total of 36 subjects completed, assuming a 10-15% drop-out rate.

6.3 Inclusion Criteria

- Males and Females, 18 to 65 years of age (committed to consistent use of an acceptable method of birth control as described in Section 10).
- In good general health as determined by a thorough medical history and physical examination, and vital signs.
- Nonsmoker or ex-smoker (stopped >1 year prior to study entry).
- Subjects willing and able to provide written informed consent.
- Is willing and able to comply with the requirements of the protocol and must be available for the full duration of the study.
- For Part 1, subjects asymptomatic from any seasonal or perennial allergens.
- For Part 1, elevated systolic blood pressure greater than 120 but less than 160 and never been on antihypertensive medications or have been off any hypertensive treatment for a period of 12 weeks or longer.
- For Part 2, subjects with a well-documented history of seasonal allergic rhinitis (specifically ragweed pollen) with documentation of sensitivity by positive skin testing and/or IgE testing to the relevant allergens 12 months prior to enrollment that correlate with clinical history.

- For Part 2, a ragweed positive skin prick test with a wheal diameter at least 5 mm larger than the negative control and/or a ragweed specific IgE greater or equal to 0.7 kU/L.
- For Part 2, subjects with confounding allergies, or sensitization to cat epithelia, dog epithelia, *Dermatophagoides farinae,* or *Dermatophagoides pteronyssinus* or prevalent and relevant seasonal allergens as per the skin prick test may be included if sensitization is not clinically relevant (ie. subject is non-symptomatic and/or can avoid the allergen during the study) at the discretion of the Investigator.
- For Part 2, subjects with a baseline TNSS at Visit 3 and a minimum qualifying TNSS score of a change of 6/12 from their baseline score after NAC #1 on at least 2 diary cards.
- For Part 2, subjects' average post diluents nasal congestion score must be 1 at admission for each study visit.

6.4 Exclusion Criteria

Deviations from exclusion criteria are not allowed because they can potentially jeopardize the scientific integrity of the study, regulatory acceptability or participant safety. Therefore, adherence to the criteria as specified in the protocol is essential

Patients who meet any of these criteria are not eligible for enrollment as study participants:

- Pregnancy or breast-feeding
- Female of childbearing potential not using adequate contraceptive measures.
- Smoking within the past year or during the protocol.
- Systemic corticosteroid or other immunosuppressive medications use in the previous three months or during the protocol.*
- Intranasal corticosteroid use in the previous month or during the protocol.*
- Intranasal antihistamine or cromolyn use in the previous week or during the study.*
- Allergen immunotherapy during previous 12 months or during the protocol.*
- Omalizumab use in previous 12 months or during the protocol.*
- Systemic antihistamine or leukotriene modifying medication use in the previous week or during the protocol.*
- Use of antibiotics, NSAIDS, antihypertensives, beta-blockers, photosensitizing medications, or vitamin D supplements during study. Antihypertensive and beta-blocker exclusion applies to Part 1 only.
- Use of any intranasally administered over-the-counter product or nasal irrigation (e.g., neti pot) during study.
- Inability to give informed consent.
- Persistent asthma or any medical condition that in the opinion of the investigator may compromise the subject's ability to safely participate in the study.

- Subjects with any significant clinical abnormalities which may interfere with study participation.
- Prior use of AO+ Mist.
- Subjects with immunodeficiencies, nasal lesions, nasal polyps, or sinus infections.
- Use of an investigational drug within 30 days before screening Visit 1.

* Exclusionary criterion due to 1) medication use as marker of persistent comorbid allergic or inflammatory condition that may increase subject risk with study participation, 2) potential of medication to interfere with study outcome measures or results.

7 PARTICIPANT ENROLLMENT

7.1 Consenting Participants

Informed consent for participation in the study must be obtained before performing any study-specific procedures.

Informed Consent Forms for enrolled participants and for participants who are not subsequently enrolled will be maintained at the study sites in secure study files. Consent will be obtained by trained research study staff trained in taking informed consent. The study will be explained with the opportunity for the participant to ask questions. If a participant wishes to enter the study, a consent form will be completed and signed.

7.2 Screening for Eligibility

For Part 1, after informed consent has been obtained, screening assessments (Visit 1) will be performed within 3 weeks (-21 days to 0) prior to the Baseline visit (Visit 2) to determine participant eligibility for enrollment in the study. All screening evaluations must be completed and reviewed to confirm that participants meet all eligibility criteria before randomization on Day 0 (Visit 2). Inclusion and exclusion criteria will be reviewed at Screening (Visit 1) and Baseline visit (Visit 2) to make sure nothing changed. The participant's medical history and medication use will be reviewed. A brief physical examination including height, weight, vital signs, nasal inspection, and 12-lead ECG will be performed. Laboratory testing, including blood sample for a comprehensive metabolic panel, complete blood count, chemistry, lipid panel, and liver function test, as well as urinalysis, will also be performed as part of screening. Urine pregnancy test will be performed on WOCBP.

For Part 2, after informed consent has been obtained, screening assessments (Visit 1) will be performed within 3 weeks (-21 days to 0) prior to the NAC titration (Visit 2) and NAC #1 (Visit 3) visits to determine participant eligibility for enrollment in the study. All screening evaluations must be completed and reviewed to confirm that participants meet all eligibility criteria before randomization on Day 14 (Visit 3). Inclusion and exclusion criteria will be reviewed at Screening (Visit 1). The participant's medical history and medication use will be reviewed. A

brief physical examination including height, weight, vital signs, and nasal inspection, will be performed. Laboratory testing, including blood sample for a comprehensive metabolic panel, complete blood count, chemistry, lipid panel, and liver function test, as well as urinalysis, will also be performed as part of screening. Urine pregnancy test will be performed on WOCBP. Additionally, ragweed pollen allergen skin test (skin prick test) and/or blood collection for ragweed pollen specific IgE will be performed as part of screening if documentation of a positive test within the past 12 months for either method cannot be provided.

All screening assessments are listed in the Schedule of Events Table (Appendix A). A participant must meet all inclusion criteria, and none of the exclusion criteria, to be enrolled and randomized in this study. The Investigator and team will maintain a screening log to record details of all persons screened and to confirm eligibility or record reasons for screening failure, as applicable.

7.3 Study Withdrawal and Withdrawal from Investigational Product and Stopping Criteria

A participant may withdraw from the study at any time at his/her own request, or may be withdrawn at any time at the discretion of the Investigator.

Reasons for withdrawal (participants who refuse to complete any remaining study visits) or discontinuation (participants who prematurely stop the application) at any time during the study may include, but are not limited, to the following:

- For safety reasons, either at the discretion of the Investigator or at the participant's request
- For protocol violations at the discretion of AOBiome
- Due to concomitant therapy that could interfere with the results of the study (the Investigator will report all such information on the CRFs and decide, in accordance with AOBiome, whether the participant is to be withdrawn).

The reason for participant study withdrawal will be recorded in the electronic Case Report Form (eCRF). Data from participants withdrawing from the study will be considered evaluable up to the point at which they are withdrawn using the same criteria for evaluability as for participants who complete the study.

For Part 1 safety evaluation and Part 2 preliminary efficacy , AOBiome will include study stopping rules to pause study for safety review in the event that t□1 subject reports a SAE, t□2 grade 3 (severe) AEs, or t□3 grade 2 (moderate) AEs considered possibly, probably, or definitely related to the investigational product.

7.4 Screen Failures

Data for screen and baseline failures will be collected in source documentation at the sites but will not be transmitted to AOBiome.

7.5 Early Termination

Participants who have discontinued the study early will be evaluated by the Investigator at the Unanticipated Visit. See the list of assessments to be performed at the Unanticipated Visit in the Schedule of Events Table (Appendix A). Participants with ongoing AEs or SAEs believed to be possibly related to investigational product (IP) will continue to be followed until resolution or for 30 days as warranted by the nature of the AE.

8 STUDY TREATMENT

8.1 Investigational Product

Under normal conditions of handling and administration, IP is not expected to pose significant safety risks to site staff. A Material Safety Data Sheet describing the occupational hazards and recommended handling precautions will be provided to site staff if required by local laws or will otherwise be available from the Sponsor upon request.

Investigational product must be stored in a secure area under the appropriate physical conditions for the product. Access to and administration of the IP will be limited to the Investigator and authorized site staff. Investigational product must be dispensed or administered only to participants enrolled in the study and in accordance with the protocol.

The Investigator is responsible for study treatment accountability, reconciliation, and record maintenance. The Investigator or designated site personnel must maintain study treatment accountability records throughout the course of the study. The responsible person(s) will document the amount of study treatment received from and returned to the sponsor and the amount administered to participants. The required accountability unit for this study will be the bottle. Discrepancies are to be reconciled or resolved.

Product name:	B244, 30ml/bottle	B244, 30ml/bottle	Placebo, 30ml/bottle
Dosage form:	B244 suspension	B244 suspension	Vehicle solution
Unit dose strength:	$1x10^9$ cells/ml	$4x10^9$ cells/ml	50nM Na_2HPO_4-

			2mM $MgCl_2$ (pH 7.6)
Route/administration/duration:	Intranasal application: Part 1: 1 pump (140ul) per nostril BID for 14 days Part 2: 1 pump (140ul) per nostril BID for 14 days	Intranasal application: Part 1: 1 pump (140ul) per nostril BID for 14 days Part 2: 1 pump (140ul) per nostril BID for 14 days	Intranasal application: Part 1: 1 pump (140ul) per nostril BID for 14 days Part 2: 1 pump (140ul) per nostril BID for 14 days
Dosing instruction:	Prime the spray with 5 actuations (pumps) into air prior to first use only. Apply 1 pump per nostril (2 pumps total) in the morning and repeat (2 additional pumps) at night for 14 days of treatment.	Prime the spray with 5 actuations (pumps) into air prior to first use only. Apply 1 pump per nostril (2 pumps total) in the morning and repeat (2 additional pumps) at night for 14 days of treatment.	Prime the spray with 5 actuations (pumps) into air prior to first use only. Apply 1 pump per nostril (2 pumps total) in the morning and repeat (2 additional pumps) at night for 14 days of treatment.
Physical description:	Odorless, cloudy, light pink suspension	Odorless, cloudy, light pink suspension	Odorless, clear, and colorless suspension
Manufacturer/source of procurement:	AOBiome, LLC	AOBiome, LLC	AOBiome, LLC

The contents of the label will be in accordance with all applicable regulatory requirements. B244 and matching placebo will be packaged in identical 30 ml white bottles.

8.2 Dose Changes

No dose changes are anticipated.

8.3 Storage Conditions

All investigational drug supplies in the study will be stored in a secure, refrigerated (2-8^{O}C) safe place, under the responsibility of the Investigator or other authorized individual prior to randomization and distribution to participants. Once subjects are sent home with investigational product at visit 2 and visit 3 for Part 1 and Part 2 phases of the study, respectively, subjects will store the investigational product (IP) in room temperature (20-25 ºC) during use at home on the counter, being mindful to avoid extreme temperatures and areas of excessive heat (e.g., inside the car or trunk during heat) or cold (i.e., do not freeze).

8.4 Description of Blinding Method

This study will be double-blind: neither Investigator(s), nor study participants, nor those involved in the conduct of the trial (including sponsor staff) will be aware of the treatment the participants are receiving.

8.5 Treatment Assignments:

This is a double blind study. Randomization will be stratified by site.

When a subject completes the screening tests and procedures, the investigator will notify the Site's pharmacist and the subject will be randomly assigned to one of three treatment groups in a 1:1:1 ratio. Randomization and treatment assignment will be verified and coordinated by the Site's pharmacist.

Each participant scheduled to receive investigational product (IP) will receive a randomization number at the time of randomization. The randomization number will be used to identify the study medication kit assigned to the participant and indicate the treatment to be administered to that participant.

8.6 Treatment Compliance

Study treatment must be dispensed or administered according to procedures described herein. Only participants enrolled in the study may receive study treatment. Only authorized site personnel may supply study treatment. Participants will record use of the study medication utilizing the study diary at the time of use each day. Participants will review study medication compliance with the Investigator or designee. Any missed doses, timing, and reason for missed dose will be recorded in the eCRF. There should be no doubling of doses to make up for missed doses. If a dose is missed, the next dose of study medication should be taken as scheduled.

Each site participating in the trial will be instructed to assess subject's compliance by weighing the investigational product and obtaining the weight of the bottle in grams pre and post application during Part 1 (at Visits 2 and 3) as well as during Part 2 (at Visits 3 and 4). Sites will be provided scales, which will be calibrated prior to each use. Study personnel will be instructed to record measurements into the eCRF.

8.7 Treatment Application

Subjects will receive one 30 ml bottle at the Baseline visit (Visit 2) for a 14 day application in Part 1. Subjects will receive one 30 ml bottle at the NAC #1 visit (Visit 3) for a 14 day application in Part 2. Subjects will be instructed in the use of the spray bottle and asked to self-administer the Investigational Product as follows:

- Spray 1 pump of medication into each nostril twice a day for 14 days

Detailed Investigational Product nasal spray instructions are as follows:

- Shake bottle gently
- Prime nozzle if unused; Pump until mist appears (5 times)
- Place nozzle straight up one nostril and pump once
- Sniff gently after spraying
- Repeat other nostril
- Wipe spray nozzle with tissue when done
- Do not wash/rinse the application site or blow their nose *for 30 min* after applying the mist

In Part 1, subjects will be asked to report for a screening visit and if all inclusion/exclusion criteria are met, subjects will be asked to report for a baseline visit and randomization. Subjects will then be trained on the use of the nasal spray, and have their first dose of treatment on site. Subjects will remain onsite for observation for 1 hour post dosing. Subjects will then be dispensed study medication for 14 days of at home dosing. While at home, subjects will dose twice daily (am and pm), and record their dosing, any occurrence of an AE, and Total Nasal Symptom Score (TNSS) using a study diary. Subjects will be asked to visit the site at Day 14 (Week 2) and at follow-up visits at Day 21 (Week 3) and Day 42 (Week 6) for safety assessments and will additionally record Peak Nasal Inspiratory Flow (PNIF), intranasal fluid, intranasal nitric oxide (nNO) and nasal inflammation score.

In Part 2, subjects will be asked to report for a screening visit (Visit 1) and if all inclusion/exclusion criteria are met, subjects will be asked to report for a NAC titration (ragweed pollen nasal allergen titration) visit to administer a single provocative challenge dose for each subject (Visit 2). Subjects will then return 14 days after the washout period for basic health checks and baseline assessments (intranasal fluid, TNSS, PNIF, intranasal nitric oxide, and have a nasal inflammation score) followed by confirmatory NAC (NAC#1) (Visit 3). During this time subjects will use a study diary to complete TNSS at -30 (30 minutes prior to NAC), 0 (baseline prior to NAC), 15, 30, 45, 60, 90, and 120 minutes. PNIF at -30, 0, 15, 30, 60, and 120 minutes will also be obtained. TNSS is the sum of four individual symptom scores (running nose, congestion, itchy nose, and sneezing) rated on a four point scale (0-3). Subjects are required to meet the minimum qualifying TNSS score of a change of 6/12 from their baseline score, on at least 2 diary cards. Subjects will also provide samples for intranasal fluid at 0, 20, and 60 minutes, measure intranasal nitric oxide (nNO) and have a nasal inflammation score assessed by the investigator using a nasal otoscope at 0 and 120 minutes, in addition to blood collection at 120 min for biomarkers. Subjects meeting the requirement will be randomized to one of the three treatment arms. Randomization will be 1:1:1 so that equal number of subjects will be treated in each arm of the study. Subjects will then be trained on the use of the nasal spray, and have their first dose of treatment on site (self-dosing). Subjects will remain onsite for observation for 1 hour post dosing. Subjects will then be dispensed study medication for 14 days of at home

dosing. While at home, subjects will dose twice daily (am and pm), and record their dosing, and any occurrence of an AE and TNSS using a study diary. Subjects will return to the clinic for the last dose following the 14 days of at home dosing for their final visit (Visit 4). During this time subjects will again have their baseline assessments and be exposed to ragweed pollen challenge (NAC#2), and will use a study diary to complete TNSS at -30, 0, 15, 30, 45, 60, 90, and 120 minutes and PNIF at -30, 0, 15, 30, 60, and 120 minutes. Prior to and 20 and 60 minutes after NAC #2, subjects will additionally provide samples for intranasal fluid, and have nNO measured and have a nasal inflammation score assessed by the investigator using a nasal otoscope at 0 and 120 min, in addition to blood collection at 120 min for biomarkers.

8.8 Treatment of Investigational Product Overdose

The sponsor does not recommend specific treatment for an overdose.

8.9 Product Accountability

In accordance with federal and local regulatory requirements, the Investigators and designated site personnel must document the amount of investigational product dispensed to study participants, the amount returned by study participants, and amount received and returned to the sponsor, when applicable. Product accountability records must be maintained throughout the course of the trial. Any quality issue noticed with the receipt or use of an IP (deficient IP in condition, appearance, pertaining documentation, labeling, expiry date, etc.) should be promptly reported to the Sponsor, who will initiate a complaint procedure.

All investigational product must be stored in a secure locked room with access limited to the Investigator and designated site personnel. Study product is to be stored in a refrigerator between 2-8 degrees C. Maintenance of a temperature log is required.

Under no circumstances will the Investigator allow IP to be used other than as directed by this Clinical Trial Protocol, or dispose of IP in any other manner.

8.10 Unblinding Procedures

The Investigator may unblind a participant's treatment assignment only in the case of emergency or in the event of a serious medical condition when knowledge of the study treatment is essential for the appropriate clinical management or welfare of the participant, as determined by the Investigator. It is preferred (but not required) that the Investigator first contacts the medical monitor to discuss options before unblinding the participant's treatment assignment. The Investigator must notify the Sponsor as soon as possible when a participant's treatment assignment is unblinded without revealing the treatment assignment of the unblinded participant unless that information is deemed important for the safety of participants currently in the study. The date and reason for the unblinding must be documented in the participant's study record.

The Medical Monitor may unblind the treatment assignment for any participant with an SAE. If the SAE requires that an expedited regulatory report be sent to one or more regulatory agencies, a copy of the report, identifying the participant's treatment assignment, may be sent to clinical Investigators in accordance with local regulations and/or sponsor policy.

8.11 Retrieval and Destruction of Investigational Product

All partially used or unused treatments will be returned to the sites as brought by study participants. A detailed treatment log of the returned IP shall be established.

The sites will not destroy unused IP unless the Sponsor provides written authorization to the contrary. All used and unused bottles will be shipped to the authorized drug depot at the end of the study.

8.12 Permitted Medications

All participants will be screened for concomitant medications prior to inclusion into the study. Any concomitant medication to treat adverse events will be recorded in the Concomitant Medication section of the eCRF.

8.13 Prohibited Medications

Participants will be prohibited from taking antihypertensives, beta blockers, photosensitising medications, systemic immunosuppressants and vitamin D supplements. NSAIDs or corticosteroids are also prohibited as they may affect BP. Additional prohibited medications include antihistamines, leukotriene modifying medications, cromolyn, environmental allergen immunotherapy, Omalizumab, antibiotics, and any intranasally administered over-the-counter product or nasal irrigation (e.g., neti pot). Complete list of prohibited medications will be provided to the site PIs conducting the study.

8.14 Rescue Medications

Rescue medications will not be allowed during the study. Subject may not start additional non-randomized medication and should immediately alert the study site PI. Subjects that need to start new rescue medication regimen should be immediately discontinued from study participation and would be considered Early Termination.

8.15 Handling of Investigational Product

Subjects will receive one 30 ml bottle for each Part of the study. Each bottle will be used for 14 days and brought to study appointment. The bottle may be placed on the counter to be used during the treatment period.

Subjects will be asked not to subject the treatment kit to conditions which are unnatural or harmful to the product, such as excessive heat (temperatures over 77°F (25°C) and freezing temperatures (at 0^{o}C)). Subjects may travel with their study medication but should not leave it in the hot car, outside in the cold temperatures etc. Subjects will also be asked not to tamper or cause damage to IP.

9 CONTRACEPTION REQUIREMENTS

Effective contraception is required for all women physiologically capable of becoming pregnant during study participation. Women of child-bearing potential must agree to use an acceptable form of contraception for up to 2 weeks after the study completion. Women of childbearing potential are defined as any female who has experienced menarche and who is NOT permanently sterile or postmenopausal. Postmenopausal is defined as 12 consecutive months with no menses without an alternative medical cause.

Effective contraception methods include:

- x Total abstinence (when this is in line with the preferred and usual lifestyle of the study participant). Periodic abstinence (i.e., calendar, ovulation, symptothermal, postovulation methods) and withdrawal are not acceptable methods of contraception.
- x Use of oral, injected or implanted hormonal methods of contraception or placement of an IUD or IUS or other forms of hormonal contraception that have comparable efficacy, for example hormone vaginal ring or transdermal hormone contraception.
- x Use of barrier methods (i.e., condom, diaphragm) used with a spermicide (i.e., foam, cream, or gel that kills sperm)

In case of use of oral contraception women should have been stable on the same pill for a minimum of 90 days before the baseline visit.

Male participants of the study who are having sexual intercourse with a woman who can become pregnant must use an acceptable form of birth control while participating in the study. Additionally, male participants are expected to let their female partners know of their participation in a research study of a drug, and that the effects of the drug on an unborn baby and on a pregnant woman are unknown. Male participants will also be expected to provide their female partners with the contraception requirements information previously described and the study doctor's contact information for questions.

Payment for all aspects of obstetrical care, child-or related care will be the study participant's responsibility.

In case of pregnancy, Investigational Product should be discontinued and the Sponsor should be informed immediately. Follow-up of the pregnancy will be mandatory until the outcome is available.

10 STUDY PROCEDURES

10.1 Screening

Prior to initiation of the recruitment phase, participating Investigator will identify a pool of potential study subjects. The Investigator will identify potentially eligible subjects in advance, by either reviewing past medical records and diagnoses, screening in clinics, referral from other physicians, or other sources of recruitment, to identify those aged 18 to 65 meeting the inclusion criteria. The accuracy of self-reported data, including height, weight and medical history, will be assessed during the initial on-site screening visit. Individuals who fail to meet study inclusion criteria will be dismissed at that time. Individuals who meet inclusion criteria will move on to on-site screening visit.

10.2 Informed Consent Procedures

Eligible participants may only be included in the study after providing a consent using the IRB-approved informed consent. Informed consent must be obtained before conducting any study-specific procedures (i.e. all of the procedures described in the protocol). The process of obtaining informed consent should be documented in the participant's source documents. The date of signing of informed consent (and withdrawal, if later withdrawn) should be documented in the eCRF.

10.3 Study Assessments

In Part 1, study assessments will occur at Baseline and Week 2 (Day 14) visit. Follow-up visit will be at Week 3 (Day 21) and Week 6 (Day 42) after treatment to perform safety assessments and clinical examination of the nasal mucosa, sinuses, and upper airway.

In Part 2, study assessments will occur at NAC titration visit (Day 0), NAC#1 visit (Day 14) and NAC#2 visit (Day 28). Preliminary efficacy assessments will be after nasal allergen challenge in a prophylaxis treatment setting.

In Part 1, subjects will be asked to report for a screening visit and if all inclusion/exclusion criteria are met, subjects will be asked to report for a baseline visit and randomization. Subjects will then be trained on the use of the nasal spray, and have their first dose of treatment on site. Subjects will remain onsite for observation for 1 hour post dosing. Subjects will then be dispensed study medication for 14 days of at home dosing. While at home, subjects will dose twice daily (am and pm), and record their dosing, any occurrence of an AE, and Total Nasal Symptom Score (TNSS) using a study diary. Subjects will be asked to visit the site at Day 14 (Week 2) and at follow-up visits at Day 21 (Week 3) and Day 42 (Week 6) for safety assessments and will additionally record Peak Nasal Inspiratory Flow (PNIF), intranasal fluid, intranasal nitric oxide (nNO) and nasal inflammation score.

In Part 2, subjects will be asked to report for a screening visit (Visit 1) and if all inclusion/exclusion criteria are met, subjects will be asked to report for a NAC titration (ragweed pollen nasal allergen titration) visit to administer a single provocative challenge dose for each subject (Visit 2). Subjects will then return 14 days after the washout period for basic health checks and baseline assessments (intranasal fluid, TNSS, PNIF, intranasal nitric oxide, and have a nasal inflammation score) followed by confirmatory NAC (NAC#1) (Visit 3). During this time subjects will use a study diary to complete TNSS at -30 (30 minutes prior to NAC), 0 (baseline prior to NAC), 15, 30, 45, 60, 90, and 120 minutes. PNIF at -30, 0, 15, 30, 60, and 120 minutes will also be obtained. TNSS is the sum of four individual symptom scores (running nose, congestion, itchy nose, and sneezing) rated on a four point scale (0-3). Subjects are required to meet the minimum qualifying TNSS score of a change of 6/12 from their baseline score, on at least 2 diary cards. Subjects will also provide samples for intranasal fluid at 0, 20, and 60 minutes, measure intranasal nitric oxide (nNO) and have a nasal inflammation score assessed by the investigator using a nasal otoscope at 0 and 120 minutes, in addition to blood collection at 120 min for biomarkers. Subjects meeting the requirement will be randomized to one of the three treatment arms. Randomization will be 1:1:1 so that equal number of subjects will be treated in each arm of the study. Subjects will then be trained on the use of the nasal spray, and have their first dose of treatment on site (self-dosing). Subjects will remain onsite for observation for 1 hour post dosing. Subjects will then be dispensed study medication for 14 days of at home dosing. While at home, subjects will dose twice daily (am and pm), and record their dosing, and any occurrence of an AE and TNSS using a study diary. Subjects will return to the clinic for the last dose following the 14 days of at home dosing for their final visit (Visit 4). During this time subjects will again have their baseline assessments and be exposed to ragweed pollen challenge (NAC#2), and will use a study diary to complete TNSS at -30, 0, 15, 30, 45, 60, 90, and 120 minutes and PNIF at -30, 0, 15, 30, 60, and 120 minutes. Prior to and 20 and 60 minutes after NAC #2, subjects will additionally provide samples for intranasal fluid, and have nNO measured and have a nasal inflammation score assessed by the investigator using a nasal otoscope at 0 and 120 min, in addition to blood collection at 120 min for biomarkers.

At the final visit for each phase of the study (Visit 5 for Part 1 and Visit 4 for Part 2), subjects may be requested to complete a subject satisfaction survey.

10.4 Inclusion Procedures

Once all inclusion/exclusion criteria are fulfilled, the patient becomes eligible for randomization and inclusion into the treatment period for each of Part 1 and Part 2 phases of the study. Treatment allocation will be performed as stated above in Section 8.5. Study medication will be delivered as stated in Section 8.7. Patients will be counseled on product application and diary completion.

10.5 Description by Type of Visit – Part 1

10.5.1 Screening Visit (Visit 1; Study Day -1) (-21 to 0 Days Visit Window)

- x informed consent completed and signed
- x inclusion and exclusion criteria
- x demographic data
- x medical/surgical history
- x record concomitant medications
- x obtain in clinic blood pressure, heart rate, respiratory rate
- x physical exam
- x body weight
- x height measurement
- x nasal inspection
- x 12-lead ECG
- x laboratory testing (blood for metabolic panel, hematology, clinical chemistry, lipid panel, liver function test, urinalysis)
- x urine pregnancy test (for women of childbearing potential)
- x safety AEs
- x study diary

10.5.2 Baseline ABPM Placement (-3 to 0 Days Visit Window)

This visit should occur 24 hours prior to baseline visit (Visit 2) between the hours of 8am +/- 2 hrs.

- x obtain in clinic blood pressure and heart rate
- x provide 24 hr ABPM device to the subject
- x train the subject on the use of the machine and provide instructions

10.5.3 Baseline Visit (Visit 2; Study Day 0) (-3 to 1 Days Visit Window)

This visit should occur between the hours of 8am +/- 2 hrs.

- x inclusion and exclusion criteria
- x medical/surgical history
- x record concomitant medications
- x obtain in clinic blood pressure, heart rate, respiratory rate (pre- and post-first dose)
- x physical exam
- x ambulatory blood pressure monitoring (ABPM) removal (after 24 hour measurement) and evaluation of technical success/failure criteria
- x randomization
- x nasal inspection (pre- and post-first dose)
- x delivery of the corresponding pack of Investigational Product
- x Investigational Product application (under medical supervision)
- x obtain study medication weight for Investigational Product compliance
- x study counseling

- safety AEs
- x nasal fluid collection for biomarkers
- x blood collection for biomarkers
- x peak nasal inspiratory flow (PNIF)
- x intranasal nitric oxide
- x nasal inflammation score
- x study diary

10.5.4 Baseline Repeat ABPM Placement (if Necessary) (-3 to 1 Days Visit Window)

If the ABPM technical acceptance criteria are not met during the baseline visit (Visit 2) reading, the ABPM session may be repeated. In the event of technical failure, study coordinators will not perform any study related activities outlined in Visit 2 prior to repeating the ABPM session.

Repeat monitoring may be scheduled up to 48 hours after the failed monitoring.

This visit should occur between the hours of 8am +/- 2 hrs.

- x provide 24 hr ABPM device to the patient
- x train the patient on the use of the machine and provide instruction

The patient will come back 24 hours later to remove ABPM machine and check on the readings to make sure the equipment performed as expected.

If the machine performed as expected, then Visit 2 activities will be performed as outlined in the section above.

10.5.5 Week 2 ABPM Placement (±3 Days Visit Window)

This visit should occur 24 hours prior to week 2 visit (Visit 3) between the hours of 8am +/- 2 hrs.

- x obtain in clinic blood pressure and heart rate
- x provide 24 hr ABPM device to the subject
- x train the subject on the use of the machine and provide instructions

10.5.6 Week 2 Visit (Visit 3; Study Day 14) (±3 Days Visit Window)

- x record concomitant medications
- x obtain in clinic blood pressure, heart rate, respiratory rate
- x physical exam
- x body weight
- x ABPM removal (after 24 hour measurement) and evaluation of technical success/failure criteria
- x nasal inspection
- x 12-lead ECG

- Investigational Product application (last dose, if needed)
- x obtain study medication weight for Investigational Product compliance
- x laboratory testing (blood for metabolic panel, hematology, clinical chemistry, lipid panel, liver function test, urinalysis)
- x safety AEs
- x nasal fluid collection for biomarkers
- x blood collection for biomarkers
- x peak nasal inspiratory flow (PNIF)
- x intranasal nitric oxide
- x nasal inflammation score
- x study diary

10.5.7 Week 2 Repeat ABPM Placement (if Necessary) (±3 Days Visit Window)

If the ABPM technical acceptance criteria are not met during the week 2 visit (Visit 3) reading, the ABPM session may be repeated. In the event of technical failure, study coordinators will not perform any study related activities outlined in Visit 3 prior to repeating the ABPM session.

Repeat monitoring may be scheduled up to 48 hours after the failed monitoring.

This visit should occur between the hours of 8am +/- 2 hrs.

- x provide 24 hr ABPM device to the patient
- x train the patient on the use of the machine and provide instruction

The patient will come back 24 hours later to remove ABPM machine and check on the readings to make sure the equipment performed as expected.

If the machine performed as expected, then Visit 3 activities will be performed as outlined in the section above.

10.5.8 Follow-up #1 Visit (Visit 4; Study Day 21) (±3 Days Visit Window)

- x record concomitant medications
- x obtain in clinic blood pressure, heart rate, respiratory rate
- x physical exam
- x body weight
- x nasal inspection
- x safety AEs
- x peak nasal inspiratory flow (PNIF)
- x intranasal nitric oxide
- x nasal inflammation score
- x study diary

10.5.9 Follow-up #2 Visit (Visit 5; Study Day 42) (±3 Days Visit Window)

- x record concomitant medications
- x obtain in clinic blood pressure, heart rate, respiratory rate
- x physical exam
- x body weight
- x nasal inspection
- x 12-lead ECG
- x laboratory testing (blood for metabolic panel, hematology, clinical chemistry, lipid panel, liver function test, urinalysis)
- x safety AEs
- x nasal fluid collection for biomarkers
- x blood collection for biomarkers
- x peak nasal inspiratory flow (PNIF)
- x intranasal nitric oxide
- x nasal inflammation score
- x study diary
- x subject satisfaction survey

10.6 Description by Type of Visit – Part 2

10.6.1 Screening Visit (Visit 1; Study Day -1) (-21 to 0 Days Visit Window)

- x informed consent completed and signed
- x inclusion and exclusion criteria
- x demographic data
- x medical/surgical history
- x record concomitant medications
- x obtain in clinic blood pressure, heart rate, respiratory rate
- x physical exam
- x body weight
- x height measurement
- x allergen skin test (skin prick test) (if documentation of a positive test within the past 12 months is not available)
- x blood collection for IgE (if documentation of a positive test within the past 12 months is not available)
- x nasal inspection
- x laboratory testing (blood for metabolic panel, hematology, clinical chemistry, lipid panel, liver function test, urinalysis)
- x urine pregnancy test (for women of childbearing potential)
- x safety AEs
- x study diary

10.6.2 NAC Titration Visit (Visit 2; Study Day 0) (±3 Days Visit Window)

- x physical exam
- x nasal inspection
- x NAC
- x safety AEs
- x peak nasal inspiratory flow (PNIF) (to be completed pre (-30 and 0 min) and post NAC (15, 30, 60, and 120 min))
- x intranasal nitric oxide
- x nasal inflammation score
- x study diary (TNSS to be completed pre (-30 and 0 min) and post NAC (15, 30, 45, 60, 90, and 120 min))

10.6.3 NAC #1 Visit (Visit 3; Study Day 14) (±3 Days Visit Window)

- x record concomitant medications
- x obtain in clinic blood pressure, heart rate, respiratory rate
- x physical exam
- x body weight
- x randomization
- x nasal inspection
- x NAC
- x delivery of the corresponding pack of Investigational Product
- x Investigational Product application (under medical supervision)
- x obtain study medication weight for Investigational Product compliance
- x study counseling
- x safety AEs
- x nasal fluid collection for biomarkers (to be completed pre (0 min) and post NAC (20 and 60 min))
- x blood collection for biomarkers (to be completed post NAC)
- x peak nasal inspiratory flow (PNIF) (to be completed pre (-30 and 0 min) and post NAC (15, 30, 60, and 120 min))
- x intranasal nitric oxide (to be completed pre (0 min) and post NAC (120 min))
- x nasal inflammation score (to be completed pre (0 min) and post NAC (120 min))
- x study diary (TNSS to be completed pre (-30 and 0 min) and post NAC (15, 30, 45, 60, 90, and 120 min))

10.6.4 NAC #2 Visit (Visit 4; Study Day 28) (±3 Days Visit Window)

- x record concomitant medications
- x obtain in clinic blood pressure, heart rate, respiratory rate
- x physical exam
- x body weight
- x nasal inspection

- NAC
- x Investigational Product application (last dose, if needed)
- x obtain study medication weight for Investigational Product compliance
- x laboratory testing (blood for metabolic panel, hematology, clinical chemistry, lipid panel, liver function test, urinalysis)
- x safety AEs
- x nasal fluid collection for biomarkers (to be completed pre (0 min) and post NAC (20 and 60 min))
- x blood collection for biomarkers (to be completed post NAC)
- x peak nasal inspiratory flow (PNIF) (to be completed pre (-30 and 0 min) and post NAC (15, 30, 60, and 120 min))
- x intranasal nitric oxide (to be completed pre (0 min) and post NAC (120 min))
- x nasal inflammation score (to be completed pre (0 min) and post NAC (120 min))
- x study diary (TNSS to be completed pre (-30 and 0 min) and post NAC (15, 30, 45, 60, 90, and 120 min))
- x subject satisfaction survey

10.6.5 Unscheduled/Unanticipated Study Visit

If an event arises that requires patient to come in to the research center, subjects should be scheduled for the Unscheduled visit.

During the visit, the following will be obtained:

- x medical/surgical history
- x record concomitant medications
- x obtain in clinic blood pressure, heart rate, respiratory rate
- x physical exam
- x body weight
- x nasal inspection
- x obtain study medication weight for Investigational Product compliance
- x study counseling
- x laboratory testing (blood for metabolic panel, hematology, clinical chemistry, liver function test, urinalysis)
- x study AEs
- x nasal fluid collection for biomarkers
- x blood collection for biomarkers
- x peak nasal inspiratory flow (PNIF)
- x intranasal nitric oxide
- x nasal inflammation score
- x study diary

11 METHODS OF ASSESSMENTS

11.1 Blood Pressure Measurement

Blood pressure readings will be obtained at every visit as described in Appendix A (schedule of events). Subject should be asked to remove all clothing that covers the location of cuff placement. The individual should be comfortably seated in the chair, with the legs uncrossed, and the back and arm supported, such that the middle of the cuff on the upper arm is at the level of the right atrium (the mid-point of the sternum). Neither the patient nor the observer should talk during the measurement. After 5 minutes sitting, serial clinic BP measurements and heart (x3) rate will be performed, using the non-dominant arm with a calibrated electronic sphygmomanometer. The average of the second and third readings will be documented. Respiratory rate (number of breaths per minute) will also be recorded.

11.2 Physical Examinations

The physical examination will be performed at Screening (Visit 1), Baseline (Visit 2), Week 2 (Visit 3), Follow-up #1 (Visit 4), and Follow-up #2 (Visit 5) during Part 1, Screening (Visit 1), NAC titration (Visit 2), NAC #1 (Visit 3), and NAC #2 (Visit 4) during Part 2, and at Unanticipated Visit should one occur. The physical examination includes an assessment of general appearance and a review of systems (dermatologic, head, eyes, ears, nose, mouth/throat/neck, thyroid, lymph nodes, respiratory, cardiovascular, gastrointestinal, extremities, musculoskeletal, neurologic systems).

11.3 Allergen Skin Test (Skin Prick Test)

For Part 2, subjects with a well-documented history of seasonal allergic rhinitis (specifically ragweed pollen) with documentation of sensitivity by positive skin testing and/or IgE testing to the relevant allergens 12 months prior to enrollment that correlate with clinical history are screened and enrolled. Subjects may be confirmed with a ragweed positive skin prick test with a wheal diameter at least 5 mm larger than the negative control and/or a ragweed specific IgE greater or equal to 0.7 kU/L.

A diluted ragweed pollen allergen is applied with a prick or a puncture on the surface of the skin. After the prick, the area of the skin is observed for about 15 minutes to see if a reaction develops. The “wheal”—a raised, red, itchy bump and surrounding “flare”—indicates the presence of the allergy antibody when the person is exposed to specific allergens. The larger the wheal and flare, the greater the sensitivity.

11.4 Blood Collection for IgE

Alternative to a ragweed pollen allergen skin test (skin prick test), blood collection for ragweed pollen specific IgE may be performed as part of screening. Subjects with a ragweed specific IgE greater or equal to 0.7 kU/L will be enrolled.

11.5 Ambulatory Blood Pressure Monitoring (ABPM)

11.5.1 ABPM Monitoring Schedule

ABPM will occur twice during the study.

Baseline ABPM placement visit should occur 24 hours prior to baseline visit (Visit 2) between the hours of 8am +/- 2 hrs. Subjects should report to the office at 8 am +/- 2 hrs such that they are hooked up and dosed as close to 8:00 am as possible (window 6:00 am – 10:00 am). At the baseline visit (Visit 2), subjects will have their ABPM machine removed and readings checked to make sure equipment performed as expected.

Week 2 ABPM placement visit should occur 24 hours prior to week 2 visit (Visit 3) between the hours of 8am +/- 2 hrs. Subjects should report to the office at 8 am +/- 2 hrs such that they are hooked up and dosed as close to 8:00 am as possible (window 6:00 am – 10:00 am). At the week 2 visit (Visit 3), subjects will have their ABPM machine removed and readings checked to make sure equipment performed as expected.

11.5.2 Office Visit

Subjects must arrive at the office for ABPM hookup NOT HAVING TAKEN that day's dose of study medication, and at an appropriate time so that ABPM hookup, dosing of study medication, and start of the ABPM test is at 8 am +/- 2 hrs (window 6:00 am – 10:00 am) following the guidelines below. Subjects who are non-compliant will be rescheduled for the following day or later within the visit window.

- Subject dosing of study medication must occur no earlier than 6:00 AM and no later than 10:00 AM.
- There should be at least one (1) manually initiated ABPM reading after dosing of study medication. If no manual post-dose reading was recorded, START TEST time will equal the 1st valid reading after dosing, provided that the reading occurs between 6:00 am and 10:00 am.
- There should be a final manual reading on or after 24 hours of recording. If a manual reading was not recorded, the first recording after 24 hours will be considered the last valid recording of the session.

11.5.3 ABPM Test

The ABPM device will be placed on subject's arm during the clinic visit and recording device will obtain 24-hour blood pressure and heart rate measurements. The ABPM device and cuff may only be removed at the office, after a final manual reading is performed using the ABPM device. The ABPM device will be automatically programmed to inflate every 20 minutes throughout the recording period. The duration of the ABPM test is 24 hrs.

ABPM data will then be transmitted to the Core Lab. Based on pre-established criteria, the ABPM will be reviewed by the Core Lab to determine if it was a technically successful monitoring. If technically unsuccessful, the monitoring may be repeated.

11.5.4 ABPM Technical Success/Failure Criteria

The following are the technical acceptance criteria against which subject ABPM data will be evaluated:

- There must be at least one (1) valid reading per hour with the following possible exception: no more than three (3) non-consecutive hours with zero (0) valid readings during the recording period and two (2) consecutive hours with zero (0) valid readings during the recording period.

Failure to achieve any one of the above criteria will result in the ABPM being declared a technical failure.

If a subject has a technical failure on their repeat recording at Baseline, those subjects will be excluded from the study. However, if subjects pass their baseline and fails the final repeat recording , data will be included in the in-clinic BP analysis but be excluded from the ABPM analysis. We would estimate that this will occur in less than 5% of patients.

11.5.5 Repeat ABPM

If the ABPM technical acceptance criteria are not met the ABPM session may be repeated. Repeat monitoring may be scheduled up to 48 hours after the failed monitoring.

11.6 Nasal Inspection

Nasal inspection will include clinical examination of the nasal mucosa, sinuses, and upper airway using an otoscope. Nasal inspection will be performed at the following visits: Screening (Visit 1), Baseline (Visit 2), Week 2 (Visit 3), Follow-up #1 (Visit 4), and Follow-up #2 (Visit 5) during Part 1 and Screening (Visit 1), NAC titration (Visit 2), NAC #1 (Visit 3), and NAC #2 (Visit 4) during Part 2, and at Unanticipated Visit should one occur.

11.7 Nasal Allergen Challenge (NAC)

The NAC model is a transient and direct allergen exposure allergic rhinitis (AR) model in which allergen is instilled directly to the nasal mucosa via a metered, intranasal spray (10). Typically, the model involves testing of diluent to exclude those patients who may respond inappropriately, perhaps even through non-allergic mechanisms. Patients who remain eligible are then tested with increasing doses of intranasal allergen in a step-wise fashion to find the provocative dose level at which a pre-defined level of allergic signs and symptoms are met. Signs can include objective measures of intranasal changes such as nasal congestion utilizing peak nasal inspiratory flow to look at nasal flow resistance. Symptoms typically include subjective report by patients on the nasal symptoms of allergic rhinitis such as nasal congestion, rhinorrhea, itchy nose and sneezing.

NAC studies have previously examined the potential shift in this dose-response or utilized one provocative dose level to test therapy efficacy. The advantages of this approach are primarily the high level of experimental control over allergen exposure and patient compliance to both treatment, as well as the ability to capture in a well-timed fashion both signs and symptoms. Typically, objective measures are not possible to record in a field type trial. A disadvantage of this model is the concentrated and supra-natural mode of allergen exposure via direct instillation of concentrated allergen directly to the nasal mucosa. The amount of allergen utilized in this model may constitute too large of an allergic stimulus for putative anti-allergic medications to abate; and as such, medications could be evaluated as too weakly effective to continue in development. Another disadvantage of the direct instillation NAC is the inability to test exposures to multiple allergens and pollutants that patients are typically exposed to in a real-world setting, as well as the inability to test on-symptom effects of drugs and thereby limiting this approach to testing prophylactic drug effects.

The NAC has the advantage of running pilot studies with a small number of participants as well as allowing biological sampling. The inflammatory response that ensues, including early and late phase reactions (in a subset of participants), is similar to that observed in people with symptoms of AR during natural exposure (11-13). Disease pathophysiology and the effect of medications can be studied through monitoring cytokines, mediators, and inflammatory cells, allowing for accurate measurements of drug efficacy and onset/duration of action for each group of participants experiencing early and/or late phase responses as applicable to the mechanism of action (14). The NAC model has proven reliable through many clinical trials of anti-histamines (15-18), intranasal corticosteroids (19-22), and subcutaneous and sublingual immunotherapies (23-26), albeit through variable methodologies.

For Part 2 of the study, aqueous extract of ragweed pollen (short ragweed; *Ambrosia artemisiifolia*; Stallergenes Greer, Lenoir, NC) at an allergen concentration (or dilution) sufficient to generate a provocative response will be prepared for the NAC titration visit. 0.1 mL

of sterile short ragweed pollen *amb a1* stock solution (338 ug/mL, 10 mL vial; diluent composition: 0.25% NaCl, 0.27% sodium bicarbonate, 0.2% phenol, 50% glycerin; stored at 2-8^{o}C; Lot 325732; expiration date 20Mar2019; Stallergenes Greer, Lenoir, NC) will be diluted in 2.5 mL of 0.9% sterile saline to prepare 96.5 micrograms/mL (or 0.0965 mg/mL) ragweed solution. Similarly, 0.1 mL of 50% glycerin saline (50% glycerin, 0.091% sodium bicarbonate, 0.166% sodium chloride, and water for injection q.s; 100 mL vial; stored at room temperature; Lot 2022817; expiration date Feb2023; Stallergenes Greer, Lenoir, NC) will be diluted in 2.5 mL of 0.9% sterile saline to prepare the diluent. Both of these solutions will be prepared fresh for each day's use using aseptic procedure to fill the Aptar Bidose System Liquid (BDSL) nasal pumps. Each sterile Aptar bidose pump is filled using 230 microliters of the ragweed solution or diluent to deliver 100 microliters per pump to each nostril (2 pumps total per NAC; 100 microliters per nostril of 9.65 micrograms ragweed; 19.3 micrograms total ragweed per subject).

Baseline PNIF and TNSS will be recorded 30 minutes and immediately prior to administration of the NAC titration dose, which is a a single provocative nasal allergen dose (0.1 mL per nostril at 0.0965 mg/mL *amb a1*). Nasal administration of diluent will be made 30, 20 and 10 minutes prior to commencing nasal allergen dosing. 100 /RIWKHGLOXWHGDOOHUJHQ will be sprayed into each nostril using a nasal pump (Aptar), and afterwards PNIF (15, 30, 60, and 120 min) and TNSS (15, 30, 45, 60, 90, and 120 min) will be reFRUGHG,ID31,)UHGXFWLRQRIIURP EDVHOLQH25D7166VFRUHRI6/12 were achieved on at least 2 diary cards, the qualifying concentration to be used at the NAC visit is established. If the target values are not reached, they will be excluded.

At the NAC #1 visit (Visit 3) the qualifying allergen concentration from the titration visit will be used as the single concentration for nasal challenge. Nasal administration of diluent will be made 30, 20 and 10 minutes prior to commencing nasal allergen dosing. TNSS will be recorded by the participants before the challenge at -30 (30 minutes prior to NAC), 0 (baseline prior to NAC), 15, 30, 45, 60, 90, and 120 minutes following the nasal challenge. PNIF at -30, 0, 15, 30, 60, and 120 minutes will also be obtained. Subjects are required to meet the minimum qualifying TNSS score of a change of 6/12 from their baseline score (must be), on at least 2 diary cards to be randomized and enrolled into the study.

Subjects will return to the clinic for the last dose following the 14 days of at home dosing for their final visit (Visit 4). Nasal administration of diluent will be made 30, 20 and 10 minutes prior to commencing nasal allergen dosing. During this time subjects will again have their baseline assessments and be exposed to ragweed pollen challenge (NAC#2), and will use a study diary to complete TNSS at -30, 0, 15, 30, 45, 60, 90, and 120 minutes and PNIF at -30, 0, 15, 30, 60, and 120 minutes. Differences in the pre-and post-treatment values for NAC-induced nasal symptoms and PNIF will be compared.

11.8 12-Lead ECG

12-lead ECG will be taken at Screening (Visit 1), Week 2 (Visit 3), and Follow-up #2 (Visit 5) during Part 1. Standard 12-lead ECGs will be performed according to the site SOPs. ECGs should be performed after the subject has been resting supine for t 5 minutes. The ECG will include all 12 standard leads and a Lead II rhythm strip on the bottom of the tracing. The ECG will be recorded at a paper speed of 25 mm/sec. The following ECG parameters will be [illegible] All ECGs must be evaluated by a qualified physician for the presence of abnormalities.

11.9 Laboratory Assessments

Blood sample will be taken for hematology and clinical chemistry, including standard metabolic panel, complete blood count (CBC), clinical chemistry, lipid panel, and liver function test. Approximately 15 ml of whole blood will be drawn. Urinalysis will be performed as part of safety labs. Laboratory assessments will be made at Screening (Visit 1), Week 2 (Visit 3), and Follow-up #2 (Visit 5) during Part 1, Screening (Visit 1) and NAC #2 (Visit 4) during Part 2, and at Unanticipated Visit should one occur.

Blood samples should be taken using standard venipuncture techniques. Blood sampling will be performed according to the site SOPs.

The following laboratory variables will be determined: Fasting glucose, Uric acid, BUN (blood urea nitrogen), Creatinine, BUN/creatinine ratio, eGFR (estimated glomerular filtration rate), Sodium, Potassium, Chloride, Calcium, Albumin, Bilirubin, Alkaline phosphatase, AST (aspartate aminotransferase), ALT (alanine transaminase), total cholesterol, HDL, LDL, triglycerides, CBC, ACE (angiotensin converting enzyme), and proteinurea.

Any value outside the normal range (except those affected by not fasting) will be flagged for the attention of the Investigator or designee at the site. The Investigator or designee will indicate whether or not the value is of clinical significance. If the result of the clinical chemistry test from the samples taken during the screening phase is indicated as clinically significant, the study subject will NOT be allowed into the study.

11.10 Urine Pregnancy Test

Standard urine pregnancy testing will be performed on WOCBP as part of screening.

11.11 Nasal Fluid Samples for Biomarkers

Nasosorption with paper and then synthetic absorptive matrices (SAM) has been used to sample nasal mucosal lining fluid (27). This technique can be considered as “precision mucosal sampling”, since it samples directly from the respiratory mucosa, and is free from the salivary contamination that occurs in breath and sputum sampling. Nasosorption sampling involves manipulating the synthetic absorptive matrix (SAM) up the lumen of the nasal cavity, and then holding it in position against the mucosa by external firm finger pressure. This is more

comfortable and less invasive than using a conventional swab, where rotation against the mucosal surface is generally required.

Nasosorption will be performed by placing strips of a hydrophilic polyester absorptive matrix (Mucosal Diagnostics, Hunt Developments Ltd., Midhurst, UK: available as a CE-marked device) measuring 7 × 35 mm into each nostril for 1 min. When the SAM strip is removed, it will be frozen at □80 °C for biostorage until elution, processing, and biomarker testing. After shipment to a designated lab, SAM strips will be DVKHGLQ3%6EXIIHUS+O□ containing BSA (1%) and Triton X 100 (1%) within the cup of a spin filter insert (Costar® Spin-X®). Mucosal lining fluid will be then eluted from the SAM by spin filter centrifugation (5 min at 16,000G at 4 °C), left and right nostril samples combined, and aliquots tested for biomarkers to evaluate the immune response.

Nasal fluid for biomarkers will be collected at Baseline visit (Visit 2), Week 2 visit (Visit 3), and Follow-up #2 (Visit 5) during Part 1 and NAC #1 (Visit 3; pre (0 min) and post NAC (20, 60 min)) and NAC #2 (Visit 4; pre (0 min) and post NAC (20, 60 min)) during Part 2 according to Appendix A. Samples will also be collected in the event of an Unanticipated Visit.

Samples will be evaluated for select biomarkers that may include a few of the following (27):

1. Nasal inflammatory cytokines/chemokines/immune cell markers: PGD2, beta-tryptase, histamine, C3a/4a/5a, IL-1beta, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-16, IL-17, IL-18, TNF-alpha, GM-CSF, MIP-1 alpha, MIP-1 beta, MCP-1, Eotaxin-1, MDC

Optionally, samples may be evaluated for nasal microbiome (e.g., PCR) to confirm presence of B244.

11.12 Blood Collection for Biomarkers

In addition to the blood drawn for the safety laboratory assessments, additional whole blood will be collected for potential biomarker analysis at Baseline visit (Visit 2), Week 2 visit (Visit 3), and Follow-up #2 (Visit 5) during Part 1 and NAC #1 (Visit 3; post NAC (120 min)) and NAC #2 (Visit 4; post NAC (120 min)) during Part 2 according to Appendix A. Samples will also be collected in the event of an Unanticipated Visit.

Approximately 15 ml of whole blood will be drawn for biomarkers at each visit. Patients will be asked to fast for at least 8 hrs before blood for biomarkers is drawn.

Samples will be processed to serum or plasma on site and stored at -80 □C until shipment to a designated central lab for biostorage. Upon Sponsor approval, biomarkers will be evaluated for immune response.

Samples will optionally be evaluated for biomarkers that may include a few of the following select nasal inflammatory cytokines and chemokines (27):

1. Nasal inflammatory cytokines/chemokines/immune cell markers: PGD2, beta-tryptase, histamine, C3a/4a/5a, IL-1beta, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-16, IL-17, IL-18, TNF-alpha, GM-CSF, MIP-1 alpha, MIP-1 beta, MCP-1, Eotaxin-1, MDC

11.13 Peak Nasal Inspiratory Flow (PNIF)

Peak Nasal Inspiratory Flow (PNIF) is another commonly used method for assessing nasal patency. PNIF provides an objective measurement of nasal airflow obstruction. It has the advantage of being simple, non-invasive and easily taught so participants can perform it on their own. The outcome is a direct representation of nasal congestion and therefore can provide objective confirmation of the subjective TNSS.

During Part 1, PNIF will be obtained during baseline visit (Visit 2), week 2 visit (Visit 3), follow-up #1 (Visit 4), and follow-up #2 (Visit 5).

During Part 2, -30 min and baseline (0 min) PNIF will be recorded prior to NAC titration. After 15, 30, 60, and 120 minutes of the single provocative NAC titration dose, PNIF will be recorded. Subjects will return 14 days after the washout period for basic health checks and baseline PNIF assessments followed by confirmatory NAC (NAC#1) (Visit 3). During this time subjects will obtain PNIF at -30 (30 minutes prior to NAC), 0 (baseline prior to NAC), 15, 30, 60, and 120 minutes. Subjects will return to the clinic for the last dose following the 14 days of at home dosing for their final visit (Visit 4). During this time subjects will be exposed to ragweed pollen challenge (NAC#2) and obtain PNIF at -30, 0, 15, 30, 60, and 120 minutes.

11.14 Intranasal Nitric Oxide

Exhaled nasal nitric oxide (NO) will be measured from the nasal cavity by a NO analyzer.
During Part 1, nasal NO will be obtained during baseline visit (Visit 2), week 2 visit (Visit 3), follow-up #1 (Visit 4), and follow-up #2 (Visit 5).

During Part 2, baseline nasal NO will be recorded prior to NAC titration. After 15 minutes of each NAC titration concentration, NO will be recorded. Subjects will return 14 days after the washout period for basic health checks and baseline NO assessments followed by confirmatory NAC (NAC#1) (Visit 3). During this time subjects will obtain NO at 0 (baseline prior to NAC), and 120 minutes. Subjects will return to the clinic for the last dose (15 min prior to NAC #2) following the 14 days of at home dosing for their final visit (Visit 4). During this time subjects will be exposed to ragweed pollen challenge (NAC#2) and obtain NO at 0 and 120 minutes.

11.15 Nasal Inflammation Score

Nasal inflammation will be graded by a clinician using a 0-3 nasal inflammation scale based on otoscope measurement where 0 = none, 1 = mild, 2 = moderate, and 3 = severe inflammation.

During Part 1, nasal inflammation score will be obtained during baseline visit (Visit 2), week 2 visit (Visit 3), follow-up #1 (Visit 4), and follow-up #2 (Visit 5).

During Part 2, baseline nasal inflammation score will be recorded prior to NAC titration. After 15 minutes of each NAC titration concentration, nasal inflammation score will be recorded. Subjects will return 14 days after the washout period for basic health checks and baseline nasal inflammation score assessments followed by confirmatory NAC (NAC#1) (Visit 3). During this time subjects will obtain nasal inflammation score at 0 (baseline prior to NAC), and 120 minutes. Subjects will return to the clinic for the last dose (15 min prior to NAC #2) following the 14 days of at home dosing for their final visit (Visit 4). During this time subjects will be exposed to ragweed pollen challenge (NAC#2) and obtain nasal inflammation score at 0 and 120 minutes.

11.16 Total Nasal Symptom Score (TNSS)

The Total Nasal Symptom Score (TNSS) is the sum of sub-scores for each of nasal congestion, sneezing, nasal itching, and rhinorrhea at each time point, using a four point scale (0–3; 0 = none; 1 = mild; 2 = moderate; 3 = severe), where 0 indicates no symptoms, a score of 1 for mild symptoms that are easily tolerated, 2 for awareness of symptoms which are bothersome but tolerable and 3 is reserved for severe symptoms that are hard to tolerate and interfere with daily activity. TNSS is calculated by adding the sub-score for each of the symptoms to a total out of 12.

During Part 1, subjects will dose twice daily (am and pm) at home for 2 weeks and record their dosing, any occurrence of an AE, and TNSS during treatment and through 28 days of follow-up using a study diary.

During Part 2, -30 min and baseline (0 min) TNSS will be recorded prior to NAC titration. After 15, 30, 45, 60, 90, and 120 minutes of the single NAC titration concentration, TNSS will be recorded. Subjects are required to meet the minimum qualifying TNSS score of a change of 6/12 from their baseline score, on at least 2 diary cards. Subjects will return 14 days after the washout period for basic health checks and baseline TNSS assessments followed by confirmatory NAC (NAC#1) (Visit 3). During this time subjects will use a study diary to complete TNSS at -30 (30 minutes prior to NAC), 0 (baseline prior to NAC), 15, 30, 45, 60, 90, and 120 minutes. TNSS is the sum of four individual symptom scores (running nose, congestion, itchy nose, and sneezing) rated on a four point scale (0-3). Subjects are required to meet the minimum qualifying TNSS score of a change of 6/12 from their baseline score, on at least 2 diary cards. Subjects meeting the requirement will be randomized to one of the three treatment arms. Randomization will be 1:1:1 so that equal number of subjects will be treated in each arm of the study. Subjects will then be trained on the use of the nasal spray, and have their first dose of treatment on site (self-dosing). Subjects will remain onsite for observation for 1 hour post dosing. Subjects will then be dispensed study medication for 14 days of at home dosing. While at home, subjects will dose twice daily (am and pm), and record their dosing, and any occurrence of an AE and TNSS using

a study diary. Subjects will return to the clinic for the last dose following the 14 days of at home dosing for their final visit (Visit 4). During this time subjects will again have their baseline assessments and be exposed to ragweed pollen challenge (NAC#2), and will use a study diary to complete TNSS at -30, 0, 15, 30, 45, 60, 90, and 120 minutes.

11.17 Study Diary

Subjects will be asked to fill out a daily treatment diary during screening, baseline, treatment, follow-up, and challenge period for dosing, nasal symptom scores (TNSS) and AEs.

Participants will record their nasal symptoms on diary cards that include symptoms of runny nose, nasal congestion, sneezing and nasal itching. Each symptom will be scored from 0–3, 0 indicating the absence of the symptom and 3 describing the symptom as severe and intolerable as described in Section 11.16.

AEs will be distinguished between immediate (onset of reaction is during the first 30 minutes after administration) and delayed (onset of action is after the first 30 minutes of administration) effects for safety reporting of adverse events.

11.18 Sample Shipment

All laboratory testing samples are to be shipped overnight in designated temperature conditions to the central laboratory where samples will be analyzed.

All frozen serum and nasal fluid for biomarkers are to be shipped monthly on dry ice to the central laboratory. Shipments should be made only on Mondays and Tuesdays to ensure receipt of the specimens by Friday.

12 SAFETY ASSESSMENTS

12.1 Compliance

Participants will be asked to bring study medication with them to selected scheduled visits (Visits 2 and 3 during Part 1; Visits 3 and 4 during Part 2). Study sites will be provided with a scale and weight of the study medication will be obtained before the first use (at Visit 2 during Part 1; at Visit 3 during Part 2) and at each visit after use (Visit 3 during Part 1; Visit 4 during Part 2).

At Baseline, study personnel will be asked to take out study medication bottle from the carton, weigh the bottle and record the weight. At each subsequent visit, study personnel will need to weigh the bottle without the carton and record weight. This procedure should be followed every time study medication is dispensed and returned. Sites will be provided scales, which will be calibrated prior to each use. Weight will be recorded in grams. Study personnel will be instructed to record measurements into the eCRF.

12.2 Safety Monitoring

Local and systemic adverse events (AEs) related to intranasal route of administration during 14 days of treatment and 28 days of follow-up (7 and 28 days after the last dose of the study) will be recorded for the safety phase of the study in healthy volunteers (Part 1). Additionally, local and systemic AEs will be recorded in subjects with a history of seasonal allergic rhinitis during the efficacy phase of the trial (Part 2).

Safety monitoring will include infectious complications related to B244 (by symptoms and/or testing; pulmonary, nasal, neurological), local and systemic AEs related to intranasal route during treatment and at follow-up. Local AEs include runny nose, nasal congestion, sneezing, nasal itching, palate itching, anosmia, nasal ulceration, nasal bleeding, sore throat, cough, Bell's palsy, other neurologic complications, fevers, chills, headache, muscle aches, decreased appetite, nausea, vomiting, and rash.

Sponsor will distinguish between immediate (onset of reaction is during the first 30 minutes after administration) and delayed (onset of action is after the first 30 minutes of administration) effects for safety reporting of adverse events.

Due to the nature of the nasal allergen challenge (in Part 2 of the study) which consists of delivering allergen directly into the airways of an allergic individual, a medical doctor trained for treating anaphylaxis must be present within the building. A crash cart and the crash team telephone number must be readily available. The nasal allergen challenge involves spraying a solution with very small amounts of ragweed antigen (allergy causing substance) into the nose. The side effects of this can be nasal blockage, excessive nasal discharge (similar to seasonal allergy symptoms), which may be delayed by several hours. There is also a risk of bronchoconstriction (wheezing), especially if one has a history of asthma. The most serious risk of having a nasal challenge test is anaphylactic reaction. This reaction is a medical emergency, causing difficulty breathing and a dangerously low blood pressure. However, anaphylactic reactions with nasal change test are rare and the physician will be monitoring subjects closely after the challenge during the time they are in clinic. If subjects notice symptoms noted above after they have left the clinic, they should call 911 immediately.

A 180 day follow up contact for collecting serious adverse events and new onset medical conditions for Part 1 will be the medical monitor described in section 15.7 (Larry Weiss, MD, lweiss@aobiome.com; 617-475-1605; or Carmen Margaritescu, MD, carmen.margaritescu@integrium.com; 714-328-7083). Alternatively, the site will contact subjects 180 days after the last visit and after study close out to collect SAEs or new onset medical conditions.

12.3 Pregnancy Reporting

Any pregnancy will be reported by study participants during their study participation.

Participants who report pregnancy or lactation during the review of inclusion/exclusion criteria prior to randomization will not be enrolled in the trial. In case of pregnancy, Investigational Product should be discontinued and the Sponsor informed. Follow-up of the pregnancy will be mandatory until the outcome is available.

12.4 Study Completion

A completed participant is one who has completed all study visits. Day 42 study visit is defined as the participant's last visit (Visit 5) for Part 1, while Day 28 study visit is defined as the participant's last visit (Visit 4) for Part 2.

12.5 Internal Safety Committee

An internal safety committee meeting will review the 2 week treatment safety data during Part 1 phase of the study and, if there are no safety concerns, will proceed to Part 2 of the study to evaluate preliminary efficacy in subjects with a history of seasonal allergic rhinitis (SAR).

12.6 Subject Withdrawal Criteria

A subject may withdraw from the study at any time at his/her own request, or may be withdrawn at any time at the discretion of the Investigator for safety, behavioral, or administrative reasons.

Reasons for withdrawal (subjects who refuse to return for any remaining study visits) or discontinuation (subjects who prematurely stop the application) at any time during the study may include, but are not limited, to the following:

- For safety reasons, either at the discretion of the Investigator or at the subject's request
- For protocol violations at the discretion of AOBiome
- Due to concomitant therapy that could interfere with the results of the study (the Investigator will report all such information on the CRFs and decide, in accordance with AOBiome, whether the subject is to be withdrawn).

All premature discontinuations and their causes must be carefully documented by the Investigator on the CRF or if needed on the AE form.

If, for any reason, a subject is withdrawn before completing the final visit, the reason for termination will be entered on the CRF. All data gathered on the subject prior to termination will be made available to AOBiome. Subjects not completing the entire study should be fully evaluated when possible. The appropriate CRFs should be completed.

If the subject chooses to withdraw before completing the study, the subject should notify the study coordinator who will instruct the subject on completion of assessments for Unanticipated

visit (Appendix A). For subjects who refuse to complete the assessments for their early termination, every attempt must be made to check on their status, using any mode of communication such as telephone, email, fax, or text.

13 EFFICACY ASSESSMENTS

Efficacy endpoints will be descriptively summarized and will include the number of observations, mean, median, standard deviation, minimum, and maximum of scores/values at all applicable time points and for all treatments in the ITT Population.

For Part 2 phase of the study, the following secondary efficacy assessments will be obtained:

- Change in TNSS from baseline to 14 days of prophylaxis treatment.
- Change in subjective nasal symptom scores of nasal congestion, rhinorrhea, nasal itching, and sneezing from baseline to 14 days of prophylaxis treatment.
- Nasal symptom-free response rate within each individual symptom scores and percentage of subjects with a 25% or 50% reduction in TNSS after 14 days of prophylaxis treatment.

The following exploratory efficacy assessments will be obtained:

- Change in Peak Nasal Inspiratory Flow (PNIF), nasal inflammation score using otoscope, intranasal nitric oxide levels, and cytokine concentration in nasal cavity and blood from baseline to 14 days of B244 application and during prophylaxis treatment setting.

Fisher's exact test will be used for efficacy parameters that report frequencies or incidence of measured events to make treatment group comparisons. For the continuous endpoints a one-sided two-sample equal-variance z-test will be used to compare treatment group means.

14 STATISTICAL CONSIDERATIONS

14.1 Sample Size

Part 1 (safety and tolerability evaluation in healthy volunteers) will enroll 24 subjects. Up to 12 subjects per dose cohort (n=8 active, n=4 vehicle) will be enrolled, with a target of 20 subjects completed (10 subjects per dose cohort; n=7 active, n=3 vehicle) assuming a 16.7% drop out rate. The two dose cohorts are $1x10^9$ cells/ml and $4x10^9$ cells/ml.

Part 2 (preliminary efficacy evaluation in subjects with a history of SAR to ragweed pollen) will enroll 42 subjects. Up to 14 subjects per arm ($1x10^9$ cells/ml; $4x10^9$ cells/ml or vehicle) will be enrolled, with a target of 12 subjects per arm for a total of 36 subjects completed, assuming a 10-15% drop-out rate.

14.2 Populations for Analysis

ITT: includes all randomized participants.

Safety: includes all subjects who received at least 1 dose of study medication.

Per Protocol: subjects who administered at least 50 % of IP, have at least one baseline and post baseline in clinic visit and did not have any major protocol violations.

14.3 Data Analysis

The analyses will be conducted on all participant data when the trial ends. Data will be presented by strata treatment and overall.

Categorical variables will be summarized by frequency distributions (number and percentages of subjects) and continuous variables will be summarized by descriptive statistics (mean, standard deviation etc).

Adverse events will be summarized by treatment using the Medical Dictionary for Regulatory Activities (MedDRA) system organ class and preferred term. Separate tabulations will be produced for all treatment-emergent AEs (TEAEs), treatment-related AEs (those considered by the Investigator as at least possibly drug related), SAEs, discontinuations due to AEs, and AEs [illegible] subject listings will be provided for any deaths, SAEs, and AEs leading to discontinuation.

Descriptive statistics for each sequence will be provided for clinical laboratory values (e.g., hematology, serum chemistry, and urinalysis) and vital signs data, presented as both actual values and changes from baseline relative to each on-study evaluation. Abnormal clinical laboratory values will be listed.

All data will be provided in by-subject listings.

14.3.1 Disposition

A tabulation of the disposition of subjects will be presented, including the number enrolled, the number randomized, the number treated, and the reasons for study discontinuation will be reported. Summaries of the number in each analysis set will be summarized. Entry criteria and protocol deviations will be listed.

14.3.2 Demographic and Baseline

Demographic and baseline characteristic data summarization will be performed in order to descriptively assess the comparability of strata and treatments. Data to be tabulated will include age, race, ethnicity, height, weight, and BMI, as well as baseline characteristics related to medical history.

14.4 Safety Analyses

14.4.1 Definitions

All adverse events recorded during the study will be coded according to Medical Dictionary for Regulatory Activities.

The primary analysis of safety will be based on the safety population, which is defined as all subjects receiving at least 1 dose of B244 or placebo. Adverse events (AEs) will be coded using the Medical Dictionary for Regulatory Activities (MedDRA®) for purposes of summarization. All AEs occurring during the study will be included in by-subject data listings and tabulated. Events leading to death, SAEs, and events resulting in study discontinuation will be summarized using standard descriptive statistics, and presented by treatment arm. Exposure to study drug and reasons for discontinuation of study treatment will be tabulated. Shift tables relative to the normal range will be provided for laboratory parameters. Additional analyses will be performed, if warranted, upon review of the data.

14.4.2 Adverse Events

All adverse events (AEs) recorded during the study through the date of randomization through 28 days after the last dose of study drug will analyzed.

All AE's will be coded according to Medical Dictionary for Regulatory Activities and summarized using System Organ Class (SOC) and Preferred Term (PT).

AE's will be summarized using incidence rates. Therefore, each subject will only contribute once for a given adverse event SOC or PT.

A summary of the incidence of any adverse event, SAE, and adverse events leading to discontinuation will be presented. Summaries will display, by strata and treatment sequence, the incidence of patients with events, the frequency of patients with events within each primary system organ class and by preferred terms. For each preferred term and each system organ class a patient will be counted only once. For summaries on severe or drug-related AE, for a given patient, the highest severity or relationship for a specific preferred term will be considered.

14.4.3 Deaths and Serious Adverse Events

Serious adverse events and events leading to death will be summarized overall and by primary system organ class and preferred term.

14.4.4 Adverse Events Leading to Treatment Discontinuation

Adverse events leading to treatment discontinuation will be summarized overall and by primary system organ class and preferred term.

14.5 Efficacy Analyses

Additional exploratory analyses will be defined in the Statistical Analysis Plan.

14.5.1 Handling of Dropouts or Missing Data

Missing data will not be imputed for analysis.

Subjects who dropout after enrollment but prior to randomization will be replaced.

14.6 Clinical Trial Protocol Deviations

All the following deviations will be summarized on the all randomized patient population:

- x Inclusion or exclusion criteria not satisfied.
- x Deviations related to the Investigational Product administration
- x Not permitted concomitant medications.

15 ADVERSE EVENTS (AE) AND SERIOUS ADVERSE EVENTS (SAE)

The Investigator and study staff are responsible for detecting and recording AEs and SAEs during scheduled safety evaluations and whenever such information is brought to their attention. This section of the protocol provides definitions and detailed procedures to be followed.

15.1 Definition of an AE

An AE is any untoward medical occurrence in a study participant which is temporally associated with the use of a medicinal product, regardless of its potential relationship to the medicinal product. An AE, therefore, can be any unfavorable or unintended sign, including an abnormal symptom, or disease (new or exacerbated), whether or not related to the investigational product (IP).

Examples of an AE include:

- Exacerbation of a pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after investigational product administration even though it may have been present prior to the start of the study.
- Signs, symptoms of a drug interaction.
- Signs, symptoms of a suspected overdose of either investigational product or a concurrent medication (overdose per se should not be reported as an AE/SAE).

AEs may include pre- or post-treatment events that occur as a result of protocol-mandated procedures (i.e., modification of participant's previous therapeutic regimen).

15.2 Definition of a SAE

A serious adverse event is any untoward medical occurrence that, at any dose:

(a) results in death.

(b) is life-threatening.

NOTE: The term 'life-threatening' in the definition of 'serious' refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.

(c) requires hospitalization or prolongation of existing hospitalization.

NOTE: In general, hospitalization signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or out -patient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious. Routine hospitalizations or elective surgeries are generally not regarded as SAEs.

(d) results in disability/incapacity, or

NOTE: The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g. sprained ankle) which may interfere or prevent everyday life functions but do not constitute a substantial disruption.

(e) is a congenital anomaly/birth defect

(f) Medical or scientific judgment should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. Examples of such events are invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

15.3 Time Period, Frequency, and Method of Detecting AEs and SAEs

All AEs occurring after administration of the first dose of study medication and on or before the final assessment must be reported as AEs. All AEs must be recorded irrespective of whether they are considered drug-related.

At each assessment in the period defined above, AEs will be evaluated by the Investigator and recorded.

Any AEs already documented at a previous assessment and designated as ongoing, should be reviewed at subsequent assessments as necessary. If these have resolved, this should be documented. Changes in intensity or frequency of AEs should be recorded as separate events (i.e., a new record started).

The recording of AEs and SAEs are described in Section 14.4 ("Recording of AEs and SAEs").

15.4 Recording of AEs and SAEs

All clinical events, including either observed or volunteered problems, complaints or symptoms are to be recorded on the Adverse Events page(s) of the CRF. The need to capture this information is not dependent upon whether the clinical event is associated with study treatment. Adverse clinical events resulting from concurrent illnesses or reactions to concurrent medications are also to be recorded. In order to avoid vague, ambiguous, or colloquial expressions, the AE should be recorded in standard medical terminology rather than the participant's own words.

Each adverse clinical event is to be evaluated for duration, intensity, and whether the event may be associated with the investigational product (IP) or other causes. Start and stop dates, relationship to investigational product (IP), medical management, and alternative causality of event must be recorded in the Adverse Events section of the CRF. AEs believed to be possibly related to investigational product (IP) must be followed until resolution.

15.5 Evaluating AEs and SAEs

15.5.1 Severity Rating

The severity of an adverse event (AE and SAE) is to be scored according to the following scale:

Mild	Awareness of sign or symptom, but easily tolerated
Moderate	Discomfort enough to cause interference with usual activity
Severe	Incapacitating with inability to work or perform usual activity

An AE that is assessed as severe should not be confused with a SAE. An event is defined as 'serious' when it meets one of the pre-defined outcomes as described in Section 15.2 "Definition of a SAE".

15.5.2 Relationship to Investigational Product (IP)

SAEs will be classified as "**definitely not related**", "**unlikely related**", "**possibly related**", "**probably related**", or "**definitely related**" (including unknown).

For AEs, the relationship to study treatment is to be assessed according to the following definitions:

> **Definitely not related:** The AE is definitely not related to the drug. This designation should be reserved for those events which occur prior to study treatment or for those events which cannot be even remotely related to study participation (e.g., injuries sustained in an automobile accident).
>
> **Unlikely related:** There is no reasonable association between the study treatment and the suspected event and the event could have been produced by the participant 's clinical state or other modes of therapy administered to the participant.
>
> **Possibly related:** The suspected adverse event may or may not follow a reasonable temporal sequence from study treatment administration but seems to be the type of reaction that cannot be dismissed as unlikely. The event could have been produced or mimicked by the participant 's clinical state or by other modes of therapy concomitantly administered to the participant.
>
> **Probably related:** The suspected adverse event follows a reasonable temporal sequence from study treatment administration, abates upon discontinuation of the treatment, and cannot be reasonably explained by the known characteristics of the participant 's clinical state.
>
> **Definitely related**: This designation should be reserved for those events which have no uncertainty in their relationship to treatment administration.

15.6 Pregnancy

Any pregnancy that occurs in a female participating in the study must be reported to the Sponsor within 3 working days of learning of the pregnancy. Follow-up must occur to determine the outcome of the pregnancy (including premature termination) and the status of mother and child. Pregnancy complications and elective terminations for medical reasons must be reported as AEs or SAEs. Spontaneous abortions must be reported as an SAE.

Any SAE occurring in association with a pregnancy and considered by the Investigator as possibly related or related to the investigational product must be promptly reported to the Sponsor, even if the event occurred after the participant completed the study.

The Investigator must attempt to collect pregnancy information on any female partners of male participants who become pregnant while the male participant is enrolled in the study. Pregnancy information must be reported to the Sponsor as described above.

15.7 Prompt Reporting of SAEs to the Sponsor

In the case of a Serious Adverse Event the Investigator must immediately:

- **SEND** (within 1 working day) a scan of the signed and dated corresponding page(s) in the Case Report Form and SAE form to the representative of the Monitoring Team whose name, phone number and email appear on the Clinical Trial Protocol (Larry Weiss, MD, lweiss@aobiome.com; 617-475-1605), or to a designated Safety contact provided by the Monitoring Team (Carmen Margaritescu, MD, carmen.margaritescu@integrium.com; 714-328-7083), as well as to the Central Database number;
- **ATTACH** a photocopy of all examinations carried out and the dates on which these examinations were performed. Care should be taken to ensure that the patient's identity is protected and the patient's identifiers in the Clinical Trial are properly documented on all copies of source documents provided to the Sponsor. For laboratory results, include the laboratory normal ranges;
- **Follow-up of any Serious Adverse Event** that is fatal or life threatening should be provided within one additional calendar week. The treatment code will be unblinded for reporting of Serious Adverse Events that are unexpected and reasonably associated with the use of the Investigational Product.

Follow-up

- The Investigator should take all appropriate measures to ensure the safety of the patients, including referral to a specialist if indicated. Notably he/she should follow up the outcome of any adverse events (clinical signs, laboratory values or other, etc) until the return to normal or stabilization of the patient's condition;
- In the case of any serious adverse event, the patient must be followed up until clinical recovery is complete and laboratory results have returned to normal, or until progression has been stabilized. This implies that follow-up may continue after the patient has left the Clinical Trial and that additional investigations may be requested by the Monitoring Team;
- In case of any serious adverse event brought to the attention of the Investigator at any time after cessation of Investigational Product and considered by him/her to be caused by the Investigational Product with a reasonable possibility, this should be reported to the Monitoring Team.

Primary Contact
AOBiome Reportable Events Hotline **24 Hour Phone: 617-475-1605** **Email: lweiss@aobiome.com** **Call medical monitor to email a scanned report**

16 ETHICAL AND REGULATORY STANDARDS

16.1 Ethical Conduct of Study

This clinical trial was designed and shall be implemented, executed, and reported in accordance with the ICH Harmonized Tripartite Guidelines for Good Clinical Practice, with applicable local regulations (including US Code of Federal Regulations Title 21), and with the ethical principles laid down in the Declaration of Helsinki.

The Investigator(s) should conduct the study in accordance with this protocol, the Declaration of Helsinki and ICH GCP guidelines and FDA regulations. The Investigator(s) and the Sponsor will sign the protocol and study contract, to confirm agreement. The Investigator(s) will not implement any amendment (deviation or changes of the protocol) without agreement by the Sponsor and IRB approval, except where necessary to eliminate immediate hazard(s) to study participants, or when change(s) involve only logistical or administrative aspects of the study.

Records that may reveal the identities of participants must be well protected, with consideration given to confidentiality and the right to privacy of participants.

16.2 Laws and Regulations

This Clinical Trial will be conducted in compliance with all international laws and regulations, and national laws and regulations of the countries in which the clinical trial is performed, as well as any applicable guidelines. The trial will be registered and updated on www.clintrials.gov and on other sites, as deemed appropriate.

16.3 Informed Consent

Each participant must be provided with a statement that the investigation involves research and that the IRB has approved solicitation of participants to participate; a fair explanation of the procedures to be followed and their purposes, including identification of any procedures which

are experimental; a description in lay language of any possible side effects; a description of any attendant discomforts and risks reasonably to be expected; a description of any benefits reasonably to be expected; a disclosure of any appropriate alternative procedures that might be advantageous for the participant; an offer to answer any inquiries concerning the procedures, and instruction that the person is free to withdraw consent and discontinue participation in the project or activity at any time without prejudice to the participant. Payment to research participants for taking part in the study is considered a benefit. All information concerning payment, including the schedule of payments, must be set forth in the informed consent, including a disclosure that the Investigator is being paid to perform the stated research.

A participant must give manual paper consent to take part in the study. Participants below the age of majority in the municipality must give written assent to participate in this study. This consent must be dated and retained by the Principal Investigator as part of the study records. A downloadable digital copy shall be given to the person signing the form. The informed consent process must be documented in the participant's source documents.

The Investigator agrees that the Sponsor, its employees or agents will have the right from time to time during the course of this study to audit and review pertinent medical records relating to this clinical trial. A statement will be obtained from each person participating in the study permitting the release of his/her medical records as necessary for inspection by authorized personnel of the Sponsor, FDA, and the staff managing the clinical study.

The release of medical records and the review of the contents will be in compliance with the Health Insurance Portability and Accountability Act of 1996 (HIPAA).

16.4 Institutional Review Board/Independent Ethics Committee (IRB/EC)

The protocol and informed consent form and the electronic version of the consent for this study must be approved by the IRB. A copy of the Letter of Approval from the Board, which contains specific identification of the documents approved, must be received by the Sponsor prior to shipment of drug supplies to the Principal Investigator.

All changes to the protocol, as well as a change of Investigator, must also be approved by the IRB and documentation of this approval provided to the study monitor. Records of the IRB's review and approval of all documents pertaining to the study must be kept on file by the Principal Investigator and are subject to FDA inspection at any time. IRB renewal for approval is required each year. The Investigator is to AOBiome, in writing, of the approval to continue the study.

16.5 Clinical Monitoring/Record Keeping

There shall be no alterations in the protocol design without the written consent and approval of the Sponsor and the approval of the IRB, except in the case that participants are at immediate risk without immediate implementation of such alterations. In the aforementioned situation, the

sites should notify the Sponsor and IRB of the deviation as soon as possible, and should seek the written consent and approval of the Sponsor and the approval of the IRB.

All results of this trial must be recorded on eCRFs. Each participant who has been randomized must have a completed eCRF. Reasons for termination must be stated in the early termination section. Study participants are not to be identified by name on eCRFs, but rather by coded identifiers and participant initials.

The study monitor will verify the accuracy of the data by reviewing pertinent source documents such as office records or hospital charts of the participants.

Study records include eCRFs, signed FDA Form 1572, original reports of test results, and digitally signed electronic informed consent forms. IRB approval letters and other documents pertaining to the conduct of the study are to be kept on file by the Investigator. If the study files are assigned to someone else or removed to another location, the Investigator is to notify the study monitor or Sponsor in writing of the change. All study records are subject to FDA inspection at any time.

All information supplied to the Investigator by the Sponsor before, during, and after the study is confidential. Such information is to be used solely in connection with the clinical study. The study protocol, IB, and any other pertinent study related materials or records provided are to be maintained in a confidential manner, reviewed carefully with attention to admonitions and returned to the Sponsor upon request. No part of these materials may be reproduced or transmitted in any form without prior written permission from the Sponsor.

17 ADMINISTRATIVE RULES

17.1 Curriculum Vitae

An updated, signed, and dated copy of the curriculum vitae limited to the experience, qualification and training for each Investigator and/or Sub-Investigator(s) will be provided to the Sponsor prior to the beginning of the Clinical Trial.

17.2 Archiving of Study Documentation

The Investigator must maintain confidential all study documentation, and take measures to prevent accidental or premature destruction of these documents.

It is recommended that the Investigator retain the study documents at least fifteen (15) years after the completion or discontinuation of the Clinical Trial. However, applicable regulatory requirements should be taken into account in the event that a longer period is required.

The Investigator must notify the Sponsor prior to destroying any study essential documents within the fifteen (15) year period following the Clinical Trial completion or discontinuation.

If the Investigator's personal situation is such that archiving can no longer be ensured by him/her, the Investigator shall inform the Sponsor and the relevant records shall be transferred to a mutually agreed upon designee.

17.3 Internal Safety Review Committee

An internal safety review committee will be set up to protect the ethical and safety interests of participants and to protect the scientific validity of the study. Adhoc safety interim analyses might be performed by an independent statistician if the safety review committee identifies potential safety signals during its routine blinded safety review. The details for the analysis plan will be documented in the trial’s Statistical Analysis Plan.

18 STUDY MONITORING

18.1 Responsibilities of the Investigator(s)

The Investigator(s) undertake(s) to perform the Clinical Trial in accordance with this Clinical Trial Protocol, ICH guidelines for Good Clinical Practice and the applicable regulatory requirements.

The Investigator is required to ensure compliance with all procedures required by the Clinical Trial Protocol and by study procedures provided by the Sponsor (including security rules). The Investigator agrees to provide reliable data and all information requested by the Clinical Trial Protocol (with the help of the Case Report Form [CRF], Discrepancy Resolution Form [DRF] or other appropriate instrument) in an accurate and legible manner according to the instructions provided and to ensure direct access to source documents to Sponsor representatives. If any particular circuits have to be defined (e.g., e-CRF, Fax), particular attention should be paid to the confidentiality of the patient's data to be transferred. The Investigator may appoint such other individuals as he/she may deem appropriate as Sub-Investigators to assist in the conduct of the Clinical Trial in accordance with the Clinical Trial Protocol. All Sub-Investigators shall be timely appointed and listed. The Sub-Investigators will be supervised by and under the responsibility of the Investigator. The Investigator will provide them with a Clinical Trial Protocol and all necessary information.

18.2 Responsibilities of the Sponsor

The Sponsor of this Clinical Trial is responsible to Health Authorities for taking all reasonable steps to ensure the proper conduct of the Clinical Trial Protocol as regards ethics, Clinical Trial Protocol compliance, integrity and validity of the data recorded on the Case Report Forms. Thus, the main duty of the Monitoring Team is to help the Investigator and the Sponsor maintain a high level of ethical, scientific, technical and regulatory quality in all aspects of the Clinical Trial. At regular intervals during the Clinical Trial, the sites will be contacted, through monitoring visits, letters or telephone calls, by a representative of the Monitoring Team to review study progress,

Investigator and patient compliance with Clinical Trial Protocol requirements, and any emergent problems. During these monitoring visits, the following but not exhaustive list of points will be scrutinized with the Investigator: patient informed consent, patient recruitment and follow-up, Serious Adverse Event documentation and reporting, outcome events documentation and reporting, Investigational Product allocation, patient compliance with the Investigational Product regimen, Investigational Product accountability, concomitant therapy use and quality of data.

18.3 Source Document Requirements

According to the ICH guidelines for Good Clinical Practice, the Monitoring Team must check the Case Report Form entries against the source documents, except for the preidentified source data directly recorded in the Case Report Form. The Informed Consent Form will include a statement by which the patient allows the Sponsor's duly authorized personnel, the Ethics Committee (IRB/EC), and the regulatory authorities to have direct access to source data which supports the data on the Case Report Forms (e.g., patient's medical file, appointment books, original laboratory records, etc.). These personnel, bound by professional secrecy, must keep confidential all personal identity or personal medical information (according to confidentiality rules).

18.4 Use and Completion of Case Report Forms (CRFs) and Additional Requests

The sponsor or CRO will be responsible for activities associated with the data management of this study. This will include setting up a relevant database and data transfer mechanisms, along with appropriate validation of data and resolution of queries. Data generated within this clinical study will be handled according to the relevant SOPs of the data management and biostatistics departments of sponsor or CRO.

For Electronic Data Capture (EDC):

Study sites will enter data directly into an EDC system by completing the eCRF via a secure internet connection. Data entered into the eCRF must be verifiable against source documents at the study sites. Any changes to the data entered into the EDC system will be recorded in an automated, secure audit trail and is Food and Drug Administration (FDA) Code of Federal Regulations (CFR) Title 21 Part 11 compliant.

Data entered into the eCRF will be validated as defined in the Data Validation Specifications (DVS). Validation includes, but is not limited to, validity checks (for example, missing data, range checks) and consistency checks (logical checks between variables) to ensure that study data are accurately reported. Additionally, CRO Data Management will perform aggregate data review as defined in the DVS to ensure that the data are complete, consistent and reasonable. The electronic edit checks will run continually throughout the course of the study and queries reviewed by CRO personnel to assure validity as compared to source records. Manual queries

may also be entered into EDC by Monitoring or Data Management personnel to address identified discrepancies.

Medical conditions/procedures will be coded using MedDRA and prior and concomitant medications will be coded using WHODrug.

At the conclusion of the study, each site will be provided with their subject CRFs in Portable Document Format (PDF) for archival. The CRF PDFs will contain subject data, audit trail information, queries including responses, and comments.

19 PUBLICATIONS

All data generated from this study are the property of AOBiome LLC and shall be held in strict confidence along with all information furnished by AOBiome. Independent analyses and/or publication of these data by the Investigator or any member of his/her staff is not permitted without prior written consent of AOBiome.

Any formal presentation or publication of data from this trial will be considered as a joint publication by the Investigator(s) and appropriate Sponsor personnel. Authorship will be determined by mutual agreement. Written permission to the Investigator will be contingent on the review by the Sponsor of the methodology and statistical analysis and any publication or presentation will provide for nondisclosure of AOBiome confidential or proprietary information. In all cases, the parties agree to submit all manuscripts or abstracts to all other parties at least 30 days prior to submission. This will enable all parties to protect proprietary information and to provide comments based on information that may not yet be available to other parties.

20 PROTOCOL ADHERENCE

This protocol defines the study objectives, the study procedures and the data to be collected on study participants. Additional assessments required to ensure safety of participants should be administered as deemed necessary on a case by case basis. Under no circumstances is an Investigator allowed to collect additional data or conduct any additional procedures for any research related purpose involving any investigational drugs under the protocol.

Investigators ascertain they will apply due diligence to avoid protocol deviations. If an Investigator feels a protocol deviation would improve the conduct of the study this must be considered a protocol amendment, and unless such an amendment is agreed upon by the sponsor and approved by the IRB/IEC and health authorities, where required, it cannot be implemented.

21 CONFIDENTIALITY

All information disclosed or provided by the Sponsor (or any company/institution acting on their behalf), or produced during the Clinical Trial, including, but not limited to, the Clinical Trial

Protocol, the CRFs, the Investigator's Brochure and the results obtained during the course of the Clinical Trial, is confidential. The Investigator or any person under his/her authority agrees to undertake to keep confidential and not to disclose the information to any third party without the prior written approval of the Sponsor. However, the submission of this Clinical Trial Protocol and other necessary documentation to the Ethics Committee (IRB/EC) is expressly permitted, the IRB/EC members having the same obligation of confidentiality. The Sub-Investigators shall be bound by the same obligation as the Investigator. The Investigator shall inform the Sub-Investigators of the confidential nature of the Clinical Trial. The Investigator and the Sub-Investigators shall use the information solely for the purposes of the Clinical Trial, to the exclusion of any use for their own or for a third party's account.

22 PROPERTY RIGHTS

All information, documents and Investigational Product provided by the Sponsor or its designee are and remain the sole property of the Sponsor. The Investigator shall not mention any information or the Product in any application for a patent or for any other intellectual property rights. All the results, data, documents and inventions, which arise directly or indirectly from the Clinical Trial in any form, shall be the immediate and exclusive property of the Sponsor. The complete verified database will be shared with the Operations Committee, which shall have full access to all data. The Sponsor may use or exploit all the results at its own discretion, without any limitation to its property right (territory, field, continuance). The Sponsor shall be under no obligation to patent, develop, market or otherwise use the results of the Clinical Trial. As the case may be, the Investigator and/or the Sub-Investigators shall provide all assistance required by the Sponsor, at the Sponsor's expense, for obtaining and defending any patent, including signature of legal documents.

23 DATA PROTECTION

The patient's personal data and Investigator's personal data which may be included in the Sponsor database shall be treated in compliance with all applicable laws and regulations;

When archiving or processing personal data pertaining to the Investigator and/or to the patients, the Sponsor shall take all appropriate measures to safeguard and prevent access to this data by any unauthorized third party.

24 SPONSOR AUDITS AND INSPECTIONS BY REGULATORY AGENCIES

For the purpose of ensuring compliance with the Clinical Trial Protocol, Good Clinical Practice and applicable regulatory requirements, the Investigator should permit auditing by or on the behalf of the Sponsor and inspection by applicable regulatory authorities.

The Investigator agrees to allow the auditors/inspectors to have direct access to his/her study records for review, being understood that this personnel is bound by professional secrecy, and as such will not disclose any personal identity or personal medical information.

The Investigator will make every effort to help with the performance of the audits and inspections, giving access to all necessary facilities, data, and documents. As soon as the Investigator is notified of a future inspection by the authorities, he will inform the Sponsor and authorize the Sponsor to participate in this inspection.

The confidentiality of the data verified and the protection of the patients should be respected during these inspections.

Any result and information arising from the inspections by the regulatory authorities will be immediately communicated by the Investigator to the Sponsor.

The Investigator shall take appropriate measures required by the Sponsor to take corrective actions for all problems found during the audit or inspections.

25 PREMATURE DISCONTINUATION OF THE STUDY OR PREMATURE CLOSE-OUT OF A SITE

25.1 Decided by the Sponsor in the Following Cases:

1. In the event the results of the Clinical Trial do not appear to be scientifically convincing to the Sponsor;
2. If the aim of the Clinical Trial has become outdated or is no longer of interest;
3. If the information on the product leads to doubt as to the benefit/risk ratio;
4. If the Investigator has received from the Sponsor all Investigational Product, means and information necessary to perform the Clinical Trial and has not included any patient after a reasonable period of time mutually agreed upon;
5. In the event of breach by the Investigator of a fundamental obligation under this agreement, including but not limited to breach of the Clinical Trial Protocol, breach of the applicable laws and regulations or breach of the ICH guidelines for GCP;
6. If the total number of patients are included earlier than expected; In any case the Sponsor will notify the Investigator of its decision by written notice.

25.2 Decided by the Investigator

The Investigator must notify (30 days' prior notice) the Sponsor of his/her decision and give the reason in writing. In all cases (decided by the sponsor or by the Investigator), the appropriate Ethics Committee(s) (IRB/EC) and Health Authorities should be informed.

26 CLINICAL TRIAL PROTOCOL AMENDMENTS

Any protocol amendments will be added as stand-alone documents. In addition, any and all revisions dictated by the amendments will be made in the protocol. Each time a protocol is amended, a new amended version date will be added to the cover page.

All appendices attached hereto and referred to herein are made part of this Clinical Trial Protocol. The Investigator should not implement any deviation from, or changes of the Clinical Trial Protocol without agreement by the sponsor and prior review and documented approval/favorable opinion from the IRB/EC of an amendment, except where necessary to eliminate an immediate hazard(s) to clinical trial patients, or when the change(s) involves only logistical or administrative aspects of the trial. Any change agreed upon will be recorded in writing, the written amendment will be signed by the Investigator and by the Sponsor and the signed amendment will be filed with this Clinical Trial Protocol. Any amendment to the Clinical Trial Protocol requires written approval/favorable opinion by the IRB/EC prior to its implementation, unless there are overriding safety reasons. In some instances, an amendment may require a change to the Informed Consent Form. The Investigator must receive an IRB/EC approval/favorable opinion concerning the revised Informed Consent Form prior to implementation of the change.

27 APPENDIX A-Schedule of Events

Visit name (Day)	Part 1					Part 2				
	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 1	Visit 2	Visit 3[13]	Visit 4[14]	
	Screening (Day -1)	Baseline (Day 0)	Week 2 (Day 14)	Follow-up #1 (Day 21)	Follow-up #2 (Day 42)	Screening (Day -1)	NAC titration (Day 0)	NAC #1 (Day 14)	NAC #2 (Day 28)	Unanticipated Visit
Visit Window, in Days	-21 to 0	-3 to 1	+/-3	+/-3	+/-3	-21 to 0	+/-3	+/-3	+/-3	
Informed Consent[1]	X					X				
Inclusion/Exclusion Criteria[2]						X				
Demographics						X				
Medical History						X				
Concomitant Medications				X	X	X		X	X	
HR, BP, and respiratory rate	X	[15]		X	X	X		X	X	
Brief Physical Exam	X	X	X	X	X	X	X	X	X	
Body Weight	X		X	X	X	X		X	X	
Height	X					X				
Allergen Skin Test (Skin Prick Test)						X[21]				
Blood Collection for IgE						X[21]				
ABPM[3]		X	X							
Randomization[4]								X		
Nasal inspection[5]	X	X[15]	X	X	X	X	X	X	X	X
NAC							X	X	X	
12-lead ECG	X		X		X					

Delivery of Investigational Product		X						X		
Investigational Product application								X	X	
Investigational Product compliance		X						X	X	X
Counseling[6]								X		X
Laboratory Testing[7]	X		X		X	X			X	X
Urinary pregnancy test[8]	X					X				
Safety AEs	X	X	X	X	X	X	X	X	X	X
Nasal fluid[9]			X		X			X[16]	X[16]	X
Blood collection[10]		X	X		X			X[17]	X[17]	X
Peak nasal inspiratory flow		X	X	X	X		X[22]	X[18]	X[18]	X
Intranasal nitric oxide		X	X	X	X		X	X[19]	X[19]	X
Nasal inflammation score[11]		X	X	X	X		X	X[19]	X[19]	X
Study diary[12]	X	X	X	X	X	X	X[22]	X[20]	X[20]	X
Subject satisfaction survey					X				X	

1. Informed consent can be obtained up to 21 days prior to the baseline visit.

2. Inclusion and exclusion criteria will be reviewed at Screening and at Baseline visit to make sure nothing changed.

3. Ambulatory Blood Pressure Monitoring for 24 hours to be performed for 24 hours prior to baseline and Week 2 visits. Subjects to visit site 24 hours prior to baseline and Week 2 visits to initiate ABPM. ABPM removal (after 24 hour measurement) at Visits 2 and 3 followed by evaluation of technical success/failure criteria.

4. Subject's demographic data and medical history are blinded to the statistician.

5. Nasal inspection will be performed by clinical examination of the nasal mucosa, sinuses, and upper airway.

6. Subjects will be counseled on the application of the nasal spray for the duration of the study.

7. Fasting blood sample for a comprehensive metabolic panel, complete blood count, chemistry, lipid panel, and liver function test-15 ml; include urinalysis. Patients should fast for at least 8 hours before the test. Blood will be shipped to the central lab for processing.

8. Urine pregnancy test to be done on WOCBP.

9. Nasal fluid will be collected at baseline, treatment, and follow-up visits for inflammatory cytokines during Part 1. Additionally, they will be collected during challenge visits per schedule of events. Optionally, samples may be evaluated for nasal microbiome (e.g., PCR) to confirm presence of B244.

10. Blood samples for biomarkers will be collected and processed on site. Serum and plasma samples will be frozen onsite and shipped to the Central lab for storage.

11. Nasal inflammation score assessment based on otoscope measurement.

12. Subjects will be asked to fill out a daily treatment diary during screening, baseline, treatment, follow-up, and challenge period for dosing, nasal symptom scores (TNSS) and AEs.

13. Subjects will have their basic health checks and baseline assessments (intranasal fluid, PNIF, intranasal nitric oxide, and nasal inflammation score), challenged with NAC #1, challenge baseline assessments made (e.g., TNSS, PNIF, nNO, intranasal fluid, nasal inflammation score), and randomized .

14. Subjects will be challenged with NAC #2 followed by assessments.

15. Pre and post first dosing.

16. To be completed pre (0 min) and post NAC (20 and 60 min).

17. To be completed post NAC (120 min).

18. To be completed pre (-30 and 0 min) and post NAC (15, 30, 60, and 120 min).

19. To be completed pre (0 min) and post NAC (120 min).

20. TNSS to be completed pre (-30 and 0 min) and post NAC (15, 30, 45, 60, 90, and 120 min).

21. Perform either skin prick test or IgE test at screening unless documentation of a positive test in the past 12 months can be provided for either method.

22. To be completed pre (-30 and 0 min) and post NAC (15, 30, 45, 60, 90, and 120 min for TNSS and 15, 30, 60, and 120 min for PNIF).

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