

A Phase IB Dose Escalating Study of the Safety, Short-Term Engraftment and Action of a Singly-Applied NB01 in Adults with Moderate Acne

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STATEMENT OF COMPLIANCE

The trial will be conducted in accordance with International Conference on Harmonization (ICH) Good Clinical Practice (GCP) and applicable United States (US) Code of Federal Regulations (CFR) for an initial Investigational New Drug (IND) study. The Principal Investigator (PI) will assure that no deviation from, or changes to the protocol will take place without prior agreement from the IND or Investigational Device Exemption sponsor, funding agency and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent forms (ICF), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the ICF must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the ICF will be IRB approved; a determination will be made regarding whether a new ICF needs to be obtained from participants who provided consent, using a previously approved ICF. Informed consent will be administered electronically (e-consent) in this study via a hand-held device.

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title:	A Phase IB Dose Escalating Study of the Safety, Short-Term Engraftment and Action of a Singly Applied NB01 in Adults with Moderate Acne
Study Description:	<p>Acne vulgaris is a multifactorial disease caused by overgrowth of <i>Propionibacterium acnes</i> (<i>P. acnes</i>), impaction of hair follicles, excessive sebum production and hormonal dysregulation. Recent literature from the Human Microbiome Project has shown there are unique microbial signatures specific to healthy and acne disease states (Fitz-Gibbon et al. 2013), (Tomida et al. 2013). Subsequent literature discovered a repressor in the porphyrin pathway in health-associated strains resulting in significantly lower porphyrin production (Johnson et al. 2016). Porphyrin is a known inflammatory mediator in acne pathogenesis (Kang et al. 2015). Porphyrin levels inversely correlate with therapeutic improvement in acne (Richter et al. 2016). Additional papers have highlighted other virulent factors such as the pIMPLE plasmid that may be associated with acne pathogenesis and were found to be more common in disease-associated strains (Kasimatis et al. 2013).</p> <p>From this data, we hypothesize that by eliminating resident disease-associated bacterial strains and replacing them with a health-associated <i>P. acnes</i> transplant, we may be able to improve, mitigate, treat clinical disease (acne) and prevent recurrences/flares of said disease. Instead of current approaches which focus on eliminating all bacteria from the skin, we aim to deliver healthy bacteria to restore the skin to a healthy state via this replacement therapy.</p> <p>We aim to test this in a Phase Ib single application study evaluating the safety, tolerability, and clinical impact that a single application of a <i>P. acnes</i> transplant, hereafter referred to as NB01, has on adult subjects with moderate acne. We want to profile the change in microbiome over the course of therapy to determine if exogenously delivered bacteria can populate the skin (engraftment) and cause a shift in the microbiome safely and subsequently impact acne</p>

	<p>biomarkers (porphyrin) that may correlate with clinical disease.</p> <p>Engraftment measurements would be more challenging to obtain in healthy individuals in which we would be trying to replace a healthy microbiome with a healthy microbiome. Our product is low risk, using bacteria native not only to human skin, but to the pilosebaceous units of the face, and <i>P. acnes</i> makes up 90% of the bacteria on the skin in both diseased and healthy individuals (Fitz-Gibbon et al. 2013), (Tomida et al. 2013).</p> <p>We intend for this therapy to eventually be used in acne subjects with ages ranging from 13-40, and all disease severities as either monotherapy for mild to mild/moderate acne and as an adjuvant therapy for moderate to severe acne at all body sites, with special attention to facial involvement.</p> <p>This approach is standard to acne therapy whereby mild disease will be treated with a monotherapy (i.e., 5.0% Benzoyl peroxide [BPO]) and moderate/severe disease will be treated with various combinatory regimens (topical antibiotics, 5.0% BPO, topical retinoids, oral antibiotics).</p>
Objectives:	<p>Primary Objectives:</p> <ol style="list-style-type: none"> 1. To determine the safety profile and tolerability of a singly applied NB01 2. To define engraftment of a singly applied topical NB01 3. Dose schedule determination based on engraftment <p>Secondary Objective: To evaluate clinical efficacy using Investigator Global Assessment (IGA) and acne lesion counts of a singly applied NB01 (FDA [Food and Drug Administration] Guidance for Industry: Acne Vulgaris, Developing Drugs for Treatment)</p> <p>Exploratory Objective: To evaluate biomarkers of treatment effects, including but not limited to porphyrin production, sebum production, and pH</p>
Study Population:	Approximately 10 male and female adult subjects combined with moderate, non-cyclical acne will be enrolled into the trial. Approximately five (5) subjects will be assigned to each dose schedule (either 10^5 CFU/cm ² or 10^6 CFU/cm ²).
Phase:	Ib
Description of Sites/Facilities Enrolling Participants:	This study will be conducted at 1 center in the state of California, within the US, under the direction of Howard L. Sofen, M.D.
Description of Study Intervention:	This is a single topical application study of a live bacteria for the study of acne in adult subjects.
Subject/Study Duration:	Subject participation in the trial will be approximately 5 weeks. Approximately 1 week pre-treatment with a daily 5.0% BPO gel 1 month of study drug: single application and 28-day follow up

1.2 SCHEDULE OF EVENTS (SOE)

Schedule of Events (SOE) - Single Application (Phase IB): NB01-P1BSA

STUDY PERIOD ►	Baseline	Treatment						End of Study
STUDY DAY (± visit window) ►	Day -5	0H (+2 days)	6H (±2 hrs)	24H (±2 hrs)	48H (±2 hrs)	7 days (±1 day)	Unscheduled Visit ⁿ	28 days ^o (±3 days)
PROCEDURE ▼ TREATMENT DAY ►	Screening	Day 1 ^b	Day 1	Day 2	Day 3	Day 7	NA	Day 28/EOS
Eligibility Criteria Review ^a	X							
Electronic Informed Consent Process	X							
Medical History	X							
Concomitant Medication Review	X	X	X	X	X	X	X	X
Directed PE ^c	X	X					X	
Cheek Swab Engraftment Sample (TaqMan® Assay)	X ^f	X	X	X	X	X	X	X
Vital Signs ^{d, e}	X			X		X	X	X
Laboratory Assessment - CBC ^d	X						X	
Pregnancy Test (urine dipstick) ^g	X	X					X	X
BPO 5.0% gel application	X ^h							
Electronic Patient Diary	X ⁱ	X	X	X	X	X	X	X
Enrollment	X							
AE Assessment	X ^k	X ^j	X	X	X	X	X	X
Assess Porphyrin Levels (Pear 3D)	X	X	X	X	X	X	X	X
Sebum Production Measurement (Pear 3D)	X	X	X	X	X	X	X	X

Schedule of Events (SOE) - Single Application (Phase IB): NB01-P1BSA (concluded)

Skin pH measurement (Skin pH Meter)	X	X	X	X	X	X		X
Assess Follicular Engraftment via Bioré® Strip (TaqMan® Assay)	X			X				
Investigator Global Assessment (IGA)	X	X	X	X	X	X	X	X
Absolute Lesion Count	X	X	X	X	X	X	X	X
Photography (Pear 3D)	X	X	X	X	X	X	X	X
Facial Cetaphil® Washing	X ^{p,q}	X ^{l,m}	X	X	X	X	X	X
Investigator Assessment of Tolerability	X	X	X	X	X	X	X	X
Single NB01 application (by Investigator)		X						

Abbreviations: AE = Adverse Event; BID = Twice Daily; BPO = Benzoyl Peroxide; CBC = Complete Blood Counts; EOS = End of Study; PE = Physical Examination.

^a Includes confirmation of contraception use for the duration of Screening prior to first application and through study participation; Principal Investigator (PI) should assess subject eligibility after e-consent and prior to remaining screening period procedures.

^b All Day 1 baseline procedures must be completed prior to the PI application of NB01 at Time Point 0 (T0H).

^c Limited PE of face, chest, arms, back (relevant acne regions).

^d Vital signs and CBC assessed at Screening Visit, prior to initiation of BPO pre-treatment. Vital signs and CBC are only repeated if clinically warranted.

^e Blood pressure, heart rate, temperature.

^f Screening sample to be obtained prior to initiation of BPO pre-treatment.

^g Urine dipstick test, assessed locally.

^h BPO gel should be applied for at least 5 days, in the AM immediately after the AM Cetaphil® wash. Final BPO pre-treatment should be completed at least 24 hours prior to PI application of NB01, thus final BPO application will most likely occur in the AM prior to the Day 1 (0H) visit.

ⁱ Trial subjects will note date and time of each BPO pre-treatment and Cetaphil® wash for the Screening period, and the date and time for each Cetaphil® wash during the 28-day treatment period.

^j Subjects will remain at the clinic for at least 30 minutes post application at T0H to be assessed for adverse reactions.

^k AEs will be assessed from the time of e-consent.

^l On Day 1, after baseline measurements have been obtained, subjects will then wash at the clinic before NB01 is applied by the PI.

^m Subjects initiate BID washing of their faces after waiting 24 hours from T0H, or 24H (Study Day 2). Subjects should withhold morning wash and morning facial shaving at home until they arrive at the clinic all clinic days. Once clinic day sampling has occurred, the subject may wash at the clinic. The only exception being that the subject should wash their face in the office with Cetaphil®, prior to the Day 2 Bioré® Strip follicular engraftment sampling

ⁿ Study procedures are completed as needed, as clinically indicated, for unscheduled study visits.

^o The EOS Visit should be completed on Day 28, or prior to the initiation of new anti-acne therapy. Should the subject discontinue the study early, the EOS should be scheduled and completed as soon as possible.

^p No other cleansers can be used throughout the study, from the Screening Visit to Day 28/EOS.

^q Subjects will hold the PM Cetaphil® wash prior to the Day 1 study drug application.

2 INTRODUCTION

2.1 STUDY RATIONALE

Acne vulgaris is a multifactorial disease affecting 85% of the global population over a lifetime and 40-50 million US people in the US currently. It impacts individuals over a large timespan ranging from 13-40 years, and has a large psychosocial impact on an individual's self-esteem. The majority of those afflicted (50%-75%) are now resistant to conventional antibiotic therapy (Walsh et al. 2016). Acne is caused by overgrowth of *P. acnes*, impaction of hair follicles, excessive sebum production and hormonal dysregulation. Current standard of care includes treatment with 5.0% BPO, retinoids, and antibiotics. However, there are significant side effects of the above medications, limiting subject compliance and leading to suboptimal long-term efficacy. The heavy dependence on long treatment courses of antibiotics combined with antibiotic resistance rising upwards of 50%-75%, is resulting in treatment failures and contributing to overall global antibiotic resistance. There has been little innovation in acne therapy in the past several decades and a significant current need to develop antibiotic alternatives.

Current therapeutic options include:

- Topical antibiotics-erythromycin/clindamycin-high resistance
- Oral Antibiotics-tetracycline (doxycycline, minocycline)-high resistance
- Topical 5.0% BPO products-irritating, drying, redness
- Topical retinoids-irritating, drying, photosensitivity, peeling,
- Oral Retinoids-high side effect profile, Ipledge system, cumbersome for subjects
- Topical acids-salicylic acid, glycolic acid
- Peels-subject out of pocket expense, not reimbursable
- Laser-Blue and red-light therapies-subject out of pocket expense, not reimbursable
- Dapsone (Aczone®)-minimal additional benefit over existing topical regimens
- Azelaic acid (Finacea®)-minimal additional benefit over existing topical regimens
- Microdermabrasion-subject out of pocket expense, not reimbursable

Recent literature from the Human Microbiome Project has shown there are unique microbial signatures specific to healthy and acne disease states (Fitz-Gibbon et al. 2013), (Tomida et al. 2013). Subsequent literature discovered a repressor in the porphyrin pathway in health-associated strains resulting in significantly lower porphyrin production (Johnson et al. 2016). Porphyrin is a known inflammatory mediator in acne pathogenesis (Kang et al. 2015). Porphyrin levels inversely correlate with therapeutic improvement in acne. (Richter et al. 2016). Additional papers have highlighted other virulent factors such as the pIMPLE plasmid that may be associated with acne pathogenesis and were found to be more common in disease-associated strains (Kasimatis et al. 2013).

From this data, we hypothesize that by eliminating resident disease-associated bacterial strains and replacing them with a health-associated *P. acnes* transplant, we may be able to improve, mitigate, treat, or prevent acne flares. We aim to test this in an initial Phase I evaluating the safety, tolerability, and clinical impact that a single dose application of NB01 has on adult subjects with moderate acne.

This initial study will be a Phase Ib single center open label dose escalation study using a single dose application with monitoring over 28 days on 10 subjects at 2 different concentrations (lower bound and upper bound). If we meet the safety criteria of this initial study, we plan to expand the IND research to a Phase Ib multi-dosed study. All subjects will be pre-treated with a daily 5.0% BPO gel (<https://www.perrigo.com/business/product.aspx?ID=88&let=B> Perrigo 5% BP Aqueous Gel) to decrease their resident microbiome bacterial load in preparation for transplantation and successful repopulation with a healthy microbiome.

2.2 BACKGROUND

We hypothesize that by eliminating resident disease-associated bacterial strains and replacing them with a health-associated *P. acnes* transplant, we may be able to improve, mitigate, treat, or prevent acne flares. We aim to test this in an initial Phase I evaluating the safety, tolerability, and clinical impact that a single dose application of NB01 has on adult subjects with moderate acne.

Our genotypic and phenotypic work identified candidate strains sourced from healthy subjects that match all our criteria for health. Genotypically, our candidate strain is a *P. acnes* ribotype 2, deoR+, lipase type II+, contains a complete CRISPR system, is absent the pIMPLE plasmid, and has no identifiable genomic evidence of antibiotic resistance. Phenotypically, our candidate strain produces low levels of porphyrin, comparable to other healthy associated ribotype 2's in the literature and was sensitive to all 5 antibiotics tested internally (Clindamycin, Doxycycline, Erythromycin, Minocycline, Tetracycline as was measured with Etest (bioMérieux).

Additional safety assessment report was performed by CHR-Hansen, Naked Biome's GMP manufacturer, who confirmed that no genomic antibiotic resistance genes were present and that our strain was sensitive to 13 out of 14 tested antibiotics, resistant only to metronidazole which *P. acnes* has known natural resistance to, and was additionally non-hemolytic and non-cytotoxic.

There is no *in vivo* data to date on the safety or clinical efficacy of a topical live *P. acnes* microbiome transplant. As described in our Pre-IND meeting, there are no well substantiated animal models for inflammatory acne and we are working with human-specific bacteria. Therefore *in vivo* animal clinical and toxicology studies will not yield clinically relevant results that would be applicable for human use. Sponsor rationale, in accordance with FDA correspondence, is that animal clinical and toxicology studies are not relevant or useful when we are using human-specific bacteria absent well-substantiated animal models for inflammatory acne for pre-human efficacy testing (Mirshahpanah and Maibach 2007). The best, most relevant, and only useful model to test our lead drug candidate is first in humans (Mirshahpanah P, Howard Maibach 2007), (Achermann et al. 2014). Additional literature on previous human studies for probiotics and acne are listed in the following references (Baquerizo et al. 2014), (Wei et al. 2016), (Thiboutot et al. 2009).

Outside of known association of *P. acnes* with acne vulgaris, below is an accrued list of rare opportunistic infections associated with *P. acnes*. *P. acnes* has been observed in other infections such as implant-associated infections, soft tissue, periodontal, periocular, cardiovascular system, deep-organ tissues infections, and may also have a role in relation to pulmonary infection as the bacteria can be isolated from lungs and lymph nodes. Routes of infection is often from the subject own commensal flora (normal skin or oral tract) (Whitman et al. 2012), (Kwon and Suh 2016). The expanded multi-locus sequence typing scheme based on 7 housekeeping genes and 2 putative virulence genes developed by McDowell et al gives a good resolution and revealed CC types which seem to have an increased capacity to cause infections and others that are rarer or found mainly among healthy individuals. (McDowell et al. 2012), (McDowell et al. 2013).

Strain HP4G1 contains the deoR repressor gene, and thus may not be associated with acne. Further, the strain has a PAp60/PAmce allele combination found in non-invasive *P. acnes* strains and lacks 2 of 4 genes involved in iron uptake, which is of importance for growth in the human body at conditions with low iron access. Notably, regarding CAMP factors 1-4, which are expected to be involved in pathogenicity and are involved in co-hemolysin with *Staphylococcus aureus* (*S. aureus*), HP4G1 had a mutation in the CAMP 2 gene leading to an extension of the gene as the stop codon was interrupted. This variant has not been observed before and as CAMP factor 2 is described as having properties for co-hemolysin and exotoxin, this may lower the pathogenic potential of the strain. Moreover, the strains belong to the CC72 clonal complex which, compared to CC5, contains strains less commonly isolated from healthy humans or individuals with infections. HP4G1 was found to be a ST100-like type not previously reported and most likely has a non-functional CAMP2 gene due to a mutation in the stop

codon. Therefore, HP4G1 is most likely less virulent than *P. acnes* in general with a low risk of causing infections.

Porphyrin is a known inflammatory metabolite involved in the pathogenesis of acne. Porphyrin is produced by the *P.acnes* bacterium and is a photosensitizing agent. Dermatologists routinely use blue and red light therapies to selectively target *P.acnes*-produced coproporphyrin III and protoporphyrin IX, respectively, thereby eliminating *P.acnes* and improving disease. As stated above, HP4G1 has a repressor of porphyrin production, called *deoR*. *In vivo*, the HP4G1 strain produces less porphyrin than acne-associated strains that lack the *deoR* repressor operon. Despite that the HP4G1 strain is a low porphyrin producer, the fact that it is applied in significant quantities (10^5 CFU/cm²) and (10^6 CFU/cm²), could lead to a temporary increase in porphyrin upon application. This hypothetical increase could be photosensitizing to subjects, as such, photosensitivity has been included as a theoretical side effect of HP4G1 application.

We are addressing the risk of these opportunistic infections through the selection of genotypic and phenotypic characteristics most associated with health and with the implementation of appropriate exclusion criteria to eliminate subjects that may have increased risk of a *P. acnes* infection.

Exclusion Criteria addressing above

1. Active bacterial, viral, or fungal skin infections.
2. Any noticeable breaks or cracks in the skin on the face, including severely excoriated skin or skin with open or weeping wounds suggestive of an active infection or increased susceptibility to infection.
3. Comorbid skin conditions in the area of application.
4. Active periodontal disease or ongoing procedures (e.g., gum grafting).
5. History/current ocular infections/surgeries within 6 months of enrollment, with the exception of any history of cataracts.
6. History of sarcoidosis.
7. History septic joints/endocarditis.
8. Participants with Netherton's syndrome or other genodermatoses that result in a defective epidermal barrier.
9. Sensitivity to or difficulty tolerating glycerin, polyethylene glycol.
10. History of isotretinoin use, with the exception of sub-therapeutic treatment within 8 weeks of enrollment.
11. Less than 80% compliance with pre-treatment BPO, or less than 5 days' worth of BPO pre-treatment (whichever is greater) during the Screening period.
12. Current major systemic comorbid conditions.
13. Currently participating in (or within 8 weeks of enrollment) in another acne trial or other investigational drug.
14. Participants with prosthetic heart valves, pacemakers, intravascular catheters, or other foreign or prosthetic devices/implantable devices/hardware.
15. Participants with close contact (e.g., spouses, children, or members in the same household) with prosthetic heart valves, pacemakers, intravascular catheters, or other foreign or prosthetic devices/implantable devices/hardware.
16. Known chronic human immunodeficiency virus (HIV), hepatitis C virus (HCV), or hepatitis B virus (HBV) infections.
17. History of malignancy (with the exception of non-melanoma skin cancer).
18. Immunosuppression (such as resulting from transplantation, immunosuppressive therapy, active HIV infection/acquired immune deficiency syndrome [AIDS], neutropenia).
19. Major surgical procedure, open biopsy, or significant traumatic injury within 14 days of initiating study drug (unless the wound has healed), or anticipation of the need for major surgery during the study.

20. The presence of a medical or psychiatric condition, history of drug or alcohol abuse that, in the opinion of the PI, makes the subject inappropriate for study inclusion.
21. Participants with close contacts (e.g., spouses, children, or members in the same household) that have severe barrier defects or are immunocompromised.
22. Inability or unwillingness of participant to comply with study protocol procedures.
23. Pregnant or lactating females, or females who desire to become pregnant and/or breast feed within the duration of study participation.
24. Imprisonment or under legal guardianship.

As was discussed at our Pre-IND meeting, *P. acnes* is most specific to facial pilosebaceous region and is the natural niche for *P. acnes*, and therefore the best region for application to appropriate the sensitivity and therapeutic impact of a topical *P. acnes* transplant (McGinley et al. 1980).

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
<ul style="list-style-type: none"> Determine the safety and tolerability of a singly applied NB01 Define engraftment of a singly applied topical NB01 Dose schedule determination based on engraftment 	<p>Safety Assessments:</p> <ul style="list-style-type: none"> Skin Dryness Skin Peeling Irritant/Contact dermatitis Hyperpigmentation Ocular Toxicity Mucosal Toxicity Skin erythema Assessment of Disease Status <p>Efficacy Assessments: Engraftment is defined as deoR/PanBac>40% and Cas5/PanBac>40%. Disease associated bacteria are typically deoR, Cas5 negative (Kang et al, 2016), therefore, ratios are expected to be low <10% in acne subjects. The NB01 strain as measured with our TaqMan assays is approximately 100% deoR/PanBac and Cas5/PanBac, so we expect the ratio to increase with successful engraftment. This will help to determine dosing regimen/schedule for multi-dosed trials where the drug will be applied in a frequency to ensure that product levels remain above these parameters. For example, if deoR/PanBac drops to <40% at or before 6 hours then the application will be twice daily (BID); every (Q) 12 hours.</p>	Disease exacerbation along with erythema, dryness, pigmentation irritant/contact dermatitis, mucosal and ocular toxicity are commonly tested safety endpoints in topical dermatologic trials.
Secondary		
To evaluate clinical efficacy using IGA and acne lesion counts of a singly applied NB01	IGA and acne lesion count	IGA and acne lesion counts are conventional measurements for assessing efficacy in acne trials
Tertiary/Exploratory		
To evaluate biomarkers of	<ul style="list-style-type: none"> Porphyryn and sebum levels 	Porphyryn is a known

<p>treatment effects, including but not limited to porphyrin production, sebum production, and pH</p>	<p>as measured using a Pear 3D Analysis System</p> <ul style="list-style-type: none"> pH as measured using pH meter 	<p>inflammatory metabolite in acne pathogenesis and levels have been shown to inversely correspond to therapy (Johnson et al. 2016). Our strains have a repressor for porphyrin production and if our strains engraft, we hypothesize that they will maintain low levels of porphyrin on the face compared to baseline screening levels.</p> <p>Sebum is “food” for <i>P. acnes</i> and we expect high sebum producers to have higher levels of resident bacteria (McGinely et al. 1980).</p> <p>Low pH is more associated with acne and conversion to anaerobic metabolism and porphyrin production (Shu et al, 2013), (Dailey et al 2015), (Kjeldstad et al. 1984).</p>
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4 STUDY DESIGN

4.1 OVERALL DESIGN

This is a first human, single-center, dose escalation, open-label trial of a single application of topically delivered NB01 in subjects with moderate, non-cyclical facial acne.

Ten subjects will be assigned to 1 of 2 dose cohorts in a stepwise fashion after a 5-day pre-treatment with 5.0% daily BPO gel (<https://www.perrigo.com/business/product.aspx?ID=88&let=B> Perrigo 5% BP Aqueous Gel).

- Cohort 1: 10⁵ CFU/cm² NB01
- Cohort 2: 10⁶ CFU/cm² NB01

This will be ascending dose finding whereby 5 initial subjects will be assigned to Cohort 1 to receive a single application of 10^5 CFU/cm² to the facial skin of the nose/cheek. If there are less than 2 dose-limiting toxicities (DLTs) during the 7-day safety assessment, a Dose Escalation Meeting will be held between the PI and the Sponsor Medical Monitor to determine whether Cohort 2 will be opened to enrollment.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

Acne vulgaris is a multifactorial disease caused by overgrowth of *P. acnes*, impaction of hair follicles, excessive sebum production and hormonal dysregulation. Recent literature from the Human Microbiome Project has shown there are unique *P. acnes* microbial signatures specific to healthy and acne disease states (Fitz-Gibbon et al. 2013), (Tomida et al. 2013). Subsequent literature discovered a repressor in the porphyrin pathway in health-associated strains resulting in significantly lower porphyrin production (Johnson et al. 2016). Porphyrin is a known inflammatory mediator in acne pathogenesis (Kang et al. 2015). Porphyrin levels inversely correlate with therapeutic improvement in acne (Richter et al. 2016). Additional papers have highlighted other virulent factors such as the pIMPLE plasmid that may be associated with acne pathogenesis and were found to be more common in disease-associated strains (Kasimatis et al. 2013).

From this data, we hypothesize that by eliminating resident disease-associated bacterial strains and replacing them with a health-associated *P. acnes* transplant, we may be able to improve, mitigate, treat clinical disease (acne) and prevent recurrences/flare-ups of said disease. Instead of current approaches which focus on eliminating all bacteria from the skin, we aim to deliver healthy bacteria to restore the skin to a healthy state via this replacement therapy.

We aim to test this in a Phase Ib single application study evaluating the safety, tolerability, and clinical impact that a single application of NB01 has on adult subjects with moderate acne. We want to profile the change in microbiome over the course of therapy to determine if exogenously delivered bacteria can populate the skin (engraftment) and cause a shift in the microbiome safely and subsequently impact acne biomarkers (porphyrin) that may correlate with clinical disease. Health-associated *P. acnes* contain both deoR and Cas5, thus the ratios of deoR/PanBac and Cas5/PanBac is approximately 1 in our formulation. Disease associated *P. acnes* typically lack deoR and Cas 5 (Kang et al, 2016) leading to a deoR/PanBac <10% on acne individuals. We expect an upwards shift in deoR/PanBac and Cas5/Panbac with the application of our strains to acne subjects. Thus, engraftment is defined as deoR/PanBac>40% and Cas5/PanBac>40%. This will help to determine dosing regimen/schedule for multi-dosed trials where the drug will be applied in a frequency to ensure that product levels remain above these parameters. For example, if deoR/PanBac drops to <40% at or before 6 hours then the application will be BID (Q12 hours). Disease-associated strains, in contrast, typically lack deoR and Cas4, but contain transposase2 (TPase2). Subjects will also be profiled with TPase2/PanBac ratios to determine how the pathogenic strain profiles are impacted over time with application of drug, and if there are certain profiles that increase or decrease likelihood of successful engraftment.

Engraftment measurements would be more challenging to obtain in healthy individuals in which we would be trying to replace a healthy microbiome with a healthy microbiome. Our product is low risk, using bacteria native not only to human skin, but to the pilosebaceous units of the face, and *P. acnes* makes up 90% of the bacteria on the skin in both diseased and healthy individuals (Fitz-Gibbon et al. 2013).

We intend for this therapy to eventually be used in all ages of acne subjects ranging from 13-40, and all disease severities as either monotherapy for mild to mild/moderate acne and as an adjuvant therapy for moderate to severe acne at all body sites, with special attention to facial involvement.

This approach is standard to acne therapy whereby mild disease will be treated with a monotherapy (i.e., 5.0% BPO) and moderate/severe disease will be treated with various combinatory regimens (topical

antibiotics, BPO, topical retinoids, oral antibiotics).

There is no precedent for topical *P. acnes* microbiome transplants in acne, but there is an analogous precedent of using autologous transplantations of coagulase-negative Staphylococcus (CoNS) species (including Staphylococcus epidermidis and Staphylococcus hominis) on 5 eczema subjects to determine their impact on colonization by *S. aureus*. In this study, CoNS species were isolated from eczema/atopic dermatitis subjects and tested for anti-staphylococcal activity against *S. aureus*. Those isolates that had antimicrobial activity against *S. aureus* were mixed in Cetaphil® (Galderma) lotion and reintroduced onto the subjects in a single application to 1 forearm at a concentration of 10^5 CFU/cm² with placebo (vehicle) applied to the contralateral forearm in a double-blinded fashion. *S. aureus* colonization was then measured 24 hours post application (Nakatsuji et al. 2017).

4.3 JUSTIFICATION FOR DOSE

P. acnes is normal, commensal bacteria on the facial skin and within the facial pilosebaceous units. *P. acnes* species make up around 90% of the bacterial species on the face, irrespective of health or disease states (Fitz-Gibbon et al. 2013), (Tomida et al. 2013). In administering our live microbiome transplant topically to the face, we are delivering our health-associated *P. acnes* strains directly into their native niche, enabling optimal engraftment and thereby any beneficial effect of restoring a microbiome population to a healthy profile.

Dose Justification:

- Lower-bound dose of 10^5 CFU/cm² determined using the average within the range of normal bacterial concentration for facial skin (10^4 - 10^7 CFU/cm²) (Kishishita et al. 1980), (Akaza et al. 2016), (Nakatsuji et al. 2017) (http://www.textbookofbacteriology.net/normalflora_3.html)
- Upper-bound dose of 10^6 CFU/cm² determined by using the upper limit of normal bacterial concentration for facial skin.

Further rationale to the study design of performing topical microbiome transplants with our dose can be extracted from the methods in (Nakatsuji et al. 2017) which studied autologous microbiome transplants of 10^5 CFU/cm² CoNS in atopic dermatitis subjects.

4.4 END OF STUDY DEFINITION

A participant is considered to have completed the study if he or she has completed all phases of the study including procedures for last visit, End of Study (EOS). See protocol section 8.1 for discontinuation criteria and visit procedure details.

5 STUDY POPULATION

Approximately 10 adult male and female subjects with moderate-noncyclical acne will be recruited for this study.

5.1 INCLUSION CRITERIA

Subjects must meet all the following inclusion criteria to be eligible for participation in the trial:

1. Ability to provide written informed e-consent.
2. Males and Females ages 18-40.
3. Acne severity: Moderate (Grade 3 on 5-point IGA scale and Moderate on an acne lesion count scale (Appendix B [Section 12.2], IGA and Lesion Count Acne Grading).
4. Acne treatment-free period (including topical or oral antibiotics, retinoids, laser therapy, topical dapsone, topical azelaic acid, facial peels, dermabrasion, sulfacetamide sulfur, and salicylic acid), of at least 3 weeks prior to e-consent (with the exception of BPO pre-treatment under this protocol).

5. Lesion count: A minimum of at least a total of 15 inflammatory lesions (papules plus pustules), with a minimum of 10 inflammatory lesions within the designated application area (cheek/nose).
6. Females with non-cyclical acne.
7. Females of childbearing potential willing to use adequate contraception (e.g., total abstinence, intrauterine device (IUD), barrier method with spermicide, surgical sterilization or surgically sterilized partner, Depo-Provera®, Norplant®, or NuvaRing® for the duration of the Screening Period and during study participation. All oral contraceptive and hormonal implants will need to have been initiated and on a stable dose for at least 3 months prior to the screening period. 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.
8. Male participants willing to use an acceptable method of contraception (e.g., total abstinence, barrier methods with spermicide, surgical sterilization or surgically sterilized partner) during study participation.

5.2 EXCLUSION CRITERIA

Subjects who meet any of the following exclusion criteria are not to be enrolled in this trial:

1. Active bacterial, viral, or fungal skin infections.
2. Any noticeable breaks or cracks in the skin on the face, including severely excoriated skin or skin with open or weeping wounds suggestive of an active infection or increased susceptibility to infection.
3. Comorbid skin conditions in the area of application.
4. Active periodontal disease or ongoing procedures (e.g., gum grafting).
5. History/current ocular infections/surgeries within 6 months of enrollment, with the exception of any history of cataracts.
6. History of sarcoidosis.
7. History septic joints/endocarditis.
8. Participants with Netherton's syndrome or other genodermatoses that result in a defective epidermal barrier.
9. Sensitivity to or difficulty tolerating glycerin, polyethylene glycol.
10. History of isotretinoin use, with the exception of sub-therapeutic treatment within 8 weeks of enrollment.
11. Less than 80% compliance with BPO, or less than 5 days' worth of BPO pre-treatment (whichever is greater) during the Screening period.
12. Current major systemic comorbid conditions.
13. Currently participating in (or within 8 weeks of enrollment) another acne trial or other investigational drug.
14. Participants with prosthetic heart valves, pacemakers, intravascular catheters, or other foreign or prosthetic devices/implantable devices/hardware.
15. Participants with close contact (e.g., spouses, children, or members in the same household) with prosthetic heart valves, pacemakers, intravascular catheters, or other foreign or prosthetic devices/implantable devices/hardware.
16. Known chronic human immunodeficiency virus (HIV), hepatitis C virus (HCV), or hepatitis B virus (HBV) infections.
17. History of malignancy (with the exception of non-melanoma skin cancer).
18. Immunosuppression (such as resulting from transplantation, immunosuppressive therapy, active HIV infection/acquired immune deficiency syndrome [AIDS], neutropenia).
19. Major surgical procedure, open biopsy, or significant traumatic injury within 14 days of initiating study drug (unless the wound has healed), or anticipation of the need for major surgery during the study.

20. The presence of a medical or psychiatric condition, history of drug or alcohol abuse that, in the opinion of the PI, makes the subject inappropriate for study inclusion.
21. Participants with close contacts (e.g., spouses, children, or members in the same household) that have severe barrier defects or are immunocompromised.
22. Inability or unwillingness of participant to comply with study protocol procedures.
23. Pregnant or lactating females, or females who desire to become pregnant and/or breast feed within the duration of study participation.
24. Imprisonment or under legal guardianship.

5.3 LIFESTYLE CONSIDERATIONS

Females of childbearing potential need to be willing to use adequate contraception (e.g., total abstinence, IUD, barrier method with spermicide, surgical sterilization or surgically sterilized partner, Depo-Provera®, Norplant®, or NuvaRing® for duration of the Screening period and during study participation. All oral contraceptive and hormonal implants will need to have been initiated and on a stable dose for at least 3 months prior to the screening period. Females must submit to a pregnancy test to ascertain that they are not pregnant before enrolling in the study.

Male participants must be willing to use an acceptable method of contraception (e.g., total abstinence, barrier methods with spermicide, surgical sterilization or surgically sterilized partner) during study participation.

5.4 SCREEN FAILURES

Screening failures are defined as participants who consent to participate in the clinical trial but are not subsequently enrolled to the study intervention or entered in the study within the Screening period. A minimal set of screen failure information is required to be collected to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any AEs.

5.5 ENROLLMENT PROCEDURES AND TREATMENT ASSIGNMENT

The screening date will be defined as the date the subject signs the ICF. Subjects will be screened within 5 days (+2 days) of enrollment to determine eligibility for participation in the study. Screening laboratory results for hematology (Complete Blood Counts [CBC]) should be obtained from the local laboratory. Screening procedures (Section 8.1) will be performed and documented, at the end of which the clinical site will complete subject and visit information on the Enrollment Request Form (ERF) for Sponsor approval. Approved subjects who meet all inclusion criteria and none of the exclusion criteria may be enrolled into the trial.

It is the responsibility of the PI to ensure that subjects are eligible for the study prior to enrolling into the study. Subjects who do not enroll within the Screening Period will be screen failed. A subject will be considered enrolled once he or she has signed the ICF, met all inclusion/exclusion criteria, executed all screening visit measurements, including daily BPO therapy, and received Sponsor approval to participate.

This is an open-label study. Subjects will be assigned a unique Screening Number at the time of e-consent. Subjects who have met all eligibility criteria will initiate an at-home daily application of a 5.0% BPO gel treatment, and BID Cetaphil® wash and complete an electronic, in-home patient diary (e-diary). They will return to the clinic on Day 1 to perform baseline assessments. Day 1 is defined as the date the first application of NB01 will occur. All baseline tests and procedures must be completed prior to the application of the first dose of study drug on Day 1, time point 0H. Once eligibility is confirmed, subjects will be assigned a unique Subject ID number and be assigned to a cohort. Once a Subject ID has been assigned to a subject, it will not be reassigned to another subject.

Each on-study visit will be scheduled relative to Day 1 0H. Enrolled subjects will complete an in-home e-diary to capture at home Cetaphil® washing through the end of the study. Visits will follow the SOE table and protocol section 8.1. The ERF and additional details can be located in the Study Reference Manual for the trial.

Re-Screening of Subjects

Subjects who screen fail may be considered for enrollment at a later date, but must re-sign the ICF and repeat screening procedures, if any screening procedures will be performed outside of the Screening period from the time of the first informed e-consent.

Replacement of Subjects

If a subject is discontinued from the study for any reason other than a dose-limiting toxicity (DLT) prior to completion of the DLT assessment window, a replacement subject will be enrolled at the same dose level as the discontinued subject. To be evaluable for the DLT observation, a subject must receive a dose of NB01 and either completes all safety procedures through Day 7 or experiences a DLT prior to Day 7. It is possible over-enrollment of subjects may occur in order to have 10 safety-evaluable subjects.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 DOSING AND ADMINISTRATION

There will be a single application of NB01, at 1 of 2 dose cohorts, administered to approximately 10 subjects:

Once a subject has completed BPO pre-treatment (with an in-home patient e-diary) and been enrolled into the trial, the subject will provide a post-BPO sample immediately prior to receiving their application of NB01 (hereafter referred to as “study drug”). The PI will apply a single dose of NB01 at one of two strengths to each subject at time point 0 hours (0H):

- Cohort 1: Initially, 5 subjects will be enrolled to receive a single topical application of NB01 at 10^5 CFU/cm² to 50 cm² of facial skin covering the nose and cheek. The lower-bound dose determined using the average range of normal bacterial concentration for facial skin (10^5 CFU/cm²).
- Cohort 2: If there are less than 2 DLTs in Cohort 1, then 5 subjects will have a single topical application of NB01 at 10^6 CFU/cm² to 50 cm² of facial skin covering the nose and cheek. The upper-bound dose was determined by upper limit of normal bacterial concentration for facial skin (10^6 CFU/cm²).

Investigators applying the drug shall wash their hands and wear non-powdered nitrile gloves for application of study drug material. Subjects will wash their face with Cetaphil® right after the time point 0H engraftment sample procurement and just before the study drug application. Subjects will document their BID Cetaphil® washing for the remainder of the study.

See Study Assessments and Procedures, for details on pre-and post-visit requirements during the treatment period.

See the Pharmacy Manual for additional study drug handling and management details for this study.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

NB01 will be supplied frozen to the PI by Naked Biome in single aliquot dosages delivered as a topical solution in a vacuum-sealed cotton applicator pad.

Naked Biome recommends that used study drug supplies be destroyed at sites if they have applicable standard operating procedure (SOP) to do so. If the site has an appropriate SOP for drug destruction as determined by Naked Biome, the site may destroy used (used, partially used or empty) study drug supplies in accordance with that site's approved SOP. A copy of the site's approved SOP will be obtained for central files. If the site does not have an acceptable SOP to destroy, or cannot due to other regulatory reasons, Naked Biome will provide instruction for the return if the used study drug for disposal/destruction. Naked Biome may request return of unused study drug for additional, long-term stability testing.

Investigators will complete product accountability for the over-the-counter products provided to trial subjects (e.g. BPO treatment, Cetaphil® wash). For further details, please see the Pharmacy Manual for this study.

6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

The clinical trial formulation comes as a clear solution dispensed using vacuumed cotton pads.

Formulation:

- Live culture of a single strain of *P. acnes*
- Purified water
- Glycerol
- Phosphate buffered saline

All excipients are generally regarded as safe (GRAS) and present in concentrations found in the FDA's Inactive Ingredient Guide <https://www.fda.gov/drugs/informationondrugs/ucm113978.htm>

Formulary breakdown for a 100 ml 25% Glycerol 1X PBS solution:

- Live culture of a single strain of *P. acnes*
- 65 ml Purified water
- 25 ml Glycerol
- 10 ml of 10X Phosphate buffered saline

6.2.3 PRODUCT STORAGE AND STABILITY

Study drug will be stored by the PI in a -80 degrees non-cycling lab freezer before application to study subjects at Day 1. A range of ± 10 degree Celsius is acceptable for storage. Please contact the sponsor in the event of temperature departure from that range. Product must be used within 15 to 30 minutes of removal from lab freezer storage, but is stable for up to 4 hours without significant drop in viability. If for some reason application is not able to occur at the planned visit within 30 minutes, the site will be instructed to discard the product according to the site's sponsor-approved standard operating procedures. Please see the Pharmacy Manual for this study for additional detail.

6.2.4 PREPARATION

Study Drug Application:

Study drug should be removed from freezer 15 minutes prior to application and allowed to thaw at room temperature.

Subjects' dose will be PI-administered at 0H study visit after samples have been procured for engraftment assay. Administers of study drug should wash their hands and wear non-powdered nitrile gloves for application. Subjects will wash their face with Cetaphil® right before in-office application, on Day 1.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

This is an open-labeled, sequential, single-ascending dose study. No randomization or blinding

procedures will be performed.

6.3.1 CONCOMITANT THERAPY

Subjects are excluded from the trial as stated above, if taking any other acne medication. BPO use is only allowed once subjects have been consented to the study and only as part of the Screening period. Subjects are to wait until the end of the study treatment period before initiating any other therapy or investigational agent for the treatment of acne. If, for any reason, the subject must be discontinued from the protocol before the Day 28 visit, the Investigator will make every effort to close out the subject's participation with a final EOS visit before any other acne therapy is initiated.

Use of Other Moisturizers, Sunscreens and Makeup

The use of moisturizers, sunscreens (including moisturizers containing sunscreens) or face makeup is prohibited on the day prior to Day 1 (the last day in the Screening Period) through completion of the Day 3 Visit. Trial participants may continue on, or introduce new moisturizers, sunscreens, or face makeup, after completion of the Day 3 Visit, as long as they do not meet the prohibited medication criteria listed in "Inclusion or Exclusion Criteria" or in protocol section 6.3.1, "Concomitant Therapy" above.

Use of Other Cleansers

The use of cleansers, other than the study-provided Cetaphil®, is prohibited throughout the study, from the Screening Visit to Day 28/EOS.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time.

Participants will remain in the study through Day 28, or may discontinue early if any of the following criteria are met:

- PI decision
- Subject withdrawal of consent
- Subject is lost to follow-up
- Unacceptable Toxicity (exacerbation of IGA 1 point higher than baseline)
- Sponsor termination of the trial

A PI may discontinue a participant from the study for the following reasons:

- Pregnancy
- Failure to comply with the study procedures, including inability to receive study drug or missing study appointments
- If any clinical AE, laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- Disease progression, which requires discontinuation of the study intervention

The reason for participant discontinuation from the study will be recorded on the Case Report Form (CRF)/electronic CRF (eCRF).

7.2 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if he or she fails to return at least 2 scheduled study visits and is unable to be contacted by the study site staff after 3 documented attempts.

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

- The site will attempt to contact the participant and reschedule the missed visit, counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the PI or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 SAFETY AND OTHER ASSESSMENTS

Subjects will come to the clinic for the following protocol-specified visits: Screening, 0H (+2-day window), 6H, 24H, 48H, 7 days (± 1 -day window) and 28 days (± 3 -day window) days post application. It is important that the 6H, 24H and 48H visits be on approximately on time (± 2 -hour window) to ensure engraftment can be properly assessed.

Screening

Once the subject has provided consent to participate, the following required procedures will be completed at the Screening Visit:

- Review of subject eligibility based on inclusion and exclusion criteria
- Vitals: Blood pressure, heart rate and temperature
- Labs:
 - CBC
 - Urine dipstick pregnancy test (females of childbearing potential)
- Screening IGA score and total lesion count
- Screening porphyrin and sebum levels (Pear 3D)
- Screening photographs (Pear 3D)
- Screening pH (pH meter)
- A pre-BPO cheek swab sample will be procured
- Pre- Bioré® sampling facial wash with Cetaphil®
- A pre-BPO Bioré® sample will be obtained

The following procedures will be completed within the Screening Period:

- Subjects will initiate 5-day, at-home pre-treatment with a 5.0% BPO gel daily application to the face (<https://www.perrigo.com/business/product.aspx?ID=88&let=B> Perrigo 5% BP Aqueous Gel) at the same time each day, immediately after the AM Cetaphil® wash. The final BPO gel application should be at least 24 hours prior to study drug application (and therefore likely the morning before the Day 1 (0H) visit)
- Subjects will initiate a 5-day at-home BID Cetaphil® wash at the same time each day, with the PM wash to be held the day before the Day 1 (0H) visit. No other cleansers can be used throughout the study, from the Screening Visit to Day 28/EOS.
- Subjects will complete an in-home patient e-diary during the pre-treatment period

Once the subject's eligibility has been confirmed and pre-treatment BPO period completed, the subject will be enrolled into the study and initiate on-study procedures, including ongoing patient e-diary for Cetaphil® washes.

Treatment Period

Post BPO-period porphyrin level, sebum production, pH, IGA assessment, total lesion count, and photos will be obtained on Day 1, followed by the engraftment assay. Urine dipstick pregnancy test will be obtained for females of childbearing potential. After these measurements are obtained, subjects will wash their face with Cetaphil® (Galderma) in the office and then the single study drug dose will be applied by the PI (OH). The application should take place at least 24 hours after the last morning BPO application. The PI will apply drug once (time point 0H) to the face: cheek/nose (approximately 50 cm²) to Cohort 1 subjects. If there are less than 2 DLTs and no possibly-related SAEs in Cohort 1 during the 7-day safety assessment, the remaining subjects will be enrolled into Cohort 2 to receive single application to the face: cheek/nose. Subjects will then be monitored for at least 30 minutes after the application for any allergic or anaphylactic reactions. Subjects should not wash or shave their face again until the 24H sampling is completed with the exception that the Bioré® strip follicular assessment will be obtained after washing face with Cetaphil® in office. Subjects may then initiate facial cleansing with Cetaphil® wash BID. However, for all office visit days, subjects should hold the morning wash and morning shaving until visit samples have been obtained.

Engraftment sample procurement: Samples for engraftment will be obtained using a Fab-Swab Filtered Air Breathable Sterile Flocked Collection Device (PurFlock® Ultra-Puritan Diagnostics). The face will be quartered into left lateral, left medial, right lateral, right medial for sampling procurement, with mid pupillary line splitting medial and lateral quarters and midline forehead/nose splitting medial quarters. Each sampling will only test a quarter of the face to ensure that sampling post drug application do not remove study drug prior to the next sampling time point and to improve standardization of sampling area. To obtain samples, the swabs should be pressed firmly and rubbed over the designated quadrant for that sample time-point with occasional rotation of the swab for a duration of a minimum of 30 seconds. Please refer to the Laboratory Manual for detailed instructions.

Subjects will be monitored for safety at all study visits: 0H, 6H, 24H, 48H, 7 days and 28 days after the initial application.

Vitals will be taken at study visits: Screening, 24H, 7 days, and 28 days.

The following will be completed at all office study visits (0H, 6H, 24H, 48H, 7 days, and 28 days), unless otherwise specified:

- Assess for any new or existing AEs or SAEs
- Review concomitant medications
- Obtain cheek samples for
 - Porphyrin presence (Pear 3D)
 - Sebum production (Pear 3D)
- Photography (Pear 3D)
- IGA
- Absolute lesion counts
- pH of skin (pH meter)
- Assess engraftment via Fab-Swab Filtered Air Breathable Sterile Flocked Collection Device (PurFlock® Ultra-Puritan Diagnostics) (TaqMan® assay)
- Assess follicular engraftment via Bioré® strip (TaqMan® assay, at 24H only)
- Urine dipstick pregnancy test (females of childbearing potential, at 0H and Day 28 only)

NOTE: Additional vital signs and CBC may be repeated, as clinically indicated.

End of Study (EOS)

Subjects will remain in the study through Day 28, or may discontinue early, as described in protocol section [7.1](#). Subjects will have completed the study upon conclusion of the Day 28/EOS visit procedures. Subjects who discontinue prior to Day 28 should have the EOS visit scheduled as soon as possible, in

order to complete final safety assessments, prior to discontinuation from the study.

Early Discontinuation of Subjects

Subjects may voluntarily withdraw from the study at any time. A subject's participation in the study may be terminated if it is deemed by the PI or Sponsor that it is unsafe for the subject to continue in the study. Subjects who discontinue prior to completing the DLT evaluation period (i.e., prior to completion of Day 7 procedures) for reasons other than a DLT, will be replaced.

Notification of early subject discontinuation from the study and the reason for discontinuation will be made to Sponsor and will be clearly documented on the appropriate eCRF.

Study Termination

The study may be stopped at his/her study site at any time by the site PI. Sponsor may stop the study (and/or the study site) for any reason with appropriate notification. Both the Sponsor and the PI reserve the right to terminate the study at any time. Should this be necessary, both parties will arrange discontinuation procedures and notify the appropriate regulatory authority(ies), and IRBs. In terminating the study, Naked Biome and the PI will assure that adequate consideration is given to the protection of the subjects' interests.

Adverse Events (AEs)

AEs occurring during the study will be recorded on an AE CRF. If AEs occur, the first concern will be the safety of the study participants.

Prior/Concomitant Therapies and Medications

At screening, all therapies and medication taken up to 2 months prior to the Screening Visit should be recorded on the eCRF. At each study visit, any therapies or medications taken by the subject since the last visit will be captured.

8.2 ADVERSE EVENTS (AEs) AND SERIOUS ADVERSE EVENTS (SAEs)

8.2.1 DEFINITION OF ADVERSE EVENT (AE) AND SERIOUS ADVERSE EVENTS (SAE)

ADVERSE EVENTS (AEs)

An AE is any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product. In addition, during the screening period, AEs will be assessed regardless of the administration of a pharmaceutical product.

NOTE: AEs must be collected once informed e-consent has been obtained, regardless of whether or not the subject has been administered study drug.

AEs will be assessed, documented, and recorded on the AE form throughout the study (i.e., after informed e-consent has been obtained). At each visit, the PI will begin by querying for AEs by asking each subject a general, non-directed question such as "How have you been feeling since the last visit?" Directed questioning and examination will then be done as appropriate. All reported AEs will be documented on the appropriate CRF/eCRF.

SERIOUS ADVERSE EVENT (SAEs)

An SAE is defined as an event that meets at least 1 of the following criteria:

- Is fatal or life-threatening.
- Requires inpatient hospitalization or prolongation of existing hospitalization, unless hospitalization is a result of:
 - A routine treatment or monitoring of the studied indication not associated with any deterioration in condition.

- An elective or preplanned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since signing the ICF.
- A treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of a SAE and not resulting in hospital admission.
- Any social reasons and respite care, in the absence of any deterioration in the subject's general condition.
- Results in persistent or significant disability, incapacity, or a substantial disruption of a person's ability to conduct normal life functions.
- Constitutes a congenital anomaly or birth defect.
- Is considered to be an important medical event or a medically significant event that may not result in death, be immediately life-threatening, or require hospitalization but may be considered serious when, based upon appropriate medical judgment, the event may jeopardize the subject or may require medical or surgical intervention to prevent 1 of the outcomes listed above.

All cancer AEs are SAEs. In addition, the Sponsor considers any abortion (spontaneous or nonspontaneous) as an SAE.

Pre-planned surgeries or procedures for pre-existing, known medical conditions for which a subject requires hospitalization is not reportable as an SAE.

Any pre-planned surgery or procedure should be clearly documented in the site source documents by the medically qualified PI at the time of the subject's entry into the study. If it has not been documented at the time of the subject's entry into the study, then it should be documented as an SAE and reported to Sponsor.

To ensure subject safety, every SAE, regardless of suspected causality (including events that may not be associated with the study drug[s] but may be associated with a study procedure or disease progression), unless otherwise specified by the Protocol, occurring after the subject has signed the e-ICF and up to the last study visit, whichever is later, must be reported to the Sponsor (or designee) within 24 hours of learning of its occurrence. Any SAEs occurring more than 30 days after the last dose of study drug should be reported to the Sponsor, or its designee, only if the PI suspects a causal relationship to the study drug. An SAE occurring at a different time interval or otherwise considered completely unrelated to a previously reported SAE should be reported separately as a new event. Previously planned (i.e., before providing informed e-consent) surgeries should not be reported as SAEs unless the underlying medical condition worsens over the course of the study.

Information about all SAEs is collected and recorded on the Adverse Event Report Form in the eCRF. The PI must assess and record the causal relationship of each SAE to each specific study drug.

The PI must also complete the Serious Adverse Event Report Form, in English, notify the Sponsor and, if necessary, send the completed and signed form to the Sponsor or designee within 24 hours of becoming aware of the SAE. The PI must provide a causality assessment, that is, assess whether there is at least a reasonable possibility that the SAE is related to the study treatment: suspected (yes) or not suspected (no).

Investigational site personnel must report any new information regarding the SAE within 24 hours of becoming aware of the information in the same manner that the initial SAE Report Form was sent. Follow-up information is recorded on an amended or new SAE Report Form, with an indication that it is follow-up to the previously reported SAE and the date of the original report. The follow-up report should include information that was not provided on the previous SAE Report Form, such as the outcome of the event (e.g., resolved or ongoing), treatment provided, action taken with study drug because of the SAE (e.g., dose reduced, interrupted, or discontinued), or subject disposition (e.g., continued or withdrew from study participation). Each recurrence, complication, or progression of the original event should be reported as follow-up to that event, regardless of when it occurs.

The treating PI will determine AEs by:

- Active query for any systemic symptoms
- Limited facial physical examination
- Utilization of Investigator Assessment of Tolerability Scale (0 = None; 1 = Slight; 2 = Moderate; 3 = Intense) for assessment of:
 - Skin Dryness
 - Skin Peeling
 - Irritant/Allergic Contact Dermatitis
 - Hyperpigmentation
 - Skin Erythema
 - Conjunctival Injection
 - Ocular Irritation
 - Mucosal Toxicity
- Assessment of disease status using IGA Score and Acne Lesion Count

Toxicities that warrant subject discontinuation from the trial, as deemed appropriate by the PI, include but are not limited to: ulceration, extreme redness, worsening of acne, extreme skin irritation, extreme ocular irritation, extreme mucosal irritation, extreme contact dermatitis.

REPORTING

AEs that begin or worsen after informed e-consent was obtained should be recorded on the Adverse Event Report Form in the eCRF. Conditions that were already present at the time of informed e-consent should be recorded on the Medical History form in the eCRF. AEs (including laboratory abnormalities that constitute AEs) should be described using a diagnosis whenever possible rather than by individual underlying signs and symptoms. When a clear diagnosis cannot be identified, each sign or symptom should be reported as a separate AE.

The term "disease progression" should be recorded as an AE/SAE itself only if there are no other identifiable AEs/SAEs associated with the disease progression at the time of reporting. For events associated with disease progression, the relevant signs and symptoms should be reported using a diagnosis whenever possible, rather than the individual underlying signs and symptoms. When a clear diagnosis cannot be identified, each sign or symptom should be reported as a separate AE. If the events resulting from disease progression meet the criteria for an SAE (e.g., resulted in hospitalization, a life-threatening event, or death), the specific event(s) should be reported as an SAE(s). In both cases (i.e., AEs or SAEs related to disease progression), for each event it should be indicated whether the event (diagnosis or signs and symptoms) is related to disease progression.

The occurrence of AEs should be sought by nondirective questioning of the subject during the screening process after signing the ICF and at each visit during the study. AEs may also be detected when they are volunteered by the subject during the screening process or between visits, or through physical examination, laboratory test, or other assessments. To the extent possible, each AE should be evaluated to determine:

- The severity.
- Whether there is at least a reasonable possibility that the AE is related to the study treatment: suspected (yes) or not suspected (no).
- The start and end dates, unless unresolved at final follow-up.
- The action taken regarding study drug.
- The event outcome (e.g., not recovered/not resolved, recovered/resolved, recovering/resolving, recovered/resolved with sequelae, fatal, unknown).
- The seriousness, as per SAE definition provided.

Unlike routine safety assessments, SAEs are monitored continuously and have special reporting requirements.

All AEs should be treated appropriately. If an AE is treated with a concomitant medication or non-drug therapy, this action should be recorded on Adverse Event Report Form in the eCRF and the treatment should be specified on the Prior/Concomitant Medications or Procedures and Non-Drug Therapy form in the CRF/eCRF.

Once an AE is detected, it should be followed until it has resolved or until it is judged to be permanent; assessment should be made at each visit (or more frequently if necessary) of any changes in severity, the suspected relationship to the study drug, the interventions required to treat the event, and the outcome.

LABORATORY TEST RESULTS

Laboratory abnormalities that constitute an AE in their own right (considered clinically meaningful, induce clinical signs or symptoms, require concomitant therapy, or require changes in study drug) should be recorded on the Adverse Event Report Form in the CRF/eCRF. Whenever possible, a diagnosis rather than a symptom should be provided (e.g., "anemia" instead of "low hemoglobin"). Laboratory abnormalities that meet the criteria for AEs should be followed until they have returned to normal or an adequate explanation of the abnormality is found. When an abnormal laboratory test result corresponds to a sign or symptom of a previously reported AE, it is not necessary to separately record the laboratory test result as an additional event.

Laboratory abnormalities that do not meet the definition of an AE should not be reported as AEs. Severity does not automatically indicate an SAE unless it meets the definition of serious, as defined, and/or per the PI's discretion.

8.2.2 DEFINITION OF DOSE-LIMITING TOXICITIES (DLTs)

Dose-limiting toxicities (DLTs) are defined as AEs occurring during the first 7 days after NB01 application that are not due to the subject's underlying disease, are not clearly attributable to other etiologies, and meet any of the following toxicity criteria:

Dermatologic Toxicities:

- Skin Dryness Score of 3
- Skin Peeling Score of 3
- Irritant/Allergic Contact Score of 3
- Hyperpigmentation Score of 3
- Skin Erythema Score of 3
- Exacerbation of disease determined by an increase in IGA score 1 point beyond baseline screening score

Ocular Toxicities:

- Conjunctival injection score of 3
- Eye irritation score of 3

Mucosal Toxicities:

- Mucosal Irritation Score of 3

If any systemic toxicities develop, the Sponsor will pause the study for further review before proceeding. If 2 or more subjects experience a DLT, or in the event of an SAE that is at least possibly-related to study treatment, the Sponsor will pause the study to discuss with the PI and regulatory authorities, if needed.

8.2.3 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

Subjects will be monitored for safety at all study visits: Screening, 0H, 6H, 24H, 48H, 7 days and 28

days after initial application.

All AEs, including local and systemic reactions not meeting the criteria for SAEs, will be captured on the appropriate CRF/eCRF. Information to be collected includes:

- event description
- time of onset
- clinician's assessment of severity
- relationship to study product (assessed only by those with the training and authority to make a diagnosis)
- time of resolution/stabilization of the event.

All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE, specifically, worsening of the pre-existing condition.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of event onset and duration of each episode.

The study site will record all reportable events with start dates occurring any time after informed e-consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, the PI will inquire about the occurrence of AE/SAE since the last visit. Events will be followed for outcome information until resolution or stabilization.

SEVERITY

A clinical determination will be made of the intensity of an AE. The severity assessment for a clinical AE must be completed using the following definitions as guidelines:

Mild; asymptomatic or mild symptoms; easily tolerated; clinical or diagnostic observations only; intervention not indicated.
Moderate; minimal. local. or noninvasive intervention indicated; limiting age□appropriate activities of daily living.
Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living.
Life-threatening consequences; urgent intervention indicated.
Death due to AE

8.3 RELATIONSHIP TO STUDY INTERVENTION

All AEs must have their causal relationship to study intervention assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be the suspect drug.

- Possibly Related – If the AE is known to have occurred with the study intervention, there is a reasonable possibility that the study intervention caused the AE, or there is a temporal relationship between the study intervention and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study intervention and the AE.
- Not Related – There is not a reasonable possibility that the administration of the study intervention caused the event and there is no temporal relationship between the study intervention and event onset, or an alternate etiology has been established. The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

OR

- Related – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals.
- Probably Related – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.
- Possibly Related – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "related", as appropriate.
- Unlikely related – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- **Not Related** – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

PREGNANCY

Pregnancy, in and of itself, is not considered as an AE unless there is suspicion that study drug may have interfered with the effectiveness of a contraceptive medication or method. Any pregnancy where the estimated date of conception occurs either prior to the last visit or within 30 days of the last study treatment or exposure to study drug (due to breast feeding) must be reported. When a pregnancy has been confirmed in a subject during maternal or paternal exposure to study drug, the following procedures should be followed to ensure subject safety:

- The study drug must be discontinued immediately (female subjects only and subject must be withdrawn from study).
- The PI must complete and submit the Clinical Trial Pregnancy form to the Sponsor or its designee within 24 hours of learning of the pregnancy.

Data on fetal outcome and breastfeeding are collected for regulatory reporting and drug safety evaluation. Follow-up should be conducted for each pregnancy to determine outcome, including spontaneous or

voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications, by following until the first well-baby visit. Pregnancy should be recorded on a Clinical Trial Pregnancy form and reported by the PI to the Sponsor or its designee. Pregnancy follow-up information should be recorded on the same form and should include an assessment of the possible causal relationship to the Sponsor's study drug to any pregnancy outcome, as well as follow-up to the first well-baby visit or the duration specified in local regulations, whichever is later.

Any SAE occurring during pregnancy must be recorded on the SAE report form and submitted to the Sponsor or designee.

8.4 EXPECTEDNESS

The PI, along with the Naked Biome Medical Monitor will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention. Consultation with FDA will occur, as necessary.

8.5 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

- The occurrence of an AE or SAE may come to the attention of the study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a Study Monitor.
- All AEs, including local and systemic reactions not meeting the criteria for SAEs, will be captured on the appropriate CRF/eCRF. Information to be collected includes: event description, time to event onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.
- Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE, more specifically, worsening of the pre-existing condition.
- Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

8.5.1 SERIOUS ADVERSE EVENT (SAE) REPORTING

The PI will immediately report to the Sponsor any SAEs no later than 24 hours after learning of a SAE, whether or not it is considered study drug-related and must also include a causal assessment of the event. All SAEs must be recorded on the Serious Adverse Event Form in the CRF/eCRF.

All SAEs will be followed until satisfactory resolution or until the site PI deems the event to be chronic or the participant is stable, and outcomes should be reported. The PI must supply the Sponsor with any additional requested documents of the event as soon as possible (e.g., discharge summaries, procedure reports/results).

In the event of a SAE, the PI must:

1. Notify Sponsor immediately of the event by email using sa-safety@nakedbiome.com.
2. Obtain and maintain in his/her files all pertinent medical records, information, and medical

- judgments from colleagues who assisted in the treatment and follow-up of the subject.
3. Provide Sponsor with a complete, written description of the AE(s) on the Serious Adverse Event Form in the CRF/eCRF describing the event chronologically, including any treatment given (e.g., medications administered, procedures performed) for the AE(s). Summarize relevant clinical information about the event: signs, symptoms, diagnosis, clinical course and relevant clinical laboratory tests, etc. Include any additional or alternative explanation(s) for the causality which includes a statement as to whether the event was or was not related to the use of the investigational drug.
 4. Promptly inform the governing IRB of the SAE as required by the IRB, local regulations, and the governing health authorities.

The study Sponsor will be responsible for notifying the FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but no later than 7 calendar days after the Sponsor's initial receipt of the information. In addition, the Sponsor must notify FDA, and all participating PIs in an IND safety report, of potential serious risks from clinical trials or any other source, as soon as possible, but no later than 15 calendar days after the Sponsor determines that the information qualifies for reporting.

PRODUCT COMPLAINTS

The Sponsor collects product complaints on study drugs and drug delivery systems used in clinical studies to ensure the safety of study participants, monitor quality, and facilitate process and product improvements.

All product complaints associated with material packaged, labeled, and released by the Sponsor or its designee will be reported to the Sponsor. All product complaints associated with other study material will be reported directly to the respective manufacturer.

The PI or his/her designee is responsible for reporting a complete description of the product complaint via email or other written communication to the Sponsor contact or respective manufacturer as noted in the packaging information. Any AE associated with a product complaint should be reported to Sponsor.

If the PI is asked to return the product for investigation, he/she will return a copy of the product complaint communication with the product.

9 EFFICACY

9.1 CONSIDERATIONS FOR EFFICACY

- **Tolerability Scale for Topical Acne Treatments, all visits:**

Reference: ClinTrials.gov: [Investigator Assessment Tolerability Scoring: A Study to Evaluate Tolerability of Two Topical Drug Products in the Treatment of Acne](#). Please see Appendix C of this protocol.

1. Skin Dryness Score: Investigator Assessment of Tolerability Scale (0 = None; 1 = Slight; 2 = Moderate; 3 = Intense)
2. Skin Peeling Score: Investigator Assessment of Tolerability Scale (0 = None; 1 = Slight; 2 = Moderate; 3 = Intense)
3. Irritant/Allergic Contact Dermatitis Score: Investigator Assessment of Tolerability Scale (0 = None; 1 = Slight; 2 = Moderate; 3 = Intense)
4. Hyperpigmentation Score: Investigator Assessment of Tolerability Scale (0 = None; 1 = Slight; 2 = Moderate; 3 = Intense)
5. Ocular Irritation: Investigator Assessment of Tolerability Scale (0 = None; 1 = Slight; 2 = Moderate; 3 = Intense)

6. Conjunctival Injection: Investigator Assessment of Tolerability Scale (0 = None; 1 = Slight; 2 = Moderate; 3 = Intense)
 7. Mucosal Toxicity: Investigator Assessment of Tolerability Scale (0 = None; 1 = Slight; 2 = Moderate; 3 = Intense)
 8. Skin Erythema Score: Investigator Assessment of Tolerability Scale (0 = None; 1 = Slight; 2 = Moderate; 3 = Intense)
- **Assessment of disease status using IGA Score and Acne Lesion Count**
 - **Engraftment and Dose Schedule Determination**

10 STATISTICAL CONSIDERATIONS

10.1 STATISTICAL HYPOTHESES

No formal statistical hypotheses will be tested. Tabulations of any adverse reactions will be examined overall and within and between cohorts. Primary and secondary efficacy endpoints will be summarized pre- and post-microbiome transplant overall and within and between cohorts.

10.2 SAMPLE SIZE DETERMINATION

No sample size and power estimates will be performed for this Phase 1 trial.

10.3 POPULATIONS FOR ANALYSES

Approximately ten (10) subjects in 2 dose cohorts will be entered sequentially, 5 DLT-evaluable subjects at the low dose followed by 5 DLT-evaluable subjects at the high dose after AE evaluation for the low dose subjects.

10.4 STATISTICAL ANALYSES

10.4.1. GENERAL APPROACH

Data will be tabulated and summarized for each subject at each time point. In addition, data for all variables will be summarized using descriptive statistics overall and by each dose cohort separately. Primary endpoints for safety will be summarized using means and standard deviation for continuous variables and frequencies and percentages for categorical variables. Plots of primary outcomes will be produced examining trends over time. Subject trajectories will be plotted for Secondary and Exploratory endpoints using spaghetti plots and changes over time in the IGA and acne lesion count will be summarized. Correlation between the efficacy biomarkers overall and by cohort and time point will be examined for changes and trends.

11 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

11.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

Good Clinical Practice (GCP)

The PI will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki (as amended in Edinburgh, Tokyo, Venice, Hong Kong, and South Africa), ICH guidelines, or with the laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the study subject. These standards are consistent with the European Union Clinical Trials Directive 2001/20/EC and GCP Directive 2005/28/EC.

The PI will ensure adherence to the basic principles of GCP, as outlined in 21 CFR 312, subpart D, "Responsibilities of Sponsors and Investigators," 21 CFR, part 50, 1998, and 21 CFR, part 56, 1998.

The PI and all applicable sub-investigators will comply with 21 CFR, Part 54, 1998, providing documentation of their financial interest or arrangements with Naked Biome, or proprietary interests in the investigational drug under study. This documentation must be provided prior to the PI's (and any sub-investigator's) participation in the study. The PI and sub-investigator agree to notify Naked Biome of any change in reportable interests during the study and for 1 year following completion of the study. Study completion is defined as the date when the last subject completes the protocol-defined activities.

11.1.1 INSTITUTIONAL REVIEW BOARD (IRB) REVIEW AND APPROVAL

The PI (or Sponsor as appropriate according to local regulations) will submit this protocol, ICF, and any accompanying material to be provided to the subject (such as advertisements, subject information sheets, or descriptions of the study used to obtain informed e-consent) to an IRB. The PI will not begin any study subject activities until approval from the IRB has been documented and provided as a letter to the PI.

Before implementation, the PI will submit to and receive documented approval from the IRB/IEC on any modifications made to the protocol or any accompanying material to be provided to the subject after initial approval, except for those necessary to reduce immediate risk to study subjects.

11.1.2 INFORMED CONSENT PROCESS

Prior to the beginning of the trial, the PI will have the IRB's written approval for the protocol and the electronically administered ICF(s) and any other information to be provided to the participants. The PI must use the most current IRB-approved ICF for documenting informed consent. Each ICF will be appropriately signed and dated by the subject and the person conducting the consent discussion, and by an impartial witness if required by local requirements.

11.1.3 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Informed consent forms (ICFs) describing in detail the study intervention, study procedures, and risks are given to the participant and written (electronic or script) documentation of informed consent is required prior to initiation of Screening procedures. The ICF will also inform subjects about research testing and any sample retention.

11.1.4 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Informed consent forms (ICFs) will be IRB-approved and the participant will be asked to read and review the document via a hand-held device and/or hard copy, as part of the electronic consent (e-consent) process. The PI will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written ICF and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant will sign the ICF prior to any procedures being done specifically for the study. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the ICF will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

11.1.5 CONFIDENTIALITY AND PRIVACY

The PI must assure that subjects' anonymity will be strictly maintained and that their identities are protected from unauthorized parties. Only subject initials, date of birth, de-identified facial photography (for internal sponsor research activities and potential publication uses), another unique identifier (as allowed by local law) and an identification code will be recorded on any form or biological sample submitted to the Sponsor, IRB, or laboratory. Laboratory specimens must be labeled in such a way as to protect subject identity while allowing the results to be recorded to the proper subject. NOTE: The PI must keep a screening log showing codes, names, and addresses for all subjects screened and for all

subjects enrolled in the trial. Subject data will be processed in accordance with all applicable regulations.

The PI agrees that all information received from Naked Biome, including but not limited to the PI investigator brochure, this protocol, CRF/eCRF, the study drug, and any other study information, remain the sole and exclusive property of Naked Biome during the conduct of the study and thereafter. This information is not to be disclosed to any third party (except employees or agents directly involved in the conduct of the study or as required by law) without prior written consent from Naked Biome. The PI further agrees to take all reasonable precautions to prevent the disclosure by any employee or agent of the study site to any third party or otherwise into the public domain.

11.1.6 SAFETY OVERSIGHT

Safety oversight will be under the direction of the Principal Investigator (PI) and the Sponsor Medical Monitor (MM).

Once the first 5 safety-evaluable subjects in Cohort 1 complete the Day 7 assessments, the PI and MM will convene a Dose Escalation call and determine whether to open enrollment into Cohort 2. The decisions will be documented in minutes, and the minutes on file as part of Sponsor's central study record.

11.1.7 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, the reported trial data are accurate, complete, and verifiable, and the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with ICH GCP, and with applicable regulatory requirement(s).

- Monitoring for this study will be performed by a Sponsor representative.
- In accordance with regulations and guidelines, the Study Monitor must have direct access to the PI's source documentation to verify the accuracy of the data recorded in the CRF/eCRF. The Monitor is responsible for routine review of the CRF/eCRF at regular intervals throughout the study to verify adherence to the protocol and the completeness, consistency, and accuracy of the data being entered on them. The Monitor should have access to any subject records needed to verify the entries on the CRF/eCRF. The PI agrees to cooperate with the Monitor to ensure that any problems detected through any type of monitoring (central or on site) are resolved.” Representatives of regulatory authorities or of Naked Biome may conduct inspections or audits of the clinical study. If the PI is notified of an inspection by a regulatory authority the PI agrees to notify the Naked Biome Medical Monitor immediately. The PI agrees to provide to representatives of a regulatory agency or Naked Biome access to records, facilities, and personnel for the effective conduct of any inspection or audit.

11.1.8 DATA HANDLING AND RECORD KEEPING

11.1.8.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

For each subject providing consent, an eCRF will be completed by an authorized study staff member whose training for this function is documented according to study procedures. An eCRF should be completed on the day of the subject visit to enable the Sponsor to perform central monitoring of safety data. Subsequent to data entry, a Study Monitor will perform source data verification within the EDC system. Original entries as well as any changes to data fields will be stored in the audit trail of the system. Prior to database lock (or any interim time points as described in the clinical data management plan), the PI will use his/her log-in credentials to confirm that the forms have been reviewed, and that the entries accurately reflect the information in the source documents. The eCRF capture the data required per the protocol SOE and procedures. System-generated or manual queries will be issued to the

investigative site staff as data discrepancies are identified by the Monitor or internal Naked Biome staff, who routinely review the data for completeness, correctness, and consistency. The site research staff is responsible for responding to the queries in a timely manner, within the system, either by confirming the data as correct or updating the original entry, and providing the reason for the update (e.g., data entry error). At the conclusion of the trial, Naked Biome will provide the site with a read-only archive copy of the data entered by that site. This archive must be stored in accordance with the records retention requirements outlined in Section 11.1.

11.1.9 FUTURE USE OF STORED SPECIMENS AND DATA

Data collected for this study will be analyzed and stored at:

Naked Biome
MBC BioLabs 953 Indiana Street
San Francisco, CA 94107

With the participant's approval and as approved by local IRBs, de-identified biological samples will be stored at Naked Biome. These samples could be used to further research the subjects with acne, its complications and other conditions for which adults with moderate acne are at increased risk, and to improve treatment. Subjects who sign the optional consent for future use of their samples can expect their research samples to be stored and used by the Sponsor for up to 15 years after the end of the study.

During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent regarding biosample storage may not be possible after the study is completed.

When the study is completed, access to study data and/or samples will reside with Naked Biome.

11.1.10 QUALITY ASSURANCE (QA) AND QUALITY CONTROL (QC)

Study conduct will be under the direction of the PI and the Study Monitor who have the appropriate expertise and training and uphold the study subjects' safety as priority. Treatment is open-label and a single application. All data will be collected on study-specific eCRFs and kept confidential to those related to this study only.

11.1.11 STUDY RECORDS RETENTION

The PI will make available all source documents and other records for this trial to Naked Biome's appointed Study Monitors, to IRBs, or to regulatory authority or health authority inspectors.

The PI must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents should be classified into at least the following 2 categories: (1) PI's study file, and (2) subject clinical source documents.

The PI's study file will contain the protocol/amendments, CRF/ eCRF and query forms, and governmental approval with correspondence, ICF, drug records, staff curriculum vitae and authorization forms, and other appropriate documents and correspondence.

The required source data should include sequential notes containing at least the following information for each subject:

- Subject identification (name, date of birth, gender)
- Documentation that subject meets eligibility criteria (i.e., history, physical examination, and confirmation of diagnosis [to support inclusion and exclusion criteria])
- Documentation for the reason(s) a subject providing consent is not enrolled
- Participation in study (including Subject ID)
- Study discussed and date of informed consent

- Dates of all visits
- Documentation that protocol-specific procedures were performed
- Results of efficacy parameters, as required by the protocol
- Start and end dates (including dose regimen) of study drug, including dates of dispensing and return
- Record of all AEs and other safety parameters (start and end date, and including causality and severity)
- Concomitant medication (including start and end date, dose [if relevant] and dose change[s])
- Date of study completion and reason for early discontinuation, if it occurs.

All clinical study documents must be retained by the PI until at least 2 years or according to local laws, whichever is longer, after the last approval of a marketing application in an ICH region (i.e., US, Europe, or Japan) and until there are no pending or planned marketing applications in an ICH region; or, if no application is filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and regulatory authorities have been notified. PIs may be required to retain documents longer if specified by regulatory requirements, local regulations, or an agreement with Naked Biome. The PI must notify Naked Biome before destroying any clinical study records.

Should the PI wish to assign the study records to another party or move them to another location, Naked Biome must be notified in advance.

If the PI cannot provide for this archiving requirement at the study site for any or all the documents, special arrangements must be made between the PI and Naked Biome to store these records securely away from the site so that they can be returned sealed to the PI in case of an inspection. When source documents are required for the continued care of the subject, appropriate copies should be made for storage away from the site.

11.1.12 PROTOCOL MODIFICATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, ICH GCP. The noncompliance may be either on the part of the participant, the PI, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH GCP:

- Compliance with Protocol
- QA and QC
- Noncompliance

The PI is responsible for ensuring the study is conducted in accordance with the procedures and evaluations described in this protocol. Protocol modifications, except those intended to reduce immediate risk to study subjects, may only be made by Naked Biome. The PI must submit all protocol modifications to the Sponsor in accordance with local requirements and receive documented approval before modifications can be implemented.

11.1.13 PUBLICATION AND DATA SHARING POLICY

A clinical study report will be prepared and provided to the regulatory agency. Naked Biome will ensure that the report meets the standards set out in the ICH Guideline for Structure and Content of Clinical Study Reports (ICH E3). NOTE: An abbreviated report may be prepared in certain cases.

PIs in this study may communicate, orally present, or publish in scientific journals or other scholarly media only after the following conditions have been met:

1. The results of the study in their entirety have been publicly disclosed by or with the consent of Naked Biome in an abstract, manuscript, or presentation form or the study has been completed at

- the study site for at least 2 years.
2. The PI will submit to Naked Biome any proposed publication or presentation along with the respective scientific journal or presentation forum at least 30 days before submission of the publication or presentation.
 3. No such communication, presentation, or publication will include Naked Biome's confidential information.
 4. The PI will comply with Naked Biome's request to delete references to its confidential information (other than the study results) in any paper or presentation and agrees to withhold publication or presentation for an additional 60 days to obtain patent protection if deemed necessary.

11.1.14 CONFLICT OF INTEREST

Any conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the PI has established procedures for all study cohort members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

12 APPENDICES**12.1 APPENDIX A: ABBREVIATIONS**

AE	Adverse Event
AIDS	acquired immune deficiency syndrome
BID	Twice Daily
BPO	5.0% Benzoyl Peroxide
CBC	Complete Blood Count
CFR	Code of Federal Regulations
CRF/eCRF	Case Report Form/electronic CRF
CoNS	coagulase-negative Staphylococcus
E-Consent, E-ICF	Electronic Informed Consent Form
eCRF	Electronic Case Report Form
e-Diary	Electronic Diary
EOS	End of Study
ERF	Enrollment Request Form
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HBV	hepatitis B virus
HCV	hepatitis C virus
HIV	human immunodeficiency virus
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IGA	Investigator Global Assessment
IND	Investigational New Drug
IRB	Institutional Review Board
IUD	Intrauterine device
NB01	<i>P. acnes</i> microbiome transplant
<i>P. acnes</i>	Propionibacterium acnes
PBS	Phosphate Buffered Saline
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event
<i>S. aureus</i>	Staphylococcus aureus
SOE	Schedule of Events
SOP	Standard Operating Procedure
TPase2	transposase2
US	United States

12.2 APPENDIX B: ACNE GRADING BY LESION COUNT AND IGA- FDA GUIDANCE FOR INDUSTRY: ACNE VULGARIS: DEVELOPING DRUGS FOR TREATMENT, DRAFT 2005

ACNE GRADING

Acne grading by lesion count: Acne may be classified as mild, moderate or severe. Comedones and inflammatory lesions are usually considered separately (DermNet).

- Mild acne
 - <20 comedones
 - <15 inflammatory lesions
 - Or, total lesion count <30
- Moderate acne
 - 20–100 comedones
 - 15–50 inflammatory lesions
 - Or, total lesion count 30–125
- Severe acne
 - >5 pseudocysts
 - Total comedone count >100
 - Total inflammatory count >50
 - Or, total lesion count >125

Investigator’s Global Assessment (IGA) 5-Point Scale		
Grade 0	Clear	Clear skin with no inflammatory or non-inflammatory lesions. (The category of clear should represent true absence of disease.)
Grade 1	Almost Clear	A few scattered comedones and no more than one small papule

Grade 2	Mild	Some comedones, some papules and pustules; no nodules
Grade 3	Moderate	Many comedones, papules and pustules; one nodule may be present
Grade 4	Severe	Covered with comedones, numerous papules and pustules and no more than a few nodular lesions

12.3 APPENDIX C: INVESTIGATOR ASSESSMENT OF TOLERABILITY SCALE, CLINICALTRIALS.GOV:
 “INVESTIGATOR ASSESSMENT TOLERABILITY SCORING: A STUDY TO EVALUATE TOLERABILITY OF
 TWO TOPICAL DRUG PRODUCTS IN THE TREATMENT OF ACNE”

Investigator Assessment of Tolerability Scale	Score			
	0	1	2	3
Skin Dryness	None	Slight	Moderate	Intense
Skin Peeling	None	Slight	Moderate	Intense
Irritant/Contact Dermatitis	None	Slight	Moderate	Intense
Hyperpigmentation	None	Slight	Moderate	Intense
Ocular Irritation	None	Slight	Moderate	Intense
Conjunctival Injection	None	Slight	Moderate	Intense
Mucosal Toxicity	None	Slight	Moderate	Intense
Skin Erythema	None	Slight	Moderate	Intense

13 REFERENCES

- Achermann Y, Goldstein EJ, Coenye T, et al. Propionibacterium acnes: from commensal to opportunistic biofilm-associated implant pathogen. *Clin Microbiol Rev*. 2014;27(3):419-440.
- Akaza N, Akamatsu H, Numata S, et al. Microorganisms inhabiting follicular contents of facial acne are not only Propionibacterium but also Malassezia spp. *J Dermatol*. 2016;43(8):906-911.
- Baquerizo Nole KL, Yim E, Keri JE. Probiotics and prebiotics in dermatology. *J Am Acad Dermatol*. 2014;71(4):814-821.
- [ClinTrials.gov] Investigator Assessment Tolerability Scoring: A Study to Evaluate Tolerability of Two Topical Drug Products in the Treatment of Facial Acne. Received: August 2009; Updated: October 2016. <https://clinicaltrials.gov/ct2/show/NCT00964223>.
- Costello EK, Lauber CL, Hamady M, et al. Bacterial community variation in human body habitats across space and time. *Science*. 2009;326(5960):1694-1697.
- Dailey HA, Gerdes S, Dailey TA. Noncanonical coproporphyrin-dependent bacterial heme biosynthesis pathway that does not use protoporphyrin. *Proc Natl Acad Sci USA*. 2015;112(7):2210-2215.
- DermNet New Zealand. Acne vulgaris: Acne Grading. <https://www.dermnetnz.org/topics/acne-vulgaris/>.
- [FDA] Guidance for Industry: Acne Vulgaris: Developing Drugs for Treatment. Draft: September 2005. <https://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM071292>.
- Fitz-Gibbon S, Tomida S, Chiu B-H, et al. Propionibacterium acnes strain populations in the in the human skin microbiome associated with acne. *J Invest Dermatol*. 2013;133(9):2152-2160.
- Gribbon EM, Cunliffe WH, Holland KT. Interaction of Propionibacterium acnes with skin lipids in vitro. *J Gen Microbiol*. 1993;139(8):1745-1751.
- Investigator Assessment Tolerability Scoring. A Study to Evaluate Tolerability of Two Topical Drug Products in the Treatment of Facial Acne, 2009; updated 2016: <https://clinicaltrials.gov/ct2/show/study/NCT00964223>
- Johnson T, Kang D, Barnard E, et al. Strain-level differences in porphyrin production and regulation in propionibacterium acnes elucidate disease associations. *mSphere*. 2016;1(1):e00023-15.
- Kang D, Shi B, Erfe MC, et al. Vitamin B12 modulates the transcriptome of the skin microbiota in acne pathogenesis. *Sci Transl Med*. 2015;7(293):293ra103.
- Kasimatis G, Fitz-Gibbon S, Tomida S. Analysis of complete genomes of Propionibacterium acnes reveals a novel plasmid and increased pseudogenes in an acne associated strain. *Biomed Res Int*. 2013:918320.
- Kishishita M, Ushijima T, Ozaki Y, et al. New medium for isolating propionibacteria and its application to assay of normal flora of human facial skin. *Appl Environ Microbiol*. 1980;40(6):1100-1105.
- Kjeldstad B, Johnsson A, Sandberg S. Influence of pH on porphyrin production in Propionibacterium acnes. *Arch Dermatol Res*. 1984;276(6):396-400.
- Kwon HH, Suh DH. Recent progress in the research about Propionibacterium acnes strain diversity and acne: pathogen or bystander? *Int J Dermatol*. 2016;55(11):1196-1204.

- Lomholt HB, Kilian M. Population genetic analysis of *Propionibacterium acnes* identifies a subpopulation and epidemic clones associated with acne. *PLoS One*. 2010;5(8):e12277.
- McDowell A, Barnard E, Nagy I, et al. An expanded multilocus sequence typing scheme for *Propionibacterium acnes*: investigation of ‘pathogenic’, ‘commensal’ and antibiotic resistant strains. *PLoS One*. 2012;7(7):e41480.
- McDowell A, Nagy I, Magyari M, et al. The opportunistic pathogen *Propionibacterium acnes*: insights into typing, human disease, clonal diversification and CAMP factor evolution. *PLoS One*. 2013;8(9):e70897.
- McDowell A, Perry AL, Lambert PA, et al. A new phylogenetic group of *Propionibacterium acnes*. *J Med Microbiol*. 2008;57(Pt2):218-224.
- McGinley KJ, Webster GF, Ruggieri MR. Regional variations in density of cutaneous propionibacteria: correlation of *Propionibacterium acnes* populations with sebaceous secretion. *J Clin Microbiol*. 1980;12(5):672-675.
- Meyer K, Pappas A, Dunn K, et al. Evaluation of Seasonal Changes in Facial Skin With and Without Acne. *J Drugs Dermatol*. 2015;14(6):593-601.
- Mirshahpanah P, Maibach HI. Models in Acnegenesis. *Cutan Ocul Toxicol*. 2007;26(3):195-202.
- Nakatsuji T, Chen TH, Narala S, et al. Antimicrobials from human skin commensal bacteria protect against *Staphylococcus aureus* and are deficient in atopic dermatitis. *Sci Transl Med*. 2017;9(378).
- Neves JR, Francesconi F, Costa A, et al. *Propionibacterium acnes* e a resistência bacteriana [Propionibacterium acnes and bacterial resistance]. *Surg Cosmet Dermatol*. 2015;7(3 Suppl 1):S27-S38.
- Nole KLB, Yim E, Keri JE. Probiotics and prebiotics in dermatology. *J Am Acad Dermatol*. 2014; 71(4): 814-821
- Patrick S, McDowell A. Order XII. Propionibacteriales ord. Nov. In: Whitman W, et al. eds. *Bergey's Manual of Systematic Bacteriology, Volume 5: The Actinobacteria*, New York, NY: Springer Verlag; 2012:1137-1145.
- Richter C, Trojahn C, Dobos G, et al. Follicular fluorescence quantity to characterize acne severity: a validation study. *Skin Res Technol*. 2016;22(4):451-459.
- Shu M, Kuo S, Wang Y, et al. Porphyrin metabolisms in human skin commensal *Propionibacterium acnes* bacteria: potential application to monitor human radiation risk. *Curr Med Chem*. 2013;20(4):562–568.
- Thiboutot D, Gollnick H, Bettoli V, et al. New insights into the management of acne: an update from the Global Alliance to Improve Outcomes in Acne Group. *J Am Acad Dermatol*. 2009;60(5 Suppl):S1-S50.
- Tomida S, Nguyen L, Chiu BH, et al. Pan-genome and comparative genome analyses of *propionibacterium acnes* reveal its genomic diversity in the healthy and diseased human skin microbiome. *mBio*. 2013;4(3):e00003-13.
- Walsh TR, Efthimiou J, Dréno B. Systematic review of antibiotic resistance in acne: an increasing topical and oral threat. *Lancet Infect Dis*. 2016;16(3):e23-e33.

Wei EX, Kirsner RS, Eaglstein WH. End points in dermatologic clinical trials: a review for clinicians. *J Am Acad Dermatol*. 2016;75(1):203-209.