CLINICAL TRIAL PROTOCOL
Phase II

COMPOUND:
B244

A Prospective, Vehicle Controlled, Double Blind, Multicenter, Randomized, Phase II Study of B244 Delivered as a Topical Spray to Determine Safety in Subjects with Mild to Moderate Atopic Dermatitis.

STUDY NUMBER: ADB244-001

VERSION DATE: October 5, 2018

Sponsor:
AOBiome Therapeutics
125 Cambridgepark Drive
Cambridge, MA 02140

Revision Chronology

Original	June 14, 2017
Amendment 1	September 1, 2017
Amendment 2	January 10, 2018
Amendment 3	October 5, 2018

SPONSOR APPROVAL

A Prospective, Vehicle Controlled, Double Blind, Multicenter, Randomized, Phase II Study of B244 Delivered as a Topical Spray to Determine Safety in Subjects with Mild to Moderate Atopic Dermatitis.

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INVESTIGATOR AGREEMENT

I have read this protocol and agree:

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

Investigator Printed Name:_____

Signature:_____ **Date:** _____

Statement of Compliance

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

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

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

PROTOCOL AMENDMENT 3 SUMMARY OF CHANGES

Section	Description of Changes
Title Page	Updated with Sponsor's new company logo and business address
Sponsor Approval Page	Updated section with up to date information
Clinical Trial Summary Section 6.2	Per Administrative Letter dated 27FEB2018, Inclusion criteria for the study has been updated as follows: increase the upper limit of subject's BSA affected by Atopic Dermatitis to 30%.
Section 11.1 Schedule of Events Table	Per Administrative Letter dated 12MAR2018: inserted clarification that that medical records from patient's dermatologist or primary care physician to confirm the diagnosis are optional. Verbal confirmation of the diagnosis present for ≥ 12 months is sufficient in order to fulfill this criterion.
Sections 7.2; 8.15.1; 11.6.2; Schedule of Events Table	Per Administrative Letter dated 26APR2018, inserted clarification language stating that the use of moisturizer in the study is not mandatory.
Sections 9; 11.6; 12.11 Schedule of Events Table	Per Administrative Letter dated 17JUL2018, subjects will be provided with two (2) actigraphy watches at Baseline period and will be asked to wear one watch on each wrist at all times through Day 28. Subjects will be asked to return both watches at Day 28 visit.
Section 8.7	Modified language around IP kits
Section 12.1	Updated shipping schedule and corrected Lab name
Section 12.11	Updated Actigraphy watch schedule and procedures
Section 12.14	Revised language around sample shipment to state that samples will be shipped according to the Lab Manual.
Section 16.7	Updated safety reporting email

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.

CLINICAL TRIAL SUMMARY

COMPOUND: B244 STUDY NUMBER: ADB244-001

TITLE:	A Prospective, Vehicle Controlled, Double Blind, Multicenter, Randomized, Phase II Study of B244 Delivered as a Topical Spray to Determine Safety in Subjects with Mild to Moderate Atopic Dermatitis.
INVESTIGATIONAL PRODUCT	B244 Topical application
STUDY ARMS	1. B244 2. Vehicle
PURPOSE:	The aim of the study is to assess the safety of B244 in the treatment of atopic dermatitis.
STUDY OBJECTIVES:	Primary: • To evaluate the safety and tolerability of B244 in participants with mild to moderate Atopic Dermatitis. Secondary: • To assess the efficacy of B244 versus vehicle by the change in Atopic Dermatitis Area Severity Index (EASI) Score from Baseline to post-baseline visits Exploratory:

	• To assess the efficacy of B244 versus vehicle by the change in Visual Analog Scale (VAS) Score from Baseline to post-baseline visits • To assess the efficacy of B244 versus vehicle by the change in Skindex 16 Score from Baseline to post-baseline visits • To assess the efficacy of B244 versus vehicle by the change in Investigator Global Assessment Score (IGA) from Baseline to post-baseline visits • To assess the efficacy of B244 versus vehicle by the change in Actigraphy Movement Count per Hour and sleep quality during nighttime from Baseline to post-baseline visits • To evaluate if B244 administration on the skin twice daily for 28 days will affect the levels of immune biomarkers. • To explore microbial content at baseline and Day 28.
STUDY DESIGN:	This is a Prospective, Vehicle Controlled, Double Blind, Multicenter, Randomized Phase II trial, comparing the effect of twice daily B244 application for 28 days vs vehicle application on treatment of mild to moderate AD At Screening and Baseline, all subjects must have atopic dermatitis, as defined by the Hanifin and Rajka criteria, which involves a minimum of 10% and a maximum of 30% body surface area, EASI score of 10 to 21 and pruritus visual analogue scale scores of ≥ 5 points on the

	VAS scale (at least moderate), IGA of 2-3. The total duration of the study will be approximately 9 weeks. Participants will report for a Screening visit and if all inclusion criteria are met, subjects will go through a two week washout phase before reporting for a Baseline visit. Subjects will come in for visits at Day 14 (Week 2), Day 28 (Week 4). A final visit will be conducted at Day 42 (Week 6). Efficacy will be assessed using Atopic Dermatitis Area and Severity Index (EASI), Visual Analog Scale (VAS) and Investigator Global Assessment (IGA) score . Blood and urine samples will be collected for standard safety laboratory tests and effect of the drug on inflammatory biomarkers. Participant's safety will be monitored throughout the study. We plan to enroll approximately 130 total patients. Assuming 20% drop out rate, we will plan to complete 104 patients. Randomization will be 1:1 so that equal number of patients will be treated in each Arm of the study. All B244 randomized subjects will be treated at the dose of $4x10^9$ cfu/ml (mid-dose of the Phase 1b/2a safety trial)
STUDY POPULATION:	Male and female subjects 18 years of age or older with clinically diagnosed mild to moderate Atopic Dermatitis.
Main Inclusion Criteria:	• Male and female subjects ≥18 years of age

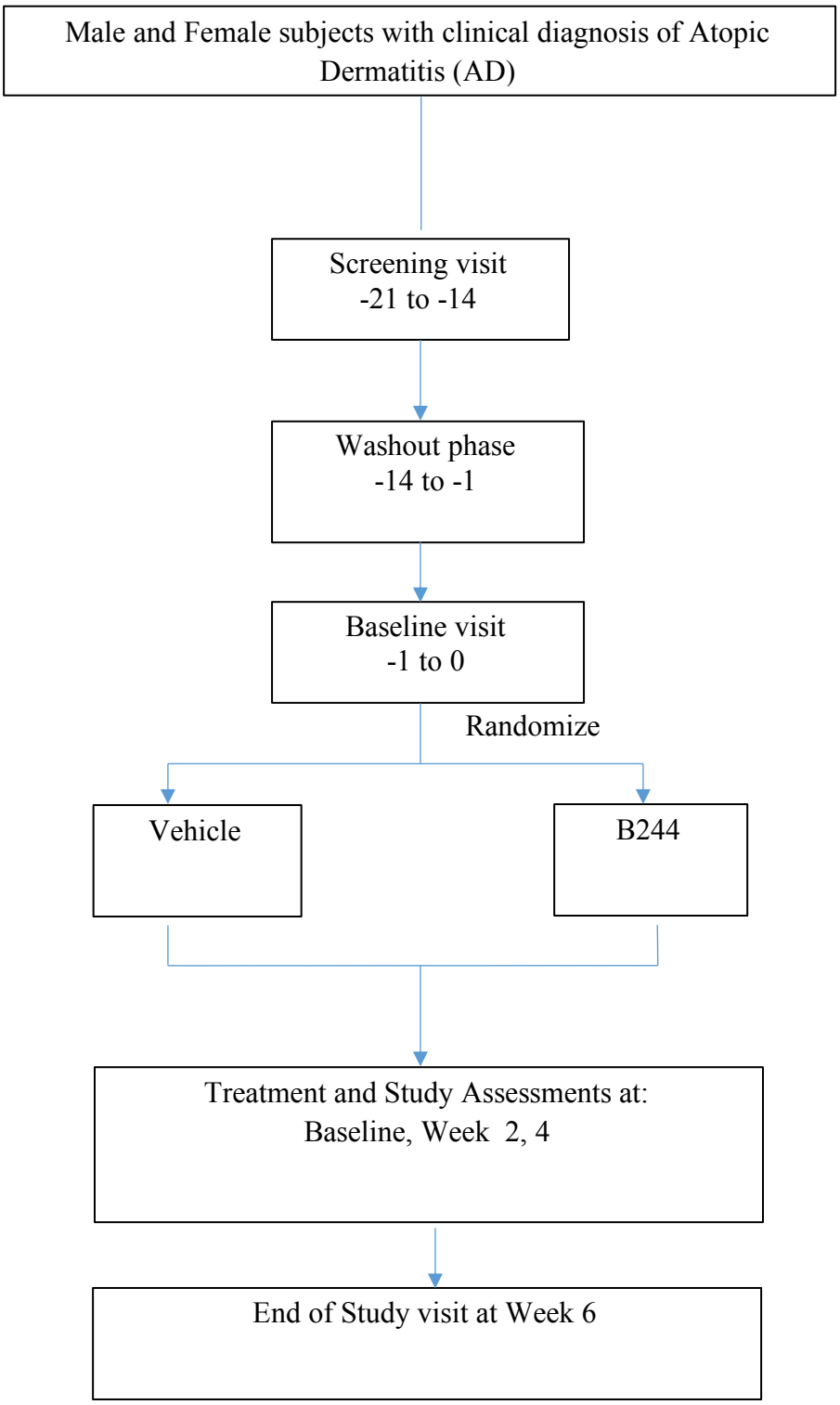
	• In good general health as determined by a thorough medical history and physical examination, and vital signs • Clinical diagnosis of mild to moderate atopic dermatitis according to the criteria of Hanifin and Rajka • Mild to moderate Atopic Dermatitis area and severity index [EASI] 10-21 • A score of at least ≥ 5 points (moderate pruritus) on the VAS for pruritus • A minimum of 10% and not more than 30% of the subjects' BSA affected by atopic dermatitis (affected is defined by physical examination findings: erythema, edema, scaling, lichenification, excoriation, with the excoriation serving as the physical examination correlate of pruritus) • An IGA score of 2-3 • Patient has a history of AD for ≥ 12 months • Ability to read and understand English and to provide written informed consent and authorization for protected health information disclosure
Main Exclusion Criteria:	• Pregnant and lactating women by urine pregnancy testing • Subjects with any significant clinical abnormalities which may interfere with study participation • Any skin condition which may interfere with evaluation of AD • Atopic dermatitis on the head or scalp

	• Subjects with Atopic dermatitis on the face • Unstable or actively infected atopic dermatitis • Patients suffering from pruritus from conditions other than AD • Patients with chronic pruritus due to systemic disease • Patients with conditions requiring inhaled steroids • Have concurrent skin disease of such severity in the study area that it could interfere with the study evaluation • Have active skin infections on the treatment area • Have received or planning to receive topical corticosteroids, topical coal tar, topical sulfur, topical PDE-4 inhibitors, topical antihistamines, topical antiseptics or antibiotics, topical antifungals, bleach baths, UVA or UVB phototherapy, oral/IV/inhaled steroids, antibiotics/antiviral/antifungal agents, oral probiotics, glucocorticoids treatment, calcineurin inhibitors, immunomodulating biologic agents, systemic glucocorticoids, systemic immunosuppressive or immunomodulatory agents within 2 weeks of Baseline visit. • Current or recent history (≤ 3 months of systemic use of Otrexup™, Rasuvo®, Rheumatrex® and Trexall™ or its generic versions such as Methotrexate • History of being seropositive for human immunodeficiency virus (HIV) at screening by laboratory testing at Screening

	• History of being positive for Hepatitis B virus surface antigen (HBsAg) or positive Hepatitis C virus antibody (HCV Ab) at screening by laboratory testing at Screening • History of renal disease • Use of any investigational drugs within the previous 30 days prior to dosing or within a period of less than five times the drug's half-life, whichever is longer • Use of any biologic within a period of 5 times its half-life • Use of vinegar or bleach baths within 2 weeks of starting the study
DOSE REGIMEN:	Subjects will apply a total of 8 pumps of IP per application to all affected areas twice-a-day (i.e. 8 pumps in the morning and 8 pumps again at night) for 28 Days.
ASSESSMENT SCHEDULE:	All subjects will attend a screening visit not more than 21 days prior to Day 1. Subjects will be required to return to the clinic at Baseline, on Day 14 (Week 2), Day 28 (Week 4). All subjects will be asked to attend a follow-up visit 2 weeks (14 (±3) days) after the last dose of study medication.
STATISTICAL CONSIDERATIONS:	• All efficacy analyses will be performed on the ITT population and PP population • The difference in treatment groups in change-from-baseline values at post-baseline visits will be analyzed using a mixed Analysis of Variance with one between-subject variable (active group vs. vehicle group) and one within-

	subject variable • One-sided two-sample equal-variance t-test will be performed as a supportive test to analyze the differences in treatment group means in change from baseline at each post-baseline visit
DURATION OF STUDY:	Subject's participation in the study will take up to 9 weeks. The estimated total study duration is 12 months. All subjects will be followed from enrollment and until the Final (End of Study Visit), which occurs 42 days following the baseline visit.

1 STUDY SCHEMA



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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

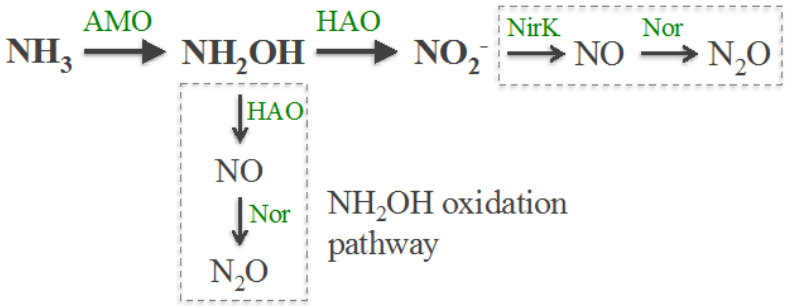
AD	Atopic Dermatitis
AE	Adverse Event
AMO	Ammonia Monooxygenase
AOB	Ammonia Oxidizing Bacteria
BID	Twice-Daily
CRF	Case Report Form
EASI	Atopic Dermatitis Area and Severity Index
E/T	Early Termination
FDA	Food and Drug Administration
HAO	NH_2OH oxidoreductase
HbsAg	Hepatitis B Virus Surface Antigen
HCV Ab	Hepatitis C Virus Antibody
HIV	Human Immunodeficiency Virus
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IGA	Investigator Global Assessment
IP	Investigational Product
IRB	Institutional Review Board
NH_2OH	Hydroxylamine
NH_3	Ammonia
NO	Nitric oxide
NO_2-	Nitrite
PCP	Primary Care Physician
SAE	Serious Adverse Event
SPM	Study Procedures Manual
VAS	Visual Analog Scale

2 INTRODUCTION

2.1 Background

Ammonia oxidizing bacteria (AOB) are essential for the initial step in environmental nitrification processes, specifically the oxidation of ammonia (NH_3) to nitrite (NO_2-). *Nitrosomonas* are Gram-negative chemolithoautotrophic betaproteobacteria that obtain energy solely from NH_3 oxidation, while fixing CO_2 for their carbon needs.[1] Oxidation of NH_3 proceeds in two steps (Figure 1) leading to sequential generation of hydroxylamine (NH_2OH) and NO_2- that require two enzyme complexes: the membrane-bound ammonia monooxygenase (AMO) comprised of subunits AmoA, AmoB and AmoC; and the periplasmic NH_2OH oxidoreductase (HAO). In addition to high NO_2- levels, NH_3 oxidation leads to nitric oxide (NO) and N_2O production through two independent pathways downstream of NH_2OH production: nitrifier denitrification and NH_2OH oxidation.[2]

Figure 1 Nitrifier Denitrification Pathway



B244 is a purified a strain of *Nitrosomonas eutropha* originally isolated from soil samples. Sequencing of the B244 genome revealed a distinct genetic profile from that of other published *Nitrosomonas* strains and AOB genomes. Based on *in vitro* co-culture studies, B244 was able to reduce survival of pathogenic bacteria. Nitrite generation from ammonia concurrently with medium acidification by B244 led to strong antibacterial effects and a marked reduction (~100-fold) in viable counts of methicillin-resistant *Staphylcoccus aureus* and *Pseudomonas aeruginosa*, two pathogens frequently isolated from infected skin and wound sites. In contrast, control cultures with B244 in the absence of ammonium or with heat-killed B244 supplemented with ammonium, had no antibacterial effects. The unique metabolic and antimicrobial activity of *Nitrosomonas*, in combination with their lack of virulence render these bacteria as attractive candidates for topical delivery of nitrite and nitric oxide on human skin with potential to improve health in both normal and abnormal skin conditions or wound sites. NO-releasing drugs or NO donors have also shown activity against *Propionibacterium acnes* and other pathogenic bacteria, anti-inflammatory activity, and inhibition of lipogenesis by insulin-stimulated immortal sebocytes.[4,5]

B244 has been developed as a topical application of a natural source of AOB and NO/NOx to the human skin. We hypothesize that application of AOB to eczematous skin may reduce *S.aureus* skin load and normalize the inflammatory response by reducing Th2 activation.

Under IND #16487, a Phase 1b/2a clinical trial was completed in 2016 where 36 participants with clinical diagnosis of facial acne vulgaris were randomized to receive twice-daily ascending doses of B244 or Vehicle

over 14 days. All 3 dose levels of B244 were well tolerated and no safety issues were identified. There were no attributable drug related SAEs reported.

In addition, a Phase IIb/III clinical trial in 372 patients with clinical diagnosis of facial acne is currently ongoing that is assessing twice-daily application of B244 or Vehicle for 3 months. To date, there have been no treatment related SAEs reported in this trial.

Eczema or Atopic dermatitis is an inflammatory skin condition that affects nearly 20% of children and 2-10% of adults[20]. Disease prevalence has steadily grown in the last 30 years, resulting in a growing field of atopic dermatitis reasearch[21]

The disease is characterized by pruritus, scratching, and eczematous lesions. The chronic condition may be associated with thickening and pigmentary changes. Onset of the disease occurs mostly between 3 and 6 months, but in 90% of children, the disease occurs by age of 5. Staphylococcus aureus is consistently found in eczematous skin lesions in patients with AD. Correlation between the severity of the disease and presence of Staphylococcus aureus has been well established and it has been shown that presence of bacteria is an important factor in skin aggravation[22]. The goal of therapy for AD is to restore the epidermal barrier function and reduce skin inflammation. However, systemic antibiotic use is controversial.

The primary purpose of this study is to evaluate the safety of B244 in treating patients with Atopic Dermatitis.

3 STUDY OBJECTIVES

3.1 Primary Objectives

- To evaluate the safety and tolerability of B244 in participants with mild to moderate Atopic Dermatitis.

3.2 Secondary Objectives

- To assess the efficacy of B244 versus vehicle by the change in Atopic Dermatitis Area Severity Index (EASI) Score from Baseline to post-baseline visits

3.3 Exploratory Objectives

- To assess the efficacy of B244 versus vehicle by the change in Visual Analog Scale (VAS) Score from Baseline to post-baseline visits
- To assess the efficacy of B244 versus vehicle by the change in Skindex 16 Score from Baseline to post-baseline visits
- To assess the efficacy of B244 versus vehicle by observing a change in the IGA score from Baseline to post-baseline visits

- To assess the efficacy of B244 versus vehicle by the change in Actigraphy Movement Count per Hour and sleep quality during nighttime from Baseline to post-baseline visits
- To evaluate if B244 administration on the skin twice daily for 28 days will affect the levels of immune biomarkers.
- To explore microbial content at baseline and Day 28.

4 ENDPOINTS

4.1 Safety & tolerability:

- Safety and tolerability endpoints will consist of all adverse events reporting during the study duration.

4.2 Efficacy:

- Difference in Atopic Dermatitis Area Severity Index (EASI) Score from Baseline to Day 28

4.3 Exploratory:

- Difference in Visual Analog Scale (VAS) Score for Pruritus from Baseline to Day 28
- Difference in the Skindex 16 Score from Baseline to Day 28
- Difference in the IGA score from Baseline to Day 28
- Difference in Actigraphy Movement Count per Hour and sleep quality during the night from Baseline to Day 28
- Difference in biomarkers between active and vehicle groups
- Composition of microbiome
- Difference in skin microbiota from baseline to Day 28 between active and vehicle groups
    - Observing a difference in Staphylococcus aureus and a more diverse microbial collection on the patient's skin.

5 STUDY DESIGN

- This is a Prospective, Vehicle Controlled, Double Blind, Multicenter, Randomized Phase II trial, comparing the effect of twice daily B244 application for 28 days vs vehicle application on treatment of mild to moderate AD
- We will enroll approximately130 patients and complete 104 patients
- After screening and recruitment, participants will be randomized to active or vehicle application for 28 days.
- Subjects will apply a total of 8pumps of IP per application to all affected areas twice-a-day (i.e. 8pumps in the morning and 8pumps again at night) for 28 days.
- Subjects will be provided with cleanser and moisturizer to use for the duration of the study
- Randomization will be 1:1 so that equal number of patients will be treated in each Arm of the study.
- All B244 randomized subjects will be treated at the dose of $4x10^9$ cfu/ml
- Screening will occur at Days -21 to -14
- Washout will occur after Screening visit (Days -14 to -1)
- Randomization will occur during the baseline period for the study
- Clinical assessments of response to treatment will be made at Baseline, Study Days 14 and 28.
- Subject will come back for the final visit on Study Day 42
- Safety evaluations will consist of review of participant's medical history at screening and on-going assessment of adverse events reported throughout the study duration.

6 SELECTION OF STUDY PARTICIPANTS

6.1 Number of Participants Planned

It is estimated that approximately 130 participants will be consented in order to provide 104 completed participants for randomization, treatment, and inclusion in the primary analysis.

6.2 Inclusion Criteria

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

Participants eligible for enrollment in the study must meet all the following criteria:

- Male and female subjects ≥18 years of age
- In good general health as determined by a thorough medical history and physical examination, and vital signs
- Clinical diagnosis of mild to moderate atopic dermatitis according to the criteria of Hanifin and Rajka
- Mild to moderate Atopic Dermatitis area and severity index [EASI] 10-21
- A score of at least ≥ 5 points (moderate pruritus) on the VAS for pruritus
- A minimum of 10% and not more than 30% of the subjects' BSA affected by atopic dermatitis (affected is defined by physical examination findings: erythema, edema, scaling, lichenification, excoriation, with the excoriation serving as the physical examination correlate of pruritus)
- An IGA score of 2-3
- Patient has a history of AD for ≥ 12 months
- The ability to understand and sign a written informed consent form, which must be obtained prior to treatment

6.3 Exclusion Criteria

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

Participants will be excluded from the study if any of the following criteria are met:

- Pregnant and lactating women by urine pregnancy testing
- Subjects with any significant clinical abnormalities which may interfere with study participation
- Any skin condition which may interfere with evaluation of AD
- Atopic dermatitis only on the head or scalp
- Subjects with Atopic dermatitis on the face
- Unstable or actively infected atopic dermatitis
- Patients suffering from pruritus from conditions other than AD
- Patients with chronic pruritus due to systemic disease
- Patients with conditions requiring inhaled steroids

- Have concurrent skin disease of such severity in the study area that it could interfere with the study evaluation
- Have active skin infections on the treatment area
- Have received or planning to receive topical corticosteroids, topical coal tar, topical sulfur, topical PDE-4 inhibitors, topical antihistamines, topical antiseptics or antibiotics, topical antifungals, bleach baths, UVA or UVB phototherapy, oral/IV/inhaled steroids, antibiotics/antiviral/antifungal agents, oral probiotics, glucocorticoids treatment, calcineurin inhibitors, immunomodulating biologic agents, systemic glucocorticoids, systemic immunosuppressive or immunomodulatory agents within 2 weeks of Baseline visit.
- Current or recent history (≤ 3 months of systemic use of Otrexup™, Rasuvo®, Rheumatrex® and Trexall™ or its generic versions such as Methotrexate
- History of being seropositive for human immunodeficiency virus (HIV) at screening by laboratory testing at Screening
- History of being positive for Hepatitis B virus surface antigen (HBsAg) or positive Hepatitis C virus antibody (HCV Ab) at screening by laboratory testing at Screening
- History of renal disease
- Use of any investigational drugs within the previous 30 days prior to dosing or within a period of less than five times the drug's half-life, whichever is longer
- Use of any biologic within a period of 5 times its half-life
- Use of vinegar or bleach baths within 2 weeks of starting the study

7 PARTICIPANT ENROLMENT

7.1 Consenting Participants

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

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

7.2 Screening for Eligibility

After informed consent has been obtained, to determine participant eligibility for enrollment in the study, screening assessments will be performed within 1 week (-21 to -14 days) prior to starting the Washout period (-14 to -1). All screening evaluations must be completed and reviewed to confirm that participants meet all eligibility criteria before Washout period and subsequent randomization on Day 1. Subjects will be asked to undergo a 2 week (-14 to -1) washout period prior to the Baseline procedure. During the washout period, subjects will be asked to stop taking bleach or vinegar baths and start using cleanser and moisturizer provided by the sponsor on as needed bases.

At least 30 days of the participant's medical history and medication use will be requested from their PCP or treating physicians and reviewed by the study staff. A complete physical examination including height, weight and vital signs will be performed.

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

7.3 Study Withdrawal and Withdrawal From Investigational Product and Stopping Criteria

A participant may withdraw from the study at any time at his/her own request, or may be withdrawn at any time at the discretion of the Investigator.
Reasons for withdrawal (participants who refuse to complete any remaining study visits) or discontinuation (participants who prematurely stop the application) at any time during the study may include, but are not limited, to the following:

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

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

7.4 Screen Failures

Data for screen and baseline failures, such as date and reason, will be collected in source documentation at the site
and will be transmitted to AOBiome.

7.5 Early Termination

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

8 STUDY TREATMENT

8.1 Investigational Product

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

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

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

Product name:	B244, 30ml/bottle	Vehicle, 30ml/bottle
Dosage form:	B244 suspension	Vehicle solution
Unit dose strength:	$4x10^9$ cfu/ml	50nM Na_2HPO_4-2mM $MgCl_2$ (pH 7.6)
Route/administration/duration:	Topical application BID for 4 weeks	Topical application BID for 4 weeks
Dosing instruction:	Subjects will apply a total of 8 pumps of IP per application to all affected areas twice-a-day (i.e. 8 pumps in the morning and 8 pumps again at night). Applications should occur in the morning and at night for 4 weeks.	Subjects will apply a total of 8 pumps of IP per application to all affected areas twice-a-day (i.e. 8 pumps in the morning and 8 pumps again at night). Applications should occur in the morning and at night for 4 weeks.
Physical description:	Odorless, cloudy, light pink suspension	Odorless, clear, and colorless suspension
Manufacturer/source of procurement:	AOBiome, LLC	AOBiome, LLC

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

8.2 Dose Changes

No dose changes are anticipated.

8.3 Storage conditions

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

8.4 Description of Blinding Method

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

8.5 Treatment Assignments:

This is a double blind study. Participants will be assigned to study treatment in accordance with the randomization schedule generated for the allocation of vehicle or B244 prior to the initiation of the trial. Randomization will be centrally-based and performed using an appropriate IWRS (an automated randomization system).

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

8.6 Treatment Compliance

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

Study staff will weigh the IP at the beginning of every visit. Weight will be recorded in the eCRF.

8.7 Treatment Application

Subjects will receive a kit containing study drug for application throughout the study. Subjects will be instructed in the use of the spray bottle and asked to self-administer the Investigational Product as follows:

- Subjects will apply a total of 8 pumps of IP per application to all affected areas twice-a-day (i.e. 8 pumps in the morning and 8 pumps again at night).
- Subject should saturate the application area well.
- Subjects may not wash the application site after applying the IP

- Subjects will be asked to let the product air dry.
- While in use, one spray bottle may be stored at ambient temperature. The bottle that is not in current use by the subject, must be stored in the refrigerator. DO NOT FREEZE.

8.8 Treatment of Investigational Product Overdose

The sponsor does not recommend specific treatment for an overdose. Washing with conventional cleanser and water will remove the product. The Investigator will use clinical judgment to treat any overdose.

8.9 Product Accountability

In accordance with federal and local regulatory requirements, the Investigator and designated site personnel must document the amount of investigational product dispensed to study participants, the amount returned by study participants, and amount received and returned to the sponsor, when applicable. Product accountability records must be maintained throughout the course of the trial. Any quality issue noticed with the receipt or use of an IP (deficient IP in condition, appearance, pertaining documentation, labeling, expiry date, etc.) should be promptly reported to the Sponsor, who will initiate a complaint procedure.
All investigational product must be stored in a secure locked room with access limited to the Investigator and designated site personnel. Study product is to be stored in a refrigerator between 2-8 degrees C. Maintenance of a temperature log is required.
Under no circumstances will the Investigator allow IP to be used other than as directed by this Clinical Trial Protocol, or dispose of IP in any other manner.

8.10 Unblinding Procedures

The Investigator may unblind a participant's treatment assignment only in the case of emergency or in the event of a serious medical condition when knowledge of the study treatment is essential for the appropriate clinical management or welfare of the participant, as determined by the Investigator. It is preferred (but not required) that the Investigator first contacts the medical monitor to discuss options before unblinding the participant's treatment assignment. The Investigator must notify the Sponsor as soon as possible when a participant's treatment assignment is unblinded without revealing the treatment assignment of the unblinded participant unless that information is deemed important for the safety of participants currently in the study. The date and reason for the unblinding must be documented in the participant's study record.
The Medical Monitor may unblind the treatment assignment for any participant with an SAE. If the SAE requires that an expedited regulatory report be sent to one or more regulatory agencies, a copy of the report, identifying the participant's treatment assignment, may be sent to clinical Investigators in accordance with local regulations and/or sponsor policy.

8.11 Retrieval and Destruction of Investigational Product

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

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

8.12 Permitted Medications

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

8.13 Prohibited Medications

Participants will be prohibited from taking any of the following therapy, including topical corticosteroids, oral/IV/inhaled steroids, topical coal tar, topical sulfur, topical PDE-4 inhibitors, topical or oral antihistamines, topical antiseptics or antibiotics, topical antifungals, bleach baths, UVA or UVB phototherapy, oral antibiotics/antiviral/antifungal/antiseptic agents, systemic immunosuppressive or immunomodulatory agents, oral or topical probiotics for the duration of the trial.

8.14 Rescue Medications

Rescue medications will not be permissible in the trial should the study subject experience acute exacerbation of symptoms related to the treated disease. Subject may not start additional non-randomized medication and should immediately alert the study site PI. Subjects that need to start a rescue medication will be discontinued from the study and will be considered an Early Termination.

8.15 Lifestyle Restrictions

8.15.1 Use of cleanser and moisturizer during the trial

At the beginning of the trial, subjects will be provided cleanser and moisturizer to use for the duration of the trial as needed. Subjects will be asked not to use any other cleansers, or moisturizers during the course of active treatment. (Baseline-Day 28). Although moisturizer is provided to subjects, its use it not mandatory in the study and may be used when and as needed only. If subjects do not have the need to use the moisturizer, they may forego its use all together.

Although study cleanser is provided, subjects will be asked to refrain from washing the treatment areas with study cleanser starting from Washout through Day 28. Subjects may use cleanser to wash other parts of their body not treated during the study. At Day 28 (end of study drug application) subjects will be asked to return cleanser that were provided to them by the sponsor and will be asked to go back to their regular regimen and use their preferred cleanser.

Provided moisturizer may be applied to the application site AFTER IP administration. Subject should apply the IP as instructed, let the site of application dry and only then apply the moisturizer.

Subjects may not wash application area with cleanser and water AFTER each application.

8.16 Handling of Investigational Product

Subjects will receive a kit containing two 30 ml white bottles. Each bottle will be used for one week and brought to all study appointments (Day 14, Day 28, Unanticipated visit, EOS visit). Subjects will be asked to refrigerate bottles that are not in use. The bottle which is being used for treatment at a given week may be placed on the counter to be used during the treatment period.

Subjects will be asked not to subject the treatment kit to conditions which are unnatural or harmful to the product, such as excessive heat (temperatures over 77°F (25°C) and freezing temperatures (at 0ºC). 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.

8.17 Treatment compliance

Each site participating in the trial will be instructed to assess subject's compliance by weighing the investigational product and obtaining the weight of the bottle in grams pre and post application (after two weeks of use). Study personnel will be asked to take out bottles from the carton, weigh both bottle and record the weight. At the Day 14 visit, study personnel will need to weigh both bottles without the carton and record weight. This procedure should be followed every time study medication is dispensed and returned. Sites will be provided scales, which will be calibrated prior to each use. Study personnel will be instructed to record measurements into the eCRF.

9 ACTIGRAPHY WATCH

At Baseline visit, subjects will be provided two actigraphy watches in order to accurately monitor subject's sleep, activity and itching patterns. Subjects will be asked to wear one watch on each wrist 24 hours a day starting with the Baseline visit through Week 4 visit. Actigraphy watches are water resistant and may be left on when bathing/showering/swimming. However, for convenience, subjects may remove watches during the above mentioned procedures but will be asked to put watches back on once these procedures have been completed.

Any deviations will be recorded in the CRF.

10 CONTRACEPTION REQUIREMENTS

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

Effective contraception methods include:

- Total abstinence (when this is in line with the preferred and usual lifestyle of the study participant). Periodic abstinence (i.e., calendar, ovulation, symptothermal, postovulation methods) and

withdrawal are not acceptable methods of contraception.

- Use of oral, injected or implanted hormonal methods of contraception or placement of an IUD or IUS or other forms of hormonal contraception that have comparable efficacy, for example hormone vaginal ring or transdermal hormone contraception.
- Use of barrier methods (i.e., condom, diaphragm) used with a spermicide (i.e., foam, cream, or gel that kills sperm)

In case of use of oral contraception women should have been stable on the same pill for a minimum of 90 days before the baseline visit.
Male participants of the study who are having sexual intercourse with a woman who can become pregnant must use an acceptable form of birth control while participating in the study. Additionally, male participants are expected to let their female partners know of their participation in a research study of a drug, and that the effects of the drug on an unborn baby and on a pregnant woman are unknown. Male participants will also be expected to provide their female partners with the contraception requirements information previously described and the study doctor's contact information for questions.

Payment for all aspects of obstetrical care, child-or related care will be the study participant's responsibility.
In case of pregnancy, Investigational Product should be discontinued and the Sponsor should be informed immediately. Follow-up of the pregnancy will be mandatory until the outcome is available.

11 STUDY PROCEDURES

11.1 Pre-screening procedures

Study subjects will be recruited from among participating hospitals, clinics, and diagnostic centers or from general population under the responsibility of a participating Investigator. Prior to initiation of the recruitment phase, participating Investigators will identify a pool of potential study subjects. Each of these centers will identify potentially eligible patients in advance, by either reviewing past medical records and diagnoses, screening in clinics, referral from other physicians, or other sources of recruitment, to identify those aged 18 or older with clinical diagnosis of mild or moderate Atopic Dermatitis. Medical records from patient's dermatologist or primary care physician to confirm the diagnosis are optional. Verbal confirmation of the diagnosis present for ≥ 12 months is sufficient in order to fulfill this criterion.

11.2 Informed Consent Procedures

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

11.3 Study Assessments

Study activities will take place according to the Time and Events table (Appendix A).

11.4 Inclusion procedures

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

11.5 Timing of patient's visits to the clinic

Patients will be asked to report to the clinic for their scheduled appointments. If a subject is unable to schedule an appointment within the required time frame, study staff will be asked to reschedule the patient to a day when they are able to come in within predetermined time frame.

11.6 Description by type of visit

11.6.1 Screening Visit (-21 to -14)

- informed consent completed and signed
- inclusion and exclusion criteria
- demographic data
- medical/surgical history
- perform Hanifin and Rajka criteria assessment
- current medications
- smoking status
- physical exam
- body weight
- height measurement
- obtain in clinic blood pressure and heart rate
- urine pregnancy test (for women of childbearing potential)
- Oral hygiene questions
- EASI assessment
- VAS assessment
- Skindex 16 Questionnaire
- IGA scoring assessment
- blood for clinical chemistry, serology and hematology
- Microbial content
- provide subjects with study cleanser to start using for the Washout period
- provide subjects with moisturizer to start using for the Washout period
- Start AE monitoring

11.6.2 Washout Phase (-14 to -1)

At the Screening visit once subjects become eligible for the study, they will be provided moisturizer and cleanser which need to be used for two weeks prior to the Baseline visit during the Washout phase of the study. Moisturizer use is not mandatory in the study and may be used when and as needed only. If subjects do not have the need to use the moisturizer, they may forego its use all together. However, subject will initiate the Washout phase once the chemistry and serology results become available from the lab. Once study staff receive those results, subjects will be notified by the telephone to start the Washout period phase and start using provided cleanser and moisturizer if needed. Subject will be asked to stop using their regular cleanser and regular moisturizers for two weeks prior to their Baseline visit. Subjects will also be asked to stop using bleach or vinegar baths.

- Call subjects to initiate Washout phase
- provide counseling as needed
- ask subjects to start using the provided cleanser
- as subjects to start using the provided moisturizer
- AE monitoring

11.6.3 Study Day 1-Baseline visit (-1 to 0)

- inclusion and exclusion criteria
- perform Hanifin and Rajka criteria assessment
- current medications
- obtain in clinic BP and HR
- EASI assessmentVAS assessment
- Skindex 16 questionnaire
- IGA scoring assessment
- dispense actigraphy watches and educate subjects on its use
- allocation of a randomized treatment kit number via IWRS
- delivery of the corresponding pack of Investigational Product
- obtain study medication weight for Investigational Product compliance
- first application of Investigational Product (under medical supervision)
- Microbial content
- study counseling
- study diary counseling
- replenish cleanser and moisturizer when needed
- obtain skin swabs
- blood for biomarkers
- AE monitoring

11.6.4 Week 2, 4 (Day 14, 28) study visit

- concomitant medications
- body weight

- obtain in clinic heart rate and blood pressure
- Oral hygiene questions
- EASI assessment
- VAS assessment
- Skindex 16 Questionnaire
- IGA scoring assessment
- obtain study medication weight for Investigational Product compliance
- collect study medication
- dispense investigational product to patient (Week 2)
- blood for biomarkers
- blood for clinical chemistry and hematology
- Microbial content (Week 4 visit only)
- study diary
- study counseling
- provide subjects with study cleanser and moisturizer (when necessary only)
- collect cleanser and moisturizer at Week 4 visit
- collect actigraphy watches returned by the subject at Week 4 visit
- recording of AEs if any
- skin swabs (Week 4 only)

11.6.5 Week 6 final visit (Day 42)-End of Study Visit

- record concomitant medications
- physical exam
- body weight
- obtain in clinic heart rate and blood pressure
- Oral hygiene questions
- EASI assessment
- VAS assessment
- Skindex 16 questionnaire
- IGA scoring assessment
- Skin swabs
- blood for clinical chemistry and hematology
- Blood for biomarkers
- recording of AEs if any

11.6.6 Unscheduled/Unanticipated Study visit

If an event arises that requires patient to come in to the research center, subjects should be scheduled for the Unscheduled visit and assessments are performed based on investigator discretion.

11.6.7 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 Week 6 final visit, will be asked to complete the Early Termination Visit. .

During the visit, the following will be obtained:

- record concomitant medications
- physical exam
- Body weight
- obtain in clinic heart rate and blood pressure
- Oral hygiene questions
- EASI assessment
- VAS assessment
- Skindex 6 questionnaire
- IGA scoring assessment
- skin swab samples
- obtain study medication weight for Investigational Product compliance ((if ET visit occurs before Week 4 visit)
- blood for biomarkers (if ET visit occurs before Week 4 visit)
- blood for clinical chemistry and hematology (if ET visit occurs before Week 4 visit)
- return Investigational product (if ET visit occurs before Week 4 visit)
- return cleanser and moisturizer (if ET visit occurs before Week 4 visit)
- return Actigraphy watch (if ET visit occurs before Week 4 visit)
- return study diary (if ET visit occurs before Week 4 visit)
- AE Monitoring

12 METHODS OF ASSESSMENTS

12.1 Skin Swab Samples

Skin swab samples will be collected as Described in Appendix A. At Baseline, study staff will examine subject's body surface area affected by AD and will be asked to identify only one area to be sampled. Once the area is identified, study staff will sample this particular treatment area. Sampling area should be noted in subject's record, so that the same treatment area is then sampled at Day 28 and Day 42. Study staff will perform swabbing procedures during subject's visit. Swab samples will need to be stored in the refrigerator until shipped at the end of the study to the SciSafe Laboratories.

Swab samples may be subjected to DNA sequencing. Samples will be used for study of the skin microbial diversity and microbiome analysis While this is not our intention to study participants genome, participants sequence may be included as part of this analysis. Samples may be stored in the freezer until future research.

We will keep the specimen for up to 5 years, or until analyzed. If we complete our research and no longer need to keep the specimens, we will destroy them. Specimens will be coded and no identifiable participant information will be used. Results will not be returned to the subjects or the investigators

12.2 Blood Pressure Measurement

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

12.3 Physical Examinations

The physical examination will be performed at Screening and at Unanticipated visit should one occur. The physical examination includes an assessment of general appearance and a review of systems (dermatologic, head, eyes, ears, nose, mouth/throat/neck, thyroid, lymph nodes, respiratory, cardiovascular, gastrointestinal, extremities, musculoskeletal, neurologic systems).

12.4 Laboratory Assessments

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

The following laboratory variables will be determined as outlined below:

The following routine clinical chemistry, hematology and Lipid Panel will be performed according to Time and Events table (Appendix A): Albumin, alkaline Phos, ALT, AST, Total Billirubin, BUN, BUN:Createnine ratio, Calcium, Chloride, Createnine, eGFR, Glucose, Potassium, Sodium, Uric Acid

Lipid Panel: HDL Cholesterol, LDL cholesterol, Total Cholesterol, Triglycerides, VLDL Cholesterol, LDL/HDL Cholesterol Ratio, Non HDL Cholesterol.

Angiotensin Converting Enzyme.

Hematology: WBC, RBC, Hemoglobin, Hematocrit, Platelets, WBC Differential (Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils), HbA1C

Serology will only be done at Screening: HIV Ab, HCV Ab, HBsAg.

Patients will be asked to fast for at least 8 hours before all blood tests are done.

The total blood volume collected for clinical labs for Screening visit will be approximately 20 ml of whole blood. Volume collected for subsequent visits would be approximately 10 ml of whole blood.

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

12.5 Oral Hygiene Questions

Subjects will be asked questions regarding their oral hygiene and use of toothpaste. This will be will be administered at the times indicated in the Time and Events Table (Appendix A)

12.6 EASI Assessment

An EASI score is used to measure the extent (area) and severity of atopic eczema (Eczema Area and Severity Index). The score will be calculated at the times indicated in the Time and Events Table (Appendix A).The investigator will assess improvement of eczema based on intensity and severity of the disease. EASI score sheet can be found in Appendix C.

The severity strata for the EASI are as follows:
0 = clear
0·1-1·0 = almost clear
1·1-7·0 = mild
7·1-21·0 = moderate
21·1-50·0 = severe
50·1-72·0 = very severe

12.7 Subject reported outcomes/Skindex16

Skindex-16 instrument will be administered at the times indicated in the Time and Events Table (Appendix A). The participant will answer a questionnaire examining the relationship between the patient's skin health and quality of life.

Skindex16 questionnaire can be found in Appendix D.

12.8 Hanifin and Rajka Diagnostic Criteria for Atopic Dermatitis (AD)

At Screening and Baseline, all subjects must have atopic dermatitis, as defined by the Hanifin and Rajka criteria, which involves a minimum of 10% and a maximum of 30% body surface area. Hanifin and Rajka criteria can be found in Appendix E

Major criteria: Must have three or more of:

1. Pruritus
2. Typical morphology and distribution

- Flexural lichenification or linearity, erythema, scaling, serum-crust

3. Chronic or chronically-relapsing dermatitis
4. Personal or family history of atopy (asthma, allergic rhinitis, atopic dermatitis)

Minor criteria: Should have three or more of:

1. Xerosis
2. Ichthyosis, palmar hyperlinearity, or keratosis pilaris
3. Immediate (type 1) skin-test reactivity
4. Raised serum IgE
5. Early age of onset
6. Tendency toward cutaneous infections (especially *S aureus* and herpes simplex) or impaired cell-mediated immunity
7. Tendency toward non-specific hand or foot dermatitis
8. Nipple eczema
9. Cheilitis
10. Recurrent conjunctivitis
11. Dennie-Morgan infraorbital fold
12. Keratoconus
13. Anterior subcapsular cataracts
14. Orbital darkening
15. Facial pallor or facial erythema
16. Pityriasis alba
17. Anterior neck folds
18. Itch when sweating
19. Intolerance to wool and lipid solvents
20. Perifollicular accentuation
21. Food intolerance
22. Course influenced by environmental or emotional factors
23. White dermographism or delayed blanch

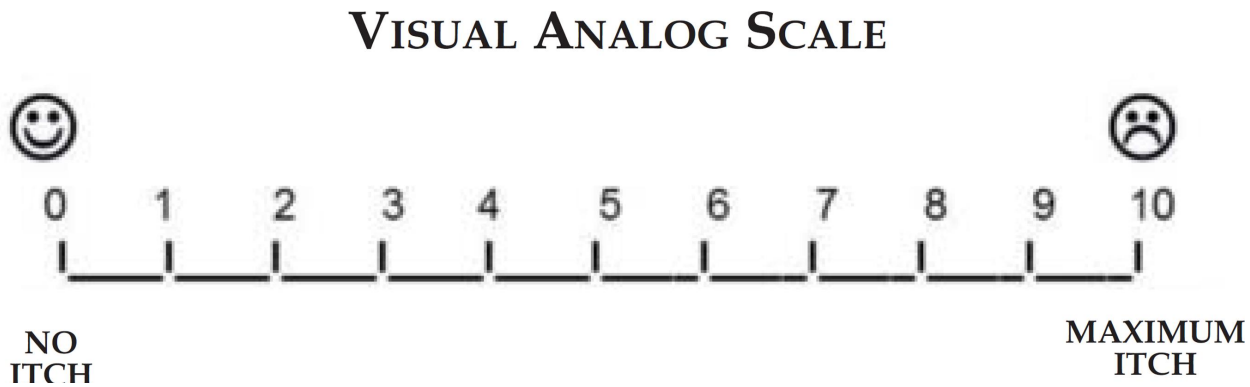
12.9 Visual Analog Scale (VAS)

VAS will be performed as a measure of pruritus at the times indicated in the Time and Events Table (Appendix A). Subjects will be asked to report their pruritus symptoms over the 24 hrs period before the visit.

VAS score can be found in Appendix F.

The following VAS categories will be used:
0=no pruritus
>0–<4 points=mild pruritus
≥4–<7 points=moderate pruritus

≥7–<9 points=severe pruritus
≥9 points=very severe pruritus



12.10 Investigator Global Assessment Score (IGA)

IGA will be performed at the times indicated (Screening, Baseline, Weeks 2, 4, and 6) in the Time and Events Table of the Protocol. The Investigator will assess improvement of Atopic Dermatitis based on the 5-point severity scale summarized below.

IGA score can be found in Appendix G.

Score	Category	Definition
0	Clear	Minor residual discoloration, no erythema or induration/papulation, no oozing/crusting
1	Almost Clear	Trace faint pink erythema with almost no induration/papulation and no oozing/crusting
2	Mild disease	Faint pink erythema with mild induration/papulation and no oozing/crusting
3	Moderate disease	Pink-red erythema with moderate induration/papulation and there may be some oozing/crusting
4	Severe disease	Deep/bright red erythema with severe induration/papulation with oozing/crusting

12.11 Actigraphy Watch

Subjects will be asked to wear two Actiwatch Spectrum watches on each wrist for the duration of the study, starting with the Baseline visit. The device is the size of a wrist watch, is water resistant, and has a plastic wrist band that is hypoallergenic so it is not likely to cause any skin problems. The device has been used by thousands of research subjects in hundreds of clinical trial with no significant problems for the subjects. Two watches will be given to subjects at Baseline visit. Subjects will be asked to put both watches on their wrists during the visit and wear both watches at all times until Day 28 visit. Subjects will be allowed to take watches off for bathing, swimming and/or work or other activities prohibiting wear of such equipment. Study site personnel will go over the procedures with each patient making sure patient understand the procedures. The actigraphy data will be used to determine the estimates of total sleep time (TST) and total wake time (TWT) each night.

Upon the start of the study, Philips will provide each site with training and provide each site with the number of devices it needs, monitor the battery status of each device and replace as needed, and provide a 24/7 help desk.

12.12 Biomarkers

In addition to the blood drawn for the safety laboratory assessments, additional blood will be collected for the biomarker analysis at Day 1B (Baseline), Day 14, Day 28 and Day 42
Subjects must be fasting for at least 8 hours Samples will be processed on site and stored in the - 80° C
Approximately 20 ml of whole blood will be drawn for biomarkers at each visit. Patients will be asked to fast for at least 8 hrs before blood for biomarkers is drawn.

Blood samples for biomarkers will be taken according to Appendix A and processed according to the lab manual. Biomarkers will be evaluated for cytokines, chemokines, inflammatory markers and immune response.

12.13 Microbial Content

Microbial content samples will be taken from one affected area and analyzed for microbial levels of Staph. aureus. Cup scrub technique will be used in recovery of microorganisms from the skin.

At Screening, study staff will examine subject's body surface area affected by AD and will be asked to identify only one area to be sampled. Once the area is identified, study staff will sample this particular treatment area. Sampling area should be noted in subject's record, so that the same treatment area is then sampled at Baseline and Week 4 visits.

Skin sampling will be conducted by the trained study staff in the study office.
Microorganisms are recovered from the site by pressing a cylinder against the affected skin with sufficient pressure to form a seal and instilling recovery buffer (phosphate buffer) into the cylinder. The surface of the skin is then mechanically 'scrubbed' with a glass rod for 2 minutes. The fluid is pipetted from the cylinder into a test tube for further analysis.

Sterile supplies will be provided by the Sponsor.

Samples may be stored frozen for up to 5 years for verification of results and possible characterization of strains recovered from subjects.

12.14 Sample Shipment

All clinical chemistry samples are to be shipped to the central laboratory where samples will be analyzed. Please refer to the Lab Manual for sample shipping temperature and Lab destination information.

13 SAFETY ASSESSMENTS

13.1 Compliance

Participants will be asked to bring study medication with them to each scheduled visit. Study site will be provided with a scale and weight of the study medication will be obtained before the first use and at each visit.

13.2 Pregnancy Reporting

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

13.3 Study Completion

A completed participant is one who has completed all study visits. Day 42 study visit is defined as the participant's last visit.

13.4 Subject Withdrawal Criteria

A subject may withdraw from the study at any time at his/her own request, or may be withdrawn at any time at the discretion of the Investigator for safety, behavioral, or administrative reasons.
Reasons for withdrawal (subjects who refuse to return for any remaining study visits) or discontinuation (subjects who prematurely stop the application) at any time during the study may include, but are not limited, to the following:

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

All premature discontinuations and their causes must be carefully documented by the Investigator on the CRF or if needed on the AE form.
If, for any reason, a subject is withdrawn before completing the final visit, the reason for termination will be entered on the CRF. All data gathered on the subject prior to termination will be made available to AOBiome. Subjects not completing the entire study should be fully evaluated when possible. The appropriate CRFs should be completed.

If the subject chooses to withdraw before completing the study, the subject should notify the study coordinator who will instruct the subject on completion of assessments for End of Study (EOS) visit Appendix A. For

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

14 EFFICACY ASSESSMENTS

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

EASI score at Baseline, Day 14, 28 and 42 will be compared to the baseline score and will be descriptively summarized as mean, median, standard deviation, minimum, and maximum of scores/values at all applicable time points and for all treatment groups.

15 STATISTICAL CONSIDERATIONS

15.1 Sample Size

A total sample size of 130 randomized subjects, allows for a dropout rate of 20%.

A sample size of 52 per group achieves >80% power to reject the null hypothesis of equal means when the population mean difference is $\mu1 - \mu2 = (-1.4) - (-5.3) = 3.9$ with a standard deviation for both groups of 7.0 and with a significance level (alpha) of 0.025 using a one-sided two-sample equal-variance t-test.

The power calculation assumes baseline treatment group means of 14 in EASI score. The change from baseline to Day 28 is assumed to be -1.4 for the vehicle group and -5.3 for the active group; this represents about 38% reduction in EASI score for the active group and 10% reduction in EASI score for the placebo group.

15.2 Populations for Analysis

ITT: includes all randomized participants.

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

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

15.3 Data Analysis

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

Categorical variables will be summarized by frequency distributions (number and percentages of subjects) and continuous variables will be summarized by descriptive statistics (mean, standard deviation etc).
Adverse events will be summarized by treatment group using the Medical Dictionary for Regulatory Activities (MedDRA) system organ class and preferred term. Separate tabulations will be produced for all treatment-emergent AEs (TEAEs), treatment-related AEs (those considered by the Investigator as at least possibly drug

related), SAEs, discontinuations due to AEs, and AEs ≥Grade 3 severity. By-subject listings will be provided for any deaths, SAEs, and AEs leading to discontinuation.
Descriptive statistics for each treatment group will be provided for clinical laboratory values (e.g., hematology, serum chemistry, and urinalysis) and vital signs data, presented as both actual values and changes from baseline relative to each on-study evaluation. Abnormal clinical laboratory values will be listed.
All data will be provided in by-subject listings.

15.3.1 Disposition

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

15.3.2 Demographic and Baseline

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

15.4 Safety Analyses

15.4.1 Definitions

All adverse events recorded during the study will be coded according to MedDRA.

15.4.2 Adverse Events

All adverse events (AEs) recorded during the study through the date of randomization through 28 days after the last dose of study drug will analyzed.
All AE's will be coded according to MEDRA and summarized using SOC and Preferred term.

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

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

15.4.3 Deaths and Serious Adverse Events

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

15.4.4 Adverse Events leading to treatment discontinuation

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

15.4.5 Efficacy Analyses

All efficacy analyses will be performed on the ITT population and PP population. P-values from analyses of efficacy endpoints must be interpreted in an exploratory fashion since there is no adjustment for multiple endpoints in this study.

The EASI score and other continuous efficacy endpoints will be summarized using descriptive statistics at Baseline, Day 14, Day 28 and Day 42 for actual values and change-from-baseline values. The difference in treatment groups in change-from-baseline values at post-baseline visits will be analyzed using a mixed Analysis of Variance with one between-subject variable (active group vs. vehicle group) and one within-subject variable (Baseline vs. Day 14, Day 28, Day 42). The interaction between within-subject and between-subject variable will be included in the mixed model to test if any difference in treatment groups at post-baseline visits. Outputs from the analysis will include the p-value for the treatment group and interaction factor separately. The p-value should be evaluated at a significance level of 0.05.

Additionally, a one-sided two-sample equal-variance t-test will be performed as a supportive test to analyze the differences in treatment group means in change from baseline at each post-baseline visit (Day 14, Day 28, Day 42). Output from the analysis will include a 95% confidence interval for the difference in treatment groups means at each visit, and the p-values from the hypothesis test of no difference in treatment group means at each visit. The p-value should be evaluated at a one-sided significance level of 0.025.

15.4.6 Handling of dropouts or missing data

Missing data will not be imputed for analysis.
Subjects who dropout after enrollment but prior to randomization will be replaced.

15.5 Clinical Trial Protocol deviations

At minimum, the following deviations will be summarized on the ITT patient population:

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

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

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

16.1 Definition of an AE

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

Examples of an AE include:

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

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

16.2 Definition of a SAE

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

a) results in death.
b) is life-threatening.

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

c) requires hospitalization or prolongation of existing hospitalization.

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

d) results in disability/incapacity

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

e) is a congenital anomaly/birth defect
f) Medical or scientific judgment should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. Examples of such events are invasive or malignant cancers, intensive treatment in

an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

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

All AEs occurring after administration of the first dose of study medication and on or before the final assessment must be reported as AEs. All AEs must be recorded irrespective of whether they are considered drug-related.
At each assessment in the period defined above, AEs will be evaluated by the Investigator and recorded. Any AEs already documented at a previous assessment and designated as ongoing, should be reviewed at subsequent assessments as necessary. If these have resolved, this should be documented. Changes in intensity or frequency of AEs should be recorded as separate events (i.e., a new record started).
The recording of AEs and SAEs are described in Section 164 ("Recording of AEs and SAEs").

16.4 Recording of AEs and SAEs

All clinical events, including either observed or volunteered problems, complaints or symptoms are to be recorded on the Adverse Events page(s) of the CRF. The need to capture this information is not dependent upon whether the clinical event is associated with study treatment. Adverse clinical events resulting from concurrent illnesses or reactions to concurrent medications are also to be recorded. In order to avoid vague, ambiguous, or colloquial expressions, the AE should be recorded in standard medical terminology rather than the participant's own words.
Each adverse clinical event is to be evaluated for duration, intensity, and whether the event may be associated with the investigational product (IP) or other causes. Start and stop dates, relationship to investigational product (IP), medical management, and alternative causality of event must be recorded in the Adverse Events section of the CRF. AEs believed to be possibly related to investigational product (IP) must be followed until resolution.

16.5 Evaluating AEs and SAEs

16.5.1 Severity Rating

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

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

An AE that is assessed as severe should not be confused with a SAE. An event is defined as 'serious' when it meets one of the pre-defined outcomes as described in Section16.2 "Definition of a SAE".

16.5.2 Relationship to Investigational product (IP)

SAEs will be classified as "**not related**" or "**related**" (including unknown).

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

Definitely not related: The AE is definitely not related to the drug. This designation should be reserved for those events which occur prior to study treatment or for those events which cannot be even remotely related to study participation (e.g., injuries sustained in an automobile accident).

Unlikely related: There is no reasonable association between the study treatment and the suspected event and the event could have been produced by the participant 's clinical state or other modes of therapy administered to the participant.

Possibly related: The suspected adverse event may or may not follow a reasonable temporal sequence from study treatment administration but seems to be the type of reaction that cannot be dismissed as unlikely. The event could have been produced or mimicked by the participant 's clinical state or by other modes of therapy concomitantly administered to the participant.

Probably related: The suspected adverse event follows a reasonable temporal sequence from study treatment administration, abates upon discontinuation of the treatment, and cannot be reasonably explained by the known characteristics of the participant 's clinical state.

Definitely related: This designation should be reserved for those events which have no uncertainty in their relationship to treatment administration.

16.6 Pregnancy

Any pregnancy that occurs in a female participating in the study must be reported to the Sponsor within 24 hrs of learning of the pregnancy. Follow-up must occur to determine the outcome of the pregnancy (including premature termination) and the status of mother and child. Pregnancy complications and elective terminations for medical reasons must be reported as AEs or SAEs. Spontaneous abortions must be reported as an SAE. Any SAE occurring in association with a pregnancy and considered by the Investigator as possibly related or related to the investigational product must be promptly reported to the Sponsor, even if the event occurred after the participant completed the study.
The Investigator must attempt to collect pregnancy information on any female partners of male participants who become pregnant while the male participant is enrolled in the study. Pregnancy information must be reported to the Sponsor as described above.

16.7 Prompt Reporting of SAEs to the Sponsor

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

- **SEND** (within 1 working day, by email) the signed and dated corresponding page(s) in the Case Report Form to the representative of the Monitoring Team whose name, address and fax number appear on the

Clinical Trial Protocol, or to a designated Safety fax number provided by the Monitoring Team, as well as to the Central Database number;

- **ATTACH** a photocopy of all examinations carried out and the dates on which these examinations were performed. Care should be taken to ensure that the patient's identity is protected and the patient's identifiers in the Clinical Trial are properly documented on all copies of source documents provided to the Sponsor. For laboratory results, include the laboratory normal ranges;
- **Follow-up of any Serious Adverse Event** that is fatal or life threatening should be provided within one additional calendar week. The treatment code will be unblinded for reporting of Serious Adverse Events that are unexpected and reasonably associated with the use of the Investigational Product.

Follow-up

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

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AOBiome Reportable Events Hotline **Email: safety@aobiome.com**

17 ETHICAL AND REGULATORY STANDARDS

17.1 Ethical Conduct of Study

This clinical trial was designed and shall be implemented, executed, and reported in accordance with the ICH Harmonized Tripartite Guidelines for Good Clinical Practice, with applicable local regulations (including US Code of Federal Regulations Title 21), and with the ethical principles laid down in the Declaration of Helsinki. The Investigator(s) should conduct the study in accordance with this protocol, the Declaration of Helsinki and ICH GCP guidelines and FDA regulations. The Investigator(s) and the Sponsor will sign the protocol and study contract, to confirm agreement. The Investigator(s) will not implement any amendment (deviation or changes of the protocol) without agreement by the Sponsor and IRB approval, except where necessary to eliminate

immediate hazard(s) to study participants, or when change(s) involve only logistical or administrative aspects of the study.
Records that may reveal the identities of participants must be well protected, with consideration given to confidentiality and the right to privacy of participants.

17.2 Laws and Regulations

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

17.3 Informed Consent

Each participant must be provided with a statement that the investigation involves research and that the IRB has approved solicitation of participants to participate; a fair explanation of the procedures to be followed and their purposes, including identification of any procedures which are experimental; a description in lay language of any possible side effects; a description of any attendant discomforts and risks reasonably to be expected; a description of any benefits reasonably to be expected; a disclosure of any appropriate alternative procedures that might be advantageous for the participant; an offer to answer any inquiries concerning the procedures, and instruction that the person is free to withdraw consent and discontinue participation in the project or activity at any time without prejudice to the participant. Payment to research participants for taking part in the study is based on time and inconvenience. All information concerning payment, including the schedule of payments, must be set forth in the informed consent, including a disclosure that the Investigator is being paid to perform the stated research.
A participant must give consent to take part in the study. Participants below the age of majority in the municipality must give written assent to participate in this study. This consent must be dated and retained by the Principal Investigator as part of the study records. A downloadable digital copy shall be given to the person signing the form. The informed consent process must be documented in the participant's source documents.
The Investigator agrees that the Sponsor, its employees or agents will have the right from time to time during the course of this study to audit and review pertinent medical records relating to this clinical trial. A statement will be obtained from each person participating in the study permitting the release of his/her medical records as necessary for inspection by authorized personnel of the Sponsor, FDA, and the staff managing the clinical study.
The release of medical records and the review of the contents will be in compliance with the Health Insurance Portability and Accountability Act of 1996 (HIPAA).

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

The protocol and informed consent form and the electronic version of the consent for this study must be approved by the IRB. A copy of the Letter of Approval from the Board, which contains specific identification of the documents approved, must be received by the Sponsor prior to shipment of drug supplies to the Principal Investigator.
All changes to the protocol, as well as a change of Investigator, must also be approved by the IRB and documentation of this approval provided to the study monitor. Records of the IRB's review and approval of all documents pertaining to the study must be kept on file by the Principal Investigator and are subject to FDA

inspection at any time. IRB renewal for approval is required each year. The Investigator is to inform AOBiome, in writing, of the approval to continue the study.

17.5 Clinical Monitoring/Record Keeping

There shall be no alterations in the protocol design without the written consent and approval of the Sponsor and the approval of the IRB, except in the case that participants are at immediate risk without immediate implementation of such alterations. In the aforementioned situation, the site should notify the Sponsor and IRB of the deviation as soon as possible, and should seek the written consent and approval of the Sponsor and the approval of the IRB.
All results of this trial must be recorded on eCRFs. Each participant who has been randomized must have a completed eCRF. Reasons for termination must be stated in the early termination section. Study participants are not to be identified by name on eCRFs, but rather by coded identifiers and participant initials.
The study monitor will verify the accuracy of the data by reviewing pertinent source documents such as office records or hospital charts of the participants.
Study records include eCRFs, signed FDA Form 1572, original reports of test results, and signed electronic informed consent forms. IRB approval letters and other documents pertaining to the conduct of the study are to be kept on file by the Investigator. If the study files are assigned to someone else or removed to another location, the Investigator is to notify the study monitor or Sponsor in writing of the change. All study records are subject to FDA inspection at any time.
All information supplied to the Investigator by the Sponsor before, during, and after the study is confidential. Such information is to be used solely in connection with the clinical study. The study protocol, IB, and any other pertinent study related materials or records provided are to be maintained in a confidential manner, reviewed carefully with attention to admonitions and returned to the Sponsor upon request. No part of these materials may be reproduced or transmitted in any form without prior written permission from the Sponsor.

18 ADMINISTRATIVE RULES

18.1 Curriculum Vitae

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

18.2 Archiving of Study Documentation

The Investigator must maintain confidential all study documentation, and take measures to prevent accidental or premature destruction of these documents.
It is recommended that the Investigator retain the study documents at least fifteen (15) years after the completion or discontinuation of the Clinical Trial. However, applicable regulatory requirements should be taken into account in the event that a longer period is required.
The Investigator must notify the Sponsor prior to destroying any study essential documents within the fifteen (15) year period following the Clinical Trial completion or discontinuation.
If the Investigator's personal situation is such that archiving can no longer be ensured by him/her, the Investigator shall inform the Sponsor and the relevant records shall be transferred to a mutually agreed upon designee.

18.3 Internal Safety Review Committee

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

19 STUDY MONITORING

19.1 Responsibilities of the Investigator(s)

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

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

19.2 Responsibilities of the Sponsor

The Sponsor of this Clinical Trial is responsible to Health Authorities for taking all reasonable steps to ensure the proper conduct of the Clinical Trial Protocol as regards ethics, Clinical Trial Protocol compliance, integrity and validity of the data recorded on the Case Report Forms. Thus, the main duty of the Monitoring Team is to help the Investigator and the Sponsor maintain a high level of ethical, scientific, technical and regulatory quality in all aspects of the Clinical Trial. At regular intervals during the Clinical Trial, the site will be contacted, through monitoring visits, letters or telephone calls, by a representative of the Monitoring Team to review study progress, Investigator and patient compliance with Clinical Trial Protocol requirements, and any emergent problems. During these monitoring visits, the following but not exhaustive list of points will be scrutinized with the Investigator: patient informed consent, patient recruitment and follow-up, Serious Adverse Event documentation and reporting, outcome events documentation and reporting, Investigational Product allocation, patient compliance with the Investigational Product regimen, Investigational Product accountability, concomitant therapy use and quality of data.

19.3 Source document requirements

According to the ICH guidelines for Good Clinical Practice, the Monitoring Team must check the Case Report Form entries against the source documents. The Informed Consent Form will include a statement by which the patient allows the Sponsor's duly authorized personnel, the Ethics Committee (IRB/EC), and the regulatory

authorities to have direct access to source data which supports the data on the Case Report Forms (e.g., patient's medical file, appointment books, original laboratory records, etc.). These personnel, bound by professional secrecy, must keep confidential all personal identity or personal medical information (according to confidentiality rules).

19.4 Use and completion of Case Report Forms (CRFs) and additional requests

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

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

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

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

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

20 PUBLICATIONS

All data generated from this study are the property of AOBiome LLC and shall be held in strict confidence along with all information furnished by AOBiome. Independent analyses and/or publication of these data by the Investigator or any member of his/her staff is not permitted without prior written consent of AOBiome. Any formal presentation or publication of data from this trial will be considered as a joint publication by the Investigator(s) and appropriate Sponsor personnel. Authorship will be determined by mutual agreement. Written permission to the Investigator will be contingent on the review by the Sponsor of the methodology and statistical analysis and any publication or presentation will provide for nondisclosure of AOBiome confidential or proprietary information. In all cases, the parties agree to submit all manuscripts or abstracts to all other

parties at least 30 days prior to submission. This will enable all parties to protect proprietary information and to provide comments based on information that may not yet be available to other parties.

21 PROTOCOL ADHERENCE

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

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

22 CLINICAL TRIAL PROTOCOL AMENDMENTS

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

The Investigator should not implement any deviation from, or changes of the Clinical Trial Protocol without agreement by the sponsor and prior review and documented approval/favorable opinion from the IRB/EC of an amendment. The Investigator is expected to take any immediate action required for the safety of any participant included in this study, even if this action represents a deviation from the protocol. In such cases, the reporting requirements identified in Section 13 must be followed and the Study Lead.

23 CONFIDENTIALITY

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

24 PROPERTY RIGHTS

All information, documents and Investigational Product provided by the Sponsor or its designee are and remain the sole property of the Sponsor. The Investigator shall not mention any information or the Product in any application for a patent or for any other intellectual property rights. All the results, data, documents and inventions, which arise directly or indirectly from the Clinical Trial in any form, shall be the immediate and exclusive property of the Sponsor. The complete verified database will be shared with the Operations Committee, which shall have full access to all data. The Sponsor may use or exploit all the results at its own

discretion, without any limitation to its property right (territory, field, continuance). The Sponsor shall be under no obligation to patent, develop, market or otherwise use the results of the Clinical Trial. As the case may be, the Investigator and/or the Sub-Investigators shall provide all assistance required by the Sponsor, at the Sponsor's expense, for obtaining and defending any patent, including signature of legal documents.

25 DATA PROTECTION

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

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

26 SPONSOR AUDITS AND INSPECTIONS BY REGULATORY AGENCIES

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

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

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

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

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

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

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

27.1 Decided by the Sponsor in the following cases:

1. In the event the results of the Clinical Trial do not appear to be scientifically convincing to the Sponsor;
2. If the aim of the Clinical Trial has become outdated or is no longer of interest;
3. If the information on the product leads to doubt as to the benefit/risk ratio;

4. If the Investigator has received from the Sponsor all Investigational Product, means and information necessary to perform the Clinical Trial and has not included any patient after a reasonable period of time mutually agreed upon;
5. In the event of breach by the Investigator of a fundamental obligation under this agreement, including but not limited to breach of the Clinical Trial Protocol, breach of the applicable laws and regulations or breach of the ICH guidelines for GCP;
6. If the total number of patients are included earlier than expected; In any case the Sponsor will notify the Investigator of its decision by written notice.

27.2 Decided by the Investigator

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

28 CLINICAL TRIAL PROTOCOL AMENDMENTS

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

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30 APPENDIX A-SCHEDULE OF EVENTS

Visit Name	Screening	Washout phase[16]	Baseline	Week 2 Day 14	Week 4 Day 28	Week 6 Final Visit (EOS) Day 42	Early Termination Visit
Visit Window in days	**-21 to -14**	**-14 to -1**	**-1 to 0**	**+/-1**	**+/-1**	**+/-1**	
Informed Consent	X						
Inclusion/Exclusion Criteria[21]	X		X				
Demographics	X						
Medical History	X						
Hanifin and Rajka criteria	X		X				
Concomitant Medications	X		X	X	X	X	X
Smoking status	X						
Physical Exam	X					X	X
Body Weight	X			X	X	X	X
Height	X						
In office BP and HR[13]	X		X	X	X	X	X
Urine pregnancy test for WOCBP[1]	X						
Oral Hygiene	X			X	X	X	X
EASI	X		X	X	X	X	X
VAS	X		X	X	X	X	X
Skindex 16 Questionnaire	X		X	X	X	X	X
IGA	X		X	X	X	X	X

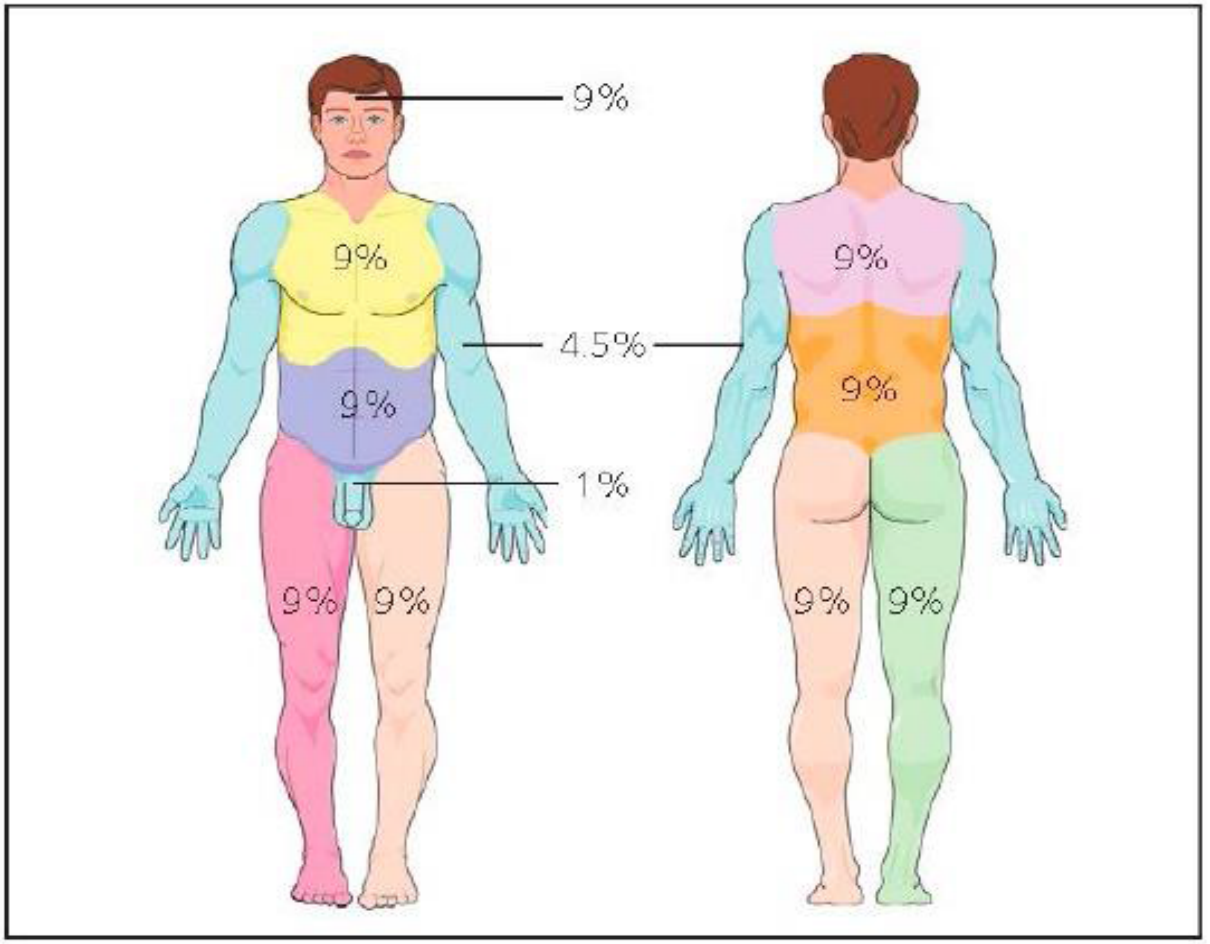
Visit Name	Screening	Washout phase[16]	Baseline	Week 2 Day 14	Week 4 Day 28	Week 6 Final Visit (EOS) Day 42	Early Termination Visit
Visit Window in days	**-21 to -14**	**-14 to -1**	**-1 to 0**	**+/-1**	**+/-1**	**+/-1**	
Clinical chemistry[11]	X			X	X	X	X[18]
Blood for biomarkers[12]			X	X	X	X	X[18]
Skin swabs			X		X	X	X[18]
Microbial composition[20]	X		X		X		
IWRS			X				
Dispense IP to patient			X	X			
Collect Investigational Product from patient				X	X		
Investigational Product application[7]			X	X	X		X[15]
Investigational Product compliance[6]			X	X	X		X[15]
Call to subjects to initiate Washout phase[16]		X[17]					
Actigraphy watch[10]			X		X[19]		X[15]
Counseling[4]		X	X	X	X		
Study cleanser [2]	X	X	X	X	X[9]		X[15]
Moisturizer[14]	X	X	X	X	X[9]		X[15]
Study diary [3]			X	X	X		X[15]
AE monitoring[8]	X	X	X	X	X	X	X

1. Urine pregnancy test for WOCBP will be done at Screening
2. Subjects are to be provided cleanser and moisturizer at Screening visit to start using it at the start of the Washout phase. However, cleanser, and moisturizer are available upon request in the event that subjects run out of their initial supplies.
3. Subjects will be asked to fill out study diary for the duration of the study (Baseline-Day 28)
4. Subjects will be counseled on the use of study medication, diary and answer any questions subject may have
5. Skin swabs will be obtained during the office visit and will be kept in the refrigerator until shipped at the end of the study
6. Weight of an IP kit will be obtained at the at the Baseline visit. Study staff will be asked to weigh 2 bottles without the carton box PRE FIRST DOSE. Weight will be recorded in grams. Subjects will be asked to bring both bottles back for the Week 2 and Week 4 visit. Upon return for the study visit, 2 bottles will be weighted again.
7. Subjects will be asked to apply the IP twice daily. First IP application will happen in the office under medical supervision.
8. AEs will be monitored throughout the trial, starting with the time ICF has been signed.
9. Cleanser and moisturizer should be returned to the study coordinator. Subjects will be asked to go back to their regular routine.
10. Actigraphy watch is dispensed at the Baseline visit. Subjects will be asked to wear one watch on each wrist starting at Baseline and through Day 28 visit. Subjects are allowed to take watches off during bathing/swimming/showering but will be asked to put watches back once these procedures are completed.
11. Patients should fast for at least 8 hours before the test. Blood for clinical chemistry will be shipped to the central lab for processing. Chemistry, Hematology, HbA1C, Lipid Panel and Angiotensin converting enzyme tests will be done at Screening, Week 2, Week 4 and Week 6. Serology will only be done at Screening. Kits and lab manuals will be provided by the Cenetron Labs.
12. Blood samples for biomarkers will be collected and processed on site within 5 minutes of collection. Patients should fast for at least 8 hours before the test. Samples will then be frozen onsite and shipped on monthly bases to the Central lab for storage.
13. Blood pressure readings will be obtained at every visit. Subject should be allowed to rest for > 5 minutes sitting, then serial clinic BP measurements and heart (x3) rate will be performed, using the non-dominant arm with a calibrated electronic sphygmomanometer. The average of the second and third readings will be documented.
14. Moisturizer to be used for the duration of the trial will be provided to all subjects starting with the Washout period. Subjects will be asked to refrain, if possible, from using any other moisturizer and apply the provided product while they are participating in the trial. Any left over moisturizer will be collected at Week 4 visit.
15. If patient's Early Termination Visit happens before Week 4, subject will be asked to return the Actigraphy watch, cleanser, moisturizer and IP to the study staff. Weigh of an IP will need to be taken.
16. Subjects will not need to come in to the clinic in order to start the Washout phase. Once all of the Screening procedures have been completed and subject becomes eligible for the study, supplies will be issued to the patient. However, the patient will be asked to hold off starting with

the Washout period until results of chemistry and serology become available. At that point, subject will then be notified via the phone that they may proceed with the Washout period. Start of the Washout period will be recorded in the CRF by the study staff. Subjects will be instructed to start using the provided cleanser and moisturizer if necessary.

17. Call to subjects to alert them to initiate the 2-week Washout phase and explain the procedures.
18. If patient's Early Termination Visit happens before Week 4, blood for biomarkers, clinical chemistry and skin swab will need to be collected.
19. Return Actigraphy watches
20. At Baseline, study staff will examine subject's body surface area affected by AD and will be asked to identify only one area to be sampled. Once the area is identified, study staff will sample this particular treatment area. Sampling area should be noted in subject's record, so that the same treatment area is then sampled at Week 4 visit.
21. Medical records from patient's dermatologist or primary care physician to confirm the diagnosis of Atopic Dermatitis are optional. Verbal confirmation of the diagnosis present for ≥ 12 months is sufficient in order to fulfill this criterion.

31 APPENDIX B-BODY SURFACE AREA AND IP APPLICATION



32 APPENDIX C EASI QUESTIONNAIRE

How to Use EASI

The EASI scoring system uses a **defined process** to grade the **severity of the signs** of eczema and the **extent affected**:

1. Select a body region

Four body regions are considered separately:

- Head and neck
- Trunk (including the genital area)
- Upper extremities
- Lower Extremities (including the buttocks)

2. Assess the extent of eczema in that body region

Each body region has potentially 100% involvement. Using the table below, give each respective body region a **score** o**f between 0 and 6** based on the percentage involvement. Precise measurements are not required.

% involvement	0	1-9%	10 - 29%	30 - 49%	50 - 69%	70 - 89%	90 - 100%
Region score	0	1	2	3	4	5	6

To aid in your body region grading you can use the **diagrams** in Appendix 1.

3. Assess the severity of each of the four signs in that body region:

1. Erythema
2. Edema/papulation
3. Excoriation
4. Lichenification

Further explanations of these terms can be found in FAQ's (Appendix 4)

Grade the severity of each sign on a scale of 0 to 3:

0	None
1	Mild
2	Moderate
3	Severe

- V Take an average of the severity across the involved region.
- V Half points may be used e.g. 2.5.
- V Palpation may be useful in assessing edema/papulation as well as lichenification

To aid your severity grading, a **photographic atlas** of suggested categories is available in **Appendix 2**

.

Remember: ***Include only inflamed areas*** *in your assessment; do not include xerosis (dryness), ichthyosis, keratosis pilaris, urticaria, infection (unless there is underlying eczema), or post inflammatory pigmentation changes.*

How to record your scores

The assessed parameters are inserted into a table (example shown below for age≥8 years). The final EASI score ranges from 0-72.

Body region	Erythema	Edema/ papulation	Excoriation	Lichenification	Area score	Multiplier	Score
Head/neck	(+	+	+	)	x	x 0.1	
Trunk	(+	+	+	)	x	x 0.3	
Upper extremities	(+	+	+	)	x	x 0.2	
Lower extremities	(+	+	+	)	x	x 0.4	
The final EASI score is the sum of the 4 region scores							———— (0-72)

Two forms of the EASI scoring system are available depending on the age of the patients. The multipliers for the region score are different in the under 8's version to reflect the relative proportion of body regions in young children:

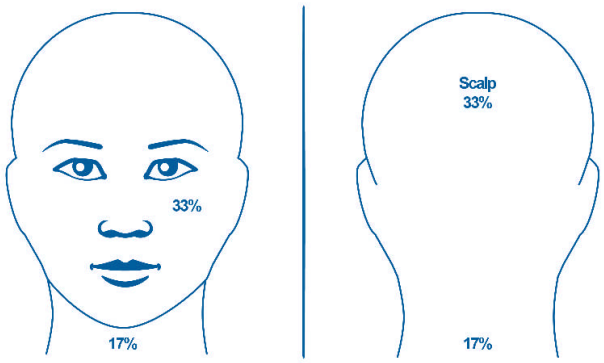
- Patients 8 years or above
- Patients under 8 years of age.

The forms can be found in appendix 3.1 and 3.2 and also as word documents on the HOME website (www.homeforeczema.org)

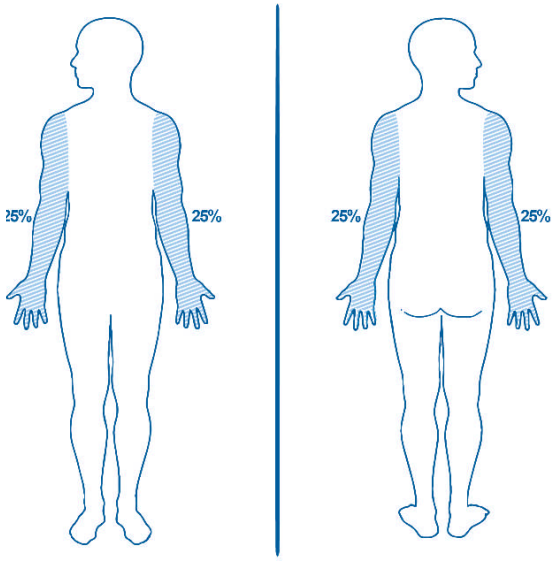
<u>Appendix 1</u>: Eczema Area and Severity Index (EASI) - Extent of eczema per body region

Score <u>each region</u> from 0 to 100%

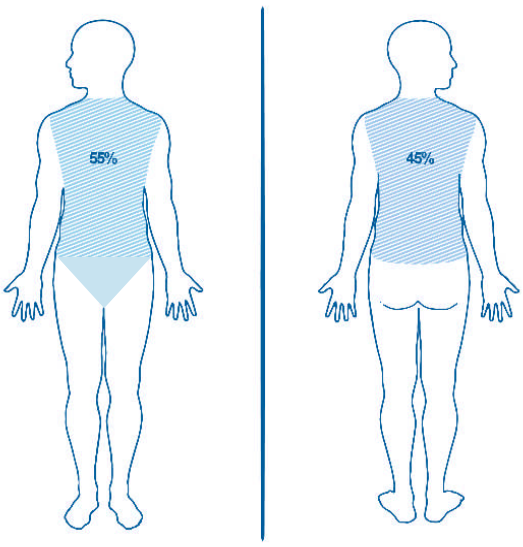
Head & neck



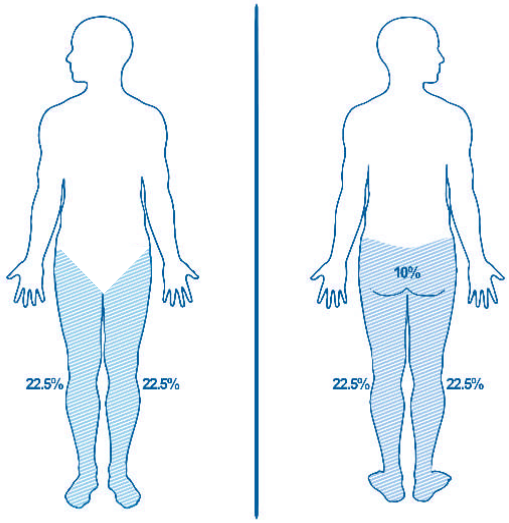
Upper extremities



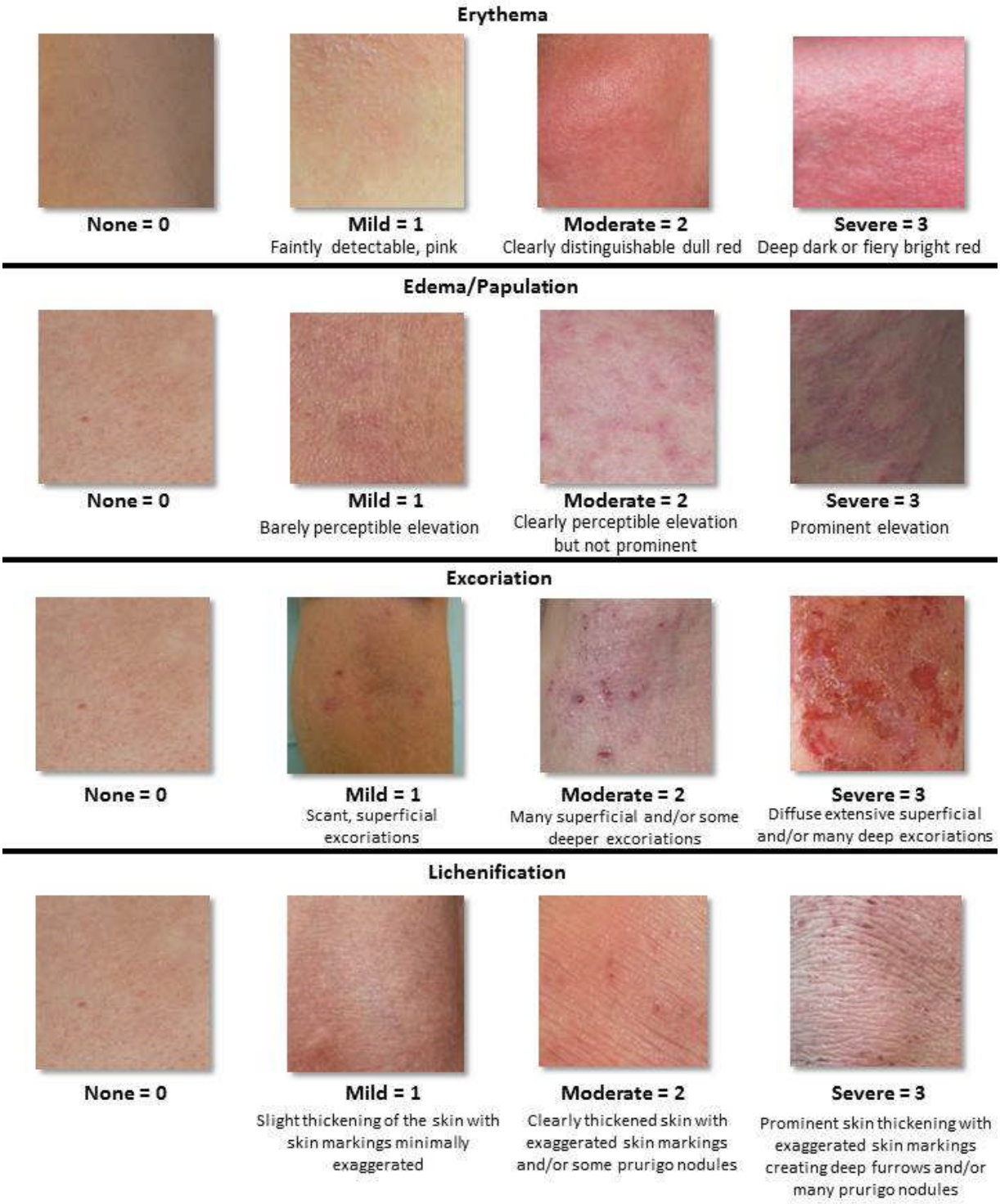
Trunk



Lower extremities



Appendix 2: Eczema Area and Severity Index (EASI) –lesion severity atlas



Appendix 3.1: Eczema Area and Severity Index (EASI) case report form – age <8 years

Area of Involvement: Each body region has potentially 100% involvement. Score **0 to 6** based on the following table:

% involvement	0	1-9%	10 - 29%	30 - 49%	50 - 69%	70 - 89%	90 - 100%
Region score	0	1	2	3	4	5	6

Severity of Signs: Grade the severity of each sign on a scale of **0 to 3**:

0	**None**
1	**Mild**
2	**Moderate**
3	**Severe**

- Take an average of the severity across the involved area.
- Half points may be used e.g. 2.5.

Scoring table:

Body region	Erythema (0-3)	Edema/ Papulation (0-3)	Excoriation (0-3)	Lichenification (0-3)	Region score (0-6)	Multiplier	Score per body region
Head/neck	(+	+	+	)	X	X 0.2	
Trunk	(+	+	+	)	X	X 0.3	
Upper extremities	(+	+	+	)	X	X 0.2	
Lower extremities	(+	+	+	)	X	X 0.3	
The final EASI score is the sum of the 4 region scores:							———————— **(0-72)**

Appendix 3.2: Eczema Area and Severity Index (EASI) case report form - age≥8 years

Area of Involvement: Each body area has potentially 100% involvement. Score **0 to 6** based on the following table:

% involvement	0	1-9%	10 - 29%	30 - 49%	50 - 69%	70 - 89%	90 - 100%
Region score	0	1	2	3	4	5	6

Severity of Signs: Grade the severity of each sign on a scale of **0 to 3**:

0	**None**
1	**Mild**
2	**Moderate**
3	**Severe**

- Take an average of the severity across the involved area.
- Half points may be used e.g. 2.5.

Scoring table:

Body region	Erythema (0-3)	Edema/ Papulation (0-3)	Excoriation (0-3)	Lichenification(0-3)	Region score (0-6)	Multiplier	Score per body region
Head/neck	(+	+	+	)	X	X 0.1	
Trunk	(+	+	+	)	X	X 0.3	
Upper extremities	(+	+	+	)	X	X 0.2	
Lower extremities	(+	+	+	)	X	X 0.4	
The final EASI score is the sum of the 4 region scores:							_____ (0-72)

Appendix 4 - Frequently Asked Questions

What is the difference between edema/papulation and lichenification?
Consider edema/papulation as corresponding to the acute signs of atopic dermatitis that reflect histological spongiosis. Lichenification are more firm thickened plaques with accentuation of the skin markings that develop as a result of prolonged scratching or rubbing in chronic disease. In darker skin types, follicular lichenification may present as firm flat-topped discrete papules. Grade these chronic lesions as lichenification.

How do I grade prurigo nodules?
Prurigo nodules are larger, deeper lesions as a result of chronic scratching and are graded as areas of lichenification.

How do I grade erythema in darker skin?
To avoid underestimating inflammation in patients with darker skin tones, take into account the underlying skin pigment when grading erythema. Often this means increasing your erythema grade by one level.

Can half-steps be used to assess lesion severity?
The original EASI validation study allowed for half steps. These may be helpful when trying to average the severity of a parameter over a region. For example, if there are some areas with an erythema grading of 2 and some areas more consistent with a severity of 3, 2.5 may be a good choice.

What if most areas in a region are a severity grade of 1, but there are some areas that are a grade 3?
Attempt to average the severity across the involved areas in that region. If these areas are close to equal in size, a score of 2 would be most appropriate. If the majority of involved areas are a grade 1, a score of 1 or 1.5 is more appropriate. Be careful not to score the highest severity in a region but the average one.

How do I grade xerosis (dryness), ichthyosis and hyperlinear palms?
Unless there is active acute or chronic eczema overlying these findings, they are not included in the EASI assessment.

How precise should my assessment of eczema extent be?
The *region scores*, which reflect the extent of eczema, were designed and validated as rough estimates of the percentage of involved skin. Each region is given a *score* ranging from 0 to 6, based on a "ballpark" estimation of extent (see region score table in page 1). If you find it difficult to provide a rough estimate of disease extent, you can use the schematics in Appendix 1 to guide you. More time-consuming methods for evaluating disease extent such as the rule of nines or the 'palm' method are generally unnecessary, as the EASI was designed to be…easy.

My patient has responded well to treatment and significantly improved since the last visit. Should I adjust the grading based on the patient's relative improvement?
No. The EASI is a static score, meaning that it is done independently at each time point to reflect current severity. You should grade the EASI per visit regardless of the previous status. Studies have shown that the EASI score has good responsiveness, meaning that overall it is sensitive to change and the improvement will be reflected in the total score.

Can the EASI be used in children?
Yes. The EASI is performed in the same method in all age groups, but the calculation of the final EASI score differs by age. When calculating the EASI, each of the 4 region scores is multiplied by a constant which reflects the relative contribution of that region to the total body surface area. For patients 8 years and older the multipliers are 0.1 for the head/neck, 0.2 for the upper extremities, 0.3 for the trunk and 0.4 for the lower extremities. Below 8 years of age the head/neck multiplier is increased to 0.2 while the lower extremities multiplier decreases to 0.3, consistent with the proportions of these regions in childhood. Refer to Appendix 3 for EASI forms by age.

What happens if a child turns 8 during the course of the study? Which EASI formula should I use?
There are no clear-cut definitions for this situation. In general, if the study is a short term study such as an RCT lasting a few months – using the same formula throughout the trial is preferred, even if the child turns 8 during these months. Keeping the EASI formula consistent in this scenario can serve to preserve the EASI sensitivity to change (e.g. its change in response to treatment) without compromising the validity of the score.
In long term studies such as cohort studies lasting a year or longer, it is important to update the EASI formula based on the physical changes children go through. Switching to the age 8+ formula once a child is older is preferred in that scenario.

What do the terms erythema, edema/papulation, excoriation and lichenification mean?
These are key signs of atopic dermatitis. Recognizing and grading them properly requires training on the visual and physical exam consistent with these signs. Generally speaking, erythema is skin redness; edema/papulation refers to an elevation or swelling of the skin (that should be differed from lichenification below); excoriations are scratch marks that have broken the skin surface; and lichenification is a leathery thickening of the skin with exaggerated skin markings.

33 APPENDIX D. SKINDEX 16 QUESTIONNAIRE

Skindex16

THESE QUESTIONS CONCERN THE SKIN CONDITION WHICH HAS BOTHERED YOU THE MOST DURING THE PAST WEEK

During the past week, how often have you been bothered by:	Never Bothered ↓			•			Always Bothered ↓
1. Your skin condition **itching**	☐0	☐1	☐2	☐3	☐4	☐5	☐6
2. Your skin condition **burning** or **stinging**	☐0	☐1	☐2	☐3	☐4	☐5	☐6
3. Your skin condition **hurting**	☐0	☐1	☐2	☐3	☐4	☐5	☐6
4. Your skin condition **being irritated**	☐0	☐1	☐2	☐3	☐4	☐5	☐6
5. The **persistence / reoccurrence** of your skin condition .	☐0	☐1	☐2	☐3	☐4	☐5	☐6
6. **Worry** about your skin condition (For example: that it will spread, get worse, scar, be unpredictable, etc)	☐0	☐1	☐2	☐3	☐4	☐5	☐6
7. The **appearance** of your skin condition	☐0	☐1	☐2	☐3	☐4	☐5	☐6
8. **Frustration** about your skin condition	☐0	☐1	☐2	☐3	☐4	☐5	☐6
9. **Embarrassment** about your skin condition.	☐0	☐1	☐2	☐3	☐4	☐5	☐6
10. **Being annoyed** about your skin condition	☐0	☐1	☐2	☐3	☐4	☐5	☐6
11. **Feeling depressed** about your skin condition	☐0	☐1	☐2	☐3	☐4	☐5	☐6
12. The effects of your skin condition on your **interactions with others** (For example: interactions with family, friends, close relationships, etc)	☐0	☐1	☐2	☐3	☐4	☐5	☐6
13. The effects of your skin condition on your **desire to be with people**	☐0	☐1	☐2	☐3	☐4	☐5	☐6
14. Your skin condition making it hard to **show affection** .	☐0	☐1	☐2	☐3	☐4	☐5	☐6
15. The effects of your skin condition on your **daily activities**.	☐0	☐1	☐2	☐3	☐4	☐5	☐6
16. Your skin condition making it hard to **work or do what you enjoy**	☐0	☐1	☐2	☐3	☐4	☐5	☐6

Have you answered every item? Yes ☐ No ☐

ESTAS PREGUNTAS SE RELACIONAN CON LA AFECCIÓN EN LA PIEL QUE MÁS LE HA MOLESTADO DURANTE LOS ÚLTIMOS 7 DÍAS

Durante los últimos 7 días, ¿con qué frecuencia le ha molestado…?	Nunca me molestó ↓			•			Siempre me molestó ↓
1. La **picazón** en la afección en la piel	☐0	☐1	☐2	☐3	☐4	☐5	☐6
2. El **ardor** o **escozor** en la afección en la piel	☐0	☐1	☐2	☐3	☐4	☐5	☐6
3. El **dolor** en la afección en la piel	☐0	☐1	☐2	☐3	☐4	☐5	☐6
4. La **irritación** en la afección en la piel	☐0	☐1	☐2	☐3	☐4	☐5	☐6
5. La **persistencia / recurrencia** de la afección en la piel	☐0	☐1	☐2	☐3	☐4	☐5	☐6
6. La **preocupación** por la afección en la piel (Por ejemplo: de que se extenderá, empeorará, dejará cicatriz, sea impredecible, etc.)	☐0	☐1	☐2	☐3	☐4	☐5	☐6
7. El **aspecto** de la afección en la piel	☐0	☐1	☐2	☐3	☐4	☐5	☐6
8. La **frustración** por la afección en la piel	☐0	☐1	☐2	☐3	☐4	☐5	☐6
9. La **vergüenza** por la afección en la piel	☐0	☐1	☐2	☐3	☐4	☐5	☐6
10. **Sentirse fastidiado** por la afección en la piel	☐0	☐1	☐2	☐3	☐4	☐5	☐6
11. **Sentirse deprimido** por la afección en la piel	☐0	☐1	☐2	☐3	☐4	☐5	☐6
12. Los efectos de la afección en la piel en sus **relaciones con los demás** (Por ejemplo: relaciones con su familia, amigos, relaciones cercanas, etc.)	☐0	☐1	☐2	☐3	☐4	☐5	☐6
13. Los efectos de la afección en la piel en su **deseo de estar con otras personas**	☐0	☐1	☐2	☐3	☐4	☐5	☐6
14. Que la afección en la piel le dificulte **demostrar afecto**	☐0	☐1	☐2	☐3	☐4	☐5	☐6
15. Los efectos de la afección en la piel en sus **actividades diarias**	☐0	☐1	☐2	☐3	☐4	☐5	☐6
16. Que la afección en la piel le dificulte **trabajar o hacer lo que disfruta**	☐0	☐1	☐2	☐3	☐4	☐5	☐6

¿Ha respondido cada pregunta? Sí ☐ No ☐

34 APPENDIX E. HANIFIN AND RAJKA CRITERIA

Hanifin and Rajka Diagnostic Criteria for Atopic Dermatitis (AD)

Major criteria: Must have three or more of:

1. Pruritus ☐
2. Typical morphology and distribution ☐

    - Flexural lichenification or linearity, erythema, scling, serum-crust

3. Chronic or chronically-relapsing dermatitis ☐
4. Personal or family history of atopy (asthma, allergic rhinitis, atopic dermatitis) ☐

Minor criteria: Should have three or more of:

1. Xerosis ☐
2. Ichthyosis, palmar hyperlinearity, or keratosis pilaris ☐
3. Immediate (type 1) skin-test reactivity ☐
4. Raised serum IgE ☐
5. Early age of onset ☐
6. Tendency toward cutaneous infections (especially *S aureus* and herpes simplex) or impaired cell-mediated immunity ☐
7. Tendency toward non-specific hand or foot dermatitis ☐
8. Nipple eczema ☐
9. Cheilitis ☐
10. Recurrent conjunctivitis ☐
11. Dennie-Morgan infraorbital fold ☐
12. Keratoconus ☐
13. Anterior subcapsular cataracts ☐
14. Orbital darkening ☐
15. Facial pallor or facial erythema ☐
16. Pityriasis alba ☐
17. Anterior neck folds ☐
18. Itch when sweating ☐
19. Intolerance to wool and lipid solvents ☐
20. Perifollicular accentuation ☐
21. Food intolerance ☐
22. Course influenced by environmental or emotional factors ☐
23. White dermographism or delayed blanch ☐

35 APPENDIX F. VISAL ANALOGUE SCALE (VAS)

Visual Analog Scale

NO ITCH

MAXIMUM ITCH

36 APPENDIX G. IGA SCALE

Investigator Global Assessment Score (IGA)

Score	Category	Definition
0	Clear	Minor residual discoloration, no erythema or induration/papulation, no oozing/crusting
1	Almost Clear	Trace faint pink erythema with almost no induration/papulation and no oozing/crusting
2	Mild Disease	Faint pink erythema with mild induration/papulation and no oozing/crusting
3	Moderate Disease	Pink-red erythema with moderate induration/papulation and there may be some oozing/crusting
4	Severe Disease	Deep/bright red erythema with severe induration/papulation with oozing/crusting