A Randomized, Double-Blinded, Aqueous Gel-Controlled Dose Escalating Trial to Study the Safety and Antimicrobial Efficacy of DBI-002 Probiotic vs. Aqueous Gel and Vehicle Gel vs. Aqueous Gel in Adults with Tinea Versicolor

NCT Number: NCT04937920

Document Date: 11/09/2020

DermBiont® Inc.

CLINICAL PROTOCOL

Protocol Number: DBI-202

A Randomized, Double-Blinded, Aqueous Gel-Controlled Dose Escalating Trial to Study the Safety and Antimicrobial Efficacy of DBI-002 Probiotic vs. Aqueous Gel and Vehicle Gel vs. Aqueous Gel in Adults with Tinea Versicolor

Development Phase of Study:	**Phase 2a**
Study Design:	**Double-Blinded**
Date of Original Protocol:	**09 November 2020**
Sponsor:	**DermBiont, Inc.**

CONFIDENTIAL

PROTOCOL REVIEW AND APPROVAL PAGE

Reviewed and approval by:

[redacted]

Signature Date

[redacted]

Signature Date

[redacted]

Signature Date

[redacted]

Signature Date

Table of Contents

Table 1. List of Definitions and Abbreviations.

TERM	DEFINITION
AE	Adverse Event
Aqueous Gel	Water made into a gel with a common gelling agent
CFU	Colony-forming unit, a unit used to estimate the number of viable bacteria or fungal cells in a sample
CRF / eCRF	Case Report Form / electronic Case Report Form
DBI-002	Probiotic investigational product (IP)
Dermatophyte	A pathogenic fungus that grows on skin, mucous membranes, hair, nails, feathers, and other body surfaces, causing ringworm and related diseases
DNA	Deoxyribonucleic Acid
Dysbiosis	Microbial imbalance on or inside the body.
FDA	Food and Drug Administration, United States
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
ICF	Informed Consent Form
IEC	Independent Ethics Committee
IP	Investigational Product
KOH	Potassium hydroxide test to diagnose fungal infection
Microbiome	Microbes, such as bacteria, fungi, and viruses, and their genes, that naturally live on human skin
PBS	Phosphate-buffered saline, an isotonic solution that is used in many biological research applications
PHI	Protected Health Information
qPCR	Quantitative polymerase chain reaction
RNA	Ribonucleic Acid
SAE	Serious Adverse Event
SAP	Statistical analysis plan
Scrub	A liquid-based, multipurpose, sample collection and preservation system that maintains viability of aerobic, anaerobic and fastidious bacteria and other fungi.

Sponsor	DermBiont Inc.
TEAEs	Treatment-emergent Adverse Events
TV	Tinea Versicolor
Vehicle	A dermatologic drug product that enhances delivery and efficacy of the active ingredient or compound

SYNOPSIS

NAME OF SPONSOR: DermBiont Inc. **NAME OF FINISHED PRODUCT**: DBI-002 Probiotic **NAME OF ACTIVE INGREDIENT(S):** DBI-002 Probiotic – [redacted] at three escalating doses (10^6, $10^8$and 10^{10} CFUs/ml)	
Title of Study	A Randomized, Double-Blinded, Aqueous Gel-Controlled Dose Escalating Trial to Study the Safety and Antimicrobial Efficacy of DBI-002 Probiotic vs. Aqueous Gel and Vehicle Gel vs. Aqueous Gel in Adults with Tinea Versicolor Aqueous
Protocol Number	DBI-202
Investigator(s)	[redacted]
Study Centre(s)	[redacted]
Publication	N/A
Phase of Development	Phase 2A
Objectives	**Primary Objective:** **Safety** • Evaluate the tolerability of a single application and five consecutive daily applications of investigational product (IP) DBI-002 and aqueous gel in patients with Tinea Versicolor (TV). **Antimicrobial Efficacy** • Evaluate the effect of a single application and five consecutive daily applications of IP DBI-002 and aqueous gel on abundance and relative abundance of *Malassezia species*. Evaluate the effect of a single application or five consecutive daily applications of aqueous gel and vehicle gel on the microbiome of the TV-affected areas.

	Secondary Objectives: • Evaluate the effect of a single application and five consecutive daily applications of DBI-002 gel on the presence and abundance of the probiotic strain [redacted] which is in the DBI-002 gel • Evaluate the effect of a single application and five consecutive daily applications of DBI-002 on the abundance and diversity of microorganisms in the cutaneous microbiome. • Evaluate the effect of a single application and five consecutive daily applications of aqueous gel and vehicle gel on the abundance and diversity of microorganisms in the cutaneous microbiome. • Evaluate the effect of a single application and five consecutive daily applications of aqueous gel and vehicle gel on the signs and symptoms of TV.
Study Design and Evaluation Methods	• A randomized, double-blinded aqueous gel-controlled, bilateral within-subject, comparison trial of IP DBI-002 probiotic vs. aqueous gel and vehicle gel vs. aqueous gel in adults with TV. • Subjects meeting the inclusion/exclusion criteria at screening will be enrolled into the study. • Three cohorts with approximately six subjects each and one cohort with approximately four subjects will be enrolled in the study. • Subjects will be enrolled to either receive a single (Day 1) treatment application dose, or daily treatment application doses for five consecutive days (Days 1 through Day 5). • Each subject will have 2 anatomical locations identified with TV: one on the chest and other on the back. • Anatomical locations will be treated with the assigned test article; one location (either on chest or back) will be treated with IP or vehicle gel, and a control site (on back or chest) will be treated with aqueous gel.

	• Microbiological samples will be collected from the designated TV locations on the chest and back (see Table 3 in section 6.6.3). • Each of the two application areas will comprise of an area of approximately 100 cm^2 which is approximately equivalent to the area of the palm of the hand. • At baseline prior to IP, vehicle gel, or aqueous gel application and then on study days 5 and 14, samples for microbiologic evaluation will be collected from designated two anatomic locations. • Clinical evaluations of **each treated TV area** will be scored for disease severity by grading the scaling, erythema, pruritus, and dyspigmentation (hypo or hyperpigmentation) as none, mild, moderate, or severe. • In addition, local tolerability will be evaluated by grading signs and symptoms (pain/burning/stinging, edema, scabbing/crusting). In the event of early termination from the study, the Day 14/final visit study procedures will be performed prior to termination, if possible.
Study Duration	Approximately 3-4 months from enrollment of the first subject until the last subject's final study visit.
Study Population	Males and females ages 18-65 years old, in general good health with TV on the trunk (chest and back).
Number of Subjects	Approximately 22 subjects to be enrolled into 4 Cohorts – with approximately 4 or 6 subjects per cohort
Number of Clinical Sites (Study Centers):	One. Additional clinical sites may be activated, if necessary.
Inclusion Criteria	Subjects must meet all of the following criteria to be included in the study: 1. Ability to understand, agree to and sign the study Informed Consent Form (ICF). If the subject is unable to provide consent him/herself, the subject's legally acceptable representative may provide written consent. 2. Male or Female Subjects of any race 18 - 65 years of age. 3. Subjects with a clinical diagnosis of Tinea versicolor (TV is a common benign superficial cutaneous fungal infection usually characterized by hypopigmented or

	hyperpigmented macules and patches on the chest and back). 4. A positive potassium hydroxide (KOH) examination consistent with *Malassezia* prior to the treatment period done at the clinical site. 5. Agree to not use soap and water on the chest and back for at least 12 hours before the study visits for Baseline/Day 1, Day 5, and Day 14, and follow all study instructions for use of soap and water on the chest and back during participation in the study.
Exclusion Criteria	Subjects with the following will be excluded from this study: 1. Females who are pregnant, planning a pregnancy, or breastfeeding. 2. Any dermatological conditions that could interfere with clinical evaluations or any disease state or physical condition which might expose the patient to an unacceptable risk by study participation. 3. Any underlying disease(s) or other dermatological conditions that require the use of exclusionary topical or systemic therapy (see below). 4. Known sensitivity to any of the components of the study medication. 5. Use of a prescription or non-prescription topical treatment on the targeted TV anatomical locations within the previous 4 weeks, for example: anti–fungal, antibacterial or anti-microbial products, selenium and anti-inflammatories (e.g., corticosteroids). 6. Use of a systemic anti-fungal or antibiotic treatment for TV within the previous 4 weeks, Use of medicated shampoos and/or soaps within the previous 4 weeks. 7. Treatment of any type of cancer within the last 6 months.

	8. History of any significant internal disease which contraindicates use of live microbiome (e.g. leukemia, liver failure, cardiovascular disease). 9. Subjects who are known to be allergic to any of the test product(s) or any components in the investigational product(s) or history of hypersensitivity or allergic reactions to any of the study preparations as described in the Investigator's Brochure (IB). 10. AIDS or AIDS related complex by medical history. 11. Known or suspected use of immunosuppressive medications and/or has a known or suspected autoimmune disease. 12. Any subject not able to meet the study attendance requirements. 13. Subjects who have participated in any other trial of an investigational /drug or device within 30 days prior to enrollment or participation in a research study concurrent with this study.
Investigational Product dosing, vehicle gel, aqueous gel and mode of administration	The IP, vehicle gel, and aqueous gel will be applied by study personnel at the investigative site. Each subject will have either a single topical application, or a daily topical application for five consecutive days of approximately 0.1 ml of each of the test articles to 100 cm^2 area of chest and back affected with TV. The IP is formulated in a low viscosity aqueous gel and approximately 0.1 ml. The three IP groups and one vehicle vs. aqueous gel group are: Cohort 1: 10^6 CFUs /ml of DBI-002 probiotic IP vs. aqueous gel Cohort 2: 10^8 CFUs /ml of DBI-002 probiotic IP vs. aqueous gel Cohort 3: 10^{10} CFUs /ml of DBI-002 probiotic IP vs. aqueous gel Cohort 4: Vehicle gel vs. aqueous gel
Subject Participation Duration	Approximately 2 weeks.

Duration of Treatment	A single (Day 1) treatment application, or once daily treatment application for five consecutive days (Day 1, 2, 3, 4, and 5) followed with a study visits at Day 5 and/or Day 14.
Statistical Methods	Descriptive statistics will be used to provide an overview of the efficacy and safety results. For categorical parameters, the number and percentage of subjects in each category will be presented. For continuous parameters, descriptive statistics will include n (number of subjects), mean, and standard deviation, median, minimum, and maximum. Appropriate inferential statistics will be used for the primary and secondary efficacy variables in an exploratory manner.
Adverse Events	During the study, subjects will be assessed for the occurrence of new and ongoing adverse events (AEs). Descriptions of AEs will include the dates of onset and resolution (if resolved), maximum severity, and seriousness, corrective treatment, outcome, and the Investigator's assessment of causality. During the study, AEs will be followed to resolution (return to normal or to the baseline state) or until clinically stable as determined by the Investigator. At the final study visit (Day 14), study-related AEs will be followed until resolution or until clinically stable as determined by the Investigator.
Primary Endpoint Safety	Tolerability will be evaluated through assessment of TV disease state severity and signs and symptoms of local tolerability (pain / burning / stinging, pruritus, erythema, edema, and scabbing / crusting) at the application locations and review of adverse events.
Primary Endpoint Antimicrobial Efficacy	• Compare the change from baseline in quantity of *Malassezia species* based on molecular diagnostic quantitative polymerase chain reaction (qPCR) performed on samples collected from test sites at baseline prior to treatment at Days 5 and 14. • Compare the change from baseline in the microbiome community and relative abundance of *Malassezia species* based on DNA sequencing performed on samples collected from test sites at baseline prior to treatment at Days 5 and 14.

Secondary Endpoints:	• Proportion of samples at each time point in which *Malassezia species* can be detected by molecular diagnosis (qPCR). • Proportion of samples at each time point in which the probiotic strain [redacted] can be detected by molecular diagnosis (qPCR). • Proportion of samples at each time point in which the probiotic strain [redacted] can be detected by bacterial culture. • Presence and abundance of live probiotic strain [redacted] on samples collected from test sites at baseline prior to treatment at Days 5 and 14. Not applicable to aqueous gel. • Changes in the signs and symptoms of TV • Change from KOH positive to KOH negative
Exploratory Endpoints	• Mean difference from baseline in individual signs and symptoms of TV at Days 5 and 14 between treatment groups and compared to baseline.

Study Design/Type
This is a single center, randomized, double-blinded, vehicle and aqueous gel -controlled, within-patient, phase 2 comparison trial.
The trial includes a Screening and Enrollment period of up to 28 days, a 1-day or 5-day Treatment Period, and a Safety Follow-Up Period to Day 14.

Subjects meeting the inclusion/exclusion criteria at screening will be enrolled into one of four cohorts of the study. Each of the three DBI-002 probiotic cohorts will have two subjects enrolled to receive a single treatment dose, and four subjects enrolled to receive a once daily treatment dose for five consecutive days. The vehicle gel vs. aqueous gel cohort will have four subjects enrolled to receive a once daily treatment dose for five consecutive days.

Each subject will be randomly assigned to have the assigned dose of DBI-002 probiotic, vehicle gel, or aqueous gel applied to the TV-affected skin location (~100 cm^2) on their chest or back. Neither the subject nor the evaluating Investigator will know to which treatment group each TV-affected skin location has been assigned.

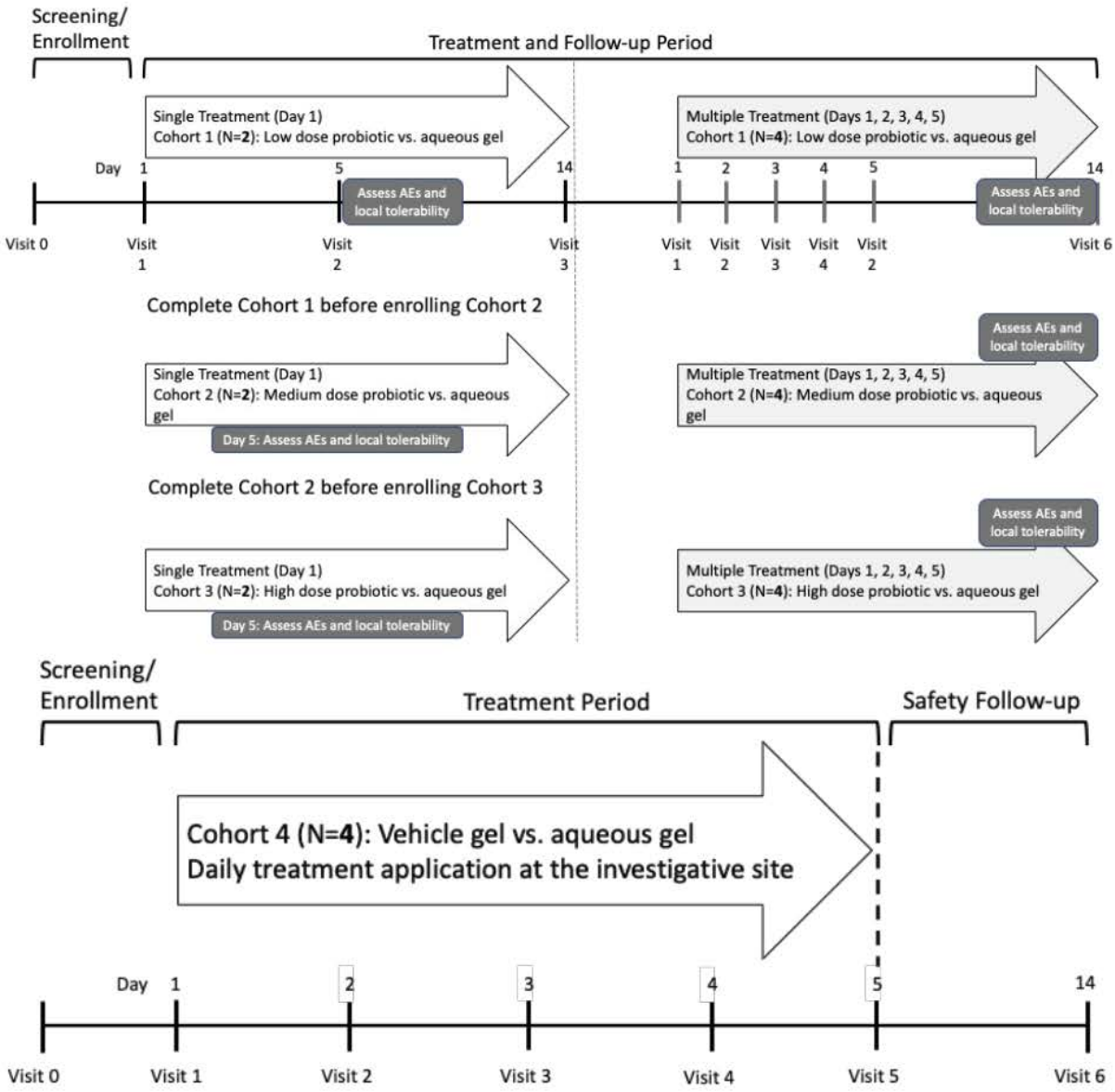
Subjects enrolled to receive a single treatment dose will have study visits scheduled to occur on Day 0 for screening and enrollment, Day 1 baseline/treatment, and Day 5 and Day 14 (±2 days) for follow-up. The screening and baseline/treatment visits may be combined if all eligibility criteria are met. There will be 3 or 4 study visits for subjects enrolled to receive a single treatment.

Subjects enrolled to receive a once daily treatment dose for five consecutive days will have study visits scheduled to occur at screening/baseline and on treatment period study days, Days 1, 2, 3, 4, and 5, and for a safety follow-up visit on Day 14 (±2 days). The screening and baseline/treatment visits may be combined if all eligibility criteria are met. There will be 6 or 7 study visits for subjects enrolled to receive multiple treatments.

For each of the three DBI-002 probiotic cohorts, the single-treatment subjects (approximately 2) will be enrolled first. After every single-treatment subject has completed the Day 5 visit, local tolerability and adverse events (AEs) will be assessed. If there are no safety signals, enrollment of the cohort's multiple treatment subjects (approximately 4) will begin. After every multiple treatment subject has completed the Day 14 visit, local tolerability and adverse events (AEs) will be assessed. If there are no safety signals, enrollment of the next cohort will begin. Cohort 1 must be completed before enrolling Cohort 2. Cohort 2 must be completed before enrolling Cohort 3. If there are safety signals specific to local tolerability and AEs from either of the first two cohorts, the following dose escalating cohort may be not be enrolled if the Investigator and sponsor are in agreement.

Cohort 4 subjects will receive multiple treatments, Enrollment of Cohort 4 is independent of the three probiotic cohorts.

Study Flowchart



Subjects enrolled to the single-treatment dose groups:
Visit 0 (may be combined with Visit 1): Screening and enrollment visit, KOH test of TV-affected chest and back skin locations

Visit 1: Baseline visit, Urine pregnancy test as applicable, clinical grading of signs and symptoms of TV, randomization of IP or aqueous gel to chest and back, scrub samples and samples for bacterial culture of IP and vehicle gel TV-affected anatomical locations prior to applications, application of IP and vehicle gel, adverse event recording

Visit 2: Clinical grading of signs and symptoms of TV, scrub samples and samples for bacterial culture of IP and aqueous gel TV-affected anatomical locations, clinical assessment of local tolerability, adverse event recording

Visit 3: Final study visit. Urine pregnancy test as applicable, clinical grading of signs and symptoms of TV, KOH test of TV-affected chest and back skin locations, scrub samples and samples for bacterial culture of IP and aqueous gel TV-affected anatomical locations, adverse event recording, standard-of-care TV treatment may be provided at the discretion of the Investigator

Subjects enrolled to the multiple treatment dose groups:
Visit 0 (may be combined with Visit 1): Screening and enrollment visit, KOH test of TV-affected chest and back skin locations

Visit 1: Baseline visit, Urine pregnancy test as applicable, clinical grading of signs and symptoms of TV, randomization of IP or vehicle gel or aqueous gel to chest and back, scrub samples and samples for bacterial culture of IP, aqueous gel, and vehicle gel TV-affected anatomical locations prior to first applications, application of IP and vehicle gel, adverse event recording

Visits 2, 3, 4: Clinical grading of signs and symptoms of TV, application of IP, aqueous gel, or vehicle gel per randomization to TV-affected locations, clinical assessment of local tolerability, adverse event recording

Visit 5: Clinical grading of signs and symptoms of TV, scrub samples and samples for bacterial culture of IP, aqueous gel, and vehicle gel TV-affected anatomical locations prior to final applications, application of IP, aqueous gel, or vehicle gel, clinical assessment of local tolerability, adverse event recording

Visit 6: Final study visit. Urine pregnancy test as applicable, clinical grading of signs and symptoms of TV, KOH test of TV-affected chest and back skin locations, scrub samples and samples for bacterial culture of IP, vehicle gel, and aqueous gel TV-affected anatomical locations, adverse event recording, standard-of-care TV treatment may be provided at the discretion of the Investigator

1 2INTRODUCTION

1.1 Background Information

The human epithelial surfaces of the skin, gut, genital tract, and respiratory tract are at the interface with the outside world and are constantly exposed to physical, chemical, bacterial, viral, and fungal challenges. Recently, the importance of healthy microbial communities on the skin to prevent and treat disease has become apparent. It is now well recognized that all human epithelial surfaces have a microbial community that can contribute to the health of the host organism, but an imbalanced or abnormal microbiome, also known as dysbiosis, can lead to a state of disease. Supplementation or replacement of a dysbiotic microbiome with beneficial or probiotic microbes has been quite successful in other systems (Van Nood et al., 2013). There is great potential for probiotics, and the metabolites they produce, to be used as therapeutics in preventing and treating skin diseases through topical application (George Kerry et al., 2018; Roudsari et al., 2015).

In addition, a postbiotic, or the dead bacterial material and metabolites that are left behind after live, active probiotic cells have been removed, can be used to improve disease symptoms through direct immunomodulation or clinical effect (Wegh et al., 2019). For example, subjects with atopic dermatitis experienced symptom relief after consuming a *Lactobacillus* postbiotic or a postbiotic in combination with a probiotic. Postbiotics often include metabolites, antimicrobial peptides, fragments of dead bacterial cells, proteins, lipids, polysaccharides, and cell lysates (Wegh et al., 2019). Probiotics, postbiotics, and a combination of both are thought to benefit the host by contributing to immune homeostasis and maintaining a healthy microbiome.

There is a third class of microbiome-based products, namely prebiotics, which are nutrients that have an effect on the microbiome. Prebiotics can be composed of anything that microbes can use as a nutritional source and include plant fibers, simple sugar carbon sources, as well as nitrogen sources. One can think of prebiotics as fertilizers for the microbial community. Formulations of prebiotics often include simple sugars used to stabilize and protect the probiotic during manufacturing and storage. These cryoprotectants are potentially prebiotics and might have therapeutic effects. This is the reason that the current protocol if comparing the vehicle against and aqueous gel. The vehicle in addition to water also contains the gelling agent CMC as well as cryoprotectants which are simple sugars and potential prebiotics. This is the reason that an aqueous gel containing just water and CMC is included as a control in this trial.

Fungi are important members of a healthy skin microbiome and *Malassezia spp*. are the most dominant fungi on most areas of the skin. *Malassezia spp*. are dependent on lipids for survival and usually grow sparsely in yeast form on the skin without causing a rash or symptoms. However, there is evidence that *Malassezia spp*. cause a number of skin disorders, including tinea versicolor (TV) (Sparber & LeibundGut-Landmann, 2017). Fourteen different species of *Malassezia* have been identified and the most common species cultured from TV are *M. globosa, M. restricta and M. sympodialis.*

The reason *Malassezia spp*. grow more actively on the skin of patients that exhibit tinea versicolor is unknown at present but may involve the activation of a tryptophan-dependent metabolic pathway. There are three common classifications of TV, white (hypopigmented), brown (hyperpigmented), and pink (inflamed). Hypopigmentation, or loss of color, is potentially caused by a fungal metabolite altering melanocyte function. Alternatively, there is evidence that *Malassezia* can also induce enlarged melanosomes in basal melanocytes, causing a brown hyperpigmented type of TV, and it is easiest to see the *Malassezia spp*. hyphae in this type. Inflammation and dermatitis from the fungus itself or its metabolites is responsible for the pink color of the third type of TV. Inflamed TV and seborrheic dermatitis may co-exist, and all three types of TV can co-exist but are usually considered distinct. TV occurs more often in teenagers and young adults and appears on the back neck, upper chest, shoulders, armpits and upper arms. TV is more likely to occur in hot, humid conditions or in patients with a suppressed immune system. Although it is not considered infectious in the conventional sense, TV sometimes affects more than one member of a family. TV may chronically recur in certain predisposed patients (Theelen et al., 2018). The most common treatment of *Malassezia* is with antifungal azoles, often ketoconazole. A more effective treatment that targets the root cause of TV is needed due to the increasing incidences of persistent cases and antifungal-resistance in fungi in general (Perlin et al., 2017), including some *Malassezia spp*. (Peano et al., 2020). In many cases, topical treatment of Malassezia using common azoles is complicated by, not only antifungal resistance, but also side effects, poor compliance, and inconsistent clinical efficacy (Angiolella et al., 2017).

The use of topical live bacterial products to treat skin diseases is a new and rapidly advancing therapeutic approach. To date, at least three live bacterial products have been studied in the clinic. An ammonia oxidizing bacterium has been used in Phase 2 trials to treat acne at a dose of 10^9 CFUs/ml (Whitlock 2017). In addition, a coagulase-negative *Staphylococcus* strain at a dose of 10^7CFUs/ml(Williams & Gallo, 2015) has been used in atopic dermatitis patients, a strain of *P. acne* has been applied to the face of acne patients at a dose of 10^8CFUs/ml (NCT03450369), and [redacted] has been applied in studies in Tinea pedis and atopic dermatitis [redacted]

A genomic analysis [redacted] using antiSmash (Blin et al., 2013; Medema et al., 2011), found genes for a number of secondary metabolites: betalactone, phosphonate, resorcinol, ectoine, terpene, among others. No virulence factors were found by genome analysis with VFDB (Virulence Factor Database; (Chen et al., 2016)) *In vitro* antibiosis assays performed by DermBiont demonstrated that the strain [redacted] used in DBI-002, strain significantly inhibited the growth of Malassezia, *S. aureus* and *Trichophyton rubrum.*

[redacted]

Given the above evidence, there is potential that the topical application of DBI-002 probiotic will improve the treatment of common diseases caused by *Malassezia*.

The purpose of the current protocol is to establish the safety and tolerability of (a) a single treatment dose application, and (b) once daily applications for five consecutive days of [redacted] to the lesions of TV. This study will explore the effect of [redacted] on the abundance of *Malassezia species* as the signs and symptoms of TV will also be evaluated. Assuming *Malassezia species* abundance is significantly decreased and there is good safety and tolerability in this trial the sponsor will then design a trial to determine if a decrease in *Malassezia species* abundance results in improvement in the signs and symptoms of TV where *Malassezia species* play an important causative role. [redacted]

1.2 Rationale

In-vitro, [redacted] has been shown to significantly inhibit the growth of *Malassezia species* most frequently associated with TV and Seborrheic Dermatitis. Based on these observations, topical application of the investigational product (IP) probiotic DBI-002 [redacted] could treat TV and other skin diseases associated with an increased abundance of *Malassezia species.* [redacted]

2 POTENTIAL RISKS AND BENEFITS

2.1 Potential Risks

[redacted] the active ingredient in IP probiotic DBI-002, has been reported to be an opportunistic pathogen. Most infections caused by this organism have been **nosocomial** (originating in a hospital) and often as a result of the contamination of hospital equipment or fluids and have occurred in **immunocompromised** hosts (Tena et al., 2015).

It is possible that once daily applications for five consecutive days of IP [redacted] could result in a worsening of the TV. It is possible, but unlikely, that the application of IP [redacted] could result in a localised infection, cellulitis, or a systemic bacterial infection. For the skin scrub sample collection, phosphate-buffered saline (PBS) with a mild detergent solution will be used and it could cause a local irritant reaction, although also unlikely.

2.2 Potential Benefits

In vitro antibiosis assays performed by DermBiont demonstrated that the [redacted] strain used in the IP probiotic DBI-002 significantly inhibited the growth of *Malassezia*. It is possible that either a single treatment or once daily applications for five consecutive days of the IP probiotic DBI-002 could result in improvement or even a clinical and microbiologic cure of the subject's TV. It should be noted that if worsening of the TV or more severe disease were to occur, the [redacted] strain is susceptible to commonly used oral antibiotics as well as topical antimicrobials.

3 STUDY OBJECTIVE AND PURPOSES

The purpose of this study is to evaluate the safety and tolerability of DBI-002 in patients with active TV. Secondly, the purpose is to observe the effect of the IP on the abundance of *Malassezia species*. Thirdly, the purpose is to evaluate presence or persistence of [redacted] strain following either a single treatment application or once daily applications for five consecutive days of DBI-002 IP gel. Fourthly, the purpose is to explore any probiotic effect of the vehicle gel. Lastly, the purpose is to observe the effect of application of DBI-002 on the signs and symptoms of active TV.

4 STUDY DESIGN AND ENDPOINTS

4.1 Description of the Study Design

4.1.1 Study Design/Type

This is a randomized, double-blinded, aqueous gel-controlled, within-patient, comparison trial examining the safety and effect of different doses of DBI-002 probiotic vs. aqueous gel and vehicle gel vs. aqueous gel in patients with Tinea versicolor.

This is a single center Phase 2A clinical trial. The trial includes a Screening and Enrollment period of up to 28 days, a 1-day or 5-day Treatment Period, and a Safety Follow-Up Period to Day 14. Study visits will occur at screening/baseline and on treatment period study days Day 1

if enrolled to receive a single treatment dose, and Days 1, 2, 3, 4, and 5 if enrolled to receive multiple treatment doses. A safety follow-up visit will occur on Day 14 (±2 days). There will be 3-4 scheduled visits for subjects enrolled to receive a single treatment dose, and 6-7 scheduled visits per subject enrolled to receive multiple treatment doses. All treatments will be performed at the investigative site during the study. Subjects meeting the inclusion/exclusion criteria at screening will be enrolled into one of three cohorts of the study. Each of the probiotic cohort groups (Cohorts 1, 2, 3) will enroll approximately 6 subjects. The vehicle gel vs. aqueous gel group (Cohort 4) will enroll approximately 4 subjects.

Each subject will be randomly assigned to have the assigned dose of DBI-002 probiotic, vehicle gel, or aqueous gel applied to the TV-affected skin location (~100 cm^2) on their chest or back. Neither the subject nor the evaluating investigator will know to which treatment group each TV-affected skin location has been assigned.

For each of the three probiotic cohorts, the single-treatment subjects (approximately 2) will be enrolled first. After every single-treatment subject has completed the Day 5 visit, local tolerability and adverse events (AEs) will be assessed. If there are no safety signals, enrollment of the cohort's multiple treatment subjects (approximately 4) will begin. After every multiple treatment subject has completed the Day 14 visit, local tolerability and adverse events (AEs) will be assessed. If there are no safety signals, enrollment the next cohort will begin. Cohort 1 must be completed before enrolling Cohort 2. Cohort 2 must be completed before enrolling Cohort 3. If there are safety signals specific to local tolerability and AEs from either of the first two cohorts, the following dose escalating cohort may be not be enrolled if the Investigator and sponsor are in agreement.

Cohort 4 subjects will receive multiple treatments, Enrollment of Cohort 4 is independent of the three probiotic cohorts, meaning that subjects may be enrolled before, in parallel with, or after the probiotic cohorts.

Subjects enrolled to the single-treatment dose groups:
Visit 0 (may be combined with Visit 1): Screening and enrollment visit, KOH test of TV-affected chest and back skin locations

Visit 1: Baseline visit, Urine pregnancy test as applicable, clinical grading of signs and symptoms of TV, randomization of IP or vehicle gel to chest and back, scrub samples and samples for bacterial culture of IP and aqueous gel TV-affected anatomical locations prior to applications, application of IP and aqueous gel, adverse event recording

Visit 2: Clinical grading of signs and symptoms of TV, scrub samples and samples for bacterial culture of IP and aqueous gel TV-affected anatomical locations, clinical assessment of local tolerability, adverse event recording

Visit 3: Final study visit. Urine pregnancy test as applicable, clinical grading of signs and symptoms of TV, KOH test of TV-affected chest and back skin locations, scrub samples and samples for bacterial culture of IP and aqueous gel TV-affected anatomical locations, adverse

event recording, standard-of-care TV treatment may be provided at the discretion of the Investigator

Subjects enrolled to the multiple treatment dose groups:
Visit 0 (may be combined with Visit 1): Screening and enrollment visit, urine pregnancy test as applicable, KOH test of TV-affected chest and back skin locations

Visit 1: Baseline visit, clinical grading of signs and symptoms of TV, randomization of IP or vehicle gel to chest and back, scrub sample and bacterial culture of IP, aqueous gel, or vehicle gel TV-affected anatomical locations prior to first applications, application of IP, aqueous gel, or vehicle gel, adverse event recording

Visits 2, 3, 4: Clinical grading of signs and symptoms of TV, application of IP, aqueous gel, or vehicle gel per randomization to TV-affected locations, clinical assessment of local tolerability, adverse event recording

Visit 5: Clinical grading of signs and symptoms of TV, scrub sample and bacterial culture of IP, aqueous gel, or vehicle gel TV-affected anatomical locations prior to final applications, application of IP, aqueous gel, or vehicle gel, clinical assessment of local tolerability, adverse event recording

Visit 6: Final study visit. Urine pregnancy test as applicable, clinical grading of signs and symptoms of TV, KOH test of TV-affected chest and back skin locations, scrub sample and bacterial culture of IP, aqueous gel, and vehicle gel TV-affected anatomical locations, adverse event recording, standard-of-care TV treatment may be provided at the discretion of the Investigator

4.2 Study Endpoints

4.2.1 Primary Endpoint

a. Safety

Tolerability will be evaluated through assessment of TV disease state severity and signs and symptoms of local tolerability (pain/burning/stinging, pruritus, erythema, edema, and scabbing/crusting) at the application locations and review of adverse events.

b. Antimicrobial Efficacy

- Compare the change from baseline in quantity of *Malassezia species* based on molecular diagnostic quantitative polymerase chain reaction (qPCR) performed on samples collected from test sites at baseline prior to treatment at Days 5 and 14.

- Compare the change from baseline in the microbiome community and relative abundance of *Malassezia species* based on DNA sequencing performed on samples collected from test sites at baseline prior to treatment at Days 5 and 14.

4.2.2 Secondary Endpoints

- Proportion of samples at each time point in which *Malassezia species* can be detected by molecular diagnosis (qPCR).
- Proportion of samples at each time point in which the probiotic strain [redacted] can be detected by molecular diagnosis (qPCR).
- Proportion of samples at each time point in which the probiotic strain [redacted] can be detected by bacterial culture.
- Presence and abundance of live probiotic strain [redacted] on samples collected from test sites at baseline prior to treatment at Days 5 and 14. Not applicable to aqueous gel.
- Changes in the signs and symptoms of TV
- Change from KOH positive to KOH negative

4.2.3 Exploratory Endpoint

- Mean difference from baseline in individual signs and symptoms of TV at Days 5 and 14 between treatment groups and compared to baseline.

5 INVESTIGATIONAL PRODUCT (IP)

Table 2. Investigational Product and Aqueous Gel Cohorts, IP descriptions, and doses.

Cohorts	Investigational Product (IP)	Doses per Subject
Cohort 1: 6 subjects	Low dose 10^6 CFUs /ml of DBI-002 probiotic vs. aqueous gel	Single treatment dose OR Once-daily application for 5 consecutive days
Cohort 2: 6 subjects	Medium dose 10^8 CFUs /ml of DBI-002 probiotic vs. aqueous gel	Single treatment dose OR Once-daily application for 5 consecutive days
Cohort 3: 6 subjects	High dose 10^{10} CFUs /ml of DBI-002 probiotic vs. aqueous gel	Single treatment dose OR Once-daily application for 5 consecutive days
Cohort 4: 4 subjects	Vehicle gel vs. aqueous (CMC) gel	Once-daily application for 5 consecutive days

6 ELIGIBILTY CRITERIA

6.1 Inclusion Criteria

Subjects must meet all of the following criteria to be included in the study:

1. Ability to understand, agree to and sign the study Informed Consent Form (ICF). If the subject is unable to provide consent him/herself, the subject's legally acceptable representative may provide written consent.
2. Male or Female Subjects of any race 18 - 65 years of age.
3. Subjects with a clinical diagnosis of Tinea versicolor (TV).
4. A positive potassium hydroxide (KOH) examination consistent with Malassezia prior to the treatment period done at the clinical site.
5. Agree to not use soap and water on the chest and back for at least 12 hours before the study visits for Baseline/Day 1, Day 5, and Day 14, and follow all study instructions for use of soap and water on the chest and back during participation in the study.

6.2 Exclusion Criteria

1. Females who are pregnant, planning a pregnancy, or breastfeeding.
2. Any dermatological conditions that could interfere with clinical evaluations or any disease state or physical condition which might expose the patient to an unacceptable risk by study participation.
3. Any underlying disease(s) or other dermatological conditions that require the use of exclusionary topical or systemic therapy (see below).
4. Known sensitivity to any of the components of the study medication.
5. Use of a prescription or nonprescription topical treatment on the targeted lesions TV anatomical locations within the previous 4 weeks, for example: anti-fungal, antibacterial or anti-microbial products, selenium and anti-inflammatories (e.g. corticosteroids).
6. Use of a systemic anti-fungal or anti-bacterial treatment for TV within the previous 4 weeks.
7. Use of medicated shampoos and/or soaps within the previous 4 weeks.
8. Treatment of any type of cancer within the last 6 months.
9. History of any significant internal disease which contraindicates use of live microbiome (e.g. leukemia, liver failure, cardiovascular disease).

10. Subjects who are known to be allergic to any of the test product(s) or any components in the investigational product(s) or history of hypersensitivity or allergic reactions to any of the study preparations as described in the Investigator's Brochure (IB).
11. AIDS or AIDS related complex by medical history.
12. Known or suspected to be taking immunosuppressive medications and/or has a known or suspected autoimmune disease.
13. Any subject not able to meet the study attendance requirements.
14. Subjects who have participated in any other trial of an investigational drug or device within 30 days prior to enrollment or participation in a research study concurrent with this study.

6.3 Recruitment and Retention

Subjects will be recruited from the Investigator's clinical dermatology practice. The investigator may also recruit subjects using Ethics Committee approved recruitment materials.

6.4 Withdrawal or Termination

6.4.1 Subject Withdrawal

Participation in the study is strictly voluntary. A subject has the right to withdraw from the study at any time for any reason and is under no obligation to disclose the reason. If a subject withdraws, the Investigator and Sponsor are to be informed immediately and the withdrawal noted on the Case Report Form. Subjects may be replaced at the discretion of the Investigator and/or Sponsor only if both are in agreement.
The Investigator has the right to terminate participation of a subject at any time for any of the following:

- Use of non-permitted concomitant medication
- Lack of subject compliance
- Protocol violation
- Disease progression
- Any perceived safety risks

6.4.2 Study Discontinuation

Study discontinuation is at the discretion of the Sponsor or the Investigator in any of, but not limited to, the following events:

- Occurrence of unusual AEs in terms of their nature, severity, causality, duration, or unexpected incidence.
- Medical or ethical reasons affecting the continued performance of the study.
- Difficulties in the recruitment of the subjects.

6.4.3 Stopping Rules

The sponsor (Medical Director/Chief Medical Officer) will halt the study for a review of the safety data if there are safety concerns noted for any reason and if one or more subjects experience two AE's assessed as a Grade 3 (severe) or higher, of the same type that is considered possibly, probably or definitely related to the IP.

6.5 Study Visits

The study will be conducted with:

1. A screening visit (Day -28 to Day 1),
2. Baseline visit (Day 1) for evaluation, sampling and treatment - may be combined with the screening visit
3. Only for subjects enrolled to receive multiple treatment doses: Treatment and evaluation visits (Days 2, 3, 4)
4. Evaluation and sampling visit (Day 5), and treatment only for subjects enrolled to receive multiple treatments
5. Safety follow-up visit (Day 14)

6.5.1 Screening Visit

Following written informed consent obtained from each subject, the Investigator will determine whether subjects are eligible to participate in the study by performing screening tests and evaluations.
Women of childbearing potential will be tested for pregnancy with a urine pregnancy test. Women who are pregnant or who have a positive pregnancy test will be excluded from the study.

(-28 Day to Day 1) Screening visit:

1. Informed consent.
2. Record demographics.
3. Review of Inclusion/Exclusion Criteria.
4. Ensure no use of soap and water on the chest and back within 12 hours before collection of Baseline scrub samples. Schedule the enrolled subject for Day 1 / Baseline visit if soap and water were used on the chest and/or back within 12 hours of the screening visit.
5. Women of childbearing potential will be tested for pregnancy with a urine pregnancy test.
6. Medical history.
7. Concomitant medication.
8. Clinical assessment of signs and symptoms of TV.
9. KOH test (TV-affected skin locations).

10. Record adverse event, if any.
11. If screening is not on the same day as all required Day / Baseline visit assessments and skin sampling, schedule an appointment for Day 1 / Baseline visit.

At the Screening visit, continuous monitoring of concomitant medications, therapies and AEs throughout the study period will begin.

6.5.2 All Other Visits

Study visit assessments will be conducted as presented in Appendix 1. The Treatment Period for subjects receiving a single treatment dose is Day/Visit 1. The Treatment Period for subjects receiving multiple treatment doses is from Day/Visit 1 through Day/Visit 5.

Day 1/Baseline visit (before the IP is applied, the following tasks will have been completed):

1. Review of Inclusion/Exclusion Criteria.
2. Ensure no direct use of soap and water for at least 12 hours on the areas where the samples are to be collected on the chest and back. After sampling and application, instruct the subject not to use soap and water on the chest and back until the morning of the next day.
3. Concomitant medications.
4. Clinical assessment of signs and symptoms of TV.
5. Score the signs and symptoms of local tolerability of the areas affected by TV.
6. Collect scrub samples from each of the TV-affected skin locations to be treated with IP, aqueous gel, and vehicle gel.
7. After collecting each scrub sample, pat the sampling location dry with wipes.
8. Collect samples for bacterial culture of the IP and vehicle gel TV-affected skin locations. Not required for vehicle gel vs. aqueous gel subjects.
9. Assigned IP, aqueous gel, and vehicle gel will then be applied to designated locations on both anterior and posterior trunk (chest or back) by investigative site staff. Approximately 0.1-0.2 ml of the IP, vehicle gel, or aqueous gel will be applied to each selected location.
10. Record any adverse events.
11. Schedule an appointment for Day 5 for subjects receiving a single treatment dose and instruct the subject to not use soap and water on the chest and back for at least 12 hours before the Day 5 visit. Schedule an appointment for the next day for the Day 2 visit for subjects receiving multiple treatment doses.
12. Instruct the subject to not wash the treated locations with soap and water on the anterior and posterior trunk (chest or back) from the time of IP, vehicle gel, and aqueous gel application at the Baseline visit until the morning of the next day.

For subjects enrolled to receive multiple treatment doses only:

Day 2 / Visit 2, Day 3 / Visit 3, Day 4 / Visit 4:

1. Clinical assessment of signs and symptoms of TV.
2. Score the signs and symptoms of local tolerability of the locations affected by TV.
3. Application of the IP, aqueous gel. and vehicle gel by investigative site staff to the assigned TV-affected skin locations.
4. Record any adverse events.
5. For the Day 2 and Day 3 visits, instruct the subject to not wash the treated locations with soap and water until the morning of the next day after IP, vehicle gel, and aqueous gel application. For the Day 4 visit, instruct the subject not to wash the treated locations with soap and water for at least 12 hours before the next day's Day 5 visit.
6. Schedule an appointment for the next day's visit.

Detailed instruction for sample collection and processing are in the Study Operation Manual, provided separately.

Day 5 / Visit 5

1. Ensure no direct use of soap and water for at least 12 hours on the areas where the scrub samples are to be collected on the chest and back. For subjects receiving multiple treatment doses, instruct the subject not to use soap and water on the chest and back for at least 12 hours after the Day 5 application.
2. Concomitant medications.
3. Clinical assessment of signs and symptoms of TV.
4. Score the signs and symptoms of local tolerability of the areas affected by TV
5. Collect scrub samples from each of the TV-affected skin locations treated with IP, vehicle gel, or aqueous gel.
6. After collecting each scrub sample, pat the sampling location dry with wipes.
7. Collect samples for bacterial culture of the IP and vehicle gel TV-affected skin locations. Not required for vehicle gel vs. aqueous gel subjects.
8. If the subject is receiving multiple treatment doses, the assigned IP, vehicle gel, or aqueous gel will then be applied to designated locations on both anterior and posterior trunk (chest or back) by investigative site staff. Approximately 0.1-0.2 ml of the IP, vehicle gel, or aqueous gel will be applied to each selected location.
9. Record any adverse events.
10. Schedule an appointment for the Day 14 visit.

Detailed instruction for sample collection and processing are in the Study Operation Manual, provided separately.

Day 14 (±2) Follow up visit (no treatment)

1. Ensure no direct use of soap and water for at least 12 hours on the areas where the scrub samples are to be collected on the chest and back.
2. Women of childbearing potential will be tested for pregnancy with a urine pregnancy test.
3. Concomitant medications.
4. Clinical assessment of signs and symptoms of TV.
5. Score the signs and symptoms of local tolerability of the areas affected by TV.
6. Collect scrub samples from each of the TV-affected skin locations that were treated with IP, vehicle gel, and aqueous gel.
7. After collecting scrub samples, pat the sampling site dry with wipes.
8. Collect samples for bacterial culture of the TV-affected skin locations that were treated with IP and vehicle gel. Not required for vehicle gel vs. aqueous gel subjects.
9. KOH test (TV-affected skin locations).
10. Record any adverse events.
11. Standard-of-care TV treatment may be provided at the discretion of the Investigator

Detailed instruction for sample collection and processing are in the Study Operation Manual, provided separately.

6.6 Study Specific Procedures

Clinical Evaluations:

All primary endpoint assessments must be done by an Investigator who is a board-certified dermatologist.

6.6.1 Grading of signs and symptoms of TV

Each treated TV lesion will be evaluated for the following component signs and symptoms of the target TV lesion: Scaling, erythema, pruritus and dyspigmentation. Each component is measured using the following scale:

Scaling score:

0	None	complete absence

1	Mild	slight
2	Moderate	definitely present
3	Severe	marked, intense

Erythema score: (redness presents specifically in the target TV lesion)

0	None	No redness
1	Mild	Mildly detectable erythema; pink
2	Moderate	Dull red; clearly distinguishable
3	Severe	Deep, dark red; marked and extensive

Pruritus score: (itching; present specifically in the target TV lesion)

0	None	No itching
1	Mild	Occasional, slight itching
2	Moderate	Constant or intermittent itching; does not disturb sleep
3	Severe	Bothersome itching that disturbs sleep or normal activity

Dyspigmentation score: (Changes in color of the target TV lesion)

0	None	No change in color
1	Mild	Minor change in color
2	Moderate	Definite change in color
3	Severe	Marked change in color

To calculate the score, the component scores are added together.

6.6.2 Local Tolerability for Areas Affected by TV (application site reaction)

Local tolerability for anatomical locations to which the IP, aqueous gel and vehicle gel are applied will be evaluated based on these signs and symptoms:
Pain/Burning: as reported by the subject as being the greatest intensity they have experienced within the last 24 hours at Baseline or since the last visit at subsequent visits.

0	None	No pain/burning
1	Mild	Slight burning/stinging sensation; not really bothersome
2	Moderate	Definite warm, burning/stinging that is somewhat bothersome
3	Severe	Hot burning/stinging sensation that causes definite discomfort and may interrupt daily activities and/or sleep.

Pruritus: as reported by the subject as being the greatest intensity they have experienced within the last 24 hours at Baseline or since the last visit at subsequent visits.

0	None	No pruritus
1	Mild	Slight pruritus, not really bothersome
2	Moderate	Definite pruritus that is somewhat bothersome
3	Severe	Intense pruritus that may interrupt daily activities and/or sleep

Erythema: as assessed by the Investigator

0	None	No erythema present
1	Mild	Slight pink coloration
2	Moderate	Definite redness
3	Severe	Marked erythema, bright red to dusky dark red in color

Edema: as assessed by the Investigator

0	None	No edema
1	Mild	Slight, but definite edema
2	Moderate	Definite edema
3	Severe	Marked edema

Scabbing/Crusting: as assessed by the Investigator

0	None	No scabbing/crusting
1	Mild	Slight, but definite scabbing/crusting
2	Moderate	Definite scabbing/crusting
3	Severe	Marked scabbing/crusting

6.6.3 Sample Collection

One representative anatomic location (chest or back), according to the grading of signs and symptoms of TV, must be classified as at least moderate to be designated as the Active Sampling (or target lesion) location on both anterior and posterior trunk (chest or back).
Subjects with both target areas affected, will have samples collected from both chest and back. One anatomical location on either chest or back will be designated as treatment site and other site as control.
KOH test will be done at the investigative site.
Scrub samples will be sent to the sponsor [redacted]
The subject's cultures will be sent to the local [redacted] microbiology laboratory.

An informational grid for sample collection and processing required for the study is shown in Table 3 below.

Table 3. Microbiological Samples.

Visit	KOH testing at the investigative site	Scrub samples for molecular diagnostics	Collect samples for bacterial culture [redacted]
Screening	Target lesion on both chest and back		
Day 1 (Baseline - Prior to dose)		Target lesion on both chest and back	Target lesion on both chest and back
Day 5		Target lesion on both chest and back	Target lesion on both chest and back
Day 14	Target lesion on both chest and back	Target lesion on both chest and back	Target lesion on both chest and back

Δ Not applicable for Cohort 4 subjects

Throughout the study, continuous monitoring of concomitant medications, therapies and AEs will occur.

6.7 Test Article (IP, vehicle gel, aqueous gel) Administration

Subjects will have either a Day 1 single treatment dose or 5 daily treatment doses (Day 1 to Day 5) of their assigned test articles (IP, vehicle gel, aqueous gel) applied by study personnel at the investigative site during the Treatment Period. Details of test article preparation will be provided in the Pharmacy Manual. Approximately 0.1 ml of the IP and vehicle gel will be applied to TV designated locations (each 100 cm^2) on treatment and control skin lesions, respectively. The test article will be allowed to air dry.

6.8 Concomitant Medication

Usage of prescription or over the counter topical or systemic medications will be reported during the subject's participation in the study. At the screening visit, the use of anti-fungal or antibacterial treatments in the past 4 weeks is an exclusion criterion.

6.9 Investigational Product

The IP, vehicle gel, or aqueous gel will be applied by study personnel at the investigative site at the baseline/Day 1 visit only for subjects receiving a single treatment dose, and once daily for 5

applications during the study for subjects receiving multiple treatment doses. Approximately 0.1 of the IP will be applied to a 100 cm^2 area of the TV-affected skin on the chest or back. The control location will be treated with 0.1ml of vehicle gel.

The doses for the three probiotic IP cohorts are 10^6 CFU/ ml, 10^8 CFU /ml, and 10^{10} CFU/ ml vs. vehicle gel.
It has been estimated that the density of bacteria on normal skin is approximately 1 x $10^5/cm^2$. At our highest dose of 10^{10} CFU/ ml applying 0.1 ml to each 100 sq.cm. site will be adding 1x10^7 CFU/cm^2 on chest or back.
The doses in this trial are comparable to the doses used in the study of other topical live bacterial products. An ammonia oxidizing bacterium has been used in Phase 2 trials to treat acne at a dose of 10^9 CFUs/ml (Whitlock 2017). In addition, a coagulase-negative *Staphylococcus* strain at a dose of 10^7/ml CFUs (Williams & Gallo, 2015) has been used in atopic dermatitis patients, and a strain of *P. acne* has been applied to the face of acne patients at a dose of 10^8/ml CFUs (NCT03450369).
Detailed information of the IP is included in the Investigator's Brochure (IB), provided separately.

6.10 Randomization

Treatment kits will be sequentially assigned to subjects. Within each kit there will be test articles (IP, vehicle gel, aqueous gel) to be applied to the chest and back. The subject and the Investigator will be blinded to the pre-defined randomization assignments of IP and vehicle gel.

6.11 Treatment Compliance

Subjects will receive either a single treatment application on one day, or once daily application of the test articles for 5 consecutive days at the investigative site by designated study personnel. This will allow for well documented compliance as the Investigator or designee will record the time, date, and other details of test article administration.

7 DATA ANALYSIS AND STATISTICAL CONSIDERATIONS

7.1 General Statistical Considerations

All statistical processing will be performed using Statistical Analysis System (SAS®) unless otherwise stated. No interim analyses are planned. Except where noted, all statistical tests will be two-sided and will be performed at the 0.05 level of significance. P-values will be provided primarily for exploratory purposes.

Descriptive statistics will be used to provide an overview of the efficacy and safety results. For categorical parameters, the number and percentage of subjects in each category will be presented. For continuous parameters, descriptive statistics will include n (number of subjects), mean, and standard deviation, median, minimum, and maximum. Appropriate inferential statistics will be used for the primary and secondary efficacy variables in an exploratory manner. Linear interpolation of efficacy variables will be performed using observed data that precedes and follows the off-schedule visit, if present. The last observation will be carried forward if all the data subsequent to the off-schedule value is missing. No adjustments will be made for multiplicity.

The number of subjects in each analysis set will be summarized. Reasons for study withdrawal will be summarized using frequencies and percentages by treatment group. Reported AEs, medical history terms and prior and concomitant procedures and therapies will be classified on the basis of [redacted]

7.2 Analysis of Efficacy Data for Objectives

7.2.1 Primary Efficacy:

The primary analysis will compare the differences at Day 5 between DBI-002 Drug Product (DP), vehicle gel, and/or aqueous gel in abundance of Malassezia based on molecular diagnostic qPCR using a 2-sided Wilcoxon sign rank test, alpha = 0.05, with a null hypothesis of median difference equal to zero.
Comparisons at baseline prior to treatment and days 5 and 14 will also be done. Additionally, a repeated measures model will be used to compare DBI-002 DP to vehicle gel, and/or aqueous gel for the primary endpoint. P-values will be provided for descriptive purposes.

7.2.2 Secondary Efficacy:

The proportion of subjects with a greater decrease in scoring of individual signs and symptoms of TV for the DBI-002 DP-treated lesion than the vehicle-treated or aqueous gel-treated lesion will be summarized descriptively for Days 5 and 14. A comparison at Day 14 of differences of abundance of live [redacted], measured by qPCR, between DBI-002 DP, vehicle gel, and/or aqueous gel treated lesioned skin will be done using a two-sided Wilcoxon sign rank test.
A comparison at Day 14, of differences of abundance and diversity of the microbiome based on DNA sequencing between DBI-002 DP, vehicle gel, and/or aqueous gel treated lesions will be done using a two-sided Wilcoxon sign rank test. Additionally, a repeated measures model will be used to compare DBI-002 DP to vehicle gel and/or aqueous gel treated lesions for the secondary endpoints. P-values will be provided for descriptive purposes.

7.2.3 Exploratory Efficacy:

A comparison of change from baseline in scoring of individual signs and symptoms of TV between DBI-002 DP, vehicle gel, and/or aqueous gel treated lesions will be done using a two-sided sign test at each of Days 5 and 14. A comparison of change from baseline in scoring of individual signs and symptoms of TV between DBI-002 DP, vehicle gel, and/or aqueous gel treated lesions will be done using a two-sided sign test at each of Days 5 and 14. Additionally, a repeated measures model will be used to compare DBI-002 DP, vehicle gel, and/or aqueous gel treated lesions for the above exploratory endpoints.

7.3 Analysis of Safety Data for Objectives

7.3.1 Local Tolerability

Signs and symptoms of local tolerability (pain / burning / stinging, pruritus, erythema, edema, and scabbing / crusting) will be summarized with changes from baseline.

7.3.2 Adverse Events

Subjects will be assessed for the occurrence of new and ongoing AEs. Descriptions of AEs will include the dates of onset and resolution (if resolved), maximum severity, seriousness, action taken regarding the investigational product, corrective treatment, outcome, and Investigator's assessment of causality. All AEs will be recorded and classified using terminology [redacted]. All reported treatment-emergent AEs (TEAEs), defined as any AE with an onset on or after the date of first study drug application, will be summarized by treatment group, the number of subjects reporting TEAEs, system organ class, preferred term, severity, and relationship to study drug. When summarizing TEAEs by severity or relationship to study drug, each subject will be counted only once within a system organ class or a preferred term using the event with the greatest severity or causality, respectively, within each category. All reported serious adverse events (SAEs) will be summarized by treatment group, the number of subjects reporting SAEs, system organ class, preferred term, severity, and relationship to study drug.

All information pertaining to AEs noted during the study will be listed by subject and will include a verbatim description of the event as reported by the Investigator, as well as the preferred term, system organ class, start date, stop date (if stopped), seriousness, severity, action taken regarding the study drug, corrective treatment, outcome and relationship to the study drug. In addition, a listing of subjects who prematurely discontinue from the study due to AEs will be provided as well as a listing of subjects who reported an SAE.

7.4 Analysis Populations

All subjects who are randomized and dispensed study drug product will be included in the Intent-to-Treat analysis set. All subjects who are randomized, receive at least one (1) confirmed dose of study drug product, and have at least one (1) post-baseline safety assessment will be included in the Safety analysis set.

7.5 Sample Size Determination

Sample size is based on clinical judgement and deemed adequate to successfully demonstrate these goals.

8 ASSESSMENT OF SAFETY

8.1 Safety Evaluations and Criteria

As detailed in section 6.6.2, local tolerability will be evaluated by recording the severity of the signs of TV as well as the signs and symptoms of local tolerability on treated areas involved with TV. In addition, adverse events will be documented.

8.2 Adverse Event

An AE is any untoward medical occurrence in a subject administered a pharmaceutical product, which does not necessarily have a causal relationship with the treatment. An AE can be any unfavorable and unintended sign (e.g., including an abnormal laboratory finding), symptom, or disease temporally associated with the use of the study drug, whether or not it is considered to be study drug related. This includes any newly occurring event or previous condition that has increased in severity or frequency since the administration of study drug (treatment-emergent).

8.3 Serious Adverse Event

A serious adverse event is any AE, occurring at any dose level and regardless of causality that:

- Results in death.
- Is life-threatening: Life-threatening means that the subject was at immediate risk of death from the reaction as it occurred, (i.e.it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form).
- Requires inpatient hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity.
- Is a congenital anomaly/birth defect.
- Is an important medical event: an important medical event is an event that may not result in death, be life-threatening, or require hospitalization but may be considered an SAE when, based upon appropriate medical judgment, it may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in the definitions for SAEs. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

A hospitalization for an elective procedure will not be considered a SAE. Any SAE occurring in the study will be reported to the IRB/IEC and Sponsor in compliance with all reporting requirements. Any SAE must be reported to the National Ethics Committee within 24 hours from the time of the investigative site's awareness.

8.4 Unexpected Adverse Event

An unexpected AE is an AE that is not reported in the Investigator's Brochure (IB).

8.5 Relationship of Adverse Events to Study Drug

The following classifications should be used when evaluating the relationship of AEs and SAEs to the investigational drug.

1. **None**: No relationship between the experience and the administration of study drug; related to other etiologies such as concomitant medications or subject's clinical state.
2. **Unlikely**: The current state of knowledge indicates that a relationship is unlikely.
3. **Possibly**: A reaction that follows a plausible temporal sequence from administration of the study drug and follows a known response pattern to the suspected study drug. The reaction might have been produced by the subject's clinical state or other modes of therapy administered to the subject.
4. **Probably**: A reaction that follows a plausible temporal sequence from administration of the study drug and follows a known response pattern to the suspected study drug. The reaction cannot be reasonably explained by the known characteristics of the subject's clinical state or other modes of therapy administered to the subject.
5. **Definitely**: A reaction that follows a plausible temporal sequence from administration of the study drug and follows a known response pattern to the suspected study drug and can be confirmed with a positive re-challenge test or supporting laboratory data.

Severity:

1. Mild: Awareness of sign or symptom, but easily tolerated
2. Moderate: Discomfort enough to cause interference with normal daily activities
3. Severe: Inability to perform normal daily activities
4. Life-threatening: Immediate risk of death from the reaction as it occurred

9 PROCEDURES FOR ADVERSE EVENT RECORDING AND SREPORTING

9.1 Emergency Sponsor Contact

In the event of a medical emergency (i.e., an event that requires immediate attention regarding the treatment of a subject, operation of the clinical study, and/or the use of investigational drug), investigational site personnel must immediately contact the Medical Monitor.

Medical Monitor Contact	
Name:	[redacted]
Telephone:	
Email:	
Name:	[redacted]
Telephone:	
Email:	

9.2 Other Required Safety Assessments

A clinically significant worsening from Baseline of any abnormal study assessment, such as physical examination, or vital signs, should be considered an AE and recorded accordingly. If possible, a diagnosis for the clinically significant study assessment should be provided by the Investigator (e.g. Urinary tract infection or anemia). In the absence of a diagnosis, the abnormal study assessment itself should be listed as the AE (e.g., bacteria in urine or decreased hemoglobin). An abnormal study assessment is considered clinically significant if the subject has one or more of the following related to the abnormal study assessment:

1. Concomitant clinical signs or symptoms.
2. Further diagnostic testing or medical/surgical intervention.
3. Discontinued from the study.

Repeat testing to determine whether the result is abnormal, in the absence of any of the above criteria, does not necessarily meet clinically significant criteria. The determination of whether the study assessment results are clinically significant must be made by the Investigator.

During the study, AEs will be followed to resolution (return to normal or to the baseline state) or until clinically stable as determined by the Investigator. At the final study visit (Day 14), study-related AEs will be followed until resolution or until clinically stable as determined by the Investigator.

10 SPECIAL REQUIREMENTS AND PROCEDURES

10.1 Study Monitoring

The Clinical Monitor and/or the Sponsor representative will arrange to visit and monitor the Investigator's site at regular intervals during the study. The monitoring visits must be conducted according to the applicable International Conference on Harmonization (ICH) and Good Clinical Practice (GCP) guidelines to ensure protocol adherence, quality of data, drug (IP) accountability, compliance with regulatory requirements, and continued adequacy of the investigational site and its facilities. During these visits, source documents to include CRFs, eCRFs and other data related to the study will be reviewed and any discrepancies or omissions will be resolved. The Clinical Monitor will be given access to study relevant source documents (including medical records) for purposes of source data verification. Remote (no visit to the investigative site) monitoring, if performed, will be conducted according to all applicable ICH and GCP guidelines.
During and/or after completion of the study, quality assurance officers named by DermBiont or the regulatory authorities may wish to perform on-site audits. The Investigator is expected to cooperate with any audit and aid and documentation (including source data) as requested.

10.2 Audits and Inspections

The Investigators and clinical sites will permit study related monitoring, audits, IRB/IEC review, and regulatory inspections as requested by FDA, DermBiont or designee, including direct access to source data/documents [i.e., original medical records, laboratory reports, hospital documents, progress reports, signed Informed Consent Forms (ICFs), etc.] in addition to eCRFs.

10.3 Data Quality Control and Quality Assurance

The Investigator is responsible for ensuring the study is conducted according to the protocol, Code of Federal Regulations (CFR), GCP, and applicable regulatory requirements. The responsibilities outlined in these documents along with the identification that a signed informed consent must be obtained prior to a subject participation in the study.

10.4 Confidentiality

To maintain subject privacy, all eCRFs, study samples, study drug accountability records, study reports and communications will identify the subject by the assigned subject identification number. The Investigator will grant monitor(s) and auditor(s) from DermBiont or designee and regulatory authority(ies) access to the subject's original medical records for verification of data gathered on the eCRFs and to audit the data collection process. The subject's confidentiality will be maintained

and will not be made publicly available to the extent permitted by the applicable laws and regulations.
All information regarding the investigational product supplied by DermBiont to the Investigator is privileged and confidential information. The Investigator agrees to use this information to accomplish the study and will not use it for other purposes without consent from DermBiont Inc. It is understood that there is an obligation to provide DermBiont with complete data obtained during the study. The information obtained from the clinical study will be used towards the development of the investigational product and may be disclosed to regulatory authority(ies), other Investigators, corporate partners, or consultants as required.

10.5 Subject Rights

Study subjects can withdraw their consent to have their clinical samples stored even after the sample has been shipped to the clinical laboratory. A study subject should contact their Investigator or the Investigator's designee and ask for his/her sample to be withdrawn from the storage and destroyed. For samples that have been partially analyzed the remaining sample will be destroyed but the clinical site and DermBiont shall be entitled to retain and use any research results obtained prior to the withdrawal of consent.

10.6 Protocol Amendments

Protocol amendments that impact subject safety, change the scope of the investigation, or affect the scientific quality of the study must be approved by the IRB/IEC and submitted to the appropriate regulatory authorities before implementation of such modifications to the study.
In the event that the protocol needs to be modified immediately to eliminate an apparent hazard to a subject, DermBiont will amend and implement the protocol change and subsequently notify the regulatory authorities and/or the IRB/IEC, as appropriate.
The Investigator must not modify the protocol without agreement from DermBiont and prior review or approval by the IRB/IEC. Any deviations from the protocol should be documented by the Investigator or designee.

10.7 Obligations of the Clinical Investigator

The Investigator will agree to be responsible for the overall conduct of the study; for completing regulatory documents and documentation of training; for ensuring that the study is conducted according to the study protocol; for protecting the rights, safety and welfare of study subjects under their care; and for insuring data quality and integrity.
If any study responsibilities are delegated, the Investigator will be responsible for maintaining written documentation of who is designate to perform the specific responsibilities.
The study will be conducted by qualified study Investigators.

The Investigator or designees are responsible for qualified subjects and collecting samples. The Investigator or designees will be responsible for maintaining records of all samples collected and investigational product administrations; recording the subject's data on the source documents and eCRFs; documenting the presence or absence of AEs following sample collection and study IP applications and following the reporting requirements of any SAEs to the Sponsor and IRB/IEC.

10.8 Institutional Review Board/Independent Ethics Committee

The Investigator must obtain written IRB/IEC approval of the protocol, approval for relevant supporting information and all types of subject recruitment and advertisement and the ICF prior to starting the study. The IRB/IEC will meet all US FDA requirements governing IRBs/IECs (21 CFR Part 56).
DermBiont or the designee must approve the ICF submitted to the investigational site's IRB/IEC. All subject recruitment and advertisements must be submitted to DermBiont or designee prior to submission to the IRB/IEC, for review.

10.9 Ethical Conduct of the Study

DermBiont and the Investigator must comply with all instructions, regulations, and agreements in this protocol and in the applicable ICH and GCP guidelines and must also conduct the study in accordance with local regulations. All protocol deviations and violations will be included in the required IEC reports in accordance with local requirements and on the appropriate forms.

10.10 Written Informed Consent

Written informed consent is required from each subject prior to any testing under this protocol, including screening tests and evaluations. The ICF, as specified by the investigational site's IRB/IEC, must follow the Protection of Human Subjects regulations listed in 21 CFR Part 50.
The background of the proposed study and the benefits and risks of the procedures and study must be explained to the subjects. It is the responsibility of the Investigator to obtain consent and to provide the subject with a copy of the signed and dated ICF. Confirmation of a subject's informed consent must also be documented in the subject's medical record prior to any testing under this protocol, including screening tests and evaluations.
All ICFs used in this study must be approved by the appropriate IRB/IEC and by DermBiont or designee. The ICF must not be altered without the prior agreement of the relevant IRB/IEC and DermBiont.

10.11 Subject Data Protection

Prior to any testing under this protocol, including screening tests and evaluations, subjects must authorize the release and use of protected health information (PHI), as described in the ICF.

11 DATA HANDLING AND RETENTION OF RECORDS

11.1 Paper and Electronic Case Report Form Completion

Paper CRF and eCRFs will be completed for each enrolled subject. It is the Investigator's responsibility to ensure the accuracy, completeness, and timeliness of the data reported in the subject's paper and eCRF. Source documentation supporting the paper and eCRF data should indicate the subject's participation in the study and should document the dates and details of study procedures, AEs, and subject status.

Investigators will maintain copies of the paper CRF and eCRFs at the clinical site. The paper CRF eCRFs will be completed as much as possible for subjects who discontinue or are terminated from the study by the investigator, and the reason for the discontinuation or termination must be clearly and concisely specified on the appropriate paper CRF and eCRF.

11.2 Retention of Records

The Investigator will maintain all study records according to ICH GCP and applicable regulatory requirements. Records will be retained for at least 2 years after the last marketing application approval or 2 years after formal discontinuation of the clinical development of the investigational product or according to applicable regulatory requirements. If the Investigator withdraws from the responsibility of keeping the study records, custody must be transferred to a person willing to accept the responsibility. DermBiont must be notified in writing if a custodial change occurs.

12 REFERENCES

[redacted]

Angiolella, L., Carradori, S., Maccallini, C., Giusiano, G., & Supuran, C. T. (2017). Targeting

Malassezia species for Novel Synthetic and Natural Antidandruff Agents. *Current Medicinal Chemistry*, *24*(22), 2392–2412. https://doi.org/10.2174/0929867324666170404110631

[redacted]

Blin, K., Medema, M. H., Kazempour, D., Fischbach, M. A., Breitling, R., Takano, E., & Weber, T. (2013). antiSMASH 2.0--a versatile platform for genome mining of secondary metabolite producers. *Nucleic Acids Research*, *41*(Web Server issue), W204-12. https://doi.org/10.1093/nar/gkt449

Chen, L., Zheng, D., Liu, B., Yang, J., & Jin, Q. (2016). VFDB 2016: hierarchical and refined dataset for big data analysis--10 years on. *Nucleic Acids Research*, *44*(D1), D694-7. https://doi.org/10.1093/nar/gkv1239

Durai, S., Vigneshwari, L., & Balamurugan, K. (2013). Caenorhabditis elegans-based in vivo screening of bioactives from marine sponge-associated bacteria against Vibrio alginolyticus. *Journal of Applied Microbiology*, *115*(6), 1329–1342. https://doi.org/10.1111/jam.12335

George Kerry, R., Patra, J. K., Gouda, S., Park, Y., Shin, H. S., & Das, G. (2018). Benefaction of probiotics for human health: A review. *Journal of Food and Drug Analysis*, *26*(3), 927–939. https://doi.org/10.1016/j.jfda.2018.01.002

[redacted]

Kalan, L. R., Meisel, J. S., Loesche, M. A., Horwinski, J., Soaita, I., Chen, X., Uberoi, A., Gardner, S. E., & Grice, E. A. (2019). Strain- and Species-Level Variation in the Microbiome of Diabetic Wounds Is Associated with Clinical Outcomes and Therapeutic Efficacy. *Cell Host and Microbe*, *25*(5), 641-655.e5. https://doi.org/10.1016/j.chom.2019.03.006

Medema, M. H., Blin, K., Cimermancic, P., de Jager, V., Zakrzewski, P., Fischbach, M. A., Weber, T., Takano, E., & Breitling, R. (2011). antiSMASH: rapid identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences. *Nucleic Acids Research*, *39*(Web Server issue), W339-46. https://doi.org/10.1093/nar/gkr466

NCT03450369. ClinicalTrials.gov. Bethesda (MD): National Library of Medicine (US). Identifier: NCT03450369. Sponsor: Naked Biome, Inc. A Study of the Safety, Engraftment, and Action of NB01 in Adults With Moderate Acne. March 1, 2018. Available From: https://clinicaltrials.gov/ct2/show/NCT03450369

Peano, A., Johnson, E., Chiavassa, E., Tizzani, P., Guillot, J., & Pasquetti, M. (2020). Antifungal

Resistance Regarding Malassezia pachydermatis: Where Are We Now? *Journal of Fungi (Basel, Switzerland)*, *6*(2). https://doi.org/10.3390/jof6020093

Perlin, D. S., Rautemaa-Richardson, R., & Alastruey-Izquierdo, A. (2017). The global problem of antifungal resistance: prevalence, mechanisms, and management. *The Lancet. Infectious Diseases*, *17*(12), e383–e392. https://doi.org/10.1016/S1473-3099(17)30316-X

Roudsari, M. R., Karimi, R., Sohrabvandi, S., & Mortazavian, A. M. (2015). Health effects of probiotics on the skin. *Critical Reviews in Food Science and Nutrition*, *55*(9), 1219–1240. https://doi.org/10.1080/10408398.2012.680078

Sparber, F., & LeibundGut-Landmann, S. (2017). Host responses to Malassezia spp. in the mammalian skin. In *Frontiers in Immunology* (Vol. 8, Issue NOV, p. 1614). https://doi.org/10.3389/fimmu.2017.01614

[redacted]

Theelen, B., Cafarchia, C., Gaitanis, G., Bassukas, I. D., Boekhout, T., & Dawson, T. L. J. (2018). Malassezia ecology, pathophysiology, and treatment. *Medical Mycology*, *56*(suppl_1), S10–S25. https://doi.org/10.1093/mmy/myx134

Van Nood, E., Vrieze, A., Nieuwdorp, M., Fuentes, S., Zoetendal, E. G., De Vos, W. M., Visser, C. E., Kuijper, E. J., Bartelsman, J. F. W. M., Tijssen, J. G. P., Speelman, P., Dijkgraaf, M. G. W., & Keller, J. J. (2013). Duodenal infusion of donor feces for recurrent clostridium difficile. *New England Journal of Medicine*, *368*(5), 407–415. https://doi.org/10.1056/NEJMoa1205037

Wegh, C. A. M., Geerlings, S. Y., Knol, J., Roeselers, G., & Belzer, C. (2019). Postbiotics and Their Potential Applications in Early Life Nutrition and Beyond. *International Journal of Molecular Sciences*, *20*(19). https://doi.org/10.3390/ijms20194673

Whitlock, D., Jamas, S., Weiss, L., inventors; AOBiome LLC, assignee. Ammonia oxidizing bacteria for treatment of acne. WO2017004557. May 1, 2017.

Williams, M. R., & Gallo, R. L. (2015). The role of the skin microbiome in atopic dermatitis. *Current Allergy and Asthma Reports*, *15*(11), 65. https://doi.org/10.1007/s11882-015-0567-4

APPENDIX 1: SCHEDULE OF EVENTS

Evaluation or Task	Screen (-28 day to day 1)	Day 1/ Baseline Prior to Application	Day 1/ Day of application	*Day 2,3,4	Day 5	Day 14
			Treatment period			Follow up
Informed consent	X					
Demographics	X					
Inclusion/Exclusion	X	X				
Ensure no use of soap and water on chest and back on previous day until scrub and culture samples are collected		X			X	X
Urine pregnancy test in women	X					X
Medical History	X					
Concomitant Medication Query	X	X		X	X	X
Clinical assessment of signs and symptoms of Tinea Versicolor (TV)	X	X	X	*X	X	X
Score signs and symptoms of local tolerability		X	X	*X	X	X
In-clinic KOH test (TV sites)	X					X
Bacterial culture all test sites		XΔ			XΔ	XΔ
Sample collection for molecular diagnosis (Scrub)		X			X	X
Record Adverse Events		X	X	*X	X	X
Application of Investigational Product (IP) ,aqueous gel and vehicle gel in the clinic (investigative site)			X	*X	X	
Instruct subject not to use soap and water on the chest and back for 12 hours after Day 1 IP ,aqueous and vehicle gel application. Use of soap and water on the chest and back is allowed on the mornings of Days 2, 3, and 4 for subjects in the multiple treatment dose groups.			X	*X		
Schedule appointment for next visit	X		X	*X	X	

*Only for subjects enrolled to receive multiple treatment doses on 5 consecutive days

Δ Not applicable to Cohort 4 subjects

Note: Screening and Day1/baseline can occur on the same day