DAIT/Rho STATISTICAL ANALYSIS PLAN For INTERIM and FINAL ANALYSIS
12 March 2024

A Pilot Study To Evaluate The Survival Of Transplanted *Staphylococcus Hominis* A9 On The Skin Of Adults With Moderate-To-Severe Atopic Dermatitis

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DAIT/Rho STATISTICAL ANALYSIS PLAN For INTERIM and FINAL ANALYSIS ACKNOWLEDGMENT AND SIGNATURE SHEET

A Pilot Study To Evaluate The Survival Of Transplanted *Staphylococcus Hominis* A9 On The Skin Of Adults With Moderate-To-Severe Atopic Dermatitis

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Document History

Version	Date	Change(s)
1.0	14 AUGUST 2023	Initial Document
2.0	12 MARCH 2024	Updated the planned analysis for secondary endpoint #1. Updated and clarified which analysis populations will be used for the planned analyses.

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1. PROTOCOL SYNOPSIS (VERSION 3.0)

Title	A Pilot Study to Evaluate the Survival of Transplanted *Staphylococcus Hominis* A9 on the Skin of Adults with Moderate-to-Severe Atopic Dermatitis
Short Title	Targeted Investigation of Microbiome Elimination (TIME-1)
Clinical Phase	Phase 1
Number of Sites	1 Clinical Site in the United States
IND Sponsor/Number	NIAID / IND # 17286
Study Objectives	**Primary Objective** To assess the duration of survival of coagulase negative staphylococcal species (CoNS) as measured by colony-forming unit (CFU) during a maximum of 24 days after the application of *Staphylococcus hominis* (*S. hominis*) A9 on the lesional ventral arm skin of Atopic Dermatitis (AD) participants positive for *Staphylococcus aureus* (*S. aureus*) (AD SA+). **Secondary Objectives** 1. To assess the duration of survival of CoNS as measured by CFU during a maximum of 24 days after the application of *S. hominis* A9 on non-lesional ventral arm skin of AD participants positive for *S. aureus* (AD SA+). 2. To assess the safety of *S. hominis* A9 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 31 per participant within each group. **Exploratory Objectives** 1. To assess the association of *S. hominis* A9 estimated CFU on the skin of the ventral arm contralateral to the arm of application and face. 2. To assess the duration of survival of CoNS as measured by CFU during a maximum of 24 days after the application of *S. hominis* A9 on lesional ventral arm skin of AD participants negative for *S. aureus* (AD SA-). 3. To assess the duration of survival of CoNS as measured by CFU during a maximum of 24 days after the application of *S. hominis* A9 on non-lesional ventral arm skin of AD participants negative for *S. aureus* (AD SA-). 4. To compare the duration of survival of CoNS as measured by CFU on lesional skin between AD SA+ vs AD SA- participants. 5. To compare the duration of survival of CoNS as measured by CFU on non-lesional skin between AD SA+ vs AD SA- participants. 6. To compare the duration of survival of CoNS as measured by CFU between lesional and non-lesional skin on AD SA+ participants. 7. To compare the duration of survival of CoNS as measured by CFU between lesional and non-lesional skin on AD SA- participants.

	8. To compare disease severity measures (local Eczema Area and Severity Index [L-EASI], SCORing Atopic Dermatitis [SCORAD] and local Pruritus Numerical Rating Scale [NRS]) between Day 0 and the last in-clinic visit among AD SA+ participants, and independently, among AD SA-. 9. To determine the antibiotic sensitivity of the skin CoNS microbiome to penicillin G, tetracycline, and erythromycin before and after treatment with ShA9.
Study Design	This is a Phase 1 pilot, open label, single center trial designed to assess the kinetics of *S. hominis* A9 survival on the skin of adults with moderate-to-severe atopic dermatitis on the ventral arms who are culture positive or negative for *S. aureus* colonization.
Primary Endpoint	The duration of survival, measured as the time needed for CoNS CFU to drop below baseline density measured before application of ShA9 + 100 CFU/cm^2 on the lesional ventral arm skin of AD participants positive for *S. aureus* (AD SA+).
Secondary Endpoints	1. The duration of survival, measured as the time needed for CoNS CFU to drop below baseline density measured before application of ShA9 + 100 CFU/cm^2 on non-lesional ventral arm skin of AD participants positive for *S. aureus* (AD SA+). 2. The count of serious and non-serious treatment-emergent AEs per participant during the time period of Day 0 to Day 31.
Exploratory Endpoints	1. Association of *S. hominis* A9 bacteria abundance estimated CFU as measured by % *S. hominis* A9 positive colonies by PCR x CoNS CFU while CoNS CFU is above baseline density measured before application of ShA9 + 100 CFU/cm^2 on the lesional and non-lesional, separately, ventral arm skin of AD participants between the arm treated with *S. hominis* A9 and the contralateral arm treated with placebo, and independently, with the participant's face. 2. The duration of survival, measured as the time needed for CoNS CFU to drop below baseline density measured before application of ShA9 + 100 CFU/cm^2 on the lesional ventral arm skin of AD participants negative for *S. aureus* (AD SA-). 3. The duration of survival, measured as the time needed for CoNS CFU to drop below baseline density measured before application of ShA9 + 100 CFU/cm^2 on the non-lesional ventral arm skin of AD participants negative for *S. aureus* (AD SA-). 4. Comparison of time needed for CoNS CFU to drop below baseline density measured before application of ShA9 + 100 CFU/cm^2 on the lesional and non-lesional, separately, ventral arm skin between AD participants positive (AD SA+) vs negative (AD SA-) for *S. aureus.* 5. Comparison of time needed for CoNS CFU to drop below baseline density measured before application of ShA9 + 100 CFU/cm^2 on lesional vs non-lesional skin of AD participants positive (AD SA+) and negative (AD SA-) for *S. aureus,* separately.

	6. Comparison of the L-EASI score of the ventral arms at Day 0 vs the last in-clinic visit among AD SA+, and independently, among AD SA-. 7. Comparison of the SCORAD score at Day 0 vs the last in-clinic visit among AD SA+, and independently, among AD SA-. 8. Comparison of the local Pruritus NRS of the ventral arms at Day 0 vs the last in-clinic visit among AD SA+, and independently, among AD SA-. 9. Comparison of the proportion of antibiotic-sensitive CoNS to penicillin G, tetracycline, and erythromycin isolated from skin swabs at baseline and after ShA9 treatment, at the last swab collection from lesional skin.
Accrual Objective	This study will enroll a minimum of 20 adult participants (a minimum of 13 *S. aureus* positive and a minimum of 7 *S. aureus* negative), 18-80 years of age, with moderate-to-severe atopic dermatitis on the ventral arms.
Study Duration	This study will take approximately 18 months to complete participant recruitment, enrollment, and follow-up. An individual participant's participation in this study will consist of up to 2 weeks of Screening, a 1-week pre-treatment phase, 1 day of treatment, and a maximum of 31 days for follow-up. Screened participants who meet all inclusion and exclusion criteria, including medication and therapy washouts, may complete Screening and Pre-treatment on the same day.
Treatment Description (Investigational Products)	Active (ShA9): 85% Phosphate-buffered saline solution (PBS) and 15% Glycerol containing healthy donor-derived (allogeneic) commensal Staph species, *S. hominis* A9 (Manufactured and packaged by University of California – San Diego [UCSD]) applied once to the right or left ventral arm (wrist to upper arm) Placebo: 85% PBS and 15% Glycerol (Manufactured and packaged by UCSD) applied once to the contralateral ventral arm
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 to 80 years of age, inclusive at time of the Screening Visit 3. Meet ADRN Standard Diagnostic Criteria (Appendix A) for active AD. 4. At least 21 cm^2 of lesional and 21 cm^2 of non-lesional skin on both the right and left ventral arms. The required area (lesional or non-lesional) may be one contiguous area or may encompass multiple areas with a total cumulative area of 21 cm^2. 5. An Investigator Global Assessment (IGA) score, on the ventral arms of at least moderate severity 6. Body surface area (BSA), as measured by Mostellar BSA Calculator, between 1.26 m^2 and 2.25 m^2.

	7. If female of childbearing potential, must agree to remain abstinent (refrain from heterosexual intercourse) or use acceptable contraception (e.g., oral contraceptives, intrauterine device (IUD), barrier method with spermicide, surgically sterilized partner, Depo-Provera, Norplant, NuvaRing, or hormonal implants) for the duration of study participation.
Exclusion Criteria	Individuals who meet any of these criteria are not eligible for enrollment as study participants: 1. Inability or unwillingness of a participant to give written informed consent or comply with study protocol. 2. Pregnant or lactating females 3. Active bacterial, viral, or fungal skin infections 4. Any noticeable breaks or cracks in the skin on the target areas of investigational product application, 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, glycerol, or soy products. 6. Participants with Netherton's syndrome or other genodermatoses that result in a defective epidermal barrier. 7. Any participant who is immunocompromised (e.g., history of lymphoma, Human Immunodeficiency Virus (HIV)/Acquired Immunodeficiency Syndrome (AIDS), Wiskott-Aldrich Syndrome), has an immune system disorder (e.g., autoimmune disease), or is using a systemic immunosuppressant (e.g., systemic corticosteroids, cyclosporine, methotrexate) 8. Any participant with current malignant disease (with the exception of non-melanoma skin cancer in an area not affected by treatment) 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 non-steroid systemic immunosuppressant within 6 months of the Screening Visit 13. Treatment with Dupilumab within 16 weeks of the Screening Visit

	14. Treatment with oral or injectable therapy for AD (excluding oral steroids) within 5 half-lives (if known) or 16 weeks before the Screening Visit, whichever is longer. 15. Participants with close contacts (e.g., spouse, children, or members in the same household) that have severe barrier defects or are immunocompromised. 16. Use of topical (including steroids and calcineurin inhibitors) AD treatments on the ventral arms or face within 7 days of the Treatment Visit; Use of topical steroids on areas outside of where investigational product is to be applied or swabbing is to be performed may be permitted, per investigator discretion. 17. Treatment with prescription moisturizers classified as medical device (e.g., Atopiclair®, MimyX®, Epiceram®, etc.) on the ventral arms or face within 7 days of the Treatment Visit; Use on areas outside of where investigational product is to be applied or swabbing is to be performed is permitted. 18. Use of any oral or topical antibiotic within 7 days of the Treatment Visit 19. Participants who have taken a bleach bath within 7 days of the Treatment Visit 20. Use of any oral steroid therapies within 28 days of the Treatment Visit 21. 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 Visit
Study Stopping Rules	Study enrollment will be suspended pending Data and Safety Monitoring Board (DSMB) expedited review of all pertinent data if any of the following occur: 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

2. INTRODUCTION

This statistical analysis plan encompasses pre-determined analyses corresponding to the study objectives delineated in the protocol. This Phase 1 pilot, open-label, single-center trial is designed to evaluate the kinetics of *S. hominis* A9 survival on the skin of adults with moderate-to-severe atopic dermatitis on the ventral arms, regardless of *S. aureus* colonization status. The statistical analysis plan (SAP) offers comprehensive information to facilitate the execution of statistical analysis (interim analysis and final analysis) and reporting of the study data for use in the 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., CoNS CFU), 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 enrolled and receive any amount of *S. hominis* A9/placebo. For analysis, *S. aureus* CFU results from swabs obtained prior to treatment application during the Treatment Visit (Day 0) will be used to define SA+ vs SA- status of participants.

4.2 Modified Intent-to-Treat (mITT) Sample

The modified intent-to-treat sample will include all participants who are enrolled and receive any amount of *S. hominis* A9/placebo. For analysis, *S. aureus* CFU results from swabs obtained prior to treatment application during the Treatment Visit (Day 0) will be used to define SA+ vs SA- status of participants.

4.3 Per-Protocol (PP) Sample

The per-protocol sample will include all mITT participants who have no major protocol deviations in the category study medications. Specifically, participants who have no major protocol deviations of the following type: (1) administered study product not approved for distribution (i.e., expired, opened, contaminated, etc.) or (2) the wrong treatment dose (dose does not equal 1 mL or 6 (six) pumps total of placebo or treatment spray per arm). For analysis, *S. aureus* CFU results from swabs obtained prior to treatment application during the Treatment Visit (Day 0) will be used to define SA+ vs SA- status of participants.

4.4 Interim Analysis Sample

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

- Positive for *S. aureus* colonization prior to treatment application at the Treatment Visit (Day 0).
- Application of the ShA9 and placebo treatments.

5. STUDY PARTICIPANTS

5.1 Disposition of Participants

The disposition (i.e., Recruited, Consented, Screened, Eligible, Enrolled, Completed, Discontinued) of all participants will be summarized in tables.

The numbers and percentages of participants enrolled, the numbers and percentages of participants who completed the End of Study Phone Visit (Day 31), as well as reasons for early termination from the study will be presented across *S. aureus* positive*/S. aureus* negative status. Moreover, the study will stratify by arm dominance, with the dominant arm being the right arm for right-handed participants, and the left arm for left-handed participants.

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 demographic characteristics will be reported using conventions from section 3.0. Demographic characteristics collected at the Screening Visit (Day -14 to Day -7) that will be summarized include age (years), sex, ethnicity, race and Body Mass Index (BMI).

A listing for vital signs will be reported. Vital signs and growth parameters are collected during all visits except the End of Study Phone Visit (Day 31) and include systolic blood pressure (mmHg), diastolic blood pressure (mmHg), heart rate (bpm), respiratory rate (respirations/min), temperature (F), height (in), weight (lbs), and body surface area (m^2).

Summary descriptive statistics for atopic dermatitis will be reported across arm dominance. Atopic dermatitis severity will be assessed during all visits except the End of Study Phone Visit (Day 31). Atopic dermatitis will be summarized by investigator's global assessment (IGA), local eczema area and severity index (EASI) score on ventral arms, scoring atopic dermatitis (SCORAD) score, and local pruritus numerical rating scale (NRS) of ventral arms.

5.3 Medical History

Medical history is collected during the Screening (Day -14 to Day -7), Pre-Treatment (Day -7), and Treatment (Day 0) visits and will be reported in a listing. An abbreviated medical history will be collected during the Pre-Treatment (Day -7) and Treatment (Day 0) visits.

6. STUDY OPERATIONS

6.1 Protocol Deviations

Protocol deviations will be listed 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 by type of deviation.

6.2 Treatment Adherence

Treatment adherence is not applicable to this study as this is a single application administered in clinic.

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.

The data will not be imputed for the primary endpoint. For more details, see Section 7.3

7.1.2 Multicenter Studies

Study participants will be recruited from 1 study site, University of California San Diego.

7.1.3 Assessment Time Windows

Allowable visit windows for all scheduled visits are provided in Section 14.2. 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

Prior to the application of the ShA9 and placebo treatment, skin swabs will be collected from the lesional and non-lesional sites on the ventral arms and one non-lesional site on the participant's face. After IP administration to each arm, additional swabs will be collected from the arms 15 minutes and 1, 2, 4, and 6 hours after application. Participants will return to the clinic 24 hours after application (24 Hour Visit) and on Days 3, 10, 17, and 24 for the collection of skin swabs from the identified target sites, as needed. However, after completion of the Day 3 Visit, a participant will not be required to complete the remaining follow up clinic visits (Day 10 through Day 24) once their lesional swabs from both arms (right and left) are CoNS negative, defined as below baseline density measured before application of ShA9 + 100 CFU/cm^2. The collected swabs will be used to assess the following:

1. *S. aureus* (CFU/cm^2)
2. Coagulase negative staphylococcal species (CoNS) (CFU/cm^2)
3. *S. hominis* A9 (CFU/cm^2)
4. Combined *S. hominis* A9 (CFU/cm^2)
5. Combined bacteria DNA (CFU/cm^2)
6. Antibiotic-sensitive CoNS

S. aureus will be measured by counting CFUs on the Baird-Parker agar plate. Coagulase negative staphylococcal species (CoNS) will be measured by counting CFUs on the mannitol salt agar with egg yolk plate. CFUs of *S. hominis A9* will be estimated from total CoNS CFU × % of *S. hominis A9* positive colonies. Abundance of bacterial DNA from total staphylococcus (G-staph), *S. aureus*, *S. hominis*, and Sh-lantibiotic will be quantified by qPCR. Antibiotic-sensitive CoNS will be estimated from [(CFU without antibiotic containing plate - CFU with antibiotic containing plate)/(CFU without antibiotic containing plate)].

Descriptive statistics and listings by participant of swab results will be presented. 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. Plots will also indicate arm dominance per participant.

7.3 Primary Objective

The primary objective for the study is to assess duration of survival of coagulase negative *staphylococcal* species (CoNS) as measured by colony-forming unit (CFU) during a maximum of 24 days after the application of *S. hominis* A9 on the lesional ventral arm skin of Atopic Dermatitis (AD) participants positive for S. aureus (AD SA+).

7.3.1 Computation of the Primary Endpoint

Time-to-CoNS elimination is defined as the time from Day 0 to the first occurrence of a value below baseline density measured before application of ShA9 + 100 CFU/cm^2. The time to event for participants who did not reach CoNS CFU below baseline density measured before application of ShA9 + 100 CFU/cm^2 during the study will be right-censored.

7.3.2 Primary Analysis of the Primary Objective

The primary endpoint for this pilot study is the time needed for CoNS CFU to drop below baseline density measured before application of ShA9 + 100 CFU/cm^2 (time-to-CoNS elimination) in AD participants positive for *S. aureus* colonization. Time-to-CoNS elimination will be estimated using Kaplan Meier procedure.

Median survival time along with 95% CI will be constructed based on a log-log transformed CI for the survivor function S(t). A graph of the Kaplan-Meier survival curve will be presented, and rates at fixed days will be derived (Therneau, et al., 2000). Furthermore, estimation of the hazard rate and 95% confidence interval will be produced using a parametric survival model.

The following SAS code will be used to execute this plan:

```
PROC LIFETEST data=data method=KM conftype=loglog plots=(survival(atrisk), hazard);
   title 'Kaplan-Meier plot of CONS CFU Below Baseline (Log-Log)';
   TIME TIME*EVENT(0);
run;
```

The primary analysis will be conducted on the modified intent to treat sample and repeated on the per-protocol sample if it differs from the modified intent to treat sample.

7.3.3 Supportive Analyses of the Primary Endpoint

We propose fitting a nonlinear time-response regression to our data set, which represents the relationship between the concentration of CoNS (Colony Forming Units per cm^2) and time. The analysis will be conducted in R, leveraging the package drc known for its robust functionality in the

area of dose-response analysis. Specifically, we will model our data using either a two-parameter or a three-parameter log-logistic regression model.

The following R code will be used to execute this plan:

```
# Load the necessary package
library(drc)

# Fit a two-parameter log-logistic model
model_2par <- drm(CoNS_CFU_per_cm2 ~ time, data = data, fct = LL.2())

# Fit a three-parameter log-logistic model
model_3par <- drm(CoNS_CFU_per_cm2 ~ time, data = data, fct = LL.3())

# Compare the AIC values of the two models
aic_2par <- AIC(model_2par)
aic_3par <- AIC(model_3par)

# Select the model with the lowest AIC
best_model <- ifelse(aic_2par < aic_3par, "Two-parameter model", "Three-parameter model")

# Compute the ED50 (or T50) from the selected model
if (best_model == "Two-parameter model") {
  ED50 <- ED(model_2par, 50)
} else {
  ED50 <- ED(model_3par, 50)
}
```

After conducting the model fits, we will evaluate the Akaike Information Criterion (AIC) for each model to compare their fit. The model yielding the lowest AIC will be selected as the primary model. From this primary model, we will determine the ED50 (or T50) - the time at which half of the maximum CoNS CFU/cm^2 is achieved. This approach follows the methodology outlined by Ritz et al., 2015. Adjustments or additional steps may be needed depending on the specifics of the data set and the research question at hand.

The supportive primary analysis will be conducted on the modified intent to treat sample and repeated on the per-protocol sample if it differs from the modified intent to treat sample.

7.4 Secondary Objectives

The secondary objectives are stated below:

1. To assess the duration of survival of CoNS as measured by CFU during a maximum of 24 days after the application of *S. hominis A9* on non-lesional ventral arm skin of AD participants positive for *S. aureus* (AD SA+).
2. To assess the safety of *S. hominis A9* 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 31 per participant within each group.

7.4.1 Secondary Endpoints

1. The duration of survival, measured as the time needed for CoNS CFU to drop below baseline density measured before application of ShA9 + 100 CFU/cm^2 on non-lesional ventral arm skin of AD participants positive for *S. aureus* (AD SA+).
2. The count of serious and non-serious treatment-emergent AEs per participant during the time period of Day 0 to Day 31.

7.4.2 Analyses of Secondary Endpoints

Secondary endpoint #1 will be analyzed using the same approach as described for the primary analysis of the primary endpoint (see Section 7.3.2). Median survival time along with 95% CI will be constructed based on a log-log transformed CI for the survivor function S(t). A graph of the Kaplan-Meier survival curve will be presented, and rates at fixed days will be derived (Therneau, et al., 2000). Furthermore, estimation of the hazard rate and 95% CI will be produced using a parametric survival model.

The summary of all treatment-emergent adverse events and treatment-emergent serious adverse events for secondary endpoint #2 will be provided by system organ class, high-level term, and preferred term in descending order of frequency.

Secondary endpoint #1 will be run on the modified intent to treat sample and re-run using the per-protocol sample if this differs from the former. Secondary endpoint #2 will be run on the safety sample.

7.5 Exploratory Objectives

The exploratory objectives are stated below:

1. To assess the association of *S. hominis A9* estimated CFU on the skin of the ventral arm contralateral to the arm of application and face.
2. To assess the duration of survival of CoNS as measured by CFU during a maximum of 24 days after the application of *S. hominis A9* on lesional ventral arm skin of AD participants negative for *S. aureus* (AD SA-).
3. To assess the duration of survival of CoNS as measured by CFU during a maximum of 24 days after the application of *S. hominis A9* on non-lesional ventral arm skin of AD participants negative for *S. aureus* (AD SA-).
4. To compare the duration of survival of CoNS as measured by CFU on lesional skin between AD SA+ vs AD SA- participants.
5. To compare the duration of survival of CoNS as measured by CFU on non-lesional skin between AD SA+ vs AD SA- participants.
6. To compare the duration of survival of CoNS as measured by CFU between lesional and non-lesional skin on AD SA+ participants.
7. To compare the duration of survival of CoNS as measured by CFU between lesional and non-lesional skin on AD SA- participants.
8. To compare disease severity measures (local Eczema Area and Severity Index [L-EASI], SCORing Atopic Dermatitis [SCORAD] and local Pruritus Numerical Rating Scale [NRS]) between Day 0 and the last in-clinic visit among AD SA+ participants, and independently, among AD SA-.
9. To determine the antibiotic sensitivity of the skin CoNS microbiome to penicillin G, tetracycline, and erythromycin before and after treatment with ShA9.

7.5.1 Exploratory Endpoints

1. Association of *S. hominis* A9 bacteria abundance estimated CFU as measured by % *S. hominis A9* positive colonies by PCR x CoNS CFU while CoNS CFU is above baseline density measured before application of ShA9 + 100 CFU/cm^2 on the lesional and non-lesional, separately, ventral arm skin of AD participants between the arm treated with *S. hominis A9* and the contralateral arm treated with placebo, and independently, with the participant's face.
2. The duration of survival, measured as the time needed for CoNS CFU to drop below baseline density measured before application of ShA9 + 100 CFU/cm^2 on the lesional ventral arm skin of AD participants negative for *S. aureus* (AD SA-).
3. The duration of survival, measured as the time needed for CoNS CFU to drop below baseline density measured before application of ShA9 + 100 CFU/cm^2 on the non-lesional ventral arm skin of AD participants negative for *S. aureus* (AD SA-).

4. Comparison of time needed for CoNS CFU to drop below baseline density measured before application of ShA9 + 100 CFU/cm^2 on the lesional and non-lesional, separately, ventral arm skin between AD participants positive (AD SA+) vs negative (AD SA-) for *S. aureus.*
5. Comparison of time needed for CoNS CFU to drop below baseline density measured before application of ShA9 + 100 CFU/cm^2 on lesional vs non-lesional skin of AD participants positive (AD SA+) and negative (AD SA-) for *S. aureus*, separately.
6. Comparison of the L-EASI score of the ventral arms at Day 0 vs the last in-clinic visit among AD SA+, and independently, among AD SA-.
7. Comparison of the SCORAD score at Day 0 vs the last in-clinic visit among AD SA+, and independently, among AD SA-.
8. Comparison of the local Pruritus NRS of the ventral arms at Day 0 vs the last in-clinic visit among AD SA+, and independently, among AD SA-.
9. Comparison of the proportion of antibiotic-sensitive CoNS to penicillin G, tetracycline, and erythromycin isolated from skin swabs at baseline and after ShA9 treatment, at the last swab collection from lesional skin.

7.5.2 Analyses of the Exploratory Endpoints

Repeated measures correlation will be calculated for determining the common within-individual association for paired (*S. hominis* treated arm and contralateral arm treated with placebo, and independently, with the participant's face) for *S. hominis A9* bacteria abundance estimated CFU as measured by % *S. hominis A9* positive colonies by PCR x CoNS CFU assessed on two or more days. The correlation coefficient, the 95% confidence interval, and the p-value will be reported. Log transformation will likely be needed for the outcome of interest to produce a less skewed distribution (Bland and Altman, 1995).

Analyses of disease severity (L-EASI, SCORAD, and local Pruritus NRS) change between Day 0 vs the last in-clinic visit among AD SA+, and independently, among AD SA- will be conducted using a repeated measures mixed-effects model with an unstructured covariance matrix. The model will estimate the pre-intervention vs. post-intervention least squares mean ± standard error and the difference reported with a 95% confidence interval and p-value.

Analyses to investigate if ShA9 application can change CoNS antibiotic sensitivity to penicillin G, tetracycline, and erythromycin will be conducted on all participants (AD SA+ and AD SA-) by comparing the proportion of antibiotic-sensitive CoNS [(CFU without antibiotic containing plate - CFU with antibiotic containing plate) / CFU without antibiotic containing plate] before and after treatment. We will model the difference in proportions of antibiotic-sensitive CoNS between swabs taken at baseline and swabs taken at the last Follow-Up Visit.

The exploratory analyses will be conducted on the modified intent-to-treat sample, and due to the exploratory nature of this endpoint analysis, no adjustments will be made for multiple comparisons.

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. Listings will be prepared for all safety measurements. All listings will be sorted in order of *S.*

aureus positive/*S. aureus* negative status, participant identifier, and the 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 25.0).
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 are defined as new AEs or an increase in grade of an AE after investigational product application (Day 0).

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 with an outcome of death
- AEs that were reported as being related to the study treatment
- AEs reported by maximum severity

In addition, AEs classified by MedDRA SOC, and preferred term will be summarized by arm dominance 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.

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 *S. aureus* positive/*S. aureus* negative participant status, vital sign parameter, and the time of assessment will be provided for vital sign measurements. Vital signs include systolic blood pressure (mmHg), diastolic blood pressure (mmHg), heart rate (bpm), respiratory rate (respirations/min), temperature (F), height (in), weight (lbs), and body surface area (m^2).

8.4.2 Pregnancy Assessment

Pregnancy tests (urine) will be performed on female participants of child-bearing potential who do not self-report as pregnant at the Screening (Day -14 to Day -7) and Treatment (Day 0) visits. If a female participant becomes pregnant prior to the Treatment Visit (Day 0), the participant will no longer be

eligible to participate in the study and will be withdrawn from all future study activities listed in the Schedule of Events.

At all other study visits, female participants of child-bearing potential will be asked if they are pregnant. If a pregnancy occurs, the pregnant participant will continue all study activities listed in the Schedule of Events. All pregnancies identified during the study shall be followed to conclusion and reported in a listing by participant. If pregnancy complications result in a congenital abnormality, birth defect, miscarriage, or medically indicated abortion, an SAE shall be reported.

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 study treatment date. During study participation, participants are permitted to use topical steroids per discretion of the investigator on areas other than the ventral arms where treatment is applied and the designated swabbing area on the face.

The number and percentage of participants receiving prior, concomitant, and post-treatment 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.

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. However, no formal interim analysis of safety data will be conducted.

10.2 Interim Analysis of Primary Endpoint

An interim analysis will be performed to inform the development of upcoming trials, especially with regards to their design and power/sample size considerations. This analysis will be performed when complete data for 50% of AD participants positive for *S. aureus* colonization becomes available. For analysis, *S. aureus* CFU results from swabs obtained prior to treatment application during the Treatment Visit will be used to define SA+ vs SA- status of participants. Duration of survival of coagulase negative *staphylococcal* species (CoNS) as measured by colony-forming unit (CFU) will be evaluated. Enrollment will not be impacted or paused while the interim analysis is being performed.

Time-to-CoNS elimination will be estimated using Kaplan Meier techniques. Median survival time along with 95% CI will be constructed based on a log-log transformed CI for the survivor function S(t). A graph of the Kaplan-Meier survival curve will be presented, and rates at fixed days will be derived (Therneau, et al., 2000). Furthermore, estimation of the hazard rate and 95% confidence interval will be produced using a parametric survival model. Summary statistics will be reported, subject-level data will not be presented for the interim analysis as only tabular group level (by arm dominance) 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 as to which arm of each participant received active treatment will be maintained for all fully blinded study staff, according to the guidelines defined in the Randomization Plan. Tabular group level (by arm dominance) summaries will be reported to the protocol chair Dr. Richard Gallo, the ADRN principal investigator Dr. Donald Leung, and staff at the NIAID and Rho.

11. SAMPLE SIZE CONSIDERATIONS

The proposed sample size for this study is 20 participants (13 participants in the *S. aureus* positive and 7 in the *S. aureus* negative arm (2:1 randomization)). The primary objective of the analysis is to estimate the duration of survival of CoNS as measured by CFU during a maximum of 24 days after the application of *S. hominis* A9 on the lesional ventral arm skin of AD participants positive for *S. aureus* (AD SA+).

The sample size was derived from ADRN-08: Targeted Microbiome Transplant in Atopic Dermatitis where, using a parametric survival model with exponential distribution, we estimated a hazard rate (λ) of approximately 0.09. This estimate was derived from a follow-up time of 11 days (shorter study) with 35 participants where nine (25%) were right-censored at trial end. The goal for this trial is to estimate the hazard rate and its 95% confidence interval with precision for the width of the confidence interval as 0.20. The 13 participants (9 events) proposed for the primary analysis were derived using these previous data as a starting point but considering more conservative estimates with a hazard rate of 0.15 and 30% right censoring, which produces a two-sided 95% CI width equal to 0.194.

12. CHANGES TO THE ANALYSES PLANNED IN THE PROTOCOL

A per-protocol (pp) analysis sample not originally defined in the protocol has been added to the statistical analysis plan.

The planned analysis for secondary endpoint #1 described in Section 13.4.4 of the Protocol does not correctly describe an appropriate analysis plan for that endpoint. Section 7.4.2 of the SAP has been updated to correctly describe the intended planned analysis for secondary endpoint #1.

13. REFERENCES

Bland, J.M., & Altman, D.G. (1995). Calculating correlation coefficients with repeated observations: Part 1 - correlation within subjects. BMJ, 310, 446.

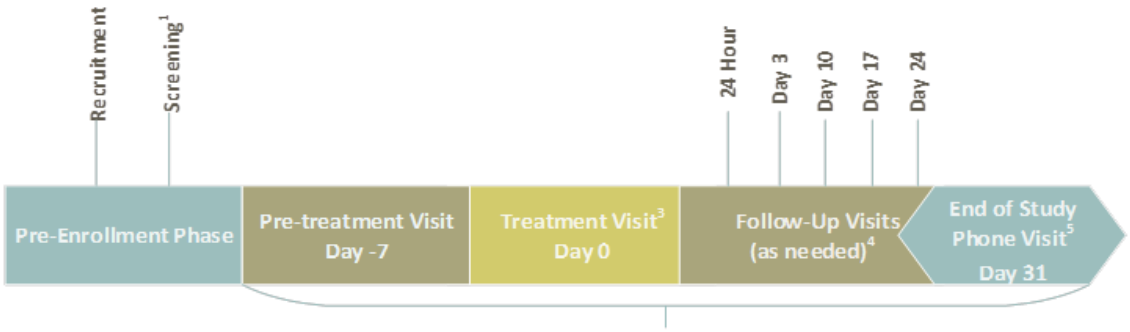
Ritz, C., Baty, F., Streibig, J. C., Gerhard, D. (2015) Dose-Response Analysis Using R PLOS ONE, 10(12), e0146021.

Therneau Terry M., Grambsch Patricia M. (2000). Modeling Survival Data: Extending the Cox Model. Springer, New York. ISBN 0-387-98784-3.

14. APPENDICES

14.1 Study Flow Chart

Figure 1 Study Flow Chart



1 Participants who meet all inclusion and exclusion criteria, including medication and therapy washouts, may complete their Pretreatment Visit on the same day as the Screening Visit.

2 Skin swabs will be collected from lesional and non-lesional areas on the ventral arms. Beginning at Day 0, two additional non-lesional swabs will be collected from the participant's face.

3 Additional skin swabs will be collected at 15 minutes, 1, 2, 4, and 6 hours post IP application on the ventral arms.

4 Participants with CoNS negative lesional skin swabs (below baseline density measured before application of ShA9 + 100 CFU/cm^2 on both their right and left arms), after the Day 3 Visit, will not be required to complete the remaining follow-up clinic visits (Day 10 through Day 24).

5 All treated participants, will be asked to complete the Day 31 End of Study Phone Visit for a final assessment of safety and disease status.

14.2 Schedule of Events

Study Visit	Recruitment[1]	Screening[2]	Pre-treatment[3]	Treatment	Follow-Up Visits[4]					End of Study Phone-Visit	Unscheduled Visit[5]
					24 Hour	Day 3	Day 10	Day 17	Day 24		
Day (D), Visit Window		Day -14 to Day -7	Day -7 ± 1 Day	Day 0	Day 1	Day 3 ± 1 Day	Day 10 ± 1 Day	Day 17 ± 2 Days	Day 24 ± 2 Days	Day 31 ± 7 Days	
Study Assessments											
Recruitment Script	X										
Informed Consent		X									
Demographics		X									
Medical History		X	X[6]	X[6]							
Physical Exam		X	X	X							X
AD Severity Assessment[7]		X	X	X	X	X	X	X	X		X
Pregnancy Status & Test[8]		X	X	X	X	X	X	X	X	X	X
Concomitant Medications		X	X	X	X	X	X	X	X	X	X
Vital Signs & Growth Parameters[9]		X	X	X	X	X	X	X	X		X
Skin Swab Collection[10]			X[11]	X[12,13]	X	X	X	X	X		X[14]
AE Assessment		X	X	X	X	X	X	X	X	X	X
Distribute Dove			X								
Distribute Cetaphil			X								
Randomization				X							
ShA9 and Placebo Application				X							
Distribute Handcard			X	X							
Blood Collection											X[14]

Table 1 Schedule of Events

1. Participants may be recruited by phone or during an in-person visit.
2. Assessment of full inclusion and exclusion criteria will occur during the Screening Visit, after participants have consented to study participation.
3. Participants who meet full inclusion and exclusion criteria including medication and therapy washouts, may complete the Screening and Pre-treatment Visits on the same day. The following assessments will not be repeated, if the Pre-treatment Visit is conducted on the same day as Screening: medical history, physical exam, AD severity assessment, concomitant medications, pregnancy test, and vitals.
4. Upon completion of the Day 3 Follow-Up Visit, a participant will be notified prior to their next scheduled follow-up visit if they need to return to the clinic. A participant will not be required to complete the remaining follow up clinic visits (Day 10 through Day 24) if the lesional swabs from both arms (right and left) collected at their most recent prior visit, have CoNS CFU below baseline density measured before application of ShA9 + 100 CFU/cm^2.
5. If disease activity increases, participants experience signs and symptoms as described on the instructional handcard, or other concerns arise between regularly scheduled visits, participants may be asked to return to the study site for an Unscheduled Visit.
6. An abbreviated Medical History will be collected.
7. AD disease severity will be assessed using the Investigator Global Assessment (Screening only), local Eczema Area and Severity Index (L-EASI) on the arms, SCORing Atopic Dermatitis (SCORAD), and local Pruritus Numerical Rating Scale (NRS).
8. A urine pregnancy test will be completed for all female participants of childbearing potential who do not self-report as pregnant at the Screening and Treatment Visits.
9. Vital signs will include temperature, heart rate, respiration rate, systolic blood pressure, and diastolic blood pressure. Growth parameters (height and weight) will only be collected at the Screening Visit.
10. Skin swab collection from the participant's ventral arms will occur during each visit, and skin swab collection from the participant's face will begin at the Treatment Visit.
11. Lesional and non-lesional sites will be identified on the participant's ventral arms for skin swab collection. 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 sites can be determined. These sites will be swabbed at subsequent visits.
12. A non-lesional site will be identified on the participant's face for skin swab collection. A digital photograph of the non-lesional swab site will be taken prior to swab collection. The photograph will include a ruler so the scale of the site can be determined. This site will be swabbed at subsequent visits.
13. Participants will remain in clinic for up to 6 hours following the application of investigational product during the Treatment Visit. Additional skin swabs will be collected from the ventral arms at 15 minutes, 1, 2, 4, and 6 hours post application.
14. Skin swabs and/or blood may be collected during an Unscheduled Visit per investigator discretion