AOBIOME

CLINICAL TRIAL PROTOCOL

Phase IIb/III

COMPOUND:

B244

A Randomized, Double Blinded, Phase IIb/III, Decentralized Study of B244 Delivered as a Topical Spray to Determine Safety and Efficacy in Participants with Mild to Moderate Acne Vulgaris

STUDY NUMBER:

AOB2016-01

VERSION DATE: October 3, 2016

PROTOCOL NUMBER: AVB244-002

Sponsor:

AOBiome LLC

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Cambridge, MA 02142-1100

Revision Chronology

Original	April 15, 2016
Amendment 1	July 22, 2016
Amendment 2	October 3, 2016

SPONSOR APPROVAL

A Randomized, Double Blinded, Phase IIb/III, Decentralized Study of B244 Delivered as a Topical Spray to Determine Safety and Efficacy in Participants with Mild to Moderate Acne Vulgaris

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Date: Oct 13th 2016

INVESTIGATOR AGREEMENT

I have read this protocol and agree:

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

Investigator Printed Name:_____

Signature:_____ **Date:** _____

Statement of Compliance

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

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

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

CLINICAL TRIAL SUMMARY

COMPOUND: B244 STUDY NUMBER: AVB244-002

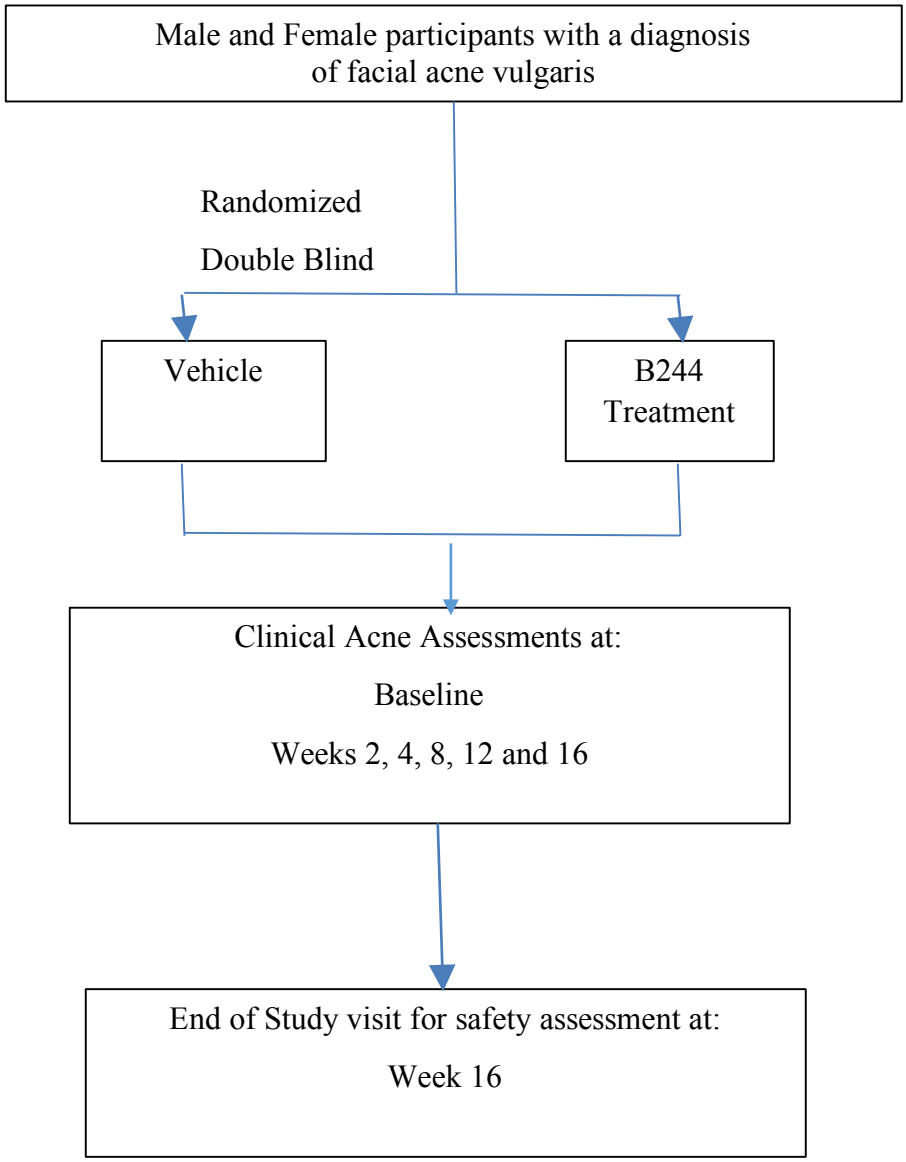
TITLE:	A Randomized, Double Blinded, Phase IIb/III, Decentralized Study of B244 Delivered as a Topical Spray to Determine Safety and Efficacy in Participants with Mild to Moderate Acne Vulgaris
INVESTIGATOR/TRIAL LOCATION:	Science 37 as a decentralized clinical trial site enrolling study participants from within the United States
STUDY OBJECTIVES:	**Primary Objectives:** • To evaluate the safety and tolerability of B244 in participants with acne vulgaris. • To assess the efficacy of B244 in participants with acne vulgaris from baseline to week 12 (end of treatment) by: o Reduction in inflammatory and non-inflammatory lesion count o IGA success **Secondary Objective:** • To evaluate the efficacy of B244 in comparison to vehicle in participants with acne vulgaris from baseline to weeks 2, 4, 8, and 16 o Reduction in inflammatory and non-inflammatory lesion counts o IGA success • Improvement in patient reported quality of life score using the Skindex-16 questionnaire in participants with acne vulgaris from baseline to weeks 2, 4, 8, 12, and 16 **Exploratory Objective:** • To evaluate facial skin microbiota in participants with acne vulgaris at baseline and weeks 2, 4, 8, 12, and 16 in B244-treated

	participants compared to vehicle
STUDY DESIGN:	Phase IIb/III, randomized, double blinded, decentralized clinical trial evaluating the safety, tolerability, and efficacy of B244 compared to vehicle in the treatment of acne vulgaris
INCLUSION CRITERIA:	1. Males and females age 18 or above 2. Clinical diagnosis of mild to moderate facial acne vulgaris defined as: a. ≥5 inflammatory lesions, and; b. ≥10 non-inflammatory lesions, and; c. IGA 2-3 3. Willing to refrain from using any treatments, other than the investigational product, for acne present on the face once consented. This includes the use of antibiotics for the treatment of acne. Topical acne treatments that do not have significant or measurable systemic absorption (e.g., benzoyl peroxide, salicylic acid) are allowed for treatment of non-facial acne 4. Willing and able to provide informed consent and to comply with the study protocol
EXCLUSION CRITERIA:	1. Pregnant and/or lactating females 2. Continuous or planned use of tanning booths or excessive sun exposure, as determined by the Investigator. 3. Active cystic acne or acne conglobata, acne fulminans, and secondary acne 4. Two or more active nodular lesions 5. Treatment with over-the-counter topical medications for the treatment of acne vulgaris including benzoyl peroxide, topical anti-inflammatory medications, corticosteroids, α-hydroxy/glycolic acid, or topical probiotics including commercially available product

	AO+Mist on the face within 7 days prior to baseline 6. Treatment with systemic corticosteroids within 28 days prior to baseline 7. Treatment with systemic antibiotics or systemic anti-acne drugs within 7 days prior to baseline 8. Prescription topical retinoid use on the face within 7 days of baseline (e.g., tretinoin, tazarotene, adapalene) 9. Commencement of new hormonal therapy or dose change to hormonal therapy within 90 days prior to baseline. Dose and frequency of use of any hormonal therapy started more than 90 days prior to baseline must remain unchanged throughout the study. Hormonal therapies include, but are not limited to, estrogenic and progestational agents such as birth control pills 10. Use of androgen receptor blockers (such as spironolactone or flutamide) in the 7 days prior to randomization 11. Oral retinoid use (e.g., isotretinoin) within 180 days prior to baseline or vitamin A supplements greater than 10,000 units/day within 180 days of baseline 12. Cosmetic facial procedures (chemical or laser peel, microdermabrasion, etc.) within the 28 days of the first dose or during the study 13. History of sensitivity to any of the study medications, or components thereof or a history of drug or other allergy that, in the opinion of the Investigator or Medical Monitor, contraindicates their participation 14. Any other condition(s) that the study Investigator feels would indicate that participation would not be in the best interest of the participant 15. The participant has been previously randomized in this study 16. The participant has received an investigational

	product within 30 days or 5 half-lives, whichever is longer prior to randomization
DOSE REGIMEN:	Participants will apply 4 pumps of spray to the face BID for 12 weeks
ASSESSMENT SCHEDULE:	Study assessments will occur at Screening, Baseline, and weeks 2, 4, 8, 12, and 16
STATISTICAL CONSIDERATIONS:	1. Modified Intent to treat analysis 2. Fisher's exact test will be used for efficacy parameters
DURATION OF STUDY:	The estimated total study duration is 12 months. All participants will be followed from consent date until the end of study visit at week 16.

STUDY SCHEMA



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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

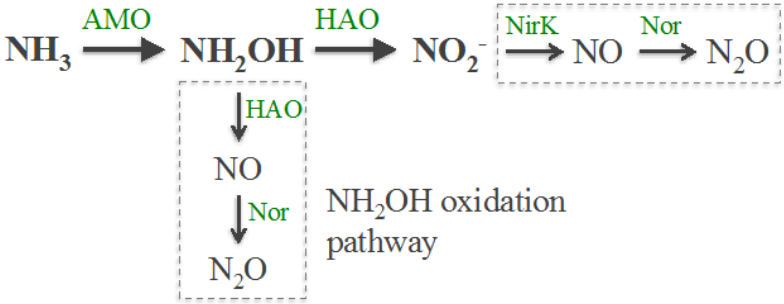
AE	Adverse Event
AMO	Ammonia Monooxygenase
AOB	Ammonia Oxidizing Bacteria
BID	Twice-Daily
CRF	Case Report Form
E/T	Early Termination
FDA	Food and Drug Administration
HAO	NH_2OH oxidoreductase
HbsAg	Hepatitis B Virus Surface Antigen
HCV Ab	Hepatitis C Virus Antibody
HIV	Human Immunodeficiency Virus
ICH	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
NORA	Network Oriented Research Assistant
QoL	Quality of Life
SAE	Serious Adverse Event
SPM	Study Procedures Manual

1. INTRODUCTION

1.1. 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/NO_x to the human skin. The active ingredient in B244 is available to consumers as a cosmetic product.

Acne vulgaris is a common disease that continues to affect many men and women of all ethnicities. Most people have had acne at some point of their life with 15-20% of people experiencing moderate to severe acne.[6] Prevalence differs from study to study but has been estimated as high as 45 million people within the United States alone and accounts for 20% of all dermatology visits.[7] Although development of effective acne treatments has come a long way, much of our understanding of the pathogenesis of acne at the cellular level is still lacking.[8]

To date, two cosmetic studies have been completed. In the cosmetic studies, a total of 24 participants applied the cosmetic product to the scalp and 83 participants applied the product to their face. In these studies, there were no reports of drug-related serious adverse events (SAEs).

A phase 1b/2a clinical trial was recently completed where 36 participants with clinical diagnosis of facial acne vulgaris were randomized to receive ascending doses of investigational product (IP) over 14 days. Safety analyses have been completed and there have been no attributable drug related SAEs reported.

1.2. Rationale for using decentralized clinical trial model

For medical science to progress, technology must advance to bridge the gap between research and the real-world patient application of medical discovery. For example, researchers are beginning to have a better understanding of the diverse human microbiome and how derangements might contribute to diseases such as acne. But to decipher the complexity of this relationship across growing and diverse populations, new technology and methodology is needed to connect with patients. Keeping pace with the advent of electronic medical records, social networking, and smartphones, new applications of technology will allow patients to interact with physicians and researchers more regularly and directly from their home in a way that will lead to better treatment and understanding of the diseases that afflict us.

The concept of using mobile smartphone technology in medicine and research is not new. Even with regard to acne, research instruments such as telemedicine, mobile electronic devices, and the internet have been used. Specifically, this technology has been used to assist with compliance, medical education, physician-patient interactions, medical access,

and evaluation of treatment efficacy in many areas including dermatology. Indeed, because of prior challenges associated with unbiased scoring of acne clinical trials, the FDA guidelines for registered trials in acne strongly suggest that a digital photographic record of all patient visits be made available as the gold standard for FDA audit of clinical outcomes.

Similarly, telemedicine and photographic technology has long been used in dermatology to bridge the access gap and to open patient-physician interactions. A limitation in telemedicine and the use of store-and-forward technology has been in the user's skill when capturing photos to be evaluated by qualified healthcare professionals. Bergman and colleagues examined the feasibility of patient mediated photographic assessment by comparing photos taken by trained staff to those taken by patients.[10] Photos were then evaluated using total inflammatory lesion count, the Burke and Cunliffe Leeds acne assessment technique, and the Investigator Global Assessment. The study showed strong inter-rater reliability for inflammatory lesion counts, Leeds assessments, and Investigator global assessments.[11] The study demonstrated that photographs taken by participants were reliable and effective to score acne lesion counts and severity.

Grading disease severity (e.g. acne severity scoring) is of utmost importance in guiding and monitoring the success of treatment. Instruments used to score disease often neglect to account for the impact of disease on livelihood. In addition, patients tend to score their acne severity higher than physicians.[12] Interestingly, there is no gold standard for scoring acne severity despite extensive acne research over the years and many attempts to create a uniform scoring system. The pleomorphic nature of acne makes it difficult to create a simple unifying grading system, yet there remains a demand for a homogenized scoring system by which acne studies can be compared.[13] The FDA has issued guidelines for acne trials that include a recommendation to keep digital photographic records of each visit so outside investigators or auditors could verify the scoring if needed.

Many different studies have employed one or more scoring methods or have created their own scoring rubric. There are two main variations of acne scoring-lesion counting and global assessment. There has been debate regarding the reliability of lesion counting compared to global assessment; important considerations include the ease and reproducibility of each scoring instrument. In a study done by Lucky et al, lesion counting showed high inter-rater reliability (.81 to .97).[14] The reliability was highest in mild cases of acne and decreased with increased number of lesions. Global assessments of acne such as the GEA have been shown to have inter-rater reliability as high as .80-.84.[15] The US FDA, American Academy of Dermatology (AAD) and European Medicines Agency (EMA) recommend a global approach.[13,15] However, FDA guidelines

also suggest the use of lesion counting in conjunction with global assessment of acne. Application of both systems of acne grading has been validated in studies done by Burke and Cunliffe.[11]

Access issues continue to limit biomedical research and healthcare. Access limits connections between patients and physicians and between researchers and needed participants. As modern medical practice shifts toward patient-centered care, a push toward greater patient engagement must also evolve. A better partnership between patients and doctors to improve understanding and management of disease will lead to better healthcare. As an example, patients will be able to enter their own data into secure electronic health records (EHR). Today, some patients enjoy access to their EHR through providers such as Kaiser Permanente and are allowed to procure certain samples for lab analysis (e.g. fecal occult blood test) in their home. This very same principle of patient empowered medical evaluation can be applied to research and made easy for patients to access. Note that physician guidance is still the foundation of medical and investigational practice. However, application of patient involvement can be as simple as using a mobile device to securely update reliable patient reported outcomes (PROs) within clinical trials. Additionally, less than 5% of clinical research participants nationally are minorities. The decentralized clinical trial model will unlock access to minorities and underserved populations that are not normally cared for at traditional clinical research sites and thus better reflect the national diversity of race and ethnicity in the United States.

New technology exists to bridge access between clinical research and the need for patient-centered care. One such application of technology is NORA (Network Oriented Research Assistant). NORA is currently being used successfully in other FDA-registered decentralized clinical trials to assess skin and mucosal disease in the home. NORA is a combination technology that includes the functionality of a telemedicine platform, an EMR, an EDC, and a mobile data collection tool. NORA was recently used to compare digital photography as a correlate of face-to-face acne scoring but has been used in other therapeutic areas as well. This approach will hopefully improve patient outcomes and advance the field of medicine by using more real world data and a better diversity of patients.

The purpose of this study is to demonstrate the safety, tolerability and efficacy of B244 administered over 12 weeks to participants with mild to moderate acne vulgaris relative to vehicle.

2. RISK ASSESSMENT

Please refer to Investigator Brochure for in depth risk assessment.

None of the potential or identified risks seen to date in participants dosed with B244 preclude further clinical development. Mitigation strategies have been implemented to promptly identify and appropriately address these risks in order to protect participant safety and to better characterize the safety profile of the drug. Careful safety monitoring should also identify any emerging safety issues in a timely fashion. There is a paucity of probiotics with novel mechanisms of action available to treat acne vulgaris, given the potential unmet medical need for treatment of acne and the risk mitigation strategies in place, the benefit to risk profile of B244 for the treatment of acne vulgaris is favorable and warrants further study.

Eligibility criteria, monitoring of clinical parameters, and stopping criteria have been chosen to mitigate identified and potential risks in this study and to better characterize the safety and tolerability of B244. Refer to Appendix A, Time and Events Table for the timing of all clinical assessments. Further, the study design in place will allow in stream review of the efficacy, safety, and tolerability data for B244 during the study.

3. STUDY OBJECTIVES

3.1. Primary Objectives

1. To evaluate the safety and tolerability of B244 in participants with acne vulgaris
2. To assess the efficacy of B244 in participants with mild to moderate acne vulgaris from baseline to week 12 (end of treatment) by:
    i) Reduction in inflammatory and non-inflammatory lesion count
    ii) IGA success

3.2. Secondary Objectives

1. Improvement in patient reported quality of life score using the Skindex-16 questionnaire in participants with acne vulgaris from baseline to weeks 2, 4, 8, 12 and 16.
2. To evaluate the efficacy of B244 in participants with mild to moderate acne vulgaris from baseline to weeks 2, 4, 8, and 16:
    i) Reduction in inflammatory and non-inflammatory lesion count
    ii) IGA success

3.3. Exploratory Objective

1. To evaluate facial skin microbiota in participants with acne vulgaris at baseline, week 4, week 8, week 12, and week 16 in B244-treated participants compared to vehicle from:

    i) Skin swabs will be taken from forehead, nose, both cheeks and chin

    All participants (vehicle and B244) will undergo skin swabs and testing.

4. ENDPOINTS

4.1. Safety & tolerability:

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

4.2. Efficacy:

1. Number of Inflammatory Lesions and Non-Inflammatory Lesions at baseline and weeks 2, 4, 8, 12, and 16.
2. Investigator Global Assessment (IGA) at baseline and weeks 2, 4, 8, 12, and 16.
3. Skindex 16 at baseline and weeks 2, 4, 8, 12, and 16.

4.3. Exploratory:

1. Microbial content, microbiota composition, and B244 presence/absence at baseline, weeks 4, 8, 12, and 16.

5. STUDY DESIGN

- This is a randomized, double blinded, Phase IIb/III decentralized study of B244 to determine safety and efficacy in participants with mild to moderate acne vulgaris
- Randomization will be 1:1 so that 186 participants will be treated with B244 and 186 participants will be treated with vehicle
- All B244 randomized participants will be treated at the dose of $4x10^9$ cfu/ml (mid-dose of the Phase Ib/IIa trial)
- FDA Guidance efficacy endpoints will be assessed and compared between treatment groups
- Screening will occur at Study Week -3 (Days -21 to 0)

- Randomization will occur during the baseline period for the study (Days -7 to 0).
- Clinical acne assessments (IGA, lesion counts) will be made at Baseline; Study Weeks 2, 4, 8, 12, and 16.
- 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.

5.1. Consenting & Screening Period

Electronic 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 virtually, using an electronic consent (eConsent) form that the participant will view on his/her home computer or tablet. The eConsent form will be identical in content to a paper consent form, but will be viewed on a computer or tablet. Participants will receive a link via to access the eConsent web portal. Participants without access to a computer or tablet with which to complete the informed consent process will be shipped a tablet to complete the eConsent form from the web portal. Site personnel will organize the return of the tablet upon completion of the informed consent process.

After informed consent has been obtained, to determine participant eligibility for enrollment in the study, screening assessments will be performed within 3 weeks (-28 days to -8) prior to the Baseline visit (-7 days to 1), randomization and first dose of the investigational product (IP). Participants will be shipped an iPhone pre-loaded, after consent, with NORA for use during their participation in the study. All screening evaluations must be completed and reviewed to confirm that participants meet all eligibility criteria before randomization. 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.

Re-screening refers to repeating the entire screening process if a participant has not yet met all the eligibility criteria within 14 days of the original screening visit. As acne is a very dynamic disease, potential participants will be allowed to be re-screened up to three times. Each participant must be re-consented before re-screening occurs.

5.2. Treatment Period

Once it has been determined that the participant meets all inclusion criteria and no exclusion criteria, they will be enrolled into the trial and randomized. Eligible participants will perform baseline assessments after randomization. All assessments and the relative timings are listed in the Time and Events Table (Appendix A).
Participants will apply the investigational product (IP) to the face BID, once in the morning and once in the evening for 12 weeks.
Participants will be assessed for safety and efficacy endpoints throughout the study at the times indicated in the Time & Events Table (Appendix A). The last assessment is at Week 16.

5.3. 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).

Reasons for withdrawal from investigational product (IP) may include the following:

- Adverse event
- Protocol deviations at the discretion of the Sponsor
- Termination of the study by AOBiome
- Investigator discretion
- Participant reached protocol-defined stopping criteria

The reason for participant study withdrawal or withdrawal from IP 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.

Any subject who develops Grade 2 or higher adverse events [according to the common toxicity criteria for adverse events (CTCAE) version 3] should be discontinued from treatment, be continued in the trial, and followed until resolution of the adverse events.

Participants who are prematurely withdrawn from IP will remain in the study. If a participant is prematurely withdrawn from IP, the Investigator must make every recorded effort to ensure the participant attends all visits as scheduled in Appendix A.

5.3.1. **Protocol-Defined Stopping Criteria**

The number, severity, type, nature and pattern of all clinical and non-clinical findings will be considered before making a decision to stop dosing of subsequent participants in the study. Based on these results and the nature of the AE, a decision may be made to adjust participant(s) to the original dose.

Treatment with the investigational product (IP) will be stopped and the participant will be withdrawn from the study if any of the following occurs during the study:

- Hypersensitivity reactions considered possibly related to the investigational product (IP) or vehicle that may include allergic (or immediate) and delayed responses
- Severe, localized inflammatory reactions and infections on the face whose signs and symptoms may include pain, pruritus, erythema, and edema
- Bacteremia or sepsis consistent with clinical signs, symptoms, and laboratory evaluations
- Serious Adverse Events (SAEs) considered possibly related to the investigational product (IP) as assessed by the Investigator
- The Investigator considers that the participant is being exposed to unacceptable, medical risk(s) for any reason while participating in this study

The study will be terminated for the following reasons:

If one or more participants experience a possibly drug-related SAE or a possibly drug-related significant non-SAE, that in the opinion of the study physician, Investigator or AOBiome Medical Monitor or designee warrants discontinuation of further dosing.

5.4. **Screen Failures**

Data for screen and baseline failures will be collected in source documentation at the site

but will not be transmitted to AOBiome.

5.5. Early Termination

Participants who have discontinued the study early will be evaluated by the Investigator at approximately 7 ± 1 days after last dose of IP for an Unscheduled Visit. See the list of assessments to be performed at the Unscheduled 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.

6. SELECTION OF STUDY PARTICIPANTS

6.1. Number of Participants Planned

It is estimated that approximately 372 participants will be enrolled & randomized in order to provide a maximum of 322 eligible participants completing the study.

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:

1. Male and females age 18 or older
2. Clinical diagnosis of mild to moderate facial acne vulgaris defined as:
    a. ≥5 inflammatory lesions, and;
    b. ≥10 non-inflammatory lesions, and;
    c. IGA 2-3
3. Willing to refrain from using any treatments, other than the investigational product, for acne present on the face. This includes the use of antibiotics for the treatment of acne. Topical acne treatments that do not have significant or measurable systemic absorption (e.g., benzoyl peroxide, salicylic acid) are allowed for treatment of non-facial acne.
4. Willing and able to provide informed consent and to comply with the study protocol.

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:

1. Pregnant and/or lactating females
2. Continuous or planned use of tanning booths or excessive sun exposure, in the opinion of the Investigator.
3. Active cystic acne or acne conglobata, acne fulminans, and secondary acne
4. Two or more active nodular lesions
5. Treatment with over-the-counter topical medications for the treatment of acne vulgaris including benzoyl peroxide, topical anti-inflammatory medications, corticosteroids, α-hydroxy/glycolic acid, or topical probiotics including commercially available product AO+Mist on the face within 7 days prior to baseline.
6. Treatment with systemic corticosteroids within 28 days prior to baseline.
7. Treatment with systemic antibiotics or systemic anti-acne drugs within 7 days prior to baseline.
8. Prescription topical retinoid use on the face within 7 days of baseline (e.g., tretinoin, tazarotene, adapalene).
9. Commencement of new hormonal therapy or dose change to hormonal therapy within 90 days prior to baseline. Dose and frequency of use of any hormonal therapy started more than 90 days prior to baseline must remain unchanged throughout the study. Hormonal therapies include, but are not limited to, estrogenic and progestational agents such as birth control pills.
10. Use of androgen receptor blockers (such as spironolactone or flutamide) in the 7 days prior to randomization.
11. Oral retinoid use (e.g., isotretinoin) within 180 days prior to baseline or vitamin A supplements greater than 10,000 units/day within 180 days of baseline.
12. Cosmetic facial procedures (chemical or laser peel, microdermabrasion, etc.) within the 28 days of the first dose or during the study.

13. History of sensitivity to any of the study medications, or components thereof or a history of drug or other allergy that, in the opinion of the Investigator or Medical Monitor, contraindicates their participation.
14. Any condition that the study Investigator feels would indicate that participation would not be in the best interest of the participant.
15. The participant has been previously randomized in this study.
16. The participant has received an investigational product within 30 days or 5 half-lives, whichever is longer prior to randomization.

7. LIFESTYLE RESTRICTIONS

1. Use of anti-aging and anti-acne products on the face will be prohibited during the study.
2. All makeup must be removed before each photography session. No other topical products should be applied to the face until all photographs have been taken.
3. Participants will be discouraged from facial washing for at least 2 hours AFTER each application of investigational product. Participants may bathe or shower with soap and water BEFORE each application.
4. Participants will be encouraged to avoid prolonged periods of sun exposure and discouraged from the use of tanning beds during the study. Extra care should be taken to wear protective clothing, including sunglasses, and avoid sun exposure from 10 AM to 2 PM.
5. 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.

8. CONTRACEPTION REQUIREMENTS

Effective contraception is required for all women physiologically capable of becoming pregnant during study participation.

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.

9. STUDY TREATMENT

9.1. 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 12 weeks	Topical application BID for 12 weeks
Dosing instruction:	4 pumps of spray to saturate the entire face applied BID. Applications should occur in the morning and at night for 12 weeks.	4 pumps of spray to saturate the entire face applied BID. Applications should occur in the morning and at night for 12 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 metered opaque bottles.

9.2. Modalities of administration

4 pumps of spray to saturate the entire face must be applied twice a day. Applications should occur in the morning and at night for the duration of the study. Participants will be instructed to close their eyes and mouth during the application.

9.3. 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.

9.4. 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 IxRS (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.

9.5. 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 mobile application 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.

9.6. Treatment of Investigational Product Overdose

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

9.7. 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 the designated refrigerator between 2-8^{o}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.

9.8. 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. Investigators have direct access to the participant's individual study treatment. 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.

9.9. Retrieval and Destruction of Investigational Product

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

The site will not destroy unused IP unless the Sponsor provides written authorization to the contrary.

10. CONCOMITANT MEDICATIONS

10.1. Permitted Medications

At study entry, participants must not be using any medication to treat acne vulgaris. Topical acne treatments that do not have significant or measurable systemic absorption (e.g., benzoyl peroxide, salicylic acid) are allowed for treatment of non-facial acne.

Any concomitant medication to treat adverse events will be recorded in the Concomitant Medication section of the eCRF.

10.2. Prohibited Medications

Participants will be asked to stop taking medications for treatment of facial acne vulgaris from 7 days prior to randomization during the washout period and throughout the study until the final follow-up visit.

11. STUDY PROCEDURES

11.1. Informed Consent Procedures

Eligible participants may only be included in the study after providing virtual electronic 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.

11.2. Study Assessments

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

11.3. Demographics

All participant demographic and baseline characteristic data will be collected.

11.4. Medical History

Relevant medical history/current medical conditions data will be collected from all participants.

11.5. Pregnancy Testing

A urine pregnancy test will be performed for women physiologically capable of becoming pregnant prior to administration of the first dose of IP.

11.6. Skin Swab Samples

All participants will self-collect a total of 5 skin swabs sampling the forehead, both cheeks, nose and chin at Baseline, Week 4, 8, 12 and Week 16. Participants will collect swabs at Baseline and then one swab at Week 4, 8, 12, 16. Patients will be instructed to collects samples before washing or applying product on their face. Patients will be instructed to send samples back to the study site using the self-addressed stamped envelope.

Swab samples may be subjected to DNA sequencing. 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 indefinitely, 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.

11.7. Safety Assessments

Participants will report adverse events immediately to the Investigator and study personnel. Participants will also be solicited for adverse events at each scheduled visit. Limited vital signs may be measured at home intermittently and recorded in the study mobile application by the participants.

11.8. Compliance

Participants will be supplied with scales and will be asked to weigh each spray bottle provided before the first use and after 7 days of use. Weight will be recorded in the study mobile application.

11.9. Pregnancy Reporting

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

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

12. EFFICACY ASSESSMENTS

12.1. Lesion Counts

Using standardized and validated facial photography modules built into the study mobile platform, participants will perform self-photography and photographs will be automatically uploaded the mobile platform. Acne lesion counts will be performed at the times indicated in the Time and Events Table (Appendix A).

Lesion counting will be performed using the photos taken by the participant and stored inside the study mobile platform by trained personnel only and every effort should be made that counting and clinical evaluation is done by the same evaluator for a given participant. The counting is to be done by lesion type and by counting area. The face will be separated into five areas: the forehead, the right cheek, the left cheek, the chin and the nose. Lesions under the jaw line or behind the hairline (including eyebrows) will not be included in the counts (Rizer 2001).The evaluator will be recorded in the eCRF.

NOTE: Papules, pustules, cysts and nodules are classified as inflammatory acne lesions while open and closed comedones are classified as non-inflammatory lesions.

12.2. Investigator Global Assessment

IGA will be performed at the times indicated in the Time and Events Table (Appendix A). The Investigator will assess improvement of acne based on the 5-point severity scale summarized below based on examination of the standardized photography digital images of the face.

Table 1 IGA Severity Scale

Grade	Description
0	Clear skin with no inflammatory or non-inflammatory lesions
1	Almost clear; rare non-inflammatory lesions with no more than one small inflammatory lesion

2	Mild severity; greater than Grade 1; some non-inflammatory lesions with no more than a few inflammatory lesions (papules/pustules only, no nodular lesions)
3	Moderate severity; greater than Grade 2; up to many non-inflammatory lesions and may have some inflammatory lesions, but no more than one small nodular lesion
4*	Severe; greater than Grade 3; up to many non-inflammatory and inflammatory lesions, but no more than a few nodular lesions

* Participants who worsen beyond Grade 4 should be described.

12.3. **Skindex-16**

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 (Appendix B). The questionnaire will be completed by the participant within the study mobile application as an electronic patient reported outcome (ePRO).

12.4. **Photography**

In order to participate in the trial, all participants will be asked to provide photos at Baseline, Weeks 2, 4, 8, 12 and 16. Digital photos will be taken using standardized procedures by study participants. Participants will use a guided facial photography module on an iPhone to take at least 5 standardized digital photos of their own face using validated standards. An online instructional video will demonstrate standard recommended photography techniques.

Each participant will take a total of 5 face images (full face, forehead, right side, left side, and chin). Investigators or their designee will confirm the photographic quality.

Photo collection is required for participation in the trial. The photographs will be used for research purposes only and will not be used for marketing or advertising purposes.

Photo collection and processing instructions will be available in a separate manual.

12.5. Study Completion

A completed participant is one who has completed all study visits. The Week 16 study visit is defined as the participant's last visit.

13. STATISTICAL CONSIDERATIONS

13.1. Sample Size

Primary efficacy power calculations assume a >25% difference between B244 treatment and vehicle and a 15% dropout. As per FDA statistical guidance for co-primary endpoints, each of the endpoints comprising the co-primary endpoint will be tested at an alpha level of $p<0.05$. In order to achieve 90% power with a 5% Type I error rate, a total of 372 participants is required to be enrolled in the trial.

13.2. Populations for Analysis

Safety Population: includes all randomized participants who receive one dose of investigational product (IP).

mITT Population: includes all randomized participants who receive one dose of investigational product (IP)

13.3. Data Analysis

The analyses will be conducted on all participant data when the trial ends. Details of the planned analysis will be presented in the trial Statistical Analysis Plan.

13.3.1. Demographic and Safety Data

Demographics and baseline characteristics will be listed and summarized descriptively by cohort and treatment. Participant disposition and incidence of AEs will also be summarized overall.

13.3.2. Safety Analyses

Safety analyses will be performed on data from all participants in the Safety Population. AEs, and other safety measures will be listed and summarized.

13.3.3. **Efficacy Analyses**

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 mITT Population.

IGA Success is defined as "Clear" (Grade 0) or "Almost clear" (Grade 1) at the pre-specified primary time points. For participants whose baseline score is Grade 2, the clinically meaningful criterion for IGA success is achieving a score of Grade 0 at the pre-specified primary time point because of limitations inherent to an ordinal scale.

Lesion counts will be enumerated as the number of inflammatory and non-inflammatory lesions separately.

Participant reported outcomes will be obtained through the Skindex-16 questionnaire.

Details of the data analysis will be defined in the statistical analysis plan.

14. **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.

14.1. **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).

14.2. **Definition of a SAE**

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

(a) results in death.

(b) is life-threatening.

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

(c) requires hospitalization or prolongation of existing hospitalization.

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

(d) results in disability/incapacity, or

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

(e) is a congenital anomaly/birth defect

(f) Medical or scientific judgment should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize

the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. Examples of such events are invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

14.3. Time Period, Frequency, and Method of Detecting AEs and SAEs

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

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

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

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

14.4. 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.

14.5. Evaluating AEs and SAEs

1.1.1. Severity Rating

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

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

An AE that is assessed as severe should not be confused with a SAE. An event is defined as 'serious' when it meets one of the pre-defined outcomes as described in Section 14.2 "Definition of a SAE".

1.1.2. 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.

1.1.3. Follow-Up of AEs and SAEs

After the initial AE/SAE report, the Investigator is required to proactively follow each participant and provide further information for clinically important AEs to the Sponsor's medical monitor or designee on the participant's condition within 24 hours as described in Section 14.7.

New or updated information will be recorded on the "SAE" Report Form. The updated SAE Report Form should be sent to Sponsor's medical monitor or designee within 24 hours as described in Section 14.7.

14.6. Pregnancy

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

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

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

14.7. Prompt Reporting of SAEs to the Sponsor

Any SAE occurring in a participant receiving treatment or any SAE that the Investigator becomes aware of post-treatment during the follow up period, must be reported by the Investigator to the medical monitor or designee **within 24 hours** even if the SAE does not appear to be drug-related. This should be done by telephone **and** by sending an email (high importance) of the Adverse Event form plus other related information. Additionally, it may be necessary for the Sponsor to directly communicate with the Investigator if additional information is required.

During both business and non-business hours, the telephone numbers and email addresses listed below should be used to notify the Sponsor

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

A Serious Adverse Event Report Form must be completed, scanned and forwarded via email to the medical monitor or designee at the number listed above within 24 hours of becoming aware of the event.

All additional follow-up evaluations must be reported to the medical monitor or designee. Such data should be sent to the Sponsor within 10 calendar days. All SAEs will be followed until the Investigator and Sponsor agree the event is satisfactorily resolved.

The Sponsor will be responsible for completing the safety report and for notifying the relevant authorities of any SAE as outlined in the International Conference on Harmonization (ICH) Guidelines and per local regulatory requirements. The Investigator will also ensure that the appropriate ethics committee is notified of the SAE.

15. ETHICAL AND REGULATORY STANDARDS

15.1. 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.

15.2. 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.

15.3. Informed Consent

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

A participant must give virtual electronic consent to take part in the 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).

15.4. Institutional Review Board/Independent Ethics Committee (IRB/EC)

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

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

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

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

16. ADMINISTRATIVE RULES

16.1. Curriculum Vitae

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

16.2. Study Documentation

The Investigator is required to prepare and maintain adequate and accurate case histories (i.e., source documents and/or Medical Record Supplement) designed to record all observations and other data pertinent to the study for each study participant. This includes accurate documentation of accountability of study medications. The medical records must contain adequate information to allow for verification of participant identity throughout the study.

eCRFs will be completed for each participant who is enrolled (randomized) in the study. A participant screening/enrollment log, noting reasons for screen failure where applicable, will be maintained for all participants who are consented.

All information recorded on the eCRFs for this study are considered to be the participant's source documentation. The source documents may include scanned copies of records from a hospital and/or the physician's chart, X-rays, or laboratory test documentation.

The original eCRFs for each participant may be periodically checked against the participant's source documents at the study site by the site monitor. Instances of missing or unclear data will be discussed with appropriate site personnel for resolution. A quality assurance audit will be performed on the eCRF database.

16.3. Archiving of Study Documentation

The Investigator shall retain records for two (2) years following the date a marketing application is approved for the indication pertaining to this clinical study; or, if the drug is planned to be terminated or if a Regulatory application is not planned to be progressed, until two (2) years after the investigation is discontinued and the Food and Drug Administration (FDA) or a competent regulatory authority is notified.

The Sponsor will inform the Investigator, in writing, as to when these documents no longer need to be maintained.

16.4. Monitoring and Quality Assurance

During the course of the study, a monitor will have access to all source documents, photographs, and eCRFs. The monitor will be able to remotely monitor the study at any time and review protocol compliance and CRFs, assess drug accountability and ensure that the study is being conducted according to pertinent regulatory requirements. The review of the participants' original medical records will be performed in a manner to ensure that participant confidentiality is maintained.

16.5. 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 suspicious safety signals during its routine blinded safety review. The details for the analysis plan will be documented in the trial's Statistical Analysis Plan.

17. 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.

18. 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.

19. 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.

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21. APPENDIX A - TIME AND EVENTS TABLES

Visit name (Day)	Screening (Day 0)	Baseline (Day 1)	Week 2 (Day 14)	Week 4 (Day 28)	Week 8 (Day 56)	Week 12 (Day 84)	Week 16 (Day 112)	Unscheduled Visit[3]
Visit Window, in Days	-28 to -8	-7 to 1	+/-3	+/-3	+/-3	+/-3	+/-3	
Informed Consent[1]	X							
Inclusion/Exclusion Criteria	X							
Demographics	X							
Medical History / Current Medical Conditions	X							X
21.1.1. Pregnancy Test[2]	X	(X)						
Microbiota swabs		X		X	X	X	X	X[2]
Skindex-16		X	X	X	X	X	X	X[2]
Investigational Product Administration		X	X	X	X	X		
Photography		X	X	X	X	X	X	X[2]
IGA		X	X	X	X	X	X	X[2]
Lesion Count		X	X	X	X	X	X	X[2]
Blood Pressure		X	X	X	X	X	X	X[2]
Concomitant Medications[4]	As required							
Adverse Events / Serious Adverse Events	As required							

1: Informed consent must be provided by all patients before any screening procedures are performed.
2: Only performed for women of childbearing potential, prior to first dose of Investigational Product.
3: If study participant has an unscheduled visit to end study participation, these study procedures will be conducted.

4: A thorough review of any concomitant medications (including medication name, dose, unit, frequency, and route) should be performed at every visit.