TITLE PAGE

Protocol Title:

An open-label, multicenter, Phase Ib study of B244 delivered as a topical spray to assess safety in pediatric subjects aged 2 to 17 years with atopic dermatitis

Protocol Number: ADB244-002

Amendment Number: Amendment 1

Product: B244 4 $\times 10^9$ cells/mL (OD600 2.0 ± 0.4)

Study Phase: Ib

Sponsor Name: AOBiome Therapeutics

Legal Registered Address: 125 Cambridgepark Drive, Cambridge, MA 02140

Regulatory Agency Identifying Number(s): IND 17485

Date of Amendment 1: February 25, 2019

NCT03775434

Sponsor Signatory:

I have read this protocol in its entirety and agree to conduct the study accordingly:

S. Jamas

Spiros Jamas, ScD
Head of Therapeutics

Feb 25th, 2019
Date

J Ng-C MD

Judith Ng-Cashin, M.D.
Chief Medical Officer

25 Feb 2019
Date

Medical Monitor name and contact information can be found in Appendix 2.

Investigator Agreement Page is provided in Appendix 7. The Investigator should retain the original in the study center study files and return a copy to the Sponsor or clinical research organization for archiving.

Each Investigator should be sent a copy of the Investigator Agreement page for completion. Signatures are obtained after the Sponsor has finalized and approved the protocol.

PROTOCOL AMENDMENT 1 SUMMARY OF CHANGES

Section	Description of Changes
Sections 1.1; 1.2; 1.3; 4.0; 8.2.2	This section of the protocol is being clarified to allow a -1 to 1 window for subjects to complete the Baseline (Day 1) visit. This clarification applies to all sections of the protocol discussing and describing the washout period
Section 5.3.1	Clarification is being added that subjects may use their own shampoo while in the study.
Section 6.1.1	Subjects will be dispensed up to 6 bottles of study medication for the duration of the trial. Actual number will depend upon the number of lesions and sprays of IP to be applied.
Section 6.5.2	This section of the protocol is being clarified to read: Sites will be provided scales, which will be "Tared" (set to "0") prior to each use.
Section 6.7	This section clarifies that subject may use up to 16 sprays per application, however this will depend on the number of lesions.
Section 8.2.2	Wording around timing of Washout period is being added for further clarification.
Section 9.3.5	This section of the protocol is being clarified that, as per the Schedule of Activities (Section 1.3), the first investigational product application will happen in the study center under clinical staff supervision, after the completion of the baseline assessments. All subsequent study application will be administered by the parent/guardian or by the subject in the setting of subject's home.
Section 9.4.2	There was a typo in this section of the protocol and it is being clarified that, as per Inclusion Criteria (Section 5.1) Number 8: Female subjects aged **≥11** years old, or female patients <11 years old who have started menstruating, will have urinary pregnancy test performed at all visits. The Baseline result must be available and must be negative before the subject apply the first application of IP. Positive pregnancy test will

	disqualify the subject from the participation in the study.
Section 9.4.4	This section of the protocol is being clarified that, as per the Schedule of Activities (Section 1.3), Body weight and height will be measured at Screening and at Day 28/Final Visit (or at Early Termination Visit, as applicable).
Appendix 5	This section of the protocol is being clarified that, as per Inclusion Criteria (Section 5.1) Number 8, females must either practice abstinence from heterosexual contact or use **one** (1) of the highly effective contraceptive options described in the Appendix 5. Females are not **required** to use two (2) highly effective methods of contraception

Administrative changes: Minor changes involving grammar, wordsmithing, punctuation, and other editorial changes have been made throughout the document. All are clearly identified in the track-changes version of the amendment.

Additional formatting and stylistic adjustments have been made to facilitate the reading process.

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1.0 PROTOCOL SUMMARY

1.1 Synopsis

Protocol Title: An open-label, multicenter, Phase Ib study of B244 delivered as a topical spray to assess safety in pediatric subjects aged 2 to 17 years with atopic dermatitis

Rationale:

Clinical studies with topical administration of B244 have been conducted in subjects with acne, hypertension, atopic dermatitis, and rosacea. There have been no safety signals observed to date.

The aim of this study is to assess the safety of B244 in pediatric subjects aged 2 to 17 years with atopic dermatitis.

Objectives and Endpoints

Objectives	Endpoints
Primary	
• Assessment of the safety and tolerability of B244 in pediatric subjects with atopic dermatitis.	• Incidence of treatment-emergent adverse events (TEAEs). • Change from Baseline in physical examination at each post baseline visit. • Change from Baseline in vital signs at each post baseline visit.
Exploratory	
• Efficacy of B244 on signs and symptoms of atopic dermatitis.	• Changes from Baseline to post baseline visits in: – Validated Investigator Global Assessment for Atopic Dermatitis (vIGA-AD) scale. – Area Severity Index (EASI) score and each component of EASI from Baseline to post baseline visits.
• Efficacy of B244 on patient oriented measure of atopic dermatitis.	• Changes is Patient Oriented Eczema Measure (POEM total score and each of the 7 components) from Baseline to post baseline visits.
• Efficacy of B244 on patient reported assessment of atopic dermatitis.	• Changes in patient reported outcome (self-reported ItchMan questionnaire).from Baseline to post baseline visits.

Overall Design:

This is a Phase 1b, open-label, multiple site study assessing twice daily B244 application for 28 days in pediatric subjects with atopic dermatitis.

The study will enroll 36 subjects in 3 cohorts of 12 subjects:

- Cohort 1: subjects aged 2 to 5 years.
- Cohort 2: subjects aged 6 to 11 years.
- Cohort 3: subjects aged 12 to 17 years.

Number of Investigators and Study Centers:

Approximately 6 Investigators and study centers are expected to participate in this study.

Number of Subjects:

The study will enroll 36 subjects in 3 cohorts of 12 subjects:

- Cohort 1: subjects aged 2 to 5 years.
- Cohort 2: subjects aged 6 to 11 years.
- Cohort 3, subjects aged 12 to 17 years.

Treatment Groups and Duration:

Subjects will attend for a Screening visit between Days -21 and -14. If all eligibility criteria and none of the exclusion criteria are met, subjects will be enrolled into the study and will be required to undergo a 14 day washout period (Days-14 to -1). Subjects will attend the study center on Day 1(-1 to 1) and the Baseline assessments will be performed before application of the first dose. On confirmation of continued eligibility the subject and parent or guardian of the subject will be coached on how to apply medication, depending on the affected areas. They will be instructed to apply B244 twice daily (approximately 12 hours apart) for 28 days. The first dose will be applied in the clinic under the supervision of clinical staff. Details of dose administration will be recorded in the study diary provided.

The subjects with their parent or guardian will return to the study center on Days 7, 14, and 21 for completion of study assessments. There will be a final study visit on Day 28, this will be defined as the end of the study for the subjects. A time window of ±2 day will be permitted for these 4 visits. There will not be a period of confinement in the study center all visits will be outpatient visits.

Safety monitoring will include review of TEAEs, vital signs and physical examination.

Efficacy will be assessed using EASI, vIGA-AD scale, POEM and ItchMan scores.

Statistical Methods:

All subjects who are enrolled and take at least 1 dose of study treatment will be included in the Intent to Treat (ITT) population. The ITT population will be the primary population for safety and efficacy assessments.

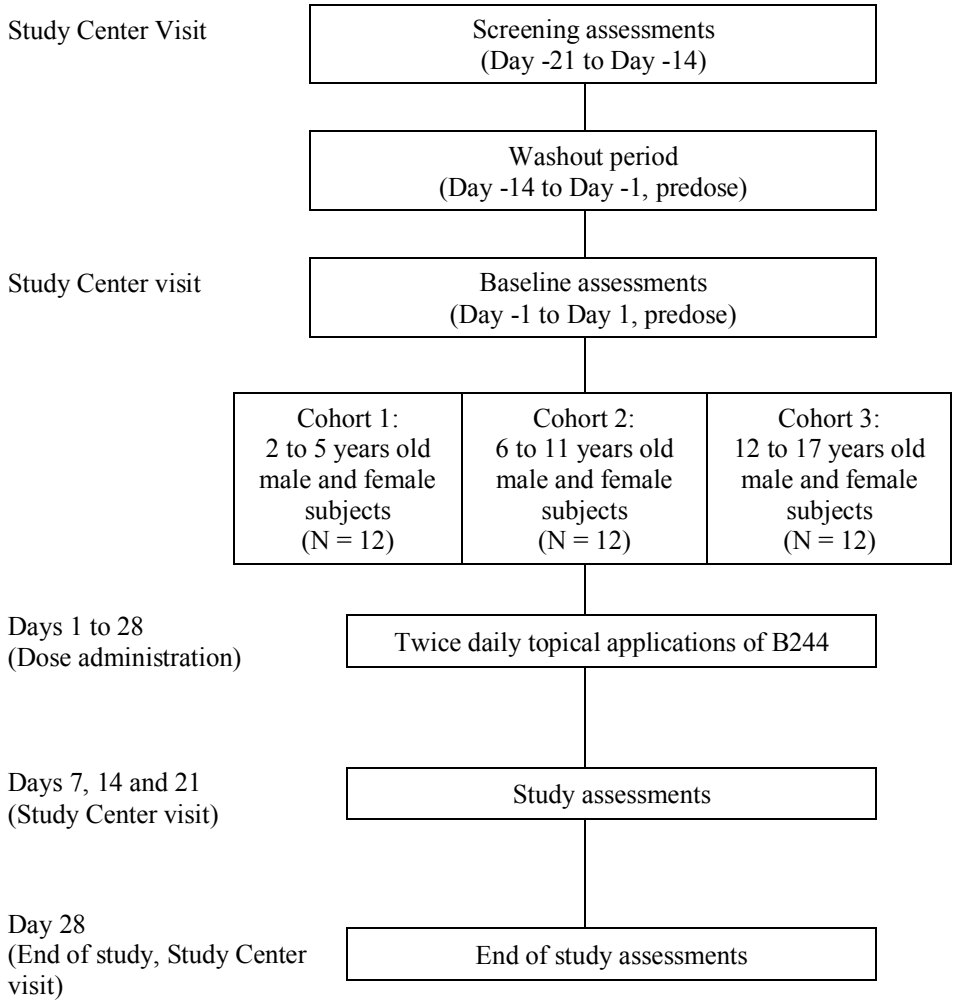
All subjects in the ITT population without any protocol deviations that may have an impact on the efficacy assessments will be included in the Per Protocol (PP) population. The PP population will be the supportive population for efficacy assessments.

The sample size is not based on statistical considerations but is typical for studies of this nature, and is considered adequate to characterize the distribution of the planned endpoints. Any statistical testing will be considered exploratory and descriptive.

Data Monitoring Committee: No

1.2 Schema

Figure 1 Study Schema



1.3 Schedule of Activities

Table 1.1 Schedule of Assessments

Visit Name	Screening	Washout	Baseline (Day 1)	Day 7	Day 14	Day 21	Day 28 Final Visit	Early Termination Visit
Study Center Visit	**1**		**2**	**3**	**4**	**5**	**6**	
Visit Window in Days	**-21 to -14**	**-14 to -1**	**-1 to 1**	**±2**	**±2**	**±2**	**±2**	
Informed Consent	X							
Inclusion/Exclusion Criteria	X		X					
Demographics	X							
Medical History	X							
Hanifin and Rajka Criteria	X		X					
Physical Examination	X		X	X	X	X	X	X
Urine Pregnancy Test[1]	X		X				X	X
Body Weight	X						X	X
Height	X						X	X
Vital Signs[2]	X		X	X	X	X	X	X
Validated Investigator Global Assessment for Atopic Dermatitis (vIGA-AD) Scale	X		X	X	X	X	X	X
Eczema Area Severity Index (EASI)	X		X	X	X	X	X	X
Patient Oriented Measure (POEM)[3]	X		X	X	X	X	X	X
ItchMan Questionnaire	X		X	X	X	X	X	X
Dispense Investigational Product to Subject[4]			X	X	X	X		

Visit Name	Screening	Washout	Baseline (Day 1)	Day 7	Day 14	Day 21	Day 28 Final Visit	Early Termination Visit
Study Center Visit	**1**		**2**	**3**	**4**	**5**	**6**	
Visit Window in Days	**-21 to -14**	**-14 to -1**	**-1 to 1**	**±2**	**±2**	**±2**	**±2**	
Investigational Product Application[5]			X	X	X	X	X	
Collect Investigational Product from Subject[6]				X	X	X	X	
Investigational Product Compliance[6]				X	X	X	X	X
Counseling[7]			X	X	X	X		
Study Cleanser[8]	X	X	X	X	X	X	X	X
Moisturizer[8]	X	X	X	X	X	X	X	X
Study Diary[9]			X	X	X	X	X	X
Adverse Events Recording[10]	X	X	X	X	X	X	X	X
Concomitant Medications	X		X	X	X	X	X	X

1. Urine pregnancy test will be administered to females 11 years of age or older.
2. Pulse rate, temperature and blood pressure will be obtained. Subject should be allowed to rest for >5 minutes sitting, then a single blood pressure and pulse rate measurement will be performed, using the non-dominant arm with a calibrated electronic sphygmomanometer.
3. Patient Oriented Measure questionnaire is to be filled out by the subject or the parent or guardian.
4. At Baseline, depending on the age of the participant, 1 or 2 bottles will be supplied. Total number of bottles to be dispensed to the subjects for the duration of the study will depend upon the body surface area affected by atopic dermatitis (Section 6.1). At each visit, study staff will determine each subject's usage and supply additional investigational product if necessary.
5. The first investigational product application will happen in the study center under clinical staff supervision after completion of the baseline assessments. Subjects and parent or guardian will be coached on how to apply the study treatment depending on the affected area and will be asked to apply the study treatment twice daily.
6. Weight of an investigational product kit will be obtained at the at the Baseline visit. Study staff will be asked to weigh dispensed bottles PRE FIRST DOSE. Weight will be recorded in grams. Subjects will be asked to bring their bottles back for every visit. Upon return for the study visit, bottles will be weighed again.

7. Subjects will be counseled on the use of the study treatment, study diary and any questions the subject, parent or guardian may have will be answered.
8. The Sponsor will provide a biome friendly, preservative-free moisturizer and cleanser to be used as needed, at the discretion of the parent or guardian. Any remaining moisturizer or cleanser will be returned to the Investigator at the Final Visit (Day 28) or the Early Termination Visit.
9. The subject, parent or guardian will be asked to fill out a daily study diary.
10. Adverse events will be monitored throughout the trial, starting with the time informed consent form has been signed.

2.0 INTRODUCTION

2.1 Study Rationale

AOBiome Therapeutics has isolated a purified strain of *Nitrosomonas eutropha*, designated *Nitrosomonas eutropha* D23, from soil samples. Sequencing of the *Nitrosomonas eutropha* D23 genome revealed a distinct genetic profile from that of other published *Nitrosomonas* strains and ammonia (NH_3) oxidizing bacteria (AOB) genomes. The unique metabolic and antimicrobial activity of *Nitrosomonas*, in combination with their complete lack of virulence, render these bacteria as attractive candidates for topical delivery of nitrite (NO_2) and nitric oxide (NO) on human skin. This has potential to improve health in both normal and abnormal skin conditions (including wound sites). The drug product has been designated the code B244.

Clinical studies with topical administration of B244 have been conducted in subjects with acne. In addition, other clinical studies, either ongoing or completed, have included the investigation of the efficacy of B244 in subjects with hypertension, atopic dermatitis, and rosacea. The results of these studies have indicated efficacy of B244 with no unexpected safety signals. The aim of this study is to assess the safety of B244 in pediatric subjects aged 2 to 17 years with atopic dermatitis.

2.2 Background

Ammonia oxidizing bacteria are ubiquitous in the environment and are essential for the initial step in nitrification processes, specifically the oxidation of NH_3 to NO. They are found in all soils and water sources (fresh, ground, and seawater). *Nitrosomonas*, as AOBs, are gram-negative chemolithoautotrophic betaproteobacteria that obtain energy solely from NH_3 oxidation, while fixing carbon dioxide (CO_2) for their carbon needs [Arp and Sayavedro-Soto, 2001].

It is hypothesized that there may be regulation of basal NO levels by AOB on the skin. In acne, for example, *Nitrosomonas eutropha* might be expected to down regulate androgen levels. Low NO causes high androgen levels. Increasing NO/NO_2 by the oxidation of NH_3 in sweat by *Nitrosomonas eutropha* may decrease androgen levels. Androgens, the male hormones present in men and women, can contribute to acne flares by over stimulating the oil glands and altering the development of skin cells that line hair follicles in the skin. Increasing NO/NO_2 production may therefore have a critical role in controlling the signs and symptoms of acne vulgaris. In this regard, the disruption of the normal AOB component of the human microbiome has the potential to disrupt the feedback regulation between acne, NO, and androgens. Nitrogen oxide 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 (Weller and Price, 2001, Rico et al, 2014).

Other important potential mechanisms through which *Nitrosomonas eutropha* D23 could positively effect acne and other skin diseases include an anti-inflammatory and antimicrobial effects

Clinical studies with topical application of B244 have been conducted, or are planned, include studies in subjects with clinical diagnosis of facial acne vulgaris (Investigational New Drug [IND] 16487) hypertension (IND 17086), atopic dermatitis (IND 17485) and mild to moderate rosacea (IND 18086). Overall, there have been no serious adverse events (SAE) or unexpected safety signals observed following treatment with B244.

Efficacy was supported by statistically significant 2-point reduction in Investigator Global Assessment (IGA) with B244 and a trend in the reduction of the number of inflammatory lesions compared with vehicle in the study conducted in subjects with facial acne vulgaris (IND16487).

B244 is comprised of *Nitrosomonas eutropha* D23 diluted in storage solution (50 mM Na_2HPO_4 and 2 mM $MgCl_2$, pH 7.6 ± 0.2). The B244 drug product is provided as a mist formulation in sterilized 7.5 or 30 mL plastic containers with appropriate sized and intended use designed spray nozzles. There are 3 concentrations, measured by optical density (OD), of the drug product: high dose (OD_{600} 4.0 ± 0.8 equivalent to 8×10^9 cells/mL), middle dose (OD_{600} 2.0 ± 0.4 equivalent to 4×10^9 cells/mL), and low dose (OD_{600} 1.0 ± 0.2 equivalent to 2×10^9 cells/mL).

A detailed description of the chemistry, pharmacology, efficacy, and safety of B244 is provided in the current Investigator's Brochure.

2.3 Benefit/Risk Assessment

To date, there have been no reported infections or health risks associated with topical application or ingestion of *Nitrosomonas* species. The absence of any known illnesses attributed to these bacteria despite widespread human exposure suggests that they are unlikely to pose significant health risk. 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, mitigating the risk of adverse effects due to high NO/NO_2 low without any adverse effects.

The Sponsor will immediately notify the Principal Investigator if any additional safety or toxicology information becomes available during the study.

This study will be performed in compliance with the protocol, International Council on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP) and applicable regulatory requirements. Aspects of the study concerned with the investigational product (IP) will meet the requirements of Good Manufacturing Practice (GMP).

3.0 OBJECTIVES AND ENDPOINTS

Table 3.1 Study Objectives and Endpoints

Objectives	Endpoints
Primary	
• Assessment of the safety and tolerability of B244 in pediatric subjects with atopic dermatitis.	• Incidence of treatment-emergent adverse events (TEAEs). • Change from Baseline in physical examination at each post baseline visit. • Change from Baseline in vital signs at each post baseline visit.
Exploratory	
• Efficacy of B244 on signs and symptoms of atopic dermatitis.	• Changes from Baseline to post baseline visits in: – Validated Investigator Global Assessment for Atopic Dermatitis (vIGA-AD) scale. – Area Severity Index (EASI) score and each component of EASI from Baseline to post baseline visits.
• Efficacy of B244 on patient oriented measure of atopic dermatitis.	• Changes is Patient Oriented Eczema Measure (POEM total score and each of the 7 components) from Baseline to post baseline visits.
• Efficacy of B244 on patient reported assessment of atopic dermatitis.	• Changes in patient reported outcome (self-reported ItchMan questionnaire) from Baseline to post baseline visits.

4.0 STUDY DESIGN

4.1 Overall Design

This is a Phase 1b, open-label, multiple site study assessing twice daily B244 application for 28 days in pediatric subjects with atopic dermatitis.

The study will enroll 36 subjects in 3 cohorts of 12 subjects:

- Cohort 1: subjects aged 2 to 5 years.
- Cohort 2: subjects aged 6 to 11 years.
- Cohort 3: subjects aged 12 to 17 years.

At Screening and Baseline, all subjects must have confirmed diagnosis of atopic dermatitis, as defined by the Hanifin and Rajka criteria, which involves a minimum of 10% but no more than 60% body surface area and a Validated Investigator Global Assessment for Atopic Dermatitis (vIGA-AD) scale of 2 or 3.

Subjects will attend for a Screening visit between Day -21 and -14. If all eligibility criteria and none of the exclusion criteria are met, subjects will be enrolled into the study and will be required to undergo a 14 day washout period (Day-14 to -1). The washout period is to ensure the subjects do not have any active systemic infections (including but not limited to, ear infections, upper respiratory tract infections, throat infections) and that they comply with the withdrawal of medications included in Exclusion Criterion 7. On completion of the washout period and confirmation of continued eligibility the subjects will be assigned to one of the cohorts based on the age of the subject.

Subjects will attend the study center on Day 1 (-1 to 1) and the Baseline assessments listed in Table 1.1 will be conducted. On confirmation of continued eligibility, the subject and parent or guardian of the subject will be coached on how to apply medication, depending on the affected areas. They will be instructed to apply B244 twice daily (approximately 12 hours apart) for 28 days and record the details of the application in the study diary provided. They will also be provided guidance on the storage of the study treatment. Guidance for the application of B244 is provided in Section 6.1. The first dose will be administered on Day 1 in the clinic, under the supervision of clinical staff, subsequent doses can be applied outside of the clinic. The importance of adherence to the medication schedule will be reinforced at each study visit.

The subjects with their parent or guardian will return to the study center on Days 7, 14 and 21 when the assessments listed in Table 1.1 will be conducted. There will be a final study visit on Day 28, this will be defined as the end of the study for the subjects. A time window of ±2 day will be permitted for these visits.

Safety monitoring will include review of TEAEs, vital signs and physical examinations as indicated in Table 1.1.

Efficacy will be assessed using EASI, vIGA-AD scale, POEM, and ItchMan scores Table 1.1.

4.2 Scientific Rationale for Study Design

AOBiome Therapeutics has developed B244 as a topical application of a natural source of AOB and NO/NO_2 to the human skin. B244 for topical delivery has the potential to improve health in both normal and abnormal skin conditions or wound sites.

A standard parallel-group, open-label design is used for this study to evaluate the safety and tolerability of B244 in pediatric subjects with atopic dermatitis.

The study will be conducted in pediatric subjects with atopic dermatitis because they are a target population.

The sample size for each cohort is based on the desire to obtain adequate safety and tolerability data to achieve the objectives of the study while exposing as few subjects as possible to B244 and study procedures.

This study will be performed in compliance with the protocol, the ICH GCP, GMP, and applicable regulatory requirements.

4.3 Justification for Dose

There are 3 concentrations of the drug product available: high dose (OD_{600} 4.0 ± 0.8 equivalent to 8×10^9 cells/mL), middle dose (OD_{600} 2.0 ± 0.4 equivalent to 4×10^9 cells/mL) and low dose (OD_{600} 1.0 ± 0.2 equivalent to 2×10^9 cells/mL).

The middle dose has been selected for this study to minimize the exposure while ensuring administration of a dose considered likely to show improvement in symptoms of atopic dermatitis.

4.4 End of Study Definition

The end of the study is defined as the date of last scheduled procedure shown in the Table 1.1 for the last subject in the study.

4.5 Dose Escalation Criteria

There will not be any dose escalation in this study.

4.6 Study Stopping Criteria

Dosing for any individual subject will be stopped if:

- The subject experiences a possibly drug-related SAE.
- The subject experiences a possibly drug-related significant non-serious adverse event (AE), which in the opinion of the Principal Investigator, Chief Investigator, Medical Monitor, or

Sponsor's medical representative, warrants discontinuation from the study for that subject's wellbeing. The occurrence of significant non-serious AEs as described above will require that further dosing of other subjects be evaluated by the Chief Investigator, the Medical Monitor and the Sponsor's Medical Advisor to determine study discontinuation.

- The subject's atopic dermatitis worsens and requires alternative or supplemental medication during the study (see Section 6.8 for allowed treatments).
- The subject or his/her parent or guardian choose to discontinue for any reason.

Dosing for a cohort will be stopped if:

- A subject experiences a possibly drug-related SAE.
- More than 2 subjects experiences a possibly drug-related significant non-serious AE, which in the opinion of the Principal Investigator, Chief Investigator, Medical Monitor, or Sponsor's medical representative, warrants discontinuation from the study for that subject's wellbeing. The occurrence of significant non-serious AEs as described above will require that further dosing of other subjects be evaluated by the Chief Investigator, the Medical Monitor and the Sponsor's Medical Advisor to determine study discontinuation.

The study will be stopped if:

- More than 1 subject experiences a drug-related SAE.

5.0 STUDY POPULATION

Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, is not permitted.

5.1 Inclusion Criteria

Subjects are eligible to be included in the study only if all of the following criteria apply:

1. Male and female subjects 2 to 17 years of age, inclusive.
2. Confirmed diagnosis of atopic dermatitis according to the Hanifin and Rajka criteria.
3. A minimum of 10% but no more than 60% of the subjects' body surface area (see Appendix 6 for guidance) is affected by atopic dermatitis (affected is defined by physical examination findings: erythema, edema, scaling, lichenification, and excoriation; with the excoriation serving as the physical examination correlate of pruritus).
4. A vIGA-AD scale of 2 or 3 at Screening and Baseline.
5. Subject, or the parent or guardian, to provide written informed consent and authorization for protected health information disclosure.
6. Subjects must be generally in good health based on Investigator's assessment (other than atopic dermatitis).
7. Normal vital signs, or with no clinically significant vital signs that in the opinion of the Investigator, would place the subject at increased risk or would confound the objectives of the study.
8. Females must not be pregnant, as confirmed by negative urine pregnancy testing. Female subjects aged ≥11 years old, or female patients <11 years old who have started menstruating, will have urinary pregnancy test performed at Screening and prior to the first dose with negative results in order to participate in the study. Females must either practice abstinence from heterosexual contact or use one of the highly effective contraceptive options described in the Appendix 5.
9. Male subjects of reproductive potential, must be willing to practice effective contraception during the study while receiving study treatment from Day 1 and for 7 days after the last study visit (Day 28).
10. Ability to comprehend and comply with study procedures.
11. Agree to commit to participate in the current protocol.
12. Provide written informed consent prior to any study procedure being performed.

5.2 Exclusion Criteria

Subjects are excluded from the study if any of the following criteria apply:

1. Clinically significant physical or mental disorder which, in the opinion of the Investigator, would place the subject at increased risk or would confound the objectives of the study.
2. Subjects with atopic dermatitis on the face only.

3. Active cutaneous bacterial, viral or fungal infection in any treatment area at Baseline (eg, clinically infected atopic dermatitis).
4. History or presence of immunological deficiencies or diseases, organ transplant, human immunodeficiency virus (HIV), diabetes, malignancy, malignant or pre-malignant skin conditions, serious active or recurrent infection, systemic immunosuppressive regimens, clinically significant renal disease severe hepatic disorders, or other severe uncontrolled conditions (eg, drug or alcohol abuse), that are significant and/or that may pose a health risk to the subject in the study or may have an impact on the study assessments.
5. Unstable atopic dermatitis or a consistent requirement for high-potency corticosteroids (class I-III steroids).
6. Active systemic or localized infection (including infected AD).
7. Subjects unable to comply with the excluded medication/therapy restriction listed in Appendix 4.
8. Known hypersensitivity to the study treatment.
9. Known to have hepatitis B, hepatitis C or HIV I or II tests. Details will be recorded in medical history, a blood sample will not be collected for confirmation.
10. Female subject who is pregnant, breastfeeding, or considering pregnancy during the study.
11. Any skin condition which in the Investigator's opinion may interfere with the evaluation of atopic dermatitis.
12. Use of any investigational drugs within the previous 30 days prior to dosing or within a period of less than 5 times the drug's half-life, whichever is longer.
13. Use of any biologic within a period of 5 times its half-life.
14. Children or relatives of the Sponsor, clinical research organization, or the Study Site personnel are excluded from participating in the study.

5.3 Lifestyle Considerations

There are no dietary or activity related restrictions for this study.

5.3.1 Use of Cleanser and Moisturizer During the Study

At the beginning of the study, subjects will be provided with cleanser and moisturizer to use for the duration of the stud as needed.

Subject should be advised to apply moisturizer 10 minutes after the application of the B244.

Subjects may not use other cleansers to wash their body for the duration of the trial. Subjects may use their preferred shampoo for the duration of the trial.

During Day 28 visit (End of B244 application) subjects will be asked to return cleanser and moisturizer that was provided to them by the Sponsor and will be asked to go back to their regular regimen and use their preferred cleanser and moisturizer.

Subjects will be encouraged not to use sunscreen during participation in the study. Sunscreen should be used minimally or used sparingly while in the active phase (Baseline to Day 28) of the study.

Subjects who do not have atopic dermatitis on their face may apply makeup as needed. However, those subjects who have atopic dermatitis on their face and require to use makeup will be advised to use it minimally.

5.4 Screen Failures

Screen failures are defined as subjects who consent/assent to participate in the clinical study but are not subsequently assigned to study treatment. A minimal set of screen failure information is required to ensure transparent reporting of screen failure subjects to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any SAE.

Individuals who do not meet the criteria for participation in this study (screen failure) may be rescreened on 1 occasion. Rescreened subjects should not be assigned the same subject number as for the initial screening.

6.0 INVESTIGATIONAL PRODUCT TREATMENT AND APPLICATION

Study treatment is defined as any investigational treatment(s), marketed product(s), placebo, or medical device(s) intended to be administered to a subject according to the study protocol.

6.1 Study Treatment(s) Administered

6.1.1 Investigational Product

Under normal conditions of handling and administration, the Investigational Product (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.

The IP 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. The IP must be dispensed or administered only to participants enrolled in the study and in accordance with the protocol.

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

Product name:	B244, 30 mL/bottle
Dosage form:	B244 suspension
Unit dose strength:	$4x10^9$ cfu/mL
Route/administration/duration:	Topical application twice daily for 4 weeks
Physical description:	Odorless, cloudy, light pink suspension
Manufacturer/source of procurement:	AOBiome Therapeutics

Up to 6 bottles total will be dispensed to the subjects and will depend upon the body surface area affected by atopic dermatitis (Appendix 6) and the age of the subject (Table 6.1).

Table 6.1 Suggested Number of Bottles of B244 Issued to the Subject and Parent or Guardian

BSA (%)	2 to 5 years old	6 to 11 years old	12 to17 years old
10	1 bottle	1 bottle	2 bottles
20	1 bottle	2 bottles	3 bottles
30	2 bottles	2 bottles	4 bottles
50	2 bottles	3 bottles	5 bottles

Table 6.2 Body Surface Area and Suggested Application of Investigational Product

Affected Body Surface Area	Approximate Number of Pumps per application		
	2 to 5 years old	6 to 11 years old	12 to- 17 years old
10%	2	2	3
20%	3	4	7
30%	4	6	10
50%	7	10	16

At Baseline visit (Day1), all subjects will be dispensed up to 2 bottles of IP, depending on the number of lesions. Upon examining the subject, study staff will instruct the patient on the number of pumps to be applied per application for the duration of the treatment. If during the study participation subject develops new lesions, those should be treated as well.

It is suggested that subjects will use up to the number of bottles indicated in Table 6.1 during participation in the trial, however this number should not exceed 6 bottles per subject per study. Study staff should monitor usage at every study visit and determine whether more IP should be dispensed to the subject based on age and usage, where the total number of pumps per application should not exceed 16.

Subjects and their guardians should use the details in Table 6.2 to guide the suggested number of pumps to be applied on each application. Subjects will apply 1 pump (0.14 mL) of B244 (OD_{600} 2.0 ± 0.4 equivalent to 4×10^9 cells/mL) per 200 cm^2 (approximately one adult palm size) of the affected area, twice a day approximately 12 hours apart, for 28 days (Day 1 to 28).

One pump should cover the surface area of skin the approximate size of one adult sized palm. One pump will be applied directly to the skin for each atopic dermatitis area that is the size of a palm of 1 adult sized hand.

The total number of bottles to be dispensed to the subjects will depend upon the body surface area affected by atopic dermatitis (Appendix 6), age of the subject and the number of areas affected by the disease.

The subject and parent or guardian of each subject will be instructed by clinical staff regarding the use of the spray pump and application of the dose to the area of atopic dermatitis. The first dose will be applied by the subject, parent or guardian in the study center under the supervision of the clinical staff.

For each dosing period, the subject under the supervision of the parent or guardian, or the parent or guardian, will dispense the required amount of study treatment using the pump. Whether the subject is likely to be able to apply the study treatment will be decided by the Investigator, or delegate, during the training session. If the subject is considered to be too young, or would be unreliable, to apply the study treatment the application will be done by the parent or guardian. Whenever the study treatment is applied by the subject the application must be under the supervision of the parent or guardian.

- The person applying the study treatment should wash his/her hands before and after applying study treatment.
- Subject should shake the bottle before each application and then saturate the application area well, holding the bottle approximately 6 inches away from the skin.
- Subjects will be asked to let the product air dry.
- Subjects may not wash for 2 hours post morning application of the IP.
- It is recommended that subjects may not bathe, shower or wash until the morning after the evening application of B244. For younger subjects who need cleaning related to diaper changes this is acceptable.
- While in use, one spray bottle may be stored at ambient temperature.
- DO NOT FREEZE
- The Sponsor will provide a biome friendly, preservative-free moisturizer and cleanser to be used as needed, at the discretion of the parent or guardian to clean the area of application before application of the treatment.
- The subject or parent or guardian will apply the study treatment to the affected area twice daily (approximately 12 hours apart) for 28 consecutive days.
- The parent or guardian will verify if the study treatment was applied as directed. Application details will be recorded in the study diary provided.
- Subjects will be asked not to expose 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°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 bottle.

6.2 Preparation/Handling/Storage

All investigational drug supplies in the study will be stored in a secure, refrigerated (2 to 8°C) safe place, under the responsibility of the Investigator or other authorized individual.

The Investigator, or designee will maintain temperature monitoring of the IP with daily temperature readings. All temperature excursions must be reported to the Sponsor using the Temperature Excursion log. If the IP was exposed to the temperature excursion outside the range of 2 to 8°C, but for the period greater than 24 hours, the IP must be quarantined until the Sponsor's approval on future use.

The Investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study treatment received and any discrepancies are reported and resolved before use of the study treatment.

The Investigator or designee must provide instruction to the subject, and parent or guardian, on the storage requirements for the study treatment.

Only subjects enrolled in the study may receive study treatment and only authorized study center staff may supply study treatment. All study treatments must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the Investigator and authorized study center staff.

The Investigator, institution, or the head of the medical institution (where applicable) is responsible for study treatment accountability, reconciliation, and record maintenance (ie, receipt, reconciliation, and final disposition records).

Further guidance and information for the final disposition of unused study treatment will be provided.

6.3 Accountability, Destruction and Return of Study Supplies

In accordance with federal and local regulatory requirements, the Investigator and designated site personnel must document the amount of IP dispensed to study subjects, the amount returned by study subjects, 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 IP 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 to 8°C. Maintenance of a temperature log is required.

Under no circumstances will the Investigator or designees allow IP to be used other than as directed by this Clinical Study Protocol, or dispose of investigational product without Sponsor direction.

The Sponsor will supply sufficient quantities of the IP for the completion of this study.

The study pharmacist or designated study personnel will maintain an Investigational Materials Accountability Record and a Subject IP Accountability Log itemizing all IP received, dispensed to and returned from each subject during the study. All dispensed bottles must be accounted for, and any discrepancies explained. Study site should contact site Clinical Research Associate (CRA) or designee in case of any dispensing errors or if discrepancies are discovered.

Prior to site closure and at appropriate intervals during the study, site CRA will perform IP accountability and reconciliation. At the end of the study, the Investigator or designee will retain all the original documentation regarding IP accountability, return, and copies will be sent to the Sponsor.

All unused and used IP tubes will be returned to the Sponsor or its designee for destruction at the end of the study.

6.4 Measures to Minimize Bias: Randomization and Blinding

Not applicable, this is an open-label study.

6.5 Study Treatment Compliance

The prescribed dosage, timing, and mode of administration may not be changed. Any departures from the intended regimen must be recorded in the electronic case report forms (CRFs).

At each visit, prior to dispensing study treatment, previously dispensed study treatment will be retrieved by the Investigator or designee and compliance assessed. Subjects exhibiting poor compliance as assessed by the returned IP and the study diary should be counseled on the importance of good compliance to the study dosing regimen.

6.5.1 Study Diary Compliance Review

The subject will be given a study diary at each study visit of the treatment period to record date and time of applications, any adverse events, and quality of life assessments. Subjects will record every application during the treatment period and will bring the subject diary to each of the post baseline study visits for compliance review. The Investigator or designee will verify that the subject complied with the application requirements and will file the completed subject diary in the study files at each study visit until the Final/Early Termination visit. Any missed application notations will be clarified with the subject and documented in the subject's CRF.

6.5.2 Treatment Compliance

Each site participating in the trial will be instructed to assess subject's compliance by weighing the IP and obtaining the weight of the bottles in grams pre- and post-application. Study personnel will be asked to weigh dispensed bottle(s) and record the weight pre first application. At each subsequent visit and then Day 28 visit, study personnel will need to weigh bottle(s) and record the weight. This procedure should be followed every time study medication is dispensed and returned. Sites will be provided scales, which will be tared prior to each use. Study personnel will be instructed to record measurements into the eCRF.

6.6 Concomitant Therapy

Any medication or vaccine (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements) that the subject is receiving within 30 days prior to the start of the IP and through the final study visit will be recorded on the appropriate CRF) along with:

- Reason for use.
- Dates of administration including start and end dates.
- Dosage information including dose and frequency.

Any medications started during the study (including "as needed" medications) will be recorded in the concomitant medication list as soon as the Investigational Site will become aware of the medication being added.

The subject, or parent or guardian, will be instructed to record the details of any medication taken by the subject outside of the study center in the study diary provided. The details will be reviewed by the Investigator or designee on each study center visit.

The Medical Monitor should be contacted if there are any questions regarding concomitant or prior therapy.

A list of excluded medications/therapy is provided in Appendix 4.

Previous treatment of atopic dermatitis must be recorded irrespectively of the term it was given.

Acetaminophen, at doses approved for the age of the subject, is permitted for use any time during the study. Other concomitant medication may be considered on a case-by-case basis by the Investigator in consultation with the Medical Monitor.

6.7 Dose Modification

The study treatment will be administered as described in this protocol. Up to 16 pumps per application may be administered, depending on the number of lesions subject has. Number of applications will be documented in each subject's chart. Any AEs or circumstances that impact the dosing of the IP will be documented and reported to the Medical Monitor.

6.8 Rescue Medication Permitted During the Study

Over the course of the study, the Investigator will monitor and evaluate subject's condition and determine whether rescue therapy may be necessary. The use of medications to treat a subject's atopic dermatitis during the study will be permitted if the parent, guardian or medical professional determines that it becomes medically necessary. Such rescue treatment should not exceed 7 days. If a subject requires treatment for more than 7 days or requires systemic therapy, they should be discontinued from the study. Medications permitted are listed in Table 6.3.

Study staff must be notified of the use of any medication as soon as feasible. The details of the medication should be recorded in the study diary and transferred to the CRF. Details recorded should include medication name, date of administration, dosage, and number of applications.

Table 6.3 Permitted Rescue Medication

Class 4 – Mid-strength	
Clocortolone pivalate (0.1%)	Cloderm® Cream
Mometasone furoate (0.1%)	Elocon® Cream
Triamcinolone acetonide (0.1%)	Aristocort® A Cream, Kenalog® Ointment
Betamethasone valerate (0.1%)	Valisone Ointment
Fluocinolone acetonide (0.025%)	Synalar® Ointment
Class 5 – Lower Mid-strength	
Fluticasone propionate (0.05%)	Cutivate® Cream/Cutivate Lotion
Prednicarbate (0.1%)	Dermatop® Cream
Hydrocortisone probutate (0.1%)	Pandel® Cream
Triamcinolone acetonide (0.1%)	Aristocort A Cream, Kenalog Lotion
Fluocinolone acetonide (0.025%)	Synalar Cream
Class 6 – Mild	
Alclometasone dipropionate (0.05%)	Aclovate® Cream/Ointment
Desonide (0.05%)	Verdeso™ Foam, Desonate Gel™
Triamcinolone acetonide (0.025%)	Aristocort A Cream, Kenalog Lotion
Hydrocortisone butyrate (0.1%)	Locoid Cream/Ointment
Fluocinolone acetonide (0.01%)	Derma-Smoothe/FS® Scalp Oil, Synalar Topical Solution
Class 7 – Least potent	
Hydrocortisone (2%/2.5%)	Nutracort® Lotion, Synacort Cream
Hydrocortisone (0.5 to 0.1%)	Cortaid® Cream/Spray/Ointment and many other over-the-counter products

6.9 Treatment after the End of the Study

The Sponsor will not provide any additional care to subjects after they leave the study because such care should not differ from what is normally expected for subjects with atopic dermatitis.

7.0 DISCONTINUATION OF STUDY TREATMENT AND SUBJECT DISCONTINUATION/WITHDRAWAL

7.1 Discontinuation of Study Treatment

The stopping criteria for the study are described in Section 4.6.

If a subject who does not meet enrollment criteria is inadvertently enrolled, that subject must be discontinued from study treatment and the Sponsor or Sponsor designee must be contacted. An exception may be granted in rare circumstances for which there is a compelling safety reason to allow the subject to continue. In these rare cases, the Investigator must obtain documented approval from the Sponsor or Sponsor designee to allow the subject to continue in the study.

Subjects who discontinue study treatment will not be replaced.

7.2 Subject Discontinuation/Withdrawal from the Study

- A subject may withdraw from the study at any time at his/her own request, or at the request of their parent or guardian, or may be withdrawn at any time at the discretion of the Investigator for safety, behavioral, compliance, or administrative reasons.
- If the subject, or their parent or guardian, withdraws consent for disclosure of future information, the Sponsor may retain and continue to use any data collected before such a withdrawal of consent.
- If a subject withdraws from the study, he/she or their parent or guardian, may request destruction of any samples taken and not tested, and the Investigator must document this in the study center study records.
- See the schedule of assessments (SoA) Table 1.1 for data to be collected at the time of study discontinuation and follow-up and for any further evaluations that need to be completed. Should a subject, or their parent or guardian request or decide to withdraw from the study, all efforts must be made to complete and report the observations as thoroughly as possible up to the date of withdrawal. Subjects withdrawing due to an AE should be followed up according to the follow-up visit.

7.3 Lost to Follow-up

A subject will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and is unable to be contacted by the study center.

The following actions must be taken if a subject fails to return to the study center for a required study visit:

- The study center must attempt to contact the subject or their parent or guardian, and reschedule the missed visit as soon as possible and counsel the subject on the importance of

maintaining the assigned visit schedule and ascertain whether or not the subject wishes to and/or should continue in the study.

- Before a subject is deemed lost to follow-up, the Investigator or designee must make every effort to regain contact with the subject, or their parent or guardian (where possible, 3 telephone calls and, if necessary, a certified letter to the subject's last known mailing address or local equivalent methods). These contact attempts should be documented in the subject's CRF.
- Should the subject continue to be unreachable, he/she will be considered to have withdrawn from the study.

Discontinuation of specific study centers or of the study as a whole are handled as part of Appendix 2.

8.0 STUDY ASSESSMENTS AND PROCEDURES

- Study procedures and their timing are summarized in the SoA (Table 1.1).
- Protocol waivers or exemptions are not allowed.
- Immediate safety concerns should be discussed with the Medical Monitor immediately upon occurrence or awareness to determine if the subject should continue or discontinue study treatment.
- Any serious or non-serious adverse event will be appropriately reported and documented.
- Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.
- All screening evaluations must be completed and reviewed to confirm that potential subjects meet all eligibility criteria. The Investigative site will record and document as applicable either confirmation of subject eligibility or screen failure for each subject within the CRF.
- Basic demographic information, including date of birth, gender, ethnicity, and race will be recorded at the Screening Visit.

8.1 Administrative Procedures

8.1.1 Subject Informed Consent

A signed and dated, study-specific, approved by Institutional Review Board (IRB and applicable regulatory authorities informed consent form must be obtained from each subject, or their parent or guardian, prior to performing any study-related procedures. No study related procedures or activities may be performed until each subject is fully informed and the consent form is signed and dated.

8.2 Study Procedures

8.2.1 Visit 1- Screening Visit (Day -21 to Day -14)

The following assessments will be performed and recorded by the Investigator or Designee:

- Informed Consent
- Evaluation of Inclusion/Exclusion Criteria.
- Review and record demographic information.
- Medical history.
- Hanifin and Rajka criteria.
- Collect concomitant medication details.
- Physical examination.
- Vital signs.
- Body weight and height measurement.
- Urine pregnancy test for female subjects ≥11 years old, or female patients <11 years old who have started menstruating.
- vIGA-AD scale.

- EASI.
- POEM.
- ItchMan Questionnaire.
- Provide study cleanser and moisturizer.
- Start AE collection and assessment.

8.2.2 Washout Phase (Day -14 to Day -1)

Washout phase may be initiated after Screening procedures have been completed. Every effort should be made for subjects to complete a 14-day Washout period. If absolutely necessary subjects may undergo Baseline on Day -1, which is also the 13th day of Washout.

The Investigator or designee will provide the subject and parent or guardian with study cleanser and moisturizer and will provide instructions for the use. Adverse event and concomitant medication will be recorded.

8.2.3 Unscheduled Visit

If an event arises that requires subject to come in to the study center, subjects should be scheduled for the unscheduled visit and assessments performed based on Investigator discretion.

Subjects will be encouraged to report any complications or adverse effects during their participation. Investigator may evaluate the subject at an unscheduled visit, if subject's condition will be considered as worsening.

8.2.4 Day 1 (Baseline) -1 to 1 day

Baseline assessments to be completed before dose administration:

- Review Inclusion/Exclusion Criteria.
- Hanifin and Rajka criteria.
- Collect concomitant medication details.
- Vital signs.
- Urine pregnancy test for female subjects ≥11 years old, or female patients <11 years old who have started menstruating.
- Physical examination
- vIGA-AD scale.
- EASI.
- POEM.
- ItchMan Questionnaire.
- Adverse event collection and assessment.

On completion of Baseline assessments:

- Dispense IP to Subject and parent or guardian.
- Provide study diary

- Provide counseling on the use of the study treatment, study diary and any questions the subject, parent or guardian may have will be answered.
- Provide study cleanser and moisturizer.
- Administer the IP under supervision of clinical staff.

8.2.5 Out Patient, Days 1 to 6, Days 8 to 14, and Days 15 to 20

The subject, or their parent or guardian, will be instructed by the Investigator or designee to:

- Administer the IP twice a day (only PM on Day 1).
- Complete the study diary.
- Use study cleanser and moisturizer as instructed.
- Record and review AEs.
- Record and review concomitant medications.

8.2.6 Study Center Visit, Day 7, Day 14 and Day 21

- The subject or parent or guardian may apply the IP at home and use study cleanser and moisturizer as instructed. The diary card should be completed as instructed.

The following assessments will be conducted by the Investigator or designee

- Vital signs.
- vIGA-AD scale.
- EASI.
- POEM.
- ItchMan questionnaire.
- Physical examination
- Dispense IP to Subject as necessary.
- Collect IP from Subject as necessary.
- Review IP compliance.
- Provide study cleanser.
- Provide study moisturizer.
- Provide counseling on the continued use of the study treatment, study diary and any questions the subject, parent or guardian may have will be answered.
- Review study diary entries.
- Record and review AEs.
- Record and review concomitant medications.

8.2.7 Final Visit (Day 28) or Early Termination Visit

Every attempt should be made to complete all visits during the defined window periods. Subjects who do not complete all required study visits and withdraw from the study before Day 28 final visit, will be asked to complete the Early Termination Visit.

- The subject or parent or guardian may apply the IP at home (not applicable for an Early Termination Visit).

The following assessments will be performed by the Investigator or designee:

- Physical examination.
- Record and review concomitant medication details.
- Record of vital signs.
- Body weight and height.
- Urine pregnancy test for female subjects ≥11 years old, or female patients <11 years old who have started menstruating.
- vIGA-AD scale.
- EASI.
- POEM.
- ItchMan questionnaire.
- Review IP compliance
- Record and review AE details.
- Review study diary entries.
- Collect used and unused IP containers, study cleanser and moisturizer.

9.0 METHODS OF ASSESSMENT

9.1 Subject Demographics

Basic demographic information, including date of birth, gender, ethnicity, and race will be recorded at the Screening Visit.

9.2 Concomitant Medication Recording

All medications (both prescription and nonprescription, and including vitamins, herbals, topicals, inhaled, and intranasal) taken within 30 days prior to the start of the IP and through the final study visit will be recorded on the appropriate CRF (using their generic and brand name, if known) with the corresponding indication, start and stop dates. At each study visit, subjects will be asked whether they have started or discontinued any medication since their previous study visit. This includes single use or PRN (as needed) medication use.

Previous treatment of atopic dermatitis must be recorded irrespectively of the term it was given. Corresponding condition shall be captured in the subject's medical history.

9.3 Efficacy Assessments

Planned timepoints for all efficacy assessments are provided in the SoA (Table 1.1).

The Investigator or designee will be responsible for providing the subjects and parent or guardian with an explanation of the purpose of the POEM and Itch assessments and training for the completion of the assessments. The Investigator or designee will provide the relevant documents for completion and assessments will be completed during visits to the clinic.

9.3.1 Validated Investigator Global Assessment for Atopic Dermatitis (vIGA-AD) Scale

The Investigator or designee will select the vIGA-AD scale that best describes the overall appearance of the atopic dermatitis lesions at each of the assessment timepoints during the study. The vIGA-AD scale allows for an assessment of overall disease severity at a specified timepoint, based on a 5 point scale. The vIGA-AD scale evaluates clinical characteristics of erythema, infiltration, papulation, oozing, and crusting as a guideline for the overall severity assessment (Table 9.1).

Table 9.1 Validated Investigator Global Assessment for Atopic Dermatitis Scale

Score	Grade	Definition
0	Clear	No inflammatory signs of atopic dermatitis (no erythema, no induration/papulation, no lichenification, no oozing/crusting). Post inflammatory hyperpigmentation and/or hypopigmentation may be present.
1	Almost Clear	Barely perceptible erythema, barely perceptible induration/papulation, and/or minimal lichenification. No oozing or crusting.
2	Mild	Slight but definite erythema (pink), slight but definite induration/papulation, and/or slight but definite lichenification. No oozing or crusting.
3	Moderate	Clearly perceptible erythema (dull red), clearly perceptible induration/papulation, and/or clearly perceptible lichenification. Oozing and crusting may be present.
4	Severe	Marked erythema (deep or bright red), marked induration/papulation, and/or marked lichenification. Disease is widespread in extent. Oozing or crusting may be present.

9.3.2 Atopic Dermatitis Eczema Area Severity Index

The EASI is a validated scoring system used to grade the physical signs of atopic dermatitis and eczema. The Investigator or designee will select the appropriate score at each of the assessment timepoints during the study.

The EASI evaluation assesses the extent of disease (% involvement) at 4 body sites:

- Head and neck.
- Trunk.
- Upper extremities.
- Lower extremities.

and measures 4 clinical signs:

- Erythema.
- Edema/papulation.
- Excoriation.
- Lichenification.

graded on a severity scale of 0 to 3 (half points may be used):

- 0 = None.
- 1 = Mild.
- 2 = Moderate.
- 3 = Severe.

Scoring information for each of the above measures will be recorded on paper.

9.3.3 Patient Oriented Eczema Measure

The POEM is a tool developed by the University of Nottingham, United Kingdom, for monitoring atopic dermatitis severity. The subject or the parent or guardian of the subject, will complete the questionnaire at each of the assessment timepoints.

Each of the 7 questions in the POEM questionnaire carries equal weight and is scored from 0 to 4:

- No days = 0.
- 1 to 2 days = 1.
- 3 to 4 days = 2.
- 5 to 6 days = 3.
- Every day = 4.

Scores are then added to yield a total score of 0 to 28; higher scores mean the greater the severity of atopic dermatitis.

9.3.4 ItchMan Questionnaire

The ItchMan Questionnaire will be used for the subject, or the parent or guardian, to indicate how itchy the area of atopic dermatitis feels at the timepoints during the study. Pictures to guide in the grading will be included in the subject diary.

- 0 = Comfortable, no itch.
- 1 = Itches a little; does not interfere with activity.
- 2 = Itches more; sometimes interferes with activity.
- 3 = Itches a lot difficult to sit still, concentrate.
- 4 = Itches most terribly; impossible to sit still, concentrate.

9.3.5 Application Site Assessment

The study treatment application sites will be evaluated at each visit, preferably by the same assessor. The first investigational product application will happen in the study center under clinical staff supervision, after the completion of the baseline assessments. All subsequent study application will be administered by the parent/guardian or by the subject in the setting of subject's home. Skin irritation will be recorded as an adverse event and followed until resolution (or stabilization, as applicable)

9.4 Safety Assessments

Planned timepoints for all safety assessments are provided in the SoA (Table 1.1).

9.4.1 Physical Examinations

A complete physical examination will include, at a minimum, assessments of the cardiovascular, respiratory, and gastrointestinal systems. Height and weight will also be measured and recorded.

9.4.2 Pregnancy Test

Female subjects aged **≥11** years old, or female patients <11 years old who have started menstruating, will have urinary pregnancy test performed at all visits. The Baseline result must be available and must be negative before the subject apply the first application of IP. Positive pregnancy test will disqualify the subject from the participation in the study.

9.4.3 Medical History

A complete medical history included evaluation (past or present) of the following: general, head and neck, eyes, ears, nose throat, chest/respiratory, heart/cardiovascular, gastrointestinal/liver, gynecological/urogenital, musculoskeletal/extremities, skin, neurological/psychiatric, endocrine/metabolic, hematologic/lymphatic, allergies/drug sensitivities, past surgeries, substance abuse, or any other diseases or disorders.

The details of the subject's atopic dermatitis history will be recorded.

9.4.4 Vital Signs

- Blood pressure, pulse and body temperature measurements will be assessed in the sitting position with a completely automated device. Manual techniques will be used only if an automated device is not available or the subject is uncomfortable or distressed with the use of an automated device.
- Blood pressure and pulse measurements should be preceded by at least 5 minutes of rest for the subject in a quiet setting without distractions (eg, television, cell phones, use of electronic games).
- Vital signs will consist of single measurement.
- Body weight and height will be measured at Screening and at Day 28/Final Visit (or at Early Termination Visit, as applicable).

9.5 Adverse Events

The definitions of an AE or SAE can be found in Appendix 3.

Adverse events will be reported by the subject, parent or guardian.

The Investigator and any designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study treatment or study procedures, or that caused the subject to discontinue the study (see Section 7.0).

9.5.1 Time Period and Frequency for Collecting AE and SAE Information

All SAEs will be collected from the signing of the informed consent form (ICF) until the final visit (or Early Termination Visit), at the timepoints specified in the SoA (Table 1.1).

All AEs will be collected from the signing of the ICF until the final visit (or Early Termination Visit), at the timepoints specified in the SoA (Table 1.1).

All SAEs will be recorded and reported to the Sponsor or designee within 24 hours, as indicated in Appendix 3. The Investigator or designee will submit any updated SAE data to the Sponsor within 24 hours of it being available.

Investigators or designees are not obligated to actively seek AE or SAE after conclusion of the study participation. However, if the Investigator or designee learns of any SAE, including a death, at any time after a subject has been discharged from the study, and he/she considers the event to be reasonably related to the study treatment or study participation, the Investigator or designee must promptly notify the Sponsor.

The method of recording, evaluating, and assessing causality of AEs and SAEs and the procedures for completing and transmitting SAE reports are provided in Appendix 3.

9.5.2 Method of Detecting AEs and SAEs

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the subject is the preferred method to inquire about AE occurrences.

9.5.3 Follow-up of AEs and SAEs

After the initial AE/SAE report, the Investigator or designee is required to proactively follow each subject at subsequent visits/contacts. All SAEs, will be followed until resolution, stabilization, the event is otherwise explained, or the subject is lost to follow-up (as defined in Section 7.3). Further information on follow-up procedures is given in Appendix 3.

9.5.4 Regulatory Reporting Requirements for SAEs

- Prompt notification by the Investigator or designee to the Sponsor of an SAE is essential so that legal obligations and ethical responsibilities toward the safety of subjects and the safety of a study treatment under clinical investigation are met.
- The Sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study treatment under clinical investigation. The Sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, IRBs, and Investigators.

- Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSARs) according to local regulatory requirements and Sponsor policy and forwarded to Investigators as necessary.
- An Investigator who receives an Investigator safety report describing an SAE or other specific safety information (eg, summary or listing of SAEs) from the Sponsor will review and then file it along with the Investigator's Brochure and will notify the IRB, if appropriate according to local requirements.

9.5.5 Pregnancy

- Details of all pregnancies in female subjects will be collected after the start of study treatment and until 1 month after the last dose.
- If a pregnancy is reported, the Investigator should inform the Sponsor within 24 hours of learning of the pregnancy and should follow the procedures outlined in Appendix 6.
- Abnormal pregnancy outcomes (eg, spontaneous abortion, fetal death, stillbirth, congenital anomalies, and ectopic pregnancy) are considered SAEs.

9.6 Treatment of Overdose

The Sponsor does not recommend specific treatment for an overdose. Washing with conventional cleanser and water will remove the product.

The subject, or the parent or guardian, should notify the Investigator of any overdose.

In the event of an overdose, the Investigator should:

1. Contact the Medical Monitor immediately.
2. Closely monitor the subject for any AE/SAE.
3. Document the quantity of the excess dose as well as the duration of the overdose in the CRF.

Decisions regarding dose interruptions will be made by the Investigator in consultation with the Medical Monitor based on the clinical evaluation of the subject.

9.7 Pharmacodynamics

Pharmacodynamics parameters are not evaluated in this study.

9.8 Genetics

Genetics are not evaluated in this study.

9.9 Biomarkers

Biomarkers are not evaluated in this study.

9.10 Health Economics/Medical Resource Utilization and Health Economics

Health Economics/Medical Resource Utilization and Health Economics parameters are not evaluated in this study.

10.0 STATISTICAL CONSIDERATIONS

10.1 Statistical Hypotheses

No formal statistical hypothesis testing is planned for this study. All analyses will be considered exploratory and descriptive.

10.2 Sample Size Determination

The study will enroll and dose 36 subjects, in 3 age cohorts of 12 subjects:

- Cohort 1: subjects aged 2 to 5 years old.
- Cohort 2: subjects aged 6 to 11 years old.
- Cohort 3: subjects aged 12 to 17 years old.

The sample size is not based on statistical considerations but is typical for studies of this nature, and is considered adequate to characterize the distribution of the planned endpoints. Any statistical testing will be considered exploratory and descriptive.

10.3 Populations for Analyses

For purposes of analysis, the analysis sets in Table 10.1 are defined.

Table 10.1 Analysis Sets

Analysis Set	Description
Intent to Treat (ITT) population	All subjects who are enrolled and take at least 1 dose of study treatment. The ITT population will be the primary population for safety and efficacy assessments.
Per Protocol (PP) population	All subjects in the ITT population without any protocol deviations that may have an impact on the efficacy assessments. The PP population will be the supportive population for efficacy assessments.

10.4 Statistical Analyses

The Statistical Analysis Plan will be developed and finalized before database lock and will describe the subject analysis sets to be included in the analyses, and procedures for accounting for missing, unused, and spurious data. This section is a summary of the planned statistical analyses of the primary and secondary endpoints.

All analyses, summaries, and listings will be performed using SAS® software (version 9.3 or higher).

The following descriptive statistics will be used as applicable to summarize the study data unless otherwise specified:

- Continuous variables: sample size (n), mean, standard deviation, median, minimum (min), and maximum (max).
- Categorical variables: frequencies and percentages.

Individual subject data will be presented in listings.

10.4.1 Efficacy Analyses

All efficacy analyses will be performed on the ITT and PP populations, for overall and by age cohorts (2 to 5 years, 6 to 11 years, and 12 to 17 years).

Summary statistics will be provided for the vIGA-AD scale, EASI score (total and by component), POEM score (total and by component) and the ItchMan scale, and changes from baseline at each visit. The frequency and percentage of subjects who have achieved clear (0) or almost clear (1) on the vIGA-AD scale will also be provided at each visit.

10.4.2 Safety Analyses

All safety analyses will be performed on the ITT population.

All safety assessments, including TEAEs, vital signs and weight, physical examinations, medical examination pregnancy test results and concomitant medication will be listed and as appropriate summarized with descriptive statistics.

Treatment-emergent adverse events are defined as AEs that first occurred or worsened in severity after the first administration of study treatment.

Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). The numbers of TEAEs and incidence rates will be tabulated by preferred term and system organ class, for overall and by cohort.

Treatment-emergent AEs by maximum severity, TEAEs by relationship to study treatment, SAEs, TEAEs leading to death, and TEAEs leading to discontinuation of study treatment will be tabulated for overall and by age cohort.

All vital signs measurements, weight, and body mass index will be summarized for overall and by age cohort, using descriptive statistics at each visit for raw numbers and change from Baseline. The incidence of abnormal physical examination results will also be summarized using descriptive statistics.

10.4.3 Missing Data

Data from subjects who withdraw from the study, including AEs and any follow-up, will be included in the analyses of all outcomes.

Missing data will not be imputed.

10.5 Interim Analyses

No interim analysis is planned.

10.6 Monitoring Committee

A Safety Monitoring Committee will not be established.

11.0 REFERENCES

1. Arp DJ, Sayavedra-Soto LA, Hommes NG. Molecular biology and biochemistry of ammonia oxidation by Nitrosomonas europaea. Arch Microbiol 2002: 178. 250-255.

2. Rico J, Quiring J, Hollenbach S, Enloe C, Stasko N. Phase 2 study of efficacy and safety of SB204 in the treatment of acne vulgaris. Novan Therapeutics, Poster Presentation, Annual Meeting of the Society for Investigative Dermatology (SID) May 7-10, 2014, Albuquerque, New Mexico.

3. Weller R, Price R. Antimicrobial effect of acidified nitrite on dermatophyte fungi, Candida and bacterial skin pathogens. J Appl Microbiol 2001: 648-652. Appendices.

4. Investigator Brochure

Appendix 1 Abbreviations

Abbreviation	Definition
AE	Adverse event
AOB	Ammonia oxidizing bacteria
CRA	Clinical Research Associate
CRF	Case report form (electronic or paper)
CRO	Clinical research organization
EASI	Eczema Area Severity Index
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HIV	Human immunodeficiency virus
ICF	Informed consent form
ICH	International Council for Harmonisation
IRB	Institutional Review Board
vIGA-AD	Validated Investigator Global Assessment for Atopic Dermatitis
ITT	Intent To Treat
IP	Investigational product
IND	Investigational New Drug
IRB	Institutional Review Board
NO	nitrite
NO_2	nitric oxide
OD	Optical density
POEM	Patient Oriented Measure
PP	Per Protocol
SAE	Serious adverse event
SoA	Schedule of assessments
TEAE	Treatment-emergent adverse events

Appendix 2 Regulatory, Ethical, and Study Oversight Considerations

Regulatory and Ethical Considerations

- This study will be conducted in accordance with the protocol and with the following:
  - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines.
  - Applicable ICH GCP Guidelines.
  - Applicable laws and regulations.
- The protocol, protocol amendments, ICF, Investigator Brochure's, and other relevant documents (eg, advertisements) must be submitted to an IRB by the Investigator and reviewed and approved by the IRB before the study is initiated.
- Any amendments to the protocol will require IRB and regulatory authority approval, when applicable, before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to subjects.
- The Investigator will be responsible for the following:
  - Providing written summaries of the status of the study to the IRB annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB.
  - Notifying the IRB of SAEs or other significant safety findings as required by IRB procedures.
  - Providing oversight of the conduct of the study at the study center and adherence to requirements of 21 Code of Federal Regulations (CFR), ICH guidelines, the IRB, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations.
- After reading the protocol, each Investigator will sign the protocol signature page and send a copy of the signed page to the Sponsor or representative (Appendix 7). The study will not start at any study center at which the Investigator has not signed the protocol.

Adequate Resources

The Investigator is responsible for supervising any individual or party to whom the Investigator delegates study-related duties and functions conducted at the study center.

If the Investigator/institution retains the services of any individual or party to perform study-related duties and functions, the Investigator/institution should ensure this individual or party is qualified to perform those study-related duties and functions and should implement procedures to ensure the integrity of the study-related duties and functions performed and any data generated.

Financial Disclosure

Investigators and sub-Investigators will provide the Sponsor with sufficient, accurate financial information as requested to allow the Sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

Insurance

Sponsor will provide insurance in accordance with local guidelines and requirements as a minimum for the subjects in this study. The terms of the insurance will be kept in the study files.

Informed Consent Process

- The Investigator or his/her representative will explain the nature of the study to the subject and their parent or guardian, and answer all questions regarding the study.
- Subjects and their parent or guardian, must be informed that their participation is voluntary. Subjects or their parent or guardian, will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act (HIPAA) requirements, where applicable, and the IRB or study center.
- The medical record must include a statement that written informed consent was obtained before the subject was entered in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.
- Subjects, and their parent or guardian, must be re-consented to the most current version of the ICF(s) during their participation in the study.
- A copy of the ICF(s) must be provided to the subject or the subject's parent or guardian.

If a subject is rescreened the subject, or their parent or guardian, are required to sign a new ICF.

Data Protection

- Subjects will be assigned a unique identifier by the Sponsor. Any subject records or datasets that are transferred to the Sponsor will contain the identifier only; subject names or any information which would make the subject identifiable will not be transferred.
- The subject must be informed that his/her personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the subject.
- The subject must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the Sponsor, by appropriate IRB members, and by inspectors from regulatory authorities.

Medical Monitor

Dr. James Milbauer

Phone: 973-265-4431
Office: 973-335-1516
Cell: 973-727-7969
Fax: 973-335-5417

Dissemination of Clinical Study Data

The results of the study should be reported within 1 year from the end of the clinical study. Irrespective of the outcome, the Sponsor will submit to the European Union database a summary of the results of the clinical study within 1 year from the end of the clinical study. It shall be accompanied by a summary written in a manner that is understandable to laypersons.

Data Quality Assurance

- All subject data relating to the study will be recorded on printed or CRFs unless transmitted to the Sponsor or designee electronically (eg, laboratory data). The Investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.
- The Investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.
- The Investigator must permit study-related monitoring, audits, IRB review, and regulatory agency inspections and provide direct access to source data documents.
- The Sponsor or designee is responsible for the data management of this study including quality checking of the data.
- Study monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized study center personnel are accurate, complete, and verifiable from source documents; that the safety and rights of subjects are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.
- Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the Investigator for 15 years after study completion unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the Sponsor. No records may be transferred to another location or party without written notification to the Sponsor.

Source Documents

The Investigator/institution should maintain adequate and accurate source documents and study records that include all pertinent observations on each of the study center's subjects. Source data should be attributable, legible, contemporaneous, original, accurate, and complete. Changes to

source data should be traceable, should not obscure the original entry, and should be explained if necessary (eg, via an audit trail).

- Source documents provide evidence for the existence of the subject and substantiate the integrity of the data collected. Source documents are filed at the Investigator's study center.
- Data reported on the CRF or entered in the CRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The Investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.
- Source documents will include, but are not limited to, screening logs, questionnaires and forms completed by the clinical staff and the subjects, or their parent or guardian.

Study and Study Center Closure

The Sponsor designee reserves the right to close the study center or terminate the study at any time for any reason at the sole discretion of the Sponsor. Study centers will be closed upon study completion. A study center is considered closed when all required documents and study supplies have been collected and a study center closure visit has been performed.

The Investigator may initiate study center closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study center by the Sponsor or Investigator may include but are not limited to:

- Failure of the Investigator to comply with the protocol, the requirements of the IRB or local health authorities, the Sponsor's procedures, or GCP guidelines.
- Inadequate recruitment of subjects by the Investigator.
- Discontinuation of further study treatment development.

Publication Policy

The data generated by this study are confidential information of the Sponsor. The Sponsor will make the results of the study publicly available. The publication policy with respect to the Investigator and study center will be set forth in the Clinical Trial Agreement.

- The results of this study may be published or presented at scientific meetings. If this is foreseen, the Investigator agrees to submit all manuscripts or abstracts to the Sponsor before submission. This allows the Sponsor to protect proprietary information and to provide comments.
- The Sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the Sponsor will generally support publication of multicenter studies only in their entirety and not as individual study center data. In this case, a Coordinating Investigator will be designated by mutual agreement.

- Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

Appendix 3 Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

Definition of AE

AE Definition
• An AE is any untoward medical occurrence in a patient or subject, temporally associated with the use of study treatment, whether or not considered related to the study treatment. • NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of study treatment.

Events Meeting the AE Definition
• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (eg, electrocardiogram, radiological scans, and vital signs measurements), including those that worsen from Baseline, considered clinically significant in the medical and scientific judgment of the Investigator (ie, not related to progression of underlying disease). • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. • New conditions detected or diagnosed after study treatment administration even though it may have been present before the start of the study. • Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study treatment or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae.

Events NOT Meeting the AE Definition
• Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the subject's condition. • The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the subject's condition. • Medical or surgical procedure (eg, endoscopy, and appendectomy): the condition that leads to the procedure is the AE. • Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital). • Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

Definition of SAE

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (eg, hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

An SAE is defined as any untoward medical occurrence that, at any dose:
a) Results in death.
b) Is life-threatening The term 'life-threatening' in the definition of "serious" refers to an event in which the subject 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 inpatient hospitalization or prolongation of existing hospitalization In general, hospitalization signifies that the subject 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 outpatient 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. Hospitalization for elective treatment of a pre-existing condition that did not worsen from Baseline is not considered an AE.
d) Results in persistent disability/incapacity • 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 (eg, sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.
e) Is a congenital anomaly/birth defect
f) Other (important medical events): • Medical or scientific judgment should be exercised in deciding whether SAE 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 subject or may require medical or surgical intervention to prevent 1 of the other outcomes listed in the above definition. These events should usually be considered serious. Examples of such events include 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.

Recording and Follow-up of AE and/or SAE

AE and SAE Recording

- When an AE/SAE occurs, it is the responsibility of the Investigator to review all documentation (eg, hospital progress notes, laboratory reports, and diagnostics reports) related to the event.
- The Investigator or designee will then record all relevant AE/SAE information in the CRF. Each event must be recorded separately.
- It is **not** acceptable for the Investigator to send photocopies of the subject's medical records in lieu of completion of the AE/SAE CRF page.
- There may be instances when copies of medical records for certain cases are requested by the Sponsor. In this case, all subject identifiers, with the exception of the subject number, will be redacted on the copies of the medical records before submission to the Sponsor.
- The Investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

Assessment of Intensity

The Investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to 1 of the following categories:

- Mild: An event that is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.
- Moderate: An event that causes sufficient discomfort and interferes with normal everyday activities.
- Severe: An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with an SAE. Severe is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe.

  An event is defined as 'serious' when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Causality

- The Investigator is obligated to assess the relationship between study treatment and each occurrence of each AE/SAE. The AE must be characterized as unrelated, unlikely to be related, or possibly related.
  - "Unrelated" is used if there is not a reasonable possibility that the study treatment caused the AE.
  - "Unlikely to be related" suggests that only a remote connection exists between the study treatment and the AE. Other conditions, including chronic illness, progression or expression of the disease state or reaction to concomitant therapy, appear to explain the reported AE.
  - "Possibly related" suggests that the association of the AE with the study treatment is unknown; however, the AE is not reasonably supported by other conditions.
  - "Probably related" conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
- The Investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study treatment administration will be considered and investigated.
- The Investigator will also consult the Investigator's Brochure and/or Product Information, for marketed products, in his/her assessment.

- For each AE/SAE, the Investigator must document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.
- There may be situations in which an SAE has occurred and the Investigator has minimal information to include in the initial report to the Sponsor. However, it is very important that the Investigator always make an assessment of causality for every event before the initial transmission of the SAE data to the Sponsor.
- The Investigator may change his/her opinion of causality in light of follow-up information and send a SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow-up of AEs and SAEs

- The Investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by the Sponsor to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.
- New or updated information will be recorded in the originally completed CRF.
- The Investigator will submit any updated SAE data to the Sponsor within 24 hours of receipt of the information.

Reporting of SAEs

SAE Reporting via Paper SAE form

- Reporting will be via paper as standard for the site. This will be using Novella template reporting form.
- To report a Safety Event, please contact the main Safety Management phone/fax/email (or your applicable country specific toll-free numbers):

Phone: 919.313.7111 (US Toll-free: 1-866-758-2798)

Fax: 919.313.1412 (US Toll-free: 1-866-761-1274)

Email: Safety-Inbox@novellaclinical.com

Appendix 4 Excluded Medications/Therapy

Excluded medication/therapy is listed below. The use of an excluded medication/therapy is a protocol violation and must be recorded in the CRF.

Within 4 weeks prior to Baseline

- Systemic immunosuppressive/immunomodulating drugs (ie, methotrexate, cyclosporine, etc).
- Immunoglobulin or blood products.

Within 2 weeks prior to Baseline

- High, or mid or low -potency topical corticosteroids. The use of inhaled or intranasal corticosteroids will be allowed[a].
- Systemic anti-inflammatory products.
- Use of crisaborole ointment.
- Systemic antibiotics.
- Bleach baths or topical coal tar.
- Topical calcineurin inhibitor use.
- New onset use of systemic antihistamines.
- Topical antihistamines.
- The use of intranasal antihistamines will be allowed[a].

a Subjects following stable regimens (≥2 weeks consistent use before study baseline) with systemic antihistamines or oral corticosteroids are permitted to continue use but should not alter the dose or stop the regimen while in the study within 1 week prior to Baseline.

Biologics

- Cell-depleting agents, including but not limited to rituximab: within 6 months of baseline.
- Infliximab, adalimumab, golimumab, certolizumab pegol, abatacept, etanercept, anakinra, and dupilumab: within 12 weeks of baseline, or 5 half-lives, whichever is longer.
- Other biologics: within 12 weeks of Baseline, or 5 half-lives (if known), whichever is longer.

Appendix 5 Contraceptive Guidance and Collection of Pregnancy Information

Definitions:

Contraception Guidance

Male subjects

- Male subjects with female partners of childbearing potential are eligible to participate if they agree to ONE of the following [during the protocol-defined time frame in Section 5.1]:
  - Are abstinent from penile-vaginal intercourse and agree to remain abstinent for duration of study and for 3 months after the final study treatment days.
  - Agree to use a male condom and have their partner use of a contraceptive method with a failure rate of <1% per year as described in the table below when having penile-vaginal intercourse with a female.
- In addition, male subjects must refrain from donating sperm for the duration of the study and for 3 months after the last dose of study treatment.
- Male subjects with a pregnant or breastfeeding partner must agree to remain abstinent from penile-vaginal intercourse or use a male condom during each episode of penile penetration during the protocol-defined time frame.

Female subjects

Female subjects of childbearing potential are eligible to participate if they practice abstinence or agree to use a highly effective method of contraception consistently and correctly as described in the table below.

Highly Effective Contraceptive Methods

Highly Effective Contraceptive Methods That Are User Dependent [a] *Failure rate of <1% per year when used consistently and correctly.*
Sexual Abstinence Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatment. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the subject.
Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation[b] • Oral. • Intravaginal. • Transdermal.
Progestogen only hormonal contraception associated with inhibition of ovulation • Oral. • Injectable.
Highly Effective Methods That Are User Independent [a]
Implantable progestogen only hormonal contraception associated with inhibition of ovulation[b] • Intrauterine device (IUD). • Intrauterine hormone-releasing system (IUS). • Bilateral tubal occlusion.
Vasectomized Partner A vasectomized partner is a highly effective birth control method provided that the partner is the sole male sexual partner of the Women of child-bearing potential and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used.
NOTES: [a] Typical use failure rates may differ from those when used consistently and correctly. Use should be consistent with local regulations regarding the use of contraceptive methods for subjects participating in clinical studies. b Hormonal contraception may be susceptible to interaction with the study treatment, which may reduce the efficacy of the contraceptive method. Females must either practice abstinence from heterosexual contact or use one (1) of the highly effective contraceptive options described in the Appendix 5. Females are not required to use two (2) highly effective methods of contraception.

Pregnancy Testing:

Pregnancy testing (urine) will be performed at times shown in Table 1.1 for all female subjects ≥11 years, or female patients <11 years old who have started menstruating.

Collection of Pregnancy Information

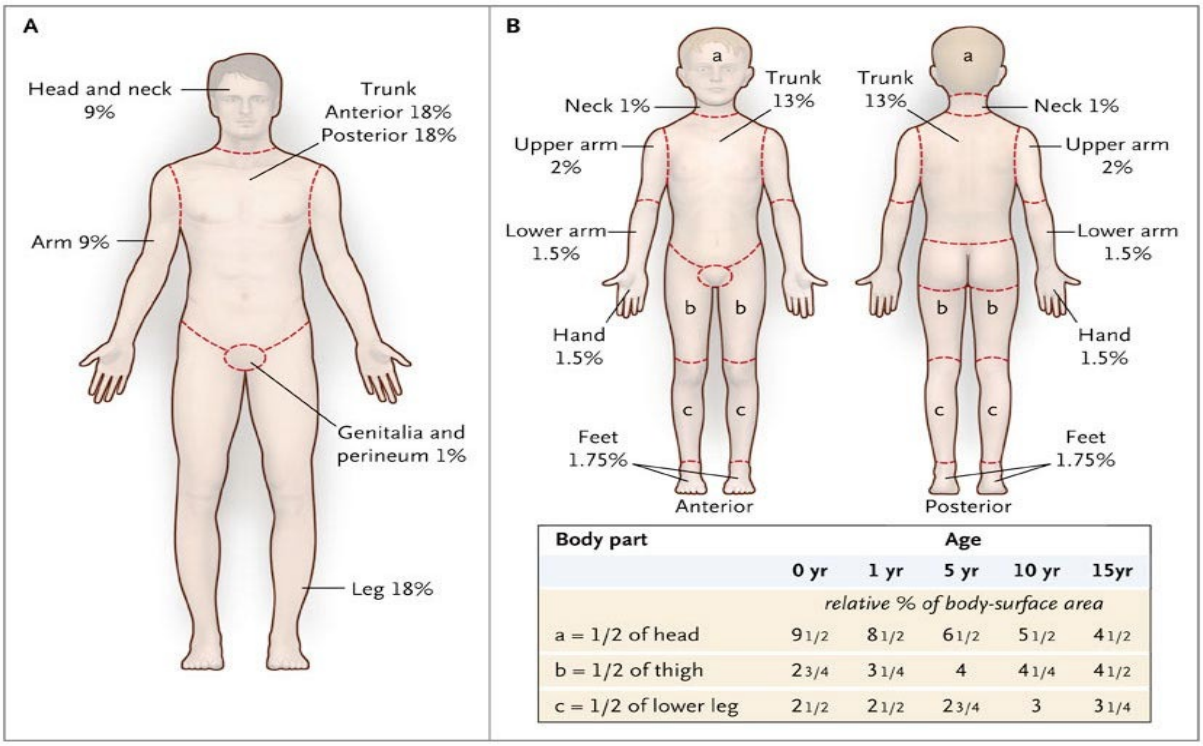
Male subjects with partners who become pregnant

- After obtaining the necessary signed informed consent from the pregnant female partner directly, the Investigator will record pregnancy information on the appropriate form and submit it to the Sponsor within 24 hours of learning of the partner's pregnancy. The female partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to the Sponsor. Generally, the follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any termination of the pregnancy will be reported regardless of fetal status (presence or absence of anomalies) or indication for the procedure.

Female Subjects who become pregnant

- The Investigator will collect pregnancy information on any female subject who becomes pregnant while participating in this study. Information will be recorded on the appropriate form and submitted to the Sponsor within 24 hours of learning of a subject's pregnancy. The subject will be followed to determine the outcome of the pregnancy. The Investigator will collect follow-up information on the subject and the neonate and the information will be forwarded to the Sponsor. Generally, follow-up will not be required for longer than 6 to 8 weeks beyond the estimated delivery date. Any termination of pregnancy will be reported, regardless of fetal status (presence or absence of anomalies) or indication for the procedure.
- While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy will be reported as an AE or SAE. A spontaneous abortion is always considered to be an SAE and will be reported as such.
- Any post study pregnancy related SAE considered reasonably related to the study treatment by the Investigator will be reported to the Sponsor as described in Section 9.5.4. While the Investigator is not obligated to actively seek this information in former subjects, he or she may learn of an SAE through spontaneous reporting.
- Any female subject who becomes pregnant while participating in the study will discontinue study treatment.

Appendix 6 Body Surface Area and Investigational Medicinal Product Application



Body part	Age				
	0 yr	1 yr	5 yr	10 yr	15yr
	relative % of body-surface area				
a = 1/2 of head	9 1/2	8 1/2	6 1/2	5 1/2	4 1/2
b = 1/2 of thigh	2 3/4	3 1/4	4	4 1/4	4 1/2
c = 1/2 of lower leg	2 1/2	2 1/2	2 3/4	3	3 1/4

Appendix 7 Signature of Investigator

PROTOCOL TITLE: An open-label, multicenter, Phase Ib study of B244 delivered as a topical spray to assess safety in pediatric subjects aged 2 to 17 years with atopic dermatitis

PROTOCOL NO: ADB244-002

VERSION: Amendment 1

This protocol is a confidential communication of [Sponsor]. I confirm that I have read this protocol, I understand it, and I will work according to this protocol. I will also work consistently with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with GCP and the applicable laws and regulations. Acceptance of this document constitutes my agreement that no unpublished information contained herein will be published or disclosed without prior written approval from the Sponsor.

Instructions to the Investigator: Please SIGN and DATE this signature page. PRINT your name, title, and the name of the study center in which the study will be conducted. Return the signed copy to [Sponsor or CRO].

I have read this protocol in its entirety and agree to conduct the study accordingly:

Print Name	
Title	
Address of Institution / Site:	_____ _____ _____ _____
Telephone Number:	

Investigator Signature: _____ Date: _____