DAIT/Rho STATISTICAL ANALYSIS PLAN
15 May 2019

A First in Man Evaluation of the Safety and Efficacy of an Allogeneic Targeted Microbiome Transplant in Adults with Moderate-to-Severe Atopic Dermatitis

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SPONSOR: Division of Allergy, Immunology, and Transplantation
National Institute of Allergy and Infectious Diseases – NIH
5601 Fishers Lane
Rockville, MD 20852
Telephone: (301) 496-5717
Fax: (301) 402-3573

PREPARED BY: Rho
Rho Building
6330 Quadrangle Drive
Chapel Hill, NC 27517
Telephone: (919) 408-8000
Fax: (919) 408-0999

DAIT/Rho STATISTICAL ANALYSIS PLAN ACKNOWLEDGMENT AND SIGNATURE SHEET

A First in Man Evaluation of the Safety and Efficacy of an Allogeneic Targeted Microbiome Transplant in Adults with Moderate-to-Severe Atopic Dermatitis

Approved: _____ Date: _____
Richard Gallo
MD, PhD
Protocol Chair

Approved: _____ Date: _____
Tissa Hata
MD
Protocol Co-Chair

Approved: _____ Date: _____
Amanda Rudman Spergel
MD
NIAID Medical Monitor

Approved: _____ Date: _____
Gloria David
PhD, MHSc
Rho Scientist

Approved: _____ Date: _____
Brett Jepson
MS
Rho Statistician

Document History

Version	Date	Change(s)	Author
1.0	24 AUG 2018	V1.0	Brett Jepson
2.0	15 MAY 2019	Added interim analysis; deleted time-to-event analyses; deleted exploratory objective and associated endpoint for microbiome transcriptome analyses; clarified specific bacteria to be analyzed; changed bacteria abundance endpoints to be longitudinal, including all available time points; added change-from-baseline longitudinal analyses as appropriate for specific bacteria abundance; changed the length of the study to 3 years based on the protocol; updated the study flow chart and schedule of events to be consistent with protocol version 2.0, updated medical monitor; clarified total expected doses; updated calculation of swab results; clarified treatment adherence.	Brett Jepson

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LIST OF ABBREVIATIONS

AD	Atopic Dermatitis
AE	Adverse Event
ANCOVA	Analysis of covariance
CFU	Colony forming units
CI	Confidence Interval
CoNS	Coagulase negative staphylococcal species
DNA	Deoxyribonucleic Acid
DSMB	Data and Safety Monitoring Board
EASI	Eczema Area and Severity Index
eCRF	Electronic Case Report Form
GMR	Geometric mean ratio
ID	Subject Identifier
IGA	Investigator's Global Assessment
IRB	Institutional Review Board
LLOQ	Lower limit of quantification
MedDRA	Medical Dictionary for Regulatory Activities
MITT	Modified Intent-to-Treat
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
PP	Per-Protocol
qPCR	Quantitative polymerase chain reaction
rCFU	Relative colony forming units
RL	Rajka-Langeland
RNA	Ribonucleic Acid
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SCORAD	Scoring Atopic Dermatitis
SOC	System Organ Class
TMT	Targeted Microbiome Transplant lotion
VAS	Visual Analog Scale
WHO	World Health Organization

1. PROTOCOL SYNOPSIS

Title	A First in Man Evaluation of the Safety and Efficacy of an Allogeneic Targeted Microbiome Transplant in Adults with Moderate-to-Severe Atopic Dermatitis
Short Title	Targeted Microbiome Transplant in Atopic Dermatitis
Clinical Phase	Phase I
Number of Sites	2 Clinical Sites in the United States
IND Sponsor/Number	NIAID / IND # 17286
Study Objectives	**Primary Objective** To assess the safety profile of 1 week of Targeted Microbiome Transplant lotion (TMT) application or placebo application, as determined by the count of serious and non-serious treatment-emergent adverse events (AEs) during the time period of Day 0 to Day 8 per participant within each group **Secondary Objectives** 1. To compare the count of serious and non-serious treatment-emergent AEs during the time period of Day 0 to Day 8 per participant between the groups receiving TMT and placebo application 2. To compare the proportion of participants experiencing at least one serious or non-serious treatment-emergent AE during the time period of Day 0 to Day 8 between the groups receiving TMT and placebo application 3. To compare the count of serious and non-serious AEs during study participation per participant between the groups receiving TMT and placebo application 4. To compare the proportion of participants experiencing at least one serious or non-serious AE during study participation between the groups receiving TMT and placebo application 5. To compare the effect of 1 week of TMT application to placebo application on disease severity measures 6. To compare the abundance of Coagulase-negative staphylococcus (CoNS) bacteria between lesional and non-lesional skin for up to 4 days after completion of 1 week of treatment separately within the groups receiving TMT or placebo application

	7. To compare the change from baseline levels of CoNS bacteria abundance between lesional and non-lesional skin for up to 4 days after completion of 1 week of treatment separately within the groups receiving TMT or placebo application 8. To compare the change from baseline levels of *S. hominis* A9 bacteria abundance between lesional and non-lesional skin for up to 4 days after completion of 1 week of treatment separately within the groups receiving TMT or placebo application 9. To compare the effect of 1 week of TMT application to placebo application separately on lesional and non-lesional skin *S. aureus* abundance for up to 4 days after completion of treatment 10. To compare the effect of 1 week of treatment separately within the groups receiving TMT or placebo application on *S. aureus* abundance between lesional and non-lesional skin for up to 4 days after completion of treatment 11. To compare the change from baseline levels of *S. aureus* abundance between lesional and non-lesional skin for up to 4 days after completion of 1 week of treatment separately within the groups receiving TMT or placebo application 12. To compare the effect of 1 week of TMT application to placebo application for up to 4 days after completion of 1 week of treatment on abundance of bacterial deoxyribonucleic acid (DNA) separately on lesional and non-lesional skin by quantitative polymerase chain reaction (qPCR) of the following: a. Combined *S. hominis* b. Combined Staphylococci c. Combined bacteria **Exploratory Objective** 1. To identify the diversity of the lesional and non-lesional skin microbiome by DNA sequencing after completion of 1 week of TMT or placebo application
Study Design	Phase I, first in man, randomized, double-blind placebo controlled multi-site trial
Primary Endpoint	The count of serious and non-serious treatment-emergent AEs per participant during the time period of Day 0 to Day 8

Secondary Endpoints	1. The occurrence of at least one serious or non-serious treatment-emergent AE during the time period of Day 0 to Day 8 2. The count of serious and non-serious AEs per participant during study participation 3. The occurrence of at least one serious or non-serious AE during study participation 4. The Eczema Area and Severity Index (EASI) score of the ventral arms at Days 0, 4, 7, 8 and 11 5. The Scoring Atopic Dermatitis (SCORAD) score at Days 0, 4, 7, 8 and 11 6. The Pruritus Visual Analog Scale (VAS) score of the ventral arms at Days 0, 4, 7, 8 and 11 7. The Rajka-Langeland (RL) score at Days 0 and 7 8. The abundance of CoNS, as measured by colony forming units per centimeter squared (CFU/cm^2) and qPCR (relative colony forming units per centimeter squared [rCFU/cm^2]) on lesional and non-lesional skin at Days 0, 4, 7, 8 and 11 9. The change from baseline levels of CoNS bacteria abundance as measured by CFU/cm^2 and qPCR (rCFU/cm^2) on lesional and non-lesional skin at Days 0 (1 hour post treatment), 4, 7, 8 and 11 10. The change from baseline levels of *S. hominis* A9 bacteria abundance as measured by qPCR (rCFU/cm^2) on lesional and non-lesional skin at Days 0 (1 hour post treatment), 4, 7, 8 and 11 11. The abundance of *S. aureus*, as measured by CFU/cm^2 and qPCR (rCFU/cm^2) on lesional and non-lesional skin at Days 0, 4, 7, 8 and 11 12. The change from baseline levels of *S. aureus* abundance, as measured by CFU/cm^2 and qPCR (rCFU/cm^2) on lesional and non-lesional skin at Days 0 (1 hour post treatment), 4, 7, 8 and 11 13. The abundance of bacterial DNA (rCFU/cm^2) on lesional and non-lesional skin at Days 0, 4, 7, 8 and 11; specific bacteria of interest are the following: • Combined *S. hominis* • Combined *Staphylococci* • Combined bacteria
Exploratory Endpoint	1. The proportion (% relative abundance) by Phylum: Class and Shannon Diversity Index of the microbiome on lesional and non-lesional skin at Day 7 after completion of 1 week of TMT or placebo application
Accrual Objective	This study will enroll approximately 54 adult participants, 18-80 years of age, with moderate-to-severe atopic dermatitis (AD) and a

	positive *S. aureus* colonized lesion (at least 15 cm^2) on the upper extremities.
Study Duration	Approximately 3 years to complete; we anticipate that participant enrollment and follow up will take approximately 2 years and data analysis and manuscript preparation will take approximately 1 additional year.
Treatment Description (Investigational Products)	Active (TMT): 50% Cetaphil® lotion and 50% Vegetable glycerin containing healthy donor-derived (allogeneic) commensal Staph species, *S. hominis* A9 (Manufactured and packaged by University of California – San Diego [UCSD]) applied to the right and left ventral upper extremities (wrist to upper arm) twice a day for 1 week Placebo: 50% Cetaphil® lotion and 50% Vegetable glycerin (Manufactured and packaged by UCSD) applied to the right and left ventral upper extremities twice a day for 1 week
Inclusion Criteria	Individuals who meet all of the following criteria are eligible for enrollment as study participants: 1. Participant must be able to understand and provide informed consent 2. Male or female participants 18-80 years of age, inclusive at time of Screening Visit 3. Meet Atopic Dermatitis Research Network (ADRN) Standard Diagnostic Criteria (Appendix A) for active AD 4. Positive *S. aureus* colonized lesion, at least 15 cm^2, on the ventral upper extremity 5. An Investigator Global Assessment (IGA) score, on the ventral arms, of at least moderate severity 6. Body surface area (BSA), as measured by Mosteller BSA Calculator, between 1.26 m^2 and 2.25 m^2 7. Females of childbearing potential who are willing to use adequate contraception 30 days prior to the Screening Visit and until participation in the study is complete. Females of childbearing potential must agree to use an acceptable method of birth control (e.g. total abstinence, oral contraceptives, intrauterine device (IUD), barrier method with spermicide, surgical sterilization or surgically sterilized partner, Depo-Provera, Norplant, NuvaRing, or hormonal implants) for the duration of study participation. 8. Male participants who are willing to use an acceptable method of contraception (e.g. barrier methods with spermicide, surgical sterilization, or surgically sterilized partner) or practice abstinence until participation in the study is complete.

Exclusion Criteria	Individuals who meet any of these criteria are not eligible for enrollment as study participants: 1. Inability or unwillingness of participant to give written informed consent or comply with study protocol 2. Pregnant or lactating females, or females who desire to become pregnant and/or breast feed within the duration of study participation 3. Active bacterial, viral, or fungal skin infections 4. Any noticeable breaks or cracks in the skin on the upper extremities, including severely excoriated skin or skin with open or weeping wounds suggestive of an active infection or increased susceptibility to infection 5. Sensitivity to or difficulty tolerating Dove fragrance-free bar soap, Cetaphil® Lotion, alcohol-based cleaners, macadamia nuts, soy, Vegetable glycerin, or palm kernels 6. Participants with prosthetic heart valves, pacemakers, intravascular catheters, or other foreign or prosthetic devices 7. Participants with Netherton's syndrome or other genodermatoses that result in a defective epidermal barrier 8. Any participant who is immunocompromised (e.g. history of lymphoma, Human Immunodeficiency Virus (HIV)/ Acquired Immune Deficiency Syndrome (AIDS), Wiskott-Aldrich Syndrome) or has a history of malignant disease (with the exception of non-melanoma skin cancer) 9. Participants with a history of psychiatric disease or history of alcohol or drug abuse that would interfere with the ability to comply with the study protocol 10. Past or current medical problems or findings from physical examination or laboratory testing that are not listed above, which, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the participant's ability to comply with study requirements or that may impact the quality or interpretation of the data obtained from the study 11. Ongoing participation in another investigational trial or use of investigational drugs within 8 weeks, or 5 half-lives (if known), whichever is longer, of the Screening Visit 12. Treatment with biologics within 16 weeks of Screening Visit

	13. Participants with close contacts (e.g. spouses, children, or members in the same household) that have severe barrier defects or are immunocompromised 14. Use of topical (including steroids and calcineurin inhibitors) AD treatments within 7 days of the Treatment Initiation Visit; Use of topical steroids on areas outside of where investigational product is to be applied may be permitted, per investigator discretion 15. Treatment of AD with prescription moisturizers classified as medical device (e.g., Atopiclair®, MimyX®, Epiceram®, Cerave®, etc.) within 7 days of the Treatment Initiation Visit 16. Use of any oral or topical antibiotics within 7 days of the Treatment Initiation Visit 17. Participants who have taken a bleach bath within 7 days of the Treatment Initiation Visit 18. Use of any oral AD therapies (steroids, immunosuppressive therapies) within 28 days of the Treatment Initiation Visit 19. Any phototherapy for skin disease (such as narrow band ultraviolet B [NBUVB], ultraviolet B [UVB], ultraviolet A1 [UVA1], psoralen + UVA [PUVA]) or regular use (more than 2 visits per week) of a tanning bed within 28 days of the Treatment Initiation Visit
Study Stopping Rules	This trial will be stopped pending immediate Data and Safety Monitoring Board (DSMB) review for the following reasons: 1. A single participant experiences any serious adverse event (SAE) for which there is a reasonable possibility that the investigational product caused the SAE 2. The development of any severe (Grade 3) AE for which attribution is defined as related or possibly related in: a. 1 out of the first 10 participants enrolled, b. 2 out of the first 20 participants enrolled, c. 3 out of the first 30 participants enrolled, d. 4 out of the first 40 participants enrolled, e. 5 out of the first 50 participants enrolled.

2. INTRODUCTION

This statistical analysis plan includes pre-planned analyses related to the study objectives outlined in the protocol. The statistical analysis plan (SAP) contains detailed information to aid in the implementation of the statistical analysis and reporting of the study data for use in the interim analysis and final Clinical Study Report (CSR). This SAP describes the populations that will be analyzed; the participant characteristic parameters, the efficacy parameters, and the safety parameters that will be evaluated; and details of the specific statistical methods that will be used. If additional analyses are required to supplement the planned analyses described in this SAP, they may be completed and will be identified in the CSR. Table, figure, and listing specifications are provided in separate documents.

3. GENERAL ANALYSIS AND REPORTING CONVENTIONS

The following analyses and reporting conventions will be used:

- Categorical variables will be summarized using counts (n) and percentages (%) and will be presented in the form "n (%)." Percentages will be rounded to one decimal place.

- Numeric variables will be summarized using n, mean, standard deviation (SD), median, minimum (min), maximum (max). For data that follow a lognormal distribution (i.e. swab data), geometric mean and geometric SD will be reported instead of mean and SD. The min/max will be reported at the same level of significance as original data. The mean and median will be reported at one more significant digit than the precision of the data, and SD will be reported at two more significant digits than the precision of the data.

- The median will be reported as the average of the two middle numbers if the dataset contains an even number of observations.

- Test statistics including *t* and *z* test statistics will be reported to two decimal places.

- *P*-values will be reported to three decimal places if greater than or equal to 0.001. If less than 0.001, the value will be reported as "<0.001." A *p*-value greater than 0.999 will be reported as ">0.999".

If departures from these general conventions are present in the specific evaluations section of this SAP, then those conventions will take precedence over these general conventions.

4. ANALYSIS SAMPLES

4.1. Safety Sample

The safety sample, which will be used for all safety analyses, will include all participants who are randomized and receive any amount of Targeted Microbiome Transplant lotion (TMT)/placebo.

Analyses using the safety sample will be done according to the treatment actually received.

4.2. Modified Intent-to-Treat (MITT) Sample

The modified intent-to-treat sample will include all participants who are randomized, provide skin swabs on Days 0 and 7 and administer at least 75% of doses (20, 23 or 26 total doses depending on actual date of the Day 7 visit; see Section 6.2) of TMT/placebo. The MITT sample will not exclude participants with lesional colony forming units (CFU) or relative colony forming units (rCFU) count of 0 at the Treatment Initiation Visit. Analyses using the MITT sample will be done according to the treatment assigned by randomization.

4.3. Per-Protocol (PP) Sample

The per-protocol sample will include all MITT participants who commit no major protocol deviations. The PP sample will not exclude participants with lesional CFU or rCFU count of 0 at the Treatment Initiation Visit.

Analyses using the PP sample will be done according to the treatment actually received.

4.4. Interim Analysis Sample

The interim analysis sample will include all participants who meet the following criteria:

- Are randomized
- Administer at least 75% of doses of TMT/placebo (20, 23 or 26 total doses depending on actual date of the Day 7 visit; see Section 6.2)
- Provide skin swabs with analyzable results at each of the Day 0, 7 and 11 visits
- Complete the Day 38 visit on or prior to the time of the interim analysis, which will be on or after the date when both of the following criteria are satisfied: 1) 10 participants given active TMT application complete the Day 38 visit and meet the 3 criteria above and 2) 5 participants given placebo complete the Day 38 visit and meet the 3 criteria above. All participants eligible at the time of the interim analysis and with analyzed samples will be included in the Interim Analysis sample.

5. STUDY PARTICIPANTS

5.1. Disposition of Participants

The disposition of all enrolled participants will be summarized in tables and listed.

The numbers and percentages of participants randomized, in each analysis sample, and completing the Day 38 visit, as well as reasons for early termination from the study will be presented. For participants discontinuing study treatment early, the reasons for discontinuing study treatment early will also be presented. Blood agar scores from the *S. aureus* skin swab used for randomization will be summarized in tables and listed. Randomization status will be determined from RhoRAND, Rho's randomization system.

For participants who fail inclusion/exclusion criteria, failed criteria will be listed by participant.

5.2. Demographic and Other Baseline Characteristics

Summary descriptive statistics for baseline and demographic characteristics will be reported for the Safety, MITT and PP samples. Characteristics at screening to be summarized include age, race, ethnicity, sex, body weight, height, and blood agar plate quadrant growth of *S. aureus*. Listings of baseline and demographic characteristics will also be prepared.

Tabular summaries will also be prepared by site.

5.3. Medical History

Medical history is collected prior to treatment initiation and will be reported in a listing for the Safety sample.

6. STUDY OPERATIONS

6.1. Protocol Deviations

Protocol deviations will be listed by site with information such as type of deviation, severity of the deviation (major or non-major), date of occurrence, and the reason for the deviation. Protocol deviations will be summarized in tabular format by type of deviation.

6.2. Treatment Adherence

TMT/placebo is prescribed to be administered on both arms twice daily beginning on Day 0 continuing through the Day 7 visit. Treatment adherence will be defined as the percentage of total doses taken out of all prescribed doses, which is expected to be 15 total doses per arm (30 total) for participants returning for the Day 7 visit on the expected visit date. For participants returning for the Day 7 visit one day prior to the expected visit date (Day 6), 13 total doses per arm (26 total) are expected. For participants returning for the Day 7 visit one day after the expected visit date (Day 8), 17 total doses per arm (34 total) are expected. Treatment adherence will be listed and summarized by treatment arm.

6.3. Atopic Dermatitis Severity Measures

Clinical characteristics of atopic dermatitis (AD) severity, such as investigator's global assessment (IGA), Eczema Area and Severity Index (EASI) score on ventral arms, scoring

atopic dermatitis (SCORAD) score, Rajka-Langeland (RL) severity score, and pruritus visual analog scale (VAS) of ventral arms, will be listed and summarized by visit.

Tabular summaries will also be prepared by site.

7. ENDPOINT EVALUATION

7.1. Overview of Efficacy Analysis Methods

7.1.1. Handling of Dropouts or Missing Data

All efforts will be made via the querying and monitoring phases to avoid missing data. In general, missing data will not be imputed.

As the primary analysis is descriptive, data will not be imputed for the primary endpoint. For more details, see Section 7.3.1.

7.1.2. Multicenter Studies

Study participants will be recruited from 2 study sites. Basic descriptive analyses of baseline demographics, medical history, and key study endpoints will be repeated for each site individually in order to allow qualitative exploration of site-to-site variability.

7.1.3. Assessment Time Windows

Allowable visit windows for all scheduled visits are provided in Section 13.2. All data will be included in analyses, regardless of time of assessment.

Unscheduled visits may also occur throughout the study. Data from unscheduled visits will be included in listings but will generally not be included in tabular or graphical summaries. The one exception is if the unscheduled visit occurs during the allowable visit window of a missed visit, any available data from the unscheduled visit will be included in the summary of the missed visit data.

7.2. Reporting of Swab Results

Swabs of lesional and non-lesional skin will be collected before and after the first IP administration on Day 0 and at all visits from treatment initiation to Day 11 to assess the following:

1. *S. aureus* (CFU/cm^2 and rCFU/cm^2)
2. Coagulase negative staphylococcal species (CoNS) (CFU/cm^2 and rCFU/cm^2)
3. *S. hominis* A9 (rCFU/cm^2)
4. Combined *S. hominis* A9 (rCFU/cm^2)
5. Combined staphylococci (rCFU/cm^2)
6. Combined bacteria (rCFU/cm^2)

Raw swab results by quantitative polymerase chain reaction (qPCR) will be multiplied by 50 to estimate the total rCFUs within the swabbed area. To account for CFU or rCFU counts of 0, one will be added to each CFU or rCFU count for analysis. The swabbed area (in cm^2) will be calculated as length (cm) x width (cm) of the swabbed area. Descriptive statistics of swab results and the change from baseline will be presented for each treatment group and overall. Swab results will also be listed for all participants.

Swab results will be plotted to show the trajectory of results over time. Data will be plotted as a spaghetti plot where each participant's values will be plotted and connected by line segments,

forming one line per participant. Quantile plots with treatment group means (or medians) as well as 25th and 75th percentiles plotted over time will be created.

7.3. Primary Objective

The primary objective for the study is to assess the safety profile of 1 week of TMT application or placebo application, as determined by the count of serious and non-serious treatment-emergent AEs during the time period of Day 0 to Day 8 per participant within each group. As such, the primary endpoint for the study is all serious and non-serious treatment-emergent adverse events (AEs) per participant during the time period of Day 0 to Day 8.

7.3.1. Computation of the Primary Endpoint

All serious and non-serious AEs that have a start date on or after the first administration of TMT/placebo (Day 0) and on or before Day 8 will be included in the primary endpoint. AEs that are present prior to Day 0 but worsen within the period from Day 0 through Day 8 will also be included in the primary endpoint. Since AEs will only be collected beginning at the time of consent, no dates will be imputed.

As the primary analysis is descriptive, data will not be imputed for the primary endpoint. To account for the decreased time in study for participants that terminate early, the regression model providing the estimates of parameters will account for the time active in the study during the Day 0 to Day 8 time period.

The primary objective will be analyzed on the safety sample. As the primary objective is descriptive, no inferential analyses are planned as part of the primary analysis.

7.3.2. Primary Analysis of the Primary Objective

The per-participant count of serious and non-serious AEs will be analyzed using a Poisson generalized linear model, with a log link function and including the natural log of the number of days active in the study during Day 0 to Day 8. Estimation will be done by residual subject-specific pseudo-likelihood. If the variance of the per-participant count of AEs is larger than the mean (i.e. overdispersion), then a Negative-Binomial model will be used instead of a Poisson model, with all other model details remaining the same. To assess overdispersion, the scale parameter will be calculated as the square root of Pearson's chi-square divided by the model degrees of freedom. A value >1 will be considered overdispersion.

Site and screening high (2-4) or low (1) *S. aureus* quadrant growth on blood agar plates will be included as covariates in the model. In the case of greater than 25% of the participants having values of 0, a zero-inflated Poisson (or Negative-Binomial if applicable) model will be produced. The zero-inflated model will use the same options as previously stated, except using a logit link function.

Estimates of the geometric mean per-participant event rate and corresponding 95% back-transformed confidence interval (CI) will be displayed for each of the treatment groups.

The safety sample will be used to analyze the primary endpoint.

7.3.3. Sensitivity Analyses of the Primary Endpoint

A similar model as specified in Section 7.3.2 adjusting for additional covariates will be generated to estimate parameters. If any additional covariates are found to be out of balance

between treatment groups at screening (e.g. severity measures, age, gender, etc.), they will be considered for inclusion in the model.

Sensitivity analyses will be performed using both the MITT and PP samples.

7.4. Secondary Objectives

For all secondary objectives, the hypotheses are as follows for each comparison:

1. H_0: there is no difference between the 2 groups being compared
2. H_A: there is a difference between the 2 groups being compared

Significance will be declared at the two-sided $\alpha=0.05$ level.

Due to the exploratory nature of these endpoint analyses, no adjustments will be made for multiple comparisons.

The secondary safety and efficacy objectives are stated below:

1. To compare the count of serious and non-serious treatment-emergent AEs during the time period of Day 0 to Day 8 per participant between the groups receiving TMT and placebo application
2. To compare the proportion of participants experiencing at least one serious or non-serious treatment-emergent AE during the time period of Day 0 to Day 8 between the groups receiving TMT and placebo application
3. To compare the count of serious and non-serious AEs during study participation per participant between the groups receiving TMT and placebo application
4. To compare the proportion of participants experiencing at least one serious or non-serious AE during study participation between the groups receiving TMT and placebo application
5. To compare the effect of 1 week of TMT application to placebo application on disease severity measures
6. To compare the abundance of CoNS bacteria between lesional and non-lesional skin after 1 week of treatment separately within the groups receiving TMT or placebo application
7. To compare the change from baseline levels of CoNS bacteria abundance between lesional and non-lesional skin for up to 4 days after completion of 1 week of TMT application
8. To compare the change from baseline levels of *S. hominis* A9 bacteria abundance between lesional and non-lesional skin for up to 4 days after completion of 1 week of treatment separately within the groups receiving TMT or placebo application
9. To compare the effect of 1 week of TMT application to placebo application separately on lesional and non-lesional skin *S. aureus* abundance for up to 4 days after completion of treatment
10. To compare the effect of 1 week of treatment separately within the groups receiving TMT or placebo application on *S. aureus* abundance between lesional and non-lesional skin for up to 4 days after completion of treatment
11. To compare the change from baseline levels of *S. aureus* abundance between lesional and non-lesional skin for up to 4 days after completion of 1 week of treatment separately within the groups receiving TMT or placebo application
12. To compare the effect of 1 week of TMT application to placebo application for up to 4 days after completion of 1 week of treatment on abundance of bacterial deoxyribonucleic acid (DNA) separately on lesional and non-lesional skin by qPCR of the following:

a. Combined *S. hominis*
b. Combined Staphylococci
c. Combined bacteria

All analyses of secondary safety objectives (objectives 1 through 4) will be done using the safety sample. All other analyses of secondary objectives will be performed on both the MITT and the PP samples.

7.4.1.1. Secondary Endpoints

1. The occurrence of at least one serious or non-serious treatment-emergent AE during the time period of Day 0 to Day 8
2. The count of serious and non-serious AEs per participant during study participation
3. The occurrence of at least one serious or non-serious AE during study participation
4. The EASI score of the ventral arms at Days 0, 4, 7, 8 and 11
5. The SCORAD score at Days 0, 4, 7, 8 and 11
6. The Pruritus VAS score of the ventral arms at Days 0, 4, 7, 8 and 11
7. The RL score at Days 0 and 7
8. The abundance of CoNS as measured by CFU/cm^2 and qPCR (rCFU/cm^2) on lesional and non-lesional skin at Days 0, 4, 7, 8 and 11
9. The change from baseline levels of CoNS bacteria abundance as measured by CFU/cm^2 and qPCR (rCFU/cm^2) on lesional and non-lesional skin at Days 0 (1 hour post treatment), 4, 7, 8 and 11
10. The change from baseline levels of *S. hominis* A9 bacteria abundance as measured by qPCR (rCFU/cm^2) on lesional and non-lesional skin at Days 0 (1 hour post treatment), 4, 7, 8 and 11
11. The abundance of *S. aureus* as measured by CFU/cm^2 and qPCR (rCFU/cm^2) on lesional and non-lesional skin at Days 0, 4, 7, 8 and 11
12. The change from baseline levels of *S. aureus* abundance as measured by CFU/cm^2 and qPCR (rCFU/cm^2) on lesional and non-lesional skin at Days 0 (1 hour post treatment), 4, 7, 8 and 11
13. The abundance of bacterial DNA (rCFU/cm^2) on lesional and non-lesional skin at Days 0, 4, 7, 8 and 11; specific bacteria of interest are the following:
    a. Combined *S. hominis*
    b. Combined Staphylococci
    c. Combined bacteria

7.4.1.2. Analyses of Secondary Endpoints

7.4.1.2.1 Secondary Safety Endpoints

For each endpoint of counts of AEs, analyses will be performed using the same model specified in Section 7.3.2. Comparisons between groups will be done using the Wald Chi-square test. Geometric mean ratios (GMRs) between groups of per-participant event rates and corresponding 95% back-transformed CIs will be displayed for each group comparison along with p-values.

For each endpoint of occurrence of at least one serious or non-serious treatment-emergent AE, comparisons will be made between groups using a Pearson Chi-square test. Proportions will be calculated as the total number of participants experiencing an event of interest divided by the total number of participants analyzed. If any expected counts of any cells are less than 5, a

Fisher's exact test will be used instead. Risk differences will be displayed with corresponding 95% CIs and *p*-values.

7.4.1.2.2 Secondary Severity Measure Endpoints

Severity measures of EASI, SCORAD, pruritus VAS of the ventral arms, and RL scores will be analyzed using analysis of covariance (ANCOVA) of the numeric response variable, comparing treatment arms at each applicable visit. Estimation will be done by restricted maximum likelihood. An identity link function will be used. The model will be adjusted for the applicable Day 0 (or Screening if Day 0 value does not exist) severity measure and site. Consideration will also be given to adjusting for any other covariates found to be out of balance between treatment groups as described in Section 7.3.3.

Estimates of the means by treatment group and corresponding 95% Wald asymptotic CIs will be displayed. Mean differences between groups and corresponding 95% *t*-type CIs at each time point will be displayed for each group comparison along with *p*-values.

7.4.1.2.3 Secondary Swab Result Endpoints

For endpoints of bacterial abundance (CoNS*, S. hominis* A9. *S. aureus*, combined *S. hominis*, combined staphylococci, and combined bacteria), $\log_{10}$ transformed abundance will be analyzed using a random effects linear model with a $\log_{10}$ transformed numeric response variable. For each objective, a single model will be produced using all available data for the applicable objective, with an interaction term of study day by treatment group or lesional status, depending on the variable of interest. Estimation will be done by restricted maximum likelihood. An identity link function will be used. The model will be adjusted for the corresponding $\log_{10}$ transformed abundance at Day 0 prior to dosing. For models comparing lesional to non-lesional abundance, a random effect of participant ID will be included in the model to account for correlation between corresponding measures within the same subject. Time in days will be included as a repeated effect in the model.

Estimates of the geometric means and corresponding 95% back-transformed Wald asymptotic CIs will be displayed. GMRs between compared groups or statuses (as applicable) and corresponding 95% back-transformed *t*-type CIs at each time point will be displayed for each comparison along with p-values.

Endpoints of the change from baseline of bacteria abundance will be analyzed with a similar model as referenced above, where the outcome variable will be the $\log_{10}$ abundance – $\log_{10}$ pre-dosing Day 0 value, adjusting for the $\log_{10}$ pre-dosing Day 0 value.

Separate models adjusting for the following values separately, instead of Day 0 pre-dosing abundance, may be considered:

1) Day 0 post-dosing abundance
2) Day 7 post-dosing abundance

7.5. Exploratory Objective

Due to the exploratory nature of this endpoint analysis, no adjustments will be made for multiple comparisons.

The exploratory objective is to identify the diversity of the lesional and non-lesional skin microbiome by DNA sequencing after completion of 1 week of TMT or placebo application.

7.5.1. Exploratory Endpoint

1. The proportion (% relative abundance) by Phylum: Class and Shannon Diversity Index of the microbiome on lesional and non-lesional skin at Day 7 after completion of 1 week of TMT or placebo application

7.5.2. Analyses of the Exploratory Endpoint

Analyses of microbiome diversity (e.g. % relative abundance by Phylum: Class and Shannon Diversity Index) will be conducted using analysis of covariance (ANCOVA) comparing treatment arms at Day 7, adjusting for pre-dosing Day 0 measure. Analyses at additional time points may be considered. Covariates similar to those used in support of the primary endpoint will be considered for inclusion in microbiome diversity analyses.

8. SAFETY EVALUATION

8.1. Overview of Safety Analysis Methods

All safety analyses will be carried out using the safety sample. Missing safety information will not be imputed. These analyses will not be stratified by site.

In addition to the reporting of primary and secondary safety endpoints, safety will be also summarized in each dose group through the reporting of vital signs, physical examination findings, and changes in routine laboratory values.

Listings will be prepared for all safety measurements. All listings will be sorted in order of treatment, participant identifier (ID), and time of assessment (e.g., visit, time, and/or event).

8.2. Adverse Events

All AEs will be classified by system organ class (SOC) and preferred term, according to a standardized thesaurus (Medical Dictionary for Regulatory Activities [MedDRA] version 20.0). Each AE is entered on the eCRF once at the highest severity. As such, no additional data manipulation is needed to identify events.

AEs will be collected from the time of consent until the participant completes study participation or until 30 days after he/she prematurely withdraws (without withdrawing consent) or is withdrawn from the study. Treatment-emergent AEs will be identified as those with an onset date on or after the first dose of study medication as well as those with onset before first dose but that continued and worsened in severity after first dose. If the start of the AE in relation to the start of study medication cannot be established (e.g., the start date for the AE is missing), then the AE will be considered treatment-emergent. All data tabulations will be of only treatment-emergent events while non-treatment-emergent AEs will be listed separately.

An overall summary table will be developed to report the number of events and the number and percentage of participants having at least one event in the following categories:

- AEs
- AEs indicated as serious
- AEs that lead to study treatment discontinuation
- AEs with an outcome of death
- AEs that were reported as being related to a study treatment

- AEs reported by maximum severity

In addition, AEs classified by MedDRA SOC and preferred term will be summarized for each treatment group and overall for each of the following:

- All AEs
- AEs by maximum severity
- AEs by relationship to study treatment

Summary tables will present the total number of events as well as the number and percentage of participants experiencing the events. If a participant experiences the same AE on multiple occasions, the event will be counted once for each occurrence when reporting the number of AEs. When reporting the number of participants experiencing the events, a participant will only be counted once if he/she experiences an event within the particular SOC or preferred term. Percentages will be based on the number of participants in the safety population.

In addition, treatment-emergent AEs classified by MedDRA system organ class and preferred term will be summarized for each treatment group and overall. The proportion of participants in each study arm experiencing each type of AE will be compared using methods described in Section 7.4.1.2.1. The frequency of treatment-emergent AEs will also be summarized by system organ class and preferred term for severity (grade) and relationship to study treatment.

A summary table of incidence rates for AEs classified by MedDRA SOC and preferred term will be provided for each treatment group and overall. The incidence rate for a SOC or preferred term will be compared between treatment groups according to Section 7.4.1.2.1.

Separate data listings will be provided for treatment-related AEs and AEs leading to study treatment discontinuation.

8.3. Deaths and Serious Adverse Events

Serious adverse events (SAEs) will be listed and summarized in the same manner described in Section 8.2. Separate displays listing and summarizing death, including time to death and cause of death, will also be created.

8.4. Vital Signs, Physical Findings, and Other Observations Related to Safety

8.4.1. Vital Signs

Data listings sorted by treatment group, participant, vital sign parameter, and time of assessment will be provided for vital sign measurements.

8.4.2. Pregnancy Assessment

Urine pregnancy assessment results will be listed for all pregnancy tests for female participants, including date, whether a test was performed and result of each pregnancy assessment. Pregnancy status questionnaire data will be reported for female participants and female partners of male participants, including date, whether a test was performed and result of each reported pregnancy test.

In the case of one or more pregnancies occurring during study participation, a data listing will be prepared to display applicable information, including date of report, method of pregnancy confirmation, delivery date (if applicable), pregnancy termination status and week (if applicable), and any problems or congenital abnormalities present.

9. OTHER ANALYSES

9.1. Use of Medications

Medications will be coded according to the World Health Organization (WHO) Drug Dictionary (version 2017.01). Medications reported on the eCRF will be categorized for analysis as prior, concomitant, or after study treatment by comparing the medication start and stop dates with the first and last dose of study treatment dates. Prior medications will have both the medication start and stop dates prior to the first dose of study treatment date. After medications will have both the medication start and stop dates after the last dose of study treatment date. All other medications will be classified as concomitant, indicating that use of the medication overlapped with use of the study treatment by at least one day.

The number and percentage of participants receiving prior, concomitant, and after medications will be presented overall and by medication class. When reporting the number of participants receiving the medication, a participant will only be counted once if they ever received the medication within the medication class. Percentages will be based on the number of participants in the analysis population.

10. INTERIM ANALYSES AND DATA MONITORING

10.1. Interim Safety Reporting to the Data and Safety Monitoring Board

The progress of the study will be monitored by the Data and Safety Monitoring Board (DSMB). The National Institute of Allergy and Infectious Diseases (NIAID) Allergy-Asthma [Alpha] DSMB will be chartered to review safety data and to make recommendations regarding continuation, termination, or modification of the study. The DSMB will formally review the safety data at least yearly. The discontinuation of study treatment will also be periodically reported to the DSMB.

In addition, safety data will be reviewed by the DSMB when an event occurs that is of sufficient concern to the NIAID medical monitor or protocol chair to warrant review, or when an event occurs that could contribute to a predefined stopping rule specified in the protocol.

Findings will be reported to Institutional Review Boards (IRBs) and health authorities.

10.2. Interim Analysis of Efficacy Data

An interim analysis will be performed based on cumulative efficacy data of all participants in the interim analysis sample (see Section 4.4). This analysis will be performed on or after the date when both of the following criteria are satisfied: 1) 10 participants given active TMT application complete the Day 38 visit and meet the criteria of the interim analysis sample and 2) 5 participants given placebo complete the Day 38 visit and meet the criteria for the interim analysis sample.

The purpose of the interim analysis is to provide critical information for future studies regarding the potency of the intervention to decrease *S. aureus* CFU as well as detect any tendency of the *S. hominis* A9 to accumulate on the skin after repeated applications. Skin total staphylococcal CFU, *S. aureus* CFU, qPCR of total DNA abundance of staphylococci, *S. aureus* specific DNA, hogocidin lantibiotic and total *S. hominis* DNA will be analyzed as part of the interim analysis. Clinical endpoints of disease severity (EASI, SCORAD, pruritus VAS and RL score) will also be analyzed.

The interim analysis will support our primary endpoint of safety to determine if there is an accumulation of bacteria during the course of treatment. This information is necessary for the design of future studies. There will be no decisions made to the conduct of this clinical trial (e.g. stopping for efficacy or futility) based on the results of the interim analysis. Enrollment will not be impacted or paused while the interim analysis is being performed.

Secondary objectives 5 through 12 will be analyzed for the interim analysis based on secondary endpoints 4 through 13, with the exception of the analyses of combined bacteria by qPCR. Summary statistics will be reported for each treatment group. Comparisons between treatment groups will be performed using a Wilcoxon rank sum test. The null hypothesis is that the mean ranks of the CFU, rCFU or disease severity measures (or change from baseline) are the same between treatment groups, while the alternative hypothesis is that the mean ranks differ between groups.

Identifying subject-level data will not be presented for the interim analysis as only tabular group level summaries will be included. Furthermore, since all participants involved in the interim analysis will have completed the study, there will be no risk in biasing site staff concerning AE reporting for these participants. AE data will not be analyzed in any fashion for the interim analysis. Blinding will be maintained for all fully blinded study staff, according to the guidelines defined in the Randomization Plan. Tabular group level summaries will be reported only to the protocol chair Dr. Richard Gallo, the ADRN PI Dr. Donald Leung, and staff at the NIAID and Rho. Since the interim analysis involves only secondary objectives related to efficacy data and this is a Phase I study, there will be no penalty in type I error adjustment of the primary analysis for an early look at the data.

11. SAMPLE SIZE CONSIDERATIONS

The proposed sample size for this study is 36 participants in the TMT arm and 18 in the placebo arm (2:1 randomization). The primary objective of the analysis is to estimate the count of serious and non-serious treatment-emergent AEs during the time period of Day 0 to Day 8 per participant for participants completing 1 week of TMT application and for participants completing 1 week of placebo application. The proposed sample size allows us to determine a safety profile of TMT application taken for 1 week, as well as to estimate parameters of secondary analyses to power for future efficacy studies.

Table 1 below shows 95% confidence intervals per treatment arm associated with a hypothetical observed mean number of serious or non-serious adverse events per participant, assuming a Poisson distribution.

Table 1 Hypothetical Observed Means of the Per Participant Adverse Event Counts and Associated 95% Confidence Intervals

Hypothetical Observed Mean of the Per Participant Adverse Event Count	**95% Confidence Interval-TMT Arm (N=36)**	**95% Confidence Interval-Placebo Arm (N=18)**
0.33	0.14, 0.52	0.07, 0.60
0.67	0.40, 0.93	0.29, 1.04
1.0	0.67, 1.33	0.54, 1.46
1.33	0.96, 1.71	0.80, 1.87
1.67	1.24, 2.09	1.07, 2.26
2.0	1.54, 2.46	1.35, 2.65
3.0	2.43, 3.57	2.20, 3.80
5.0	4.27, 5.73	3.97, 6.03
10.0	8.97, 11.03	8.54, 11.46

12. CHANGES TO THE ANALYSES PLANNED IN THE PROTOCOL

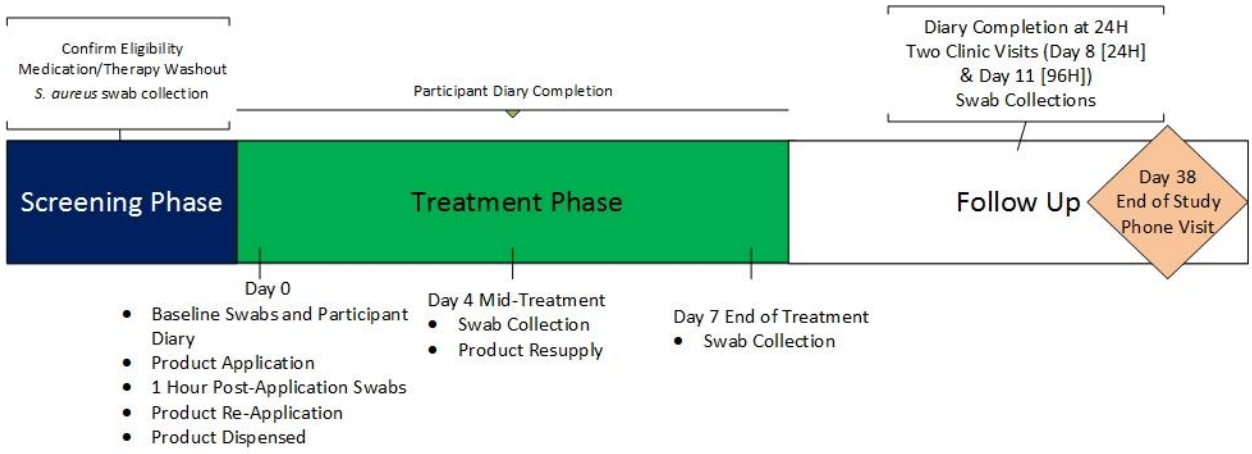
Protocol Section 13.5.1 indicated that the interim analyses include secondary objectives 5 through 12 and secondary endpoints 4 through 13, and that the interim analyses will be performed in the same manner as the final analyses of secondary objectives. Due to the small number of subjects included in the interim analysis, model-based analyses may be inappropriate. As a result, a non-parametric test will be used to compare treatment groups for the interim analysis, as specified in Section 10.2.

Also, due to the short period of time between the interim analysis and final database lock, combined bacteria as measured by qPCR (secondary endpoint 13c) will not be analyzed as part of the interim analysis.

13. APPENDICES

13.1. Study Flow Chart

Figure 1 Study Flow Chart



13.2. Schedule of Events

Table 2 Schedule of Events

Study Visit	**Recruitment**	**Screening[1]**	**Post-Screening Phone[2]**	**Repeat Culture[3]**	**Post-Culture Phone[4]**	**Treatment Initiation**	**Mid-Treatment[5]**	**End of Treatment[5]**	**24 Hour Follow-Up**	**96 Hour Follow-Up**	**End of Study Phone**	**Unscheduled Visit[6]**
Day (D), Visit Window		Day -38 to -2	Day -37 to -1	Day -15 to -2	Day -14 to -1	Day 0	Day 4 ±1 Day	Day 7 ±1 Day	Day 8[7]	Day 11 ±1 Day	Day 38 ±7 Days	
Study Assessments												
Recruitment Script	X											
Informed Consent		X										
Demographics		X										
Medical History		X		X[8]		X[8]						
Physical Exam		X		X		X						X
AD Severity Assessment		X[9]		X[9]		X[10]	X[11]	X[10]	X[11]	X[11]		X[11]
Pregnancy Test[12]		X		X		X	X	X		X	X[13]	
Concomitant Medications		X		X		X	X	X	X	X	X	X

Vital Signs[14]		X[15]		X		X	X	X	X	X		X
Participant Randomization						X[16]						
AD Lesion Assessment[17]						X	X	X	X	X		X
Skin Swab Collection		X		X		X[18]	X	X	X	X		X[19]
AE Assessment		X	X	X	X	X	X	X	X	X	X	X
IP Application						X						
IP Dispensation						X	X					
Review of Participant Diary						X[20]	X[21]	X[21]				
Paper Participant Diary Dispensation[22]						X	X	X				
IP Collection							X[23]	X[23]				
Blood Collection												X[1]

Study Visit	**Recruitment**	**Screening[1]**	**Post-Screening Phone[2]**	**Repeat Culture[3]**	**Post-Culture Phone[4]**	**Treatment Initiation**	**Mid-Treatment[5]**	**End of Treatment[5]**	**96 Hour Follow-Up**	**End of Study Phone**	**Unscheduled Visit[6]**

Day (D), Visit Window		Day -38 to -2	Day -37 to -1	Day -15 to -2	Day -14 to -1	Day 0	Day 4 ± 1 Day	Day 7 ± 1 Day	Day 11 ± 1 Day	Day 38 ± 7 Days	
Study Assessments											
Recruitment Script	X										
Informed Consent		X									
Demographics		X									
Medical History		X		X[7]		X[7]					
Physical Exam		X		X		X					X
AD Severity Assessment		X[8]		X[8]		X[9]	X[10]	X[9]	X[10]		X[10]
Pregnancy Test[11]		X		X		X	X	X	X	X[12]	
Concomitant Medications		X		X		X	X	X	X	X	X
Vital Signs[13]		X[14]		X		X	X	X	X		X
Participant Randomization						X[15]					
AD Lesion Assessment[16]						X	X	X	X		X

Skin Swab Collection		X		X		X[17]	X	X	X		X[18]
AE Assessment		X	X	X	X	X	X	X	X	X	X
IP Application						X					
IP Dispensation						X	X				
Review of Participant Diary						X[19]	X[20]	X[20]			
Paper Participant Diary Dispensation[21]						X	X	X			
IP Collection							X[22]	X[22]			
Blood Collection											X[18]

1. Assessment of full inclusion and exclusion criteria will occur during the Screening Visit, after participants have consented to study participation.
2. Screened participants who test positive for *S. aureus* on their lesional skin swab and require a medication/therapy washout of 14 days or less will be contacted via telephone to schedule their Treatment Initiation (Day 0) Visit. Participants who test positive for *S. aureus* on their lesional swab and require a medication/therapy washout of more than 14 days will be scheduled for a Repeat Culture Visit, following their washout.
3. Only participants who test positive for *S. aureus* on their Screening lesional swab and require a medication/therapy washout of more than 14 days will be scheduled for a Repeat Culture Visit to confirm they are still *S. aureus* positive prior to treatment initiation.
4. Only participants who complete the Repeat Culture Visit will have a Post-Culture Phone Visit.
5. Participants will be requested to return for their Mid-Treatment and End of Treatment Visits on Day 4 and Day 7 approximately four hours after their last TMT or placebo application.
6. If disease activity increases, participants experience signs and symptoms as described on the instructional hand card, or other concerns arise between regularly scheduled visits, participants may be asked to return to the study site for an Unscheduled Visit.
7. If participants cannot return on Day 8 for the 24 Hour Follow -Up visit, this data will be treated as missing, and the participant will be asked to continue with any remaining visits, as required.
8. An abbreviated Medical History will be collected at the Repeat Culture and Treatment Initiation visits to confirm participant still meets eligibility.
9. AD disease severity will be assessed using the Investigator Global Assessment of the ventral arms, Eczema Area and Severity Index of the ventral arms, SCORing Atopic Dermatitis, and Pruritus VAS of the ventral arms standardized scales.
10. AD disease severity will be assessed using the Eczema Area and Severity Index of the ventral arms, Rajka-Langeland, SCORing Atopic Dermatitis, and Pruritus VAS of the ventral arms standardized scales.
11. AD disease severity will be assessed using the Eczema Area and Severity Index of the ventral arms, SCORing Atopic Dermatitis, and Pruritus VAS of the ventral arms standardized scales.
12. A urine pregnancy test will be completed for all female participants of child bearing potential who do not self-report as pregnant.
13. During the End of Study Phone Visit, female participants of child-bearing potential will be asked whether they have tested positive to a pregnancy test, since their last study visit. Male participants will be asked whether their partner has tested positive to a pregnancy test, since their last study visit.
14. Vital signs will include temperature, heart rate, respiration, systolic blood pressure, and diastolic blood pressure.
15. Vital signs, including temperature, heart rate, respiration, systolic blood pressure, diastolic blood pressure, plus height and weight, will be collected at the Screening Visit.
16. Participants who meet all inclusion and exclusion criteria and require a medication/therapy washout of 14 days or less must be randomized within 14 days of their Screening Visit.
17. Digital photographs of the lesional and non-lesional swab sites will be taken prior to swab collection. Each photograph will include a ruler so the scale of the site can be determined.
18. Participants will remain in clinic for up to 1 hour following the application of investigational product during the Treatment Initiation Visit. Additional skin swabs will be collected at 1 hour post application.
19. Skin swabs and/or blood may be collected during an Unscheduled Visit per investigator discretion.
20. Participants will complete a baseline diary entry during their Treatment Initiation (Day 0) Visit.
21. Paper diaries will be collected, during the in clinic review of the participant diary, if the participant completed a paper diary in lieu of the electronic diary.
22. Paper diaries will be provided to track symptoms and compliance in the event participants do not have access to a device with internet access.

The study team will collect all dispensed packets of investigational product from participants, including empty and unused investigational product.