ATOPIC DERMATITIS RESEARCH NETWORK

PROTOCOL ADRN-08

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

Version Number 2.0 / Version Date 14 February 2019

Investigational New Drug (IND) # 17286

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INSTRUCTIONS: *The site Principal Investigator (PI) should print, sign, and date at the indicated location below. A copy should be kept for your records and the original signature page sent. After signature, please return the original of this form by surface mail to:*

DAIT Regulatory Management Center (RMC)
Pharmaceutical Product Development (PPD)
3900 Paramount Parkway
Morrisville, NC 27560

I confirm that I have read the above protocol in the latest version. I understand it, and I will work according to the principles of Good Clinical Practice (GCP) as described in the United States Code of Federal Regulations (CFR) – 45 CFR part 46 and 21 CFR parts 50, 56, and 312, and in the most current International Conference on Harmonization (ICH) document *Guidance for Industry: E6 Good Clinical Practice: Consolidated Guidance*. Further, I will conduct the study in keeping with local legal and regulatory requirements.

As the site Principal Investigator, I agree to carry out the study by the criteria written in the protocol and understand that no changes can be made to this protocol without the written permission of the Institutional Review Board (IRB) and NIAID.

_____

Site Principal Investigator (Print)

_____ _____

Site Principal Investigator (Signature) **Date**

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.

Table of Contents

Glossary of Abbreviations

AD	Atopic Dermatitis
ADRN	Atopic Dermatitis Research Network
AE	Adverse Event
AIDS	Acquired Immune Deficiency Syndrome
AMP(s)	Antimicrobial Peptides
AMT	Autologous Microbiome Transplant
ANCOVA	Analysis of Covariance
APGAR	Appearance, Pulse, Grimace, Activity, and Respiration
BSA	Body Surface Area
CBC	Complete Blood Count
cDNA	Complementary Deoxyribonucleic Acid
CFR	Code of Federal Regulations
CFU	Colony Forming Units
CoNS	Coagulase Negative Staphylococcal Species
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
DAIT	Division of Allergy, Immunology, and Transplantation
DNA	Deoxyribonucleic Acid
DSMB	Data and Safety Monitoring Board
EASI	Eczema Area Severity Index
eCRF	Electronic Case Report Form
EC	Ethics Committee
EDC	Electronic Data Capture
EH	Eczema Herpeticum
EV	Eczema Vaccinatum

FDA	Food and Drug Administration
FLG	Filaggrin
GAS	Group A Streptococcus
GBS	Group B Streptococcus
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
HMW	High-Molecular-Weight
HPLC	High-Performance Liquid Chromatography
HSV	Herpes Simplex Virus
ICH	International Conference on Harmonization
IGA	Investigator Global Assessment
IgE	Immunoglobulin E
IND	Investigational New Drug
IP	Investigational Product
IRB	Institutional Review Board
IUD	Intrauterine Device
MCB	Master Cell Bank
MCV	Molluscum Contagiosum Virus
MITT	Modified-Intent-to-Treat
MOP	Manual of Procedures
mRNA	Messenger Ribonucleic Acid
MRSA	Methicillin-Resistant *Staphylococcus aureus*
MSSA	Methicillin-Sensitive *Staphylococcus aureus*
NA	Non-Atopic
NBUVB	Narrow Band Ultraviolet B

NCI	National Cancer Institute
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NS	Normal Saline
OHRP	Office for Human Research Protections
OTU	Operational Taxonomic Unit
PI	[Site] Principal Investigator
PCR	Polymerase Chain Reaction
PHI	Personal Health Identifiers
PP	Per Protocol
PSM	Phenol-Soluble Modulin
PUVA	Psoralen Ultraviolet A
QIIME	Quantitative Insights Into Microbial Energy
qPCR	Quantitative Polymerase Chain Reaction
rCFU	Relative Colony Forming Units
RL	Rajka-Langeland
RNA	Ribonucleic Acid
rRNA	Ribosomal Ribonucleic Acid
SACCC	Statistical and Clinical Coordinating Center
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Suspected Adverse Reaction
SCORAD	Scoring Atopic Dermatitis
SOP	Standard Operating Procedure
SUSAR	Serious Unexpected Suspected Adverse Reaction
TSB	Tryptic Soy Broth

TMT	Targeted Microbiome Transplant Lotion
UVA	Ultraviolet A
UVB	Ultraviolet B
UCSD	University of California – San Diego
USP	U.S. Pharmacopeial Convention
VAS	Visual Analog Scale
VV	Vaccinia Virus
WCB	Working Cell Bank

1. Background and Rationale

1.1. Background and Scientific Rationale

Atopic Dermatitis (AD) is a common, chronic, inflammatory skin disease complicated by recurrent bacterial as well as viral skin infections (Higaki et al., 1999; Travers et al., 2001; Ricci et al., 2003; Sugimoto et al., 2006; De Bendetto et al., 2009). The skin of AD patients is highly susceptible to infections by viruses, bacteria, and fungi. These microorganisms interact with the human immune system to trigger the onset of, or exacerbate the disease. Studies suggest that 7-10% of AD participants have difficulty containing infections caused by viruses including herpes simplex virus (HSV), vaccinia virus (VV); causing eczema herpeticum (EH), eczema vaccinatum (EV), and molluscum contagiosum virus (MCV) (60). Preliminary data in the Atopic Dermatitis Research Network (ADRN) Registry study indicate 40% of AD participants and only 2% of non-atopics (NA) are *S. aureus* colonized on lesional and/or non-lesional skin. These observations are consistent with the understanding that patients with AD have defects in innate and adaptive immune responses and thus have a general defect in the control of skin infections.

Several lines of experimental evidence support the concept that the multiple defects in epithelial barrier function in AD promote dysbiosis, a state of microbial imbalance, and that this dysbiosis then promotes the immunological disorder characteristic of AD. Dysbiosis in AD has been shown to be closely associated with disease severity (Kong et al., 2012a). Antibiotic therapy is a mainstay of treatment for normalization of dysbiosis; however, antibiotic treatment nonspecifically kills bacteria and may induce additional dysbiosis. In addition, long-term treatments generate resistant bacteria. Antimicrobial peptides (AMPs) such as defensins and cathelicidins are important mediators to maintain the balance of epithelial microflora. Our studies indicate that the innate immune defense of the skin is mediated not only by AMPs produced by an individual's own cells, but also by antimicrobial molecules contributed by commensal staphylococcus species, such as *S. epidermidis* and *S. hominis* (Lai et al., 2009; Lai et al., 2010; Cogen et al., 2010a; Cogen et al., 2010b). Previously published data (Nakatsuji et al., 2017) suggest that AD skin colonized by *S. aureus* has a lower frequency of these antimicrobial Staph species compared to normal skin. Studies have also shown that AD patients colonized by *S. aureus* have more severe disease states and are prone to more frequent flares of their AD (Kong et al., 2012a; Schnopp et al., 2010; Ong et al., 2002; Kong & Segre, 2012b). More recently, to further examine if topical application of antimicrobial coagulase negative staphylococcal species (CoNS) can decrease *S. aureus* survival on the skin of AD participants, a pilot study of an autologous microbiome transplant (AMT) was conducted by the University of California – San Diego (UCSD) as the Investigational New Drug (IND) Sponsor under IND# 15786. Antimicrobial CoNS strain(s) were isolated from the non-lesional skin of each of the 5 AD participants who were *S. aureus* culture positive (see Table 1.1 for active strains used for each patient). To transiently increase their abundance, the active strain(s) of each participant were expanded, mixed with Cetaphil® lotion at $1x10^7$ colony forming units per gram (CFU/g), and then reapplied to the lesional forearm skin of the same participant to get $1x10^5 CFU/cm^2$, from which the active colonies were isolated. After 24 hours of a single application of AMT lotion, *S. aureus* CFU decreased; whereas, vehicle (Cetaphil® lotion) applied to the contralateral arm in a double-blind comparison did not (Figure 1.4, a-b). From these results, we hypothesize that an increase in the ratio of antimicrobial Staph to the level found on normal skin would normalize the balance of microflora in AD participants, thereby improving abnormal immune reactions in AD skin. The goal of this study is to develop a therapy of healthy donor-derived (allogeneic) targeted microbiome transplant lotion (TMT) of antimicrobial Staph strain, *S. hominis* A9, to help normalization of skin dysbiosis in moderate-to-severe AD. By decreasing *S. aureus*, and possibly inflammation, we propose TMT as a novel therapy for AD.

Table 1.1 AMT formula transplanted to each AD participant

Participant	CoNS species	Active clones used for AMT
AMT 1	*S. epidermidis*	AMT1-A9
AMT 2	*S. hominis*	AMT2-A12
AMT 3	*S. hominis*	AMT3-A12
AMT 4	*S. hominis*	AMT4-C2, AMT4-D12, AMT4-G1
AMT 5	*S. epidermidis*	AMT5-C5, AMT5-G6

[1.] The total CFU in AMT formula for each participant was 1×10^7 CFU/g.
[2.] The total CFU applied on the skin of each participant was 1×10^5 CFU/cm^2.

1.2. Rationale for Selection of Investigational Product or Intervention

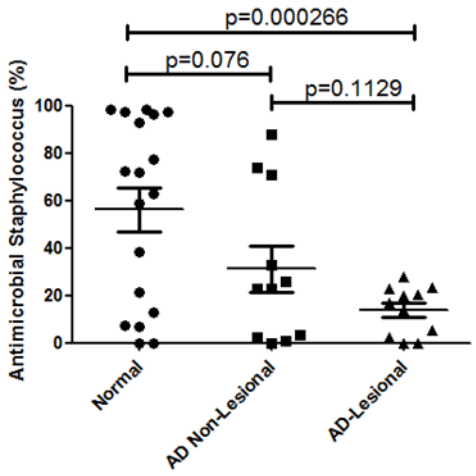
We have made a highly innovative discovery of newly recognized species such as *S. hominis*, as well as newly identified strains of *S. epidermidis* from the human skin microbiome that produce previously unknown antimicrobials that will selectively kill pathogens such as *S. aureus* while not harming the resident commensals. Because of this, these commensal bacteria can provide selective antimicrobial protection against pathogens on the skin surface yet maintain diversity. One example of such protective bacteria is a strain of *S. epidermidis* that produces Phenol Soluble Modulins (PSMs) gamma and delta (Cogen et al., 2010a). We have shown that these helical peptides are potent against *S. aureus in vitro* and on human skin. More recently, with the prior support of National Institute of Allergy and Infectious Diseases (NIAID), we have discovered a novel *S. epidermidis*-derived antibiotic we have named Firmocidin that selectively kills *S. aureus* [including methicillin-resistant *S. aureus* (MRSA) strains], group A Streptococcus (GAS), group B Streptococcus (GBS) and *Pseudomonas aeruginosa*. Another commensal strain of *S. epidermidis* produces a specific protease (esp) that disrupts *S. aureus* biofilms and has been used successfully in a human clinical trial to decrease nasal colonization (Iwase et al., 2010). We have also discovered that *S. hominis*, which is also a major commensal on the human skin, produces two novel AMPs we have named Hogocidin alpha and Hogocidin beta that kills *S. aureus* by a different mechanism. These findings indicate that multiple bacteria inhabiting the surface of normal human skin contribute directly to the skin's antimicrobial defenses. Importantly, we have now identified 4 specific strains in the microbiome with beneficial antimicrobial function that can be used in a new paradigm for host defense whereby the commensal bacteria defend their host by producing AMPs. Stability testing of these 4 specific strains in Cetaphil® lotion has revealed *S. hominis* and its antimicrobial, hogocidin alpha, to be the best formulation for our targeted microbiome transplant. This will be a Phase I, first in human, clinical trial evaluating the safety and efficacy of a novel therapy for AD, a targeted microbiome transplant lotion. The allogeneic targeted microbiome transplant lotion or TMT will be composed of 50% Cetaphil® lotion, 50% Vegetable glycerin, and commensal Staph species, *S. hominis* A9.

1.3. Preclinical Experience

It has been known for decades that patients with AD have increased colonization with bacteria and are susceptible to infections with *S. aureus* and viruses such as herpes and vaccinia. An alteration in the physical barrier by mutations in *filaggrin* (*FLG*) (Irvine 2007) and our findings of a decreased antimicrobial barrier from altered expression of antimicrobial proteins (Ong et al., 2002) have been hypothesized to contribute to the abnormal microbial colonization. Once the skin barrier is impaired and the expression of AMPs is reduced, it is likely that the homeostasis between host and microbe is shifted. This is consistent with more recent observations that show the whole skin microbial community is abnormal in AD. Several recent findings in patients with AD reveal a dramatic change in microbial community

structures compared to healthy volunteers (Kong & Serge, 2012b; Hata & Gallo, 2008). Worsening disease and lower skin bacterial diversity are associated with each other. Microbial shifts are localized to sites of disease predilection. This suggests that ecological niches such as the antecubital and popliteal creases are important for the initiation of disease and are influenced by the microbial communities that live in them.

Figure 1.3a AD skin contains a microbiome that is deficient in the ability to inhibit *S. aureus.*



Clinical improvement in AD is associated with higher bacterial diversity, indicating that current treatments that promote bacterial diversity promote improvement of the disease. Increases of specific bacterial genera, such as *Corynebacterium*, *Streptococcus,* and *Propionibacterium*, are observed during therapy indicating more complex species relationships during AD than known from culture-based methods (Kong et al., 2012a). Our prior work in the ADRN has further extended the understanding of the microbiome by demonstrating that the microbiome of AD participants is deficient in bacterial strains that provide anti-*S. aureus* activity. Figure 1.3a shows the percentage of coagulase negative Staph that secretes activity that will inhibit *S. aureus* growth. The data are conservatively biased since it eliminates coagulase positive bacteria from the calculation.

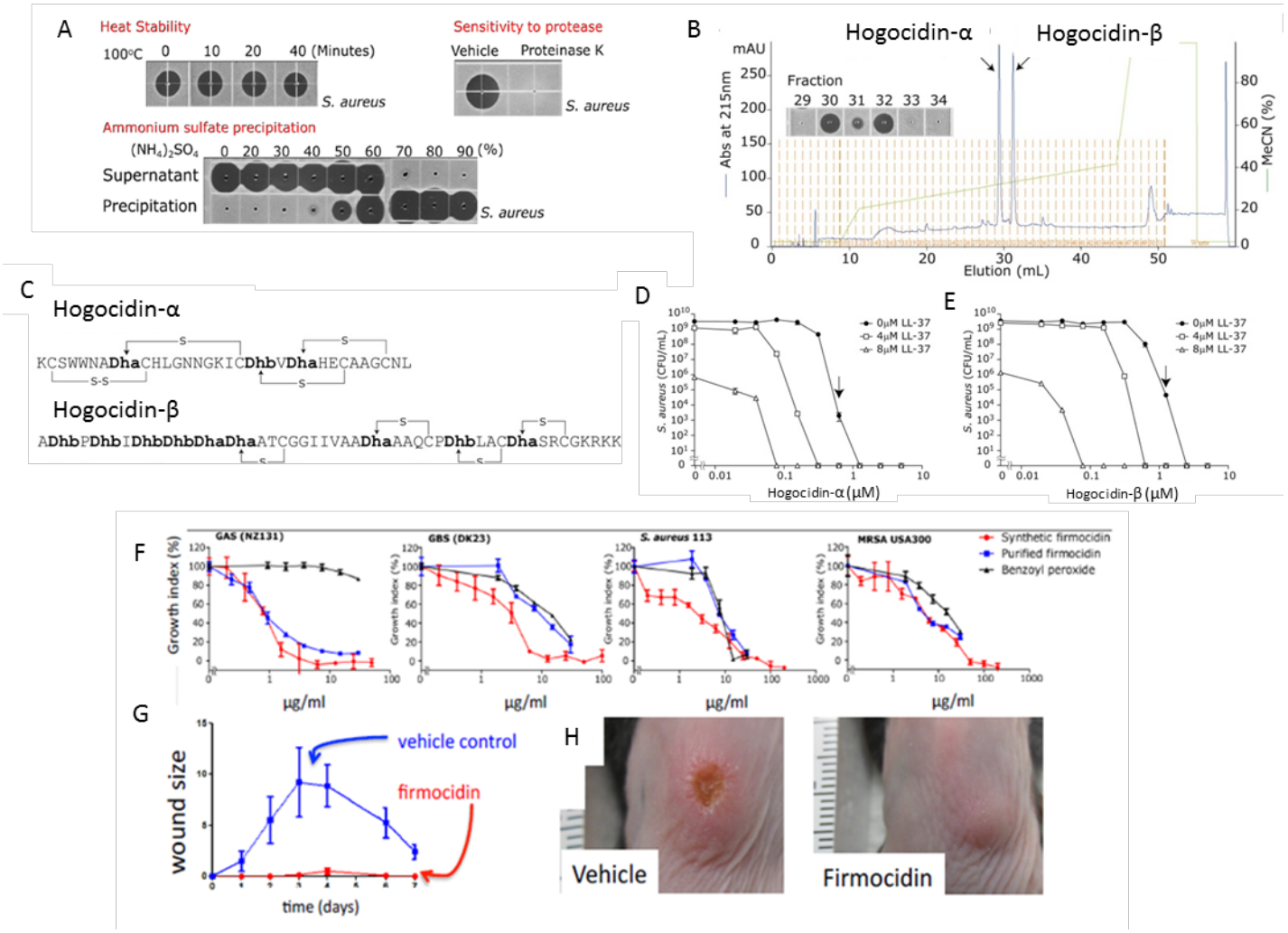
Each data point represents the proportion of coagulase negative Staph isolates recovered from the surface of an individual participant's forearm skin. One hundred isolates were measured from each individual. Bacterial colonies were cultured and sterile supernatant of that culture was added to *S. aureus* strain 113. Suppression of the growth of *S. aureus* was assessed in each assay by comparison with internal controls to evaluate growth in conditioned medium from a well-defined control strain of *S. epidermidis* that does not produce anti-Staph activity (negative control) and comparison with strains of *S. epidermidis* that do produce anti-Staph activity (positive control).

These observations show that not only is the skin of AD patients defective in host-derived protective factors (FLG, β-defensins, cathelicidins, etc.) but it also has a defect in protection from the microbiome. This is important to AD because of the risk that increased abundance of *S. aureus* has to infection and because of recent observations suggesting that the presence of δ-toxin produced by *S. aureus* will directly enhance allergic responses in mice (Nakamura et al., 2013). Recognition that AD has a lack of specific strains of bacteria supports the rationale behind our current proposal. Our microbiome transplant will inform how to replace some of the bacteria that are necessary for normal host defense of the skin.

To understand how the microbiome can directly inhibit *S. aureus*, we have sought to identify specific strains of the commensal microbiome that contribute to this activity. This work has been highly successful, and we have discovered

three novel antibiotics. All are common on normal skin but are relatively absent in AD. The activity of the products of two of these antimicrobial strains is illustrated in Figure 1.3b below.

Figure 1.3b Identification of molecules from the skin microbiome that inhibit growth of *S. aureus*.

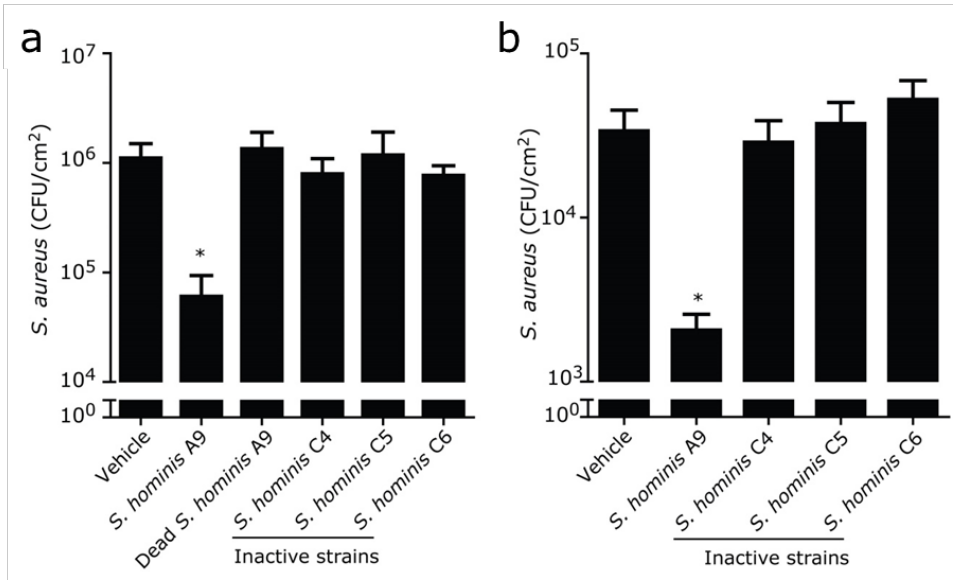


Part A illustrates the capacity of *S. hominis* A9 (culture supernatant) to inhibit *S. aureus*. Zones of inhibition of *S. aureus* are shown to illustrate heat stability, protease sensitivity, and ammonium sulfate precipitation of the purified native peptide from *S. hominis*. **Parts B and C** indicate *S. hominis* A9 strain produces two antimicrobial components. Part B illustrates the high-performance liquid chromatography (HPLC) purification of two antimicrobial peptides from culture supernatant of *S. hominis* A9. Part C illustrates the structure of purified antimicrobial peptides, Hogocidin-α and Hogocidin-β. **Parts D and** E illustrate potency of each hogocidin to kill *S. aureus*. The minimal bactericidal concentration of Hogocidin-α and Hogocidin-β was 0.63 and 1.25 μm, respectively, much lower than host antimicrobial peptides. The hogocidins exerted synergistic antimicrobial activity when combined with skin antimicrobial peptide, LL-37. **Part F** illustrates the antimicrobial dose response of Firmocidin identified in *S. epidermidis*. Comparisons of potency between synthetic and naturally purified Firmocidin and activity of a highly potent topical antimicrobial (Benzyl peroxide) to inhibit GAS, GBS, Methicillin-Sensitive *S. aureus* (MSSA) and MRSA are also shown. **Parts G and H** show data from a single IV dose of Firmocidin (20 mg/kg) given to mice 24 hours after starting a deep skin infection by subcutaneous injection of 10^8 CFU of GAS. Three days later mice given only one dose of Firmocidin showed no open wounds and 3 logs fewer bacteria at the injection site (not shown). We confirmed that these compounds are active on the skin. To test this,

we developed a method for stable formulation of commensal bacteria in a lotion base. The lotion base Cetaphil® is well tolerated by participants with AD.

Figure 1.3c below shows results of an experiment where survival of *S. aureus* was measured on the skin of mice after a single application of bacteria shown in Figure 1.3b. It shows that our transplant approach has a high likelihood to succeed in achieving the endpoint of decreasing *S. aureus*.

Figure 1.3c A bacterial transplant inhibits the survival of *S. aureus* on *ex vivo* pig skin (a) and mouse skin (b).



Having established an association between AMP-producing commensal CoNS and colonization with *S. aureus* (Figure 1.3a), and having identified active peptides produced by these strains (Figure 1.3b), we next sought to directly test if the presence of *S. hominis* producing Hogocidins (A9 strain) will reduce colonization by *S. aureus*. First, we employed an *ex vivo* pig skin colonization model. In this model, peptides or bacteria were applied to the surface of sanitized pig skin on which defined amounts of *S. aureus* had first been applied ($1x10^5$ CFU/cm^2). A significant decrease in survival of *S. aureus* was seen after a single application of *S. hominis* A9 at a final application density consistent with estimates of the density of bacteria on normal human skin ($1×10^5$ CFU/cm^2) (Figure 1.3c, a). A similar density of *S. hominis* A9 that were killed and rinsed prior to application, or use of multiple *S. hominis* isolates that did not show antimicrobial activity in culture (C4, C5 and C6 strains) did not affect *S. aureus* survival. Similarly, a single application of active *S. hominis* A9 to mice dorsal skin on which defined amounts of *S. aureus* had been applied ($1×10^5$ CFU/cm^2) reduced the survival of *S. aureus* on the skin (Figure 1.3c, b). In contrast, application of inactive strains (C4, C5, and C6 strains) at the similar density did not show this activity.

We have also published additional evidence that this approach can be successful using another beneficial *S. epidermidis* strain on human skin (Cogen et al., 2010a; Cogen et al., 2010b). In these studies, we demonstrated that application of these bacteria to human fingertips will inhibit > 99.9% survival of GAS, and application of only nanomoles of the purified AMP will inhibit growth of GAS abundance on the forearm of volunteer participants with a p value of <0.001. Thus, we have strong preliminary evidence in animals and on normal human skin that only a single application of a single antimicrobial will be effective to inhibit *S. aureus.*

Our approach will build on this preliminary data by using a strategy similar to what we have observed in normal skin. Multiple antimicrobial strains of commensal bacteria are present at the same time. We have chosen one strain based on

its stability, potency, complementary activity, and prior experience *in vivo*. This approach will permit us to collect vital new information on the interactions of the microbiome with AD patients, to better understand the mechanistic factors that influence the stability and effects of the microbiome, and evaluate a potential therapeutic intervention that may be a major modifier of clinical outcome.

1.4. Clinical Studies

In order to further examine if topical application of antimicrobial CoNS can decrease *S. aureus* survival on the skin of AD participants, a preliminary double-blind, placebo-controlled AMT study was conducted under IND# 15786 (IND Sponsor: UCSD). An antimicrobial CoNS strain was isolated from the non-lesional skin of each of 5 AD participants who were *S. aureus* culture positive. To transiently increase their abundance on each participant, the active strain(s) were expanded and then reapplied to the lesional forearm skin of the same participant, from which the active colonies were isolated (Table 1.1), to a final concentration of 1×10^5 CFU/cm^2, a density similar to previous assessments of the abundance of bacteria on normal human skin. A single application of the functionally defined and autologously-derived CoNS strain(s) decreased *S. aureus* CFU within 24 hours; whereas, vehicle applied to the contralateral arm in a double-blind comparison did not (Figure 1.4, a-b). However, CoNS survival on the arm 24 hours after AMT application was similar to the baseline. These results suggest that a single application of CoNS at a dose of 1×10^5 CFU/cm^2 was sufficient to transiently decrease survival of *S. aureus*, but the majority of transplanted CoNS died within 24 hours. Autologous derived *S. hominis* A9 (AMT) did not survive beyond 24 hours on AD lesional skin; it is unknown how long *S. hominis* A9 as applied in the allogeneic TMT product will remain alive and active on lesional or non-lesional AD skin. Therefore, we propose to apply the allogeneic TMT at a final concentration of bacteria that ranges from 1 x 10^6 CFU of bacteria/ gram Vegetable glycerin-Cetaphil® to 2 x 10^9 CFU of bacteria/ gram Vegetable glycerin-Cetaphil®, and for multiple applications (twice a day for 1 week) for better survival of transplanted CoNS.

Figure 1.4. Transplantation of antimicrobial CoNS reduces survival of *S. aureus* colonized on the skin surface (The manuscript including this data has not yet been published.

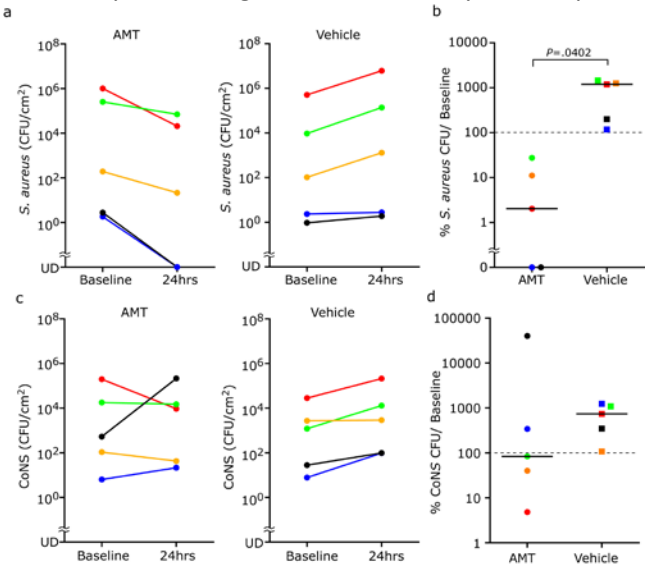


Figure 1.4 panel (a) demonstrates the effect of transplantation of antimicrobial CoNS strain(s) on survival of *S. aureus* on the skin surface of participants with AD. *S. aureus* survival was measured on the lesional sites in both antecubital fossa of the AD participant before and 24 hours after either treatment with antimicrobial CoNS strain(s) autologously screened from the same participant or vehicle. Panel (b) illustrates *S. aureus* survival on the forearm 24 hours after treatment with autologous strain(s) of CoNS or treatment with vehicle. Relative CFU (rCFU) of *S. aureus* is shown as

percent of baseline and different color symbols indicate data from each individual participant. In panel (c) CFUs of CoNS on the skin surface of participants with AD were simultaneously counted as shown in panel (a). Panel (d) shows CoNS survival on the forearm 24 hours after treatment with autologous strain(s) of CoNS or treatment with vehicle. Relative CFU of CoNS is shown as percent of baseline.

In the pilot study of 5 participants evaluating an AMT lotion (IND#15786), no serious adverse events (SAEs) were noted. Two participants noted mild worsening of AD, which was deemed unrelated to the AMT lotion, given AMT was not applied on the area with worsening AD. One participant noted mild worsening of AD with an increase in pruritus and erythema on both arms. Since AMT was applied to only one arm, it is less likely that the adverse event (AE) was related to the AMT given the bilateral worsening of AD on the upper extremities. Overall, the AMT was very well tolerated and had no significant AEs.

2. Study Hypotheses/Objectives

2.1. Hypotheses

We propose an interventional clinical trial that will apply *S. hominis* A9 to *S. aureus* colonized skin of participants with moderate-to-severe atopic dermatitis. *S. hominis* A9 is a specific strain of bacteria commonly found on normal human skin but deficient in patients with AD. This strain has been selected based on its capacity to kill *S. aureus* in pre-clinical models.

Our central hypothesis is that this intervention will be safe and well tolerated by participants with AD. Furthermore, we hypothesize that the reintroduction of this commensal microbe to the skin of AD participants will decrease colonization of *S. aureus*.

Testing these hypotheses will allow us to proceed with development of an intervention that will successfully treat *S. aureus* colonization in clinical patients with AD and eliminate the negative consequences caused by *S. aureus* on the health of skin.

2.2. Primary Objective

The primary objective of this trial 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.

2.3. 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 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

2.4. 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

3. Study Design

3.1. Description of Study Design

This is a phase I, first in man, randomized, double-blind placebo controlled multi-site trial designed to assess the safety, efficacy, and steady-state of allogeneic TMT in adults with moderate-to-severe AD. Approximately 54 adult AD participants, 18 to 80 years of age, will complete 1 week of TMT or placebo applications to non-lesional and *S. aureus* colonized lesional skin on the right and left ventral upper extremities (wrist to upper arm). Participants who withdraw or are discontinued from the study before completing the End of Treatment (Day 7) Visit will be replaced.

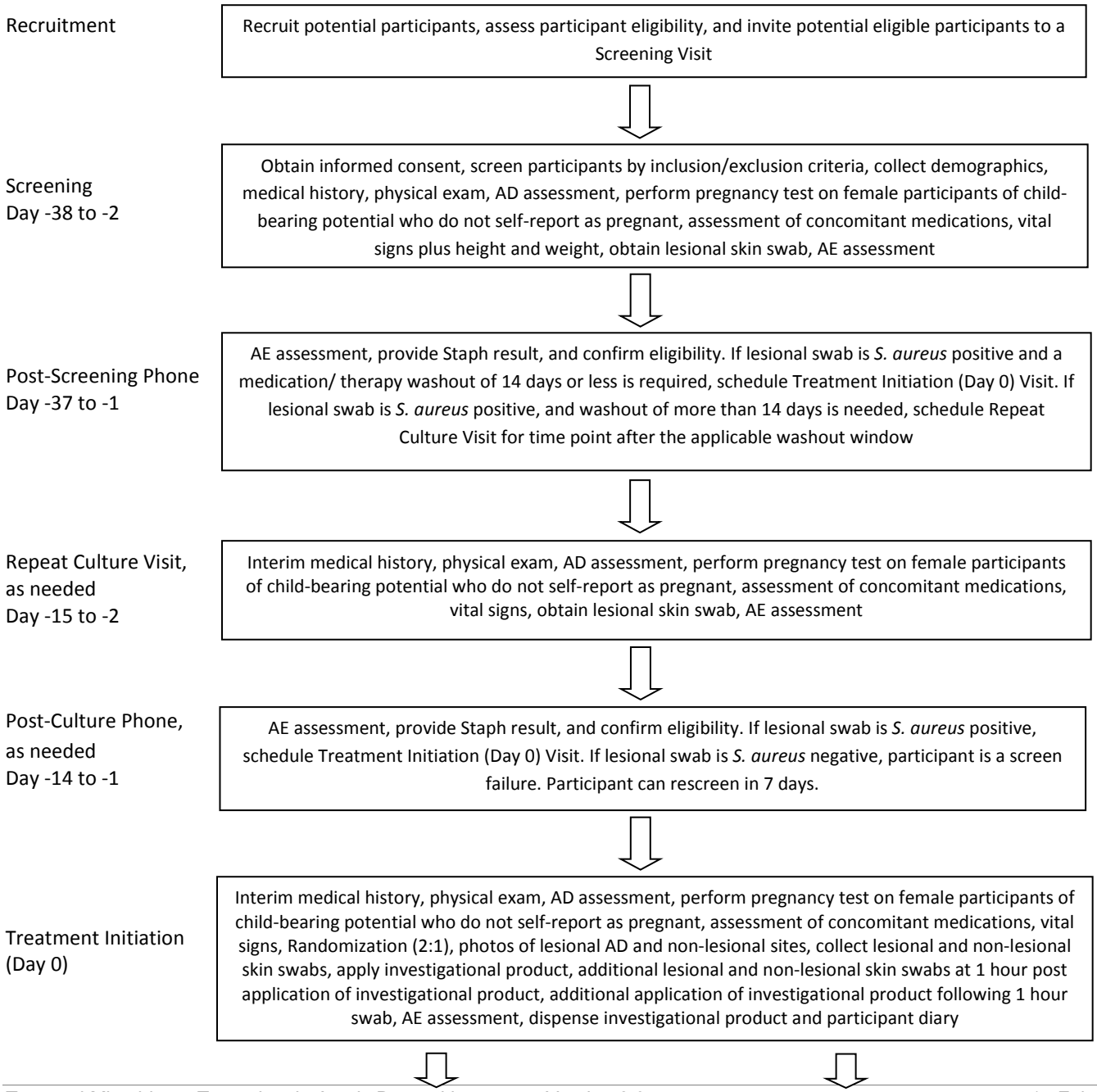
During the Screening Visit, participants will provide informed consent for study participation. Consented participants will then be further assessed for study eligibility through the collection of medical history, including medication/therapy use, a physical exam, assessment of AD severity, and swab collection for *S. aureus* screening. Participants who require a washout period of more than 14 days will be required to return to clinic for a repeat Staph culture.

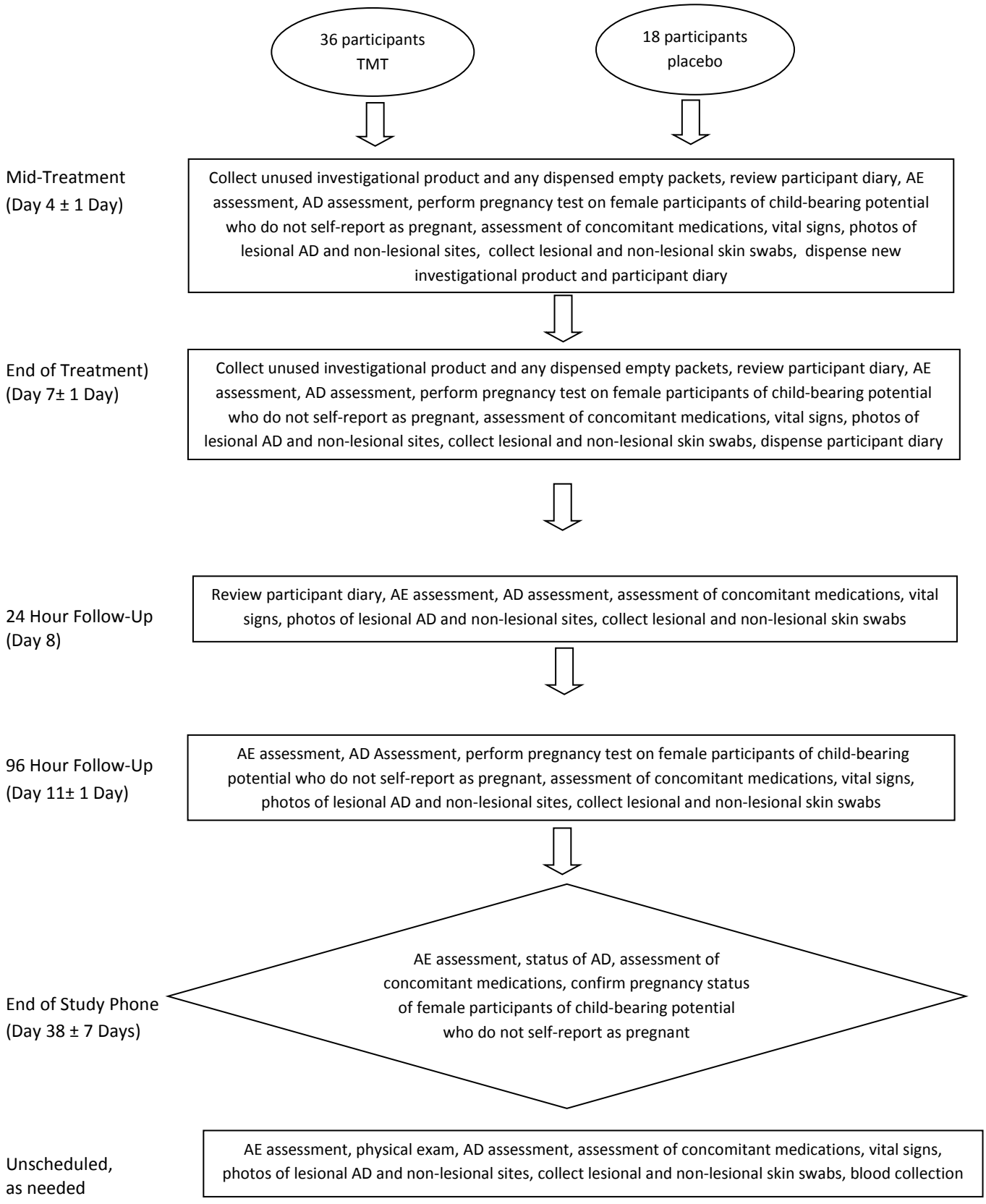
Participants who are eligible based on their positive Staph culture results will be randomized 2:1 active to placebo. One lesional site measuring at least 15 cm^2 and one non-lesional site of equal size will be identified on the participant's ventral upper extremities as the target swabbing areas. These sites will be photographed and marked for swabbing for reference at the participant's future visits. Participants will be instructed to apply investigational product with gloved hands to their ventral upper extremities bilaterally from the wrist to the upper arm, which will include the identified lesional and non-lesional swabbing sites twice a day for 1 week starting on Day 0. Participants will return to clinic on Day 4 for the assessment of AEs, the collection of skin swabs from the identified target sites, and to obtain additional

investigational product and gloves. Participants will complete an additional clinic visit on Day 7 to correspond with the end of their 1 week treatment. During this visit, participants will be assessed for AEs and provide skin swab samples. All unused product and empty packets will be returned during the Day 4 and Day 7 visits. Two additional clinic visits on Days 8 and 11 will be scheduled for additional skin swabs to assess safety and the abundance of CoNS bacteria and *S. aureus* colonization. Participants will be followed through Day 38 to assess for safety and disease status.

Approximately 54 moderate-to-severe AD participants with a positive *S. aureus* colonized lesion on the upper extremities will be enrolled for this trial. Based on preliminary data from the ADRN Registry protocol, we estimate that approximately 40% of AD participants will be *S. aureus* positive on their lesional skin, and we may need to screen up to 133 participants to meet enrollment goals. We anticipate it will take approximately 2 years to reach recruitment and enrollment goals and to complete participant follow up for this trial. The study flow diagram is provided in Figure 3.1.

Figure 3.1 Study Visit Flow Diagram




36 participants
TMT
18 participants
placebo
Mid-Treatment
(Day 4 ± 1 Day)
Collect unused investigational product and any dispensed empty packets, review participant diary, AE assessment, AD assessment, perform pregnancy test on female participants of child-bearing potential who do not self-report as pregnant, assessment of concomitant medications, vital signs, photos of lesional AD and non-lesional sites, collect lesional and non-lesional skin swabs, dispense new investigational product and participant diary
End of Treatment)
(Day 7± 1 Day)
Collect unused investigational product and any dispensed empty packets, review participant diary, AE assessment, AD assessment, perform pregnancy test on female participants of child-bearing potential who do not self-report as pregnant, assessment of concomitant medications, vital signs, photos of lesional AD and non-lesional sites, collect lesional and non-lesional skin swabs, dispense participant diary
24 Hour Follow-Up
(Day 8)
Review participant diary, AE assessment, AD assessment, assessment of concomitant medications, vital signs, photos of lesional AD and non-lesional sites, collect lesional and non-lesional skin swabs
96 Hour Follow-Up
(Day 11± 1 Day)
AE assessment, AD Assessment, perform pregnancy test on female participants of child-bearing potential who do not self-report as pregnant, assessment of concomitant medications, vital signs, photos of lesional AD and non-lesional sites, collect lesional and non-lesional skin swabs
End of Study Phone
(Day 38 ± 7 Days)
AE assessment, status of AD, assessment of concomitant medications, confirm pregnancy status of female participants of child-bearing potential who do not self-report as pregnant
Unscheduled,
as needed
AE assessment, physical exam, AD assessment, assessment of concomitant medications, vital signs, photos of lesional AD and non-lesional sites, collect lesional and non-lesional skin swabs, blood collection

3.2. Primary Endpoint

The count of serious and non-serious treatment-emergent AEs per participant during the time period of Day 0 to Day 8

3.3. 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 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

3.4. 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

3.5. Stratification, Randomization, and Blinding/Masking

Approximately 54 participants with a positive *S. aureus* colonized lesion on their ventral upper extremity will be randomized 2:1 to receive either TMT or placebo for 1 week. Randomization will be performed centrally at the Statistical and Clinical Coordinating Center (SACCC) using a stratified block randomization design. The design will stratify by *S. aureus* abundance (low or high abundance) between treatment arms by clinical site. *S. aureus* abundance will be assessed at Screening, or at the Repeat Culture Visit for those requiring a medication/ therapy washout of more than 14 days, and will be based on quadrant growth on blood agar plates (score 1-4). A score of 1 will be classified as low abundance and scores 2-4 will be classified as high abundance.

Clinical staff and laboratory staff processing and analyzing samples will be blinded to participant treatment assignments. Since analysis of samples can be unblinding, samples from a participant will only be analyzed after completion of Day 38 or early termination from the study. Samples from a pre-selected minimum number of participants, across time points, will be batched for analysis. The CFU analyses will batch lesional and non-lesional skin swabs separately. The qPCR analyses will collectively batch lesional and non-lesional skin swabs. Within each batch, all applicable samples will be analyzed in a random order to ensure that laboratory staff cannot associate sample results to a single participant.

An unblinded pharmacist will dispense the assigned treatment. To maintain study blind the packaging and appearance of the TMT and placebo will be identical.

3.5.1. Procedure for Unblinding

If a clinically significant event occurs and knowledge of the treatment assignment is required, the study treatment may be unblinded. Unblinding must be approved by the Division of Allergy, Immunology, and Transplantation (DAIT) /NIAID Medical Monitor unless an immediate life threatening condition has developed and the DAIT/NIAID Medical Monitor is not accessible. The site investigator will notify DAIT/NIAID Medical Monitor of the unblinding event on the next business day, following the emergency unblinding. The emergency unblinding will also be reported to the Data and Safety Monitoring Board (DSMB).

A full account of the event will be recorded, including the date and time of the unblinding, the reason for the decision to unblind, and the name of the individual who made the decision, and the names of the DAIT/NIAID Medical Monitor, and others who were notified. The reasons for unblinding of a participant's treatment will be documented.

Unblinding will also occur for IND Safety Reports that will be reported to the Food and Drug Administration (FDA), DSMB, and Institutional Review Board (IRB) as specified in the current FDA IND Safety Reporting guidance.

Unblinding the study due to an approved interim analysis, final analysis, or study termination will require written approval from NIAID.

4. Selection of Participants and Clinical Sites/Laboratories

4.1. Rationale for Study Population

In AD, a state of microbial imbalance known as dysbiosis occurs and is closely associated with disease severity. An important characteristic of the dysbiosis in AD is an increased abundance of *S. aureus* and a decrease in overall bacterial diversity. Several lines of experimental evidence support the concept that the multiple defects in epithelial barrier function in AD promote dysbiosis and that this dysbiosis then promotes the immunological disorder characteristic of AD. These characteristics, dysbiosis and immunological disorder, make AD patients most suitable for the transplant of beneficial skin microbiome species to help regulate the imbalance. AD patients will also be used as the control population. This study will be limited to adult moderate-to-severe AD participants, ages 18-80 years.

4.2. 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 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.

4.3. 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

5. Known and Potential Risks and Benefits to Participants

5.1. Risks of Investigational Product or Intervention as cited in Investigator Brochure

There is a theoretical risk of skin infection associated with the application of allogeneic TMT, although the likelihood of participants developing a skin infection is low given that we will only be transplanting non-pathogenic bacteria that have AMP-producing abilities onto the skin of participants. Furthermore, we will be applying these bacteria topically and will examine all participants prior to application to ensure that they do not have any cracks or excoriated skin on their arms. Theoretical risks of contact dermatitis to Vegetable glycerin or Cetaphil® lotion are also possible both with the TMT product and the placebo. Participants will be instructed to avoid applying lotion to mucosal surfaces such as the conjunctiva. If applied to mucosal surfaces, there may be a higher risk of irritation or infection. We will also avoid applying the investigational product (IP) to the hand since the hand would be a likely source of spreading these bacteria onto other surfaces that other people may contact. Disposable nitrile gloves will be provided to participants to use during application of the allogeneic TMT or placebo. Participants with close contacts (e.g. spouses, children, or members in the same household) that have severe barrier defects or are immunocompromised will be excluded from participating, per study exclusion criteria.

Should an infection occur, the participant would be treated according to standard treatment including antibiotics (for infection) and ointment (for irritation and dryness). Refer to Section 7.4 for more information on rescue medications. Contact allergic reactions to Cetaphil® lotion or Vegetable glycerin will be treated with mid-potency topical steroids such as fluocinonide ointment 0.05%.

5.2. Risks of Investigational Product or Intervention cited in Medical Literature

Not Applicable

5.3. Risks of Other Protocol Specified Medications

In the event of infection, the elected rescue treatment will follow guidelines specified in Section 7.4. There is theoretical risk associated with the use of topical or oral antibiotics as a rescue medication. Specific risks will vary between treatments. In general risks of antibiotics are hypersensitivity, urticaria, nausea, vomiting, diarrhea, sun sensitivity, anaphylaxis, and death.

5.4. Risks of Study Procedures

5.4.1. Risks Associated with Stopping the Use of Protocol Prohibited Medications/Therapies

AEs associated with stopping the use of protocol-prohibited medications/therapies may include worsening of the condition being treated and will be reported as such. In an effort to minimize these risks, participants with severe AD who may have difficulty tolerating periods without medication/therapy use will be excluded from participating, per study exclusion criteria.

5.4.2. Risks Associated with Skin Swab Collection

There are no significant risks associated with skin swab collection.

5.4.3. Risks Associated with Physical Exam

There are no known risks associated with the physical exam.

5.4.4. Risks associated with Questionnaires

There is a possibility that participants may find questions too personal. Participants may refuse to answer any questions that make them feel uncomfortable. There is also a possibility that a participant's

answers may be read by others; however, participants' records are carefully protected so this is very unlikely. See section 16.4 for more information on confidentiality.

5.4.5. Risks associated with Blood Collection

Risks associated with drawing blood include possible pain when the needle is inserted, as well as bleeding, bruising and/or infection at the puncture site. Some people may experience lightheadedness, nausea, or fainting. A numbing agent may be placed on the skin before the blood draw to reduce the pain of the stick. Side effects from this cream (mainly skin rash) may occur. There is a potential for slight psychological stress from the procedure. If psychological stress is too much in the opinion of the participant or the physician/nurse the procedures will be halted. National Institutes of Health (NIH) guidelines for blood collection (amount and frequency based on age) will be followed.

5.5. Potential Benefits

There may or may not be any direct benefits for the participants who elect to enroll in this study. One potential benefit is that the investigational product may improve the participant's AD; however, there is no guarantee that the product will help the participant's condition. The participant's skin condition may even get worse by withholding his/her previous/regular AD treatment, or the participant could be in the placebo group and not receive active treatment, in which case his/her AD may also flare.

The results of this study may also provide identification and/or validation of new targets for the future development of therapeutics for participants with AD, as well as increase current knowledge on the ability to transform the cutaneous microbiome, which could lead to potential therapeutic strategies for treating a variety of inflammatory skin conditions.

6. Investigational Agent

6.1. Investigational Agent

6.1.1. Targeted Microbiome Transplant Lotion (TMT)

6.1.1.1. Formulation, Packaging, and Labeling

The allogeneic TMT product will be manufactured and packaged at UCSD. The UCSD Manufacturing Lab has isolated *Staphylococcus hominis* strain A9 from the skin surface of a healthy donor. This *S. hominis* A9 strain produces potent antimicrobial activity against *S. aureus*. UCSD has characterized antimicrobial peptides produced by this strain with protein purification, protein sequencing, and genomic sequencing approaches. The UCSD Manufacturing Lab will establish the Master Cell Bank (MCB) of the *S. hominis* A9 strain, and it will be stored in a locked -80^{o}C freezer at UCSD. The Working Cell Bank (WCB) will be prepared from a single colony of the MCB and stored in a locked -80^{o}C freezer at UCSD.

Each batch of the allogeneic TMT product will be prepared from a single-use vial from the WCB. A vial of the *S. hominis* A9 from the WCB will be thawed and cultured in animal-free tryptic soy broth (TSB) media at 37°C to expand the number of *S. hominis* A9. Bacterial concentration will be assessed, after culturing, by OD_{600}. The cultured bacteria will then be washed twice with an equal volume of normal saline (NS) to remove the animal-free TSB. Based on pre-clinical data, proteins are undetectable in the supernatant after the second wash. Bacterial density of the resuspension will be measured by OD_{600}.

Washed bacteria will be sterilely mixed in 50% Cetaphil® lotion and 50% Vegetable glycerin using a sterile bowl and sterile spatula. Each batch will be assigned a unique lot number and will undergo a visual test for

appearance, Microbial Limits Testing in accordance with U. S. Pharmacopeial Convention (USP) <61> and <62> testing, potency testing by bacterial concentration testing and radial diffusion testing, and identity testing by polymerase chain reaction (PCR) for the hogocidin gene. Two gram aliquots of final allogeneic TMT product will be transferred into sterile heat-sealing pouches with a sterile dispenser under the sterile hood. The packet will be sealed with a heat-sealer. Twenty-two single-use packets will be grouped in boxes and labeled with the lot number and box ID. Pre-labeled boxes will be provided to the UCSD Manufacturing Lab by Eminent. Packaged boxes will be stored in a -80°C freezer at UCSD until shipment to clinical sites. After packaging, one percent of single-use packets will be selected and potential pathogens will be screened for Microbial Limits Testing in accordance with USP <61> and <62> testing, visual appearance testing, and also undergo potency testing by bacterial concentration testing and radial diffusion testing, and identity testing by PCR for the hogocidin gene. The final TMT product will be formulated at a *S. hominis* A9 concentration range from 1 x 10^6 CFU of bacteria/gram Vegetable glycerin-Cetaphil® to 2 x 10^9 CFU of bacteria/gram Vegetable glycerin-Cetaphil®

Potency testing will be performed by both the radial diffusion assay and bacterial concentration testing. Potency of TMT is acceptable if 4 out of 5 *S. hominis* A9 colonies grown from the TMT product show *S. aureus* inhibition zones greater than 1 mm in diameter. Bacterial concentration of the TMT will be checked by plating a known quantity of the TMT on an agar plate and incubating the plate overnight at 37° C. The number of viable colonies will be counted the following day. All colonies will be checked to ensure they show the same morphology. Only TMT that has a final concentration of bacteria that ranges from 1 x 10^6 CFU of bacteria/gram Vegetable glycerin-Cetaphil® to 2 x 10^9 CFU of bacteria/gram Vegetable glycerin-Cetaphil® will be used in the clinical trial.

Identity testing will be confirmed by the presence of the Hogocidin- α gene by PCR.

Any TMT that does not meet these requirements will be discarded as per UCSD's hazardous waste protocol.

6.1.1.2. Dosage, Preparation, and Administration

6.1.1.2.1. Dosage

6.1.1.2.1.1. Targeted Density of Transplanted CoNS

There is considerable variability in the abundance of CoNS bacteria on human skin. A recent unpublished study by the UCSD laboratory has found CoNS bacteria with antimicrobial activity can be detected at a range of abundance between 1 x10^2 CFU/cm^2 and 1 x10^7CFU/cm^2. No deleterious effects were observed in participants colonized at this density, but participants with this range of antimicrobial CoNS abundance were not colonized by *S. aureus*. Therefore, the application of CoNS in a lotion formulation to a final density of as low as 10^2 CFU/cm^2 can be predicted to be effective at inhibiting *S. aureus*, and a density as high as 1 x 10^7CFU/cm^2 is within the density typically observed on human skin and can be considered safe.

Using the body surface area calculations of Mosteller (Mosteller 1987), we estimate that the smallest potential participant of 4'10" and 85 pounds has a BSA of 1.26 m^2. The largest participant of 6'3" and 210 pounds has a BSA of 2.25 m^2. Using the Rule of Nines, each arm is estimated as 9% of the total BSA. Therefore, the ventral surface would be 4.5% of the total BSA. Since one hand is 1% of

BSA, the ventral surface of one arm not including the hand would be 3.5% of BSA. Thus the smallest participant has an area on their ventral arm of 441 cm^2, and the largest participant 787 cm^2.

We seek to achieve a final concentration of 1x 10^5 CFU/cm^2 of transplanted bacteria on the skin which mimics the concentration on normal skin. To achieve this, the investigational product will be formulated at a final concentration of bacteria that ranges from 1 x 10^6 CFU of bacteria/gram Vegetable glycerin-Cetaphil® to 2 x 10^9 CFU of bacteria/gram Vegetable glycerin-Cetaphil®, and a total mass of 2 g of product will be applied to each participant's arm twice a day. The acceptable dosage range will be between 2 x 10^3 CFU/cm^2 and 1 x 10^7 CFU/cm^2 to account for variability in participant surface area, bacteria survival, and loss of product during application. This range is within the predicted effective and safe concentration of 10^2 CFU/cm^2 to 10^7 CFU/cm^2.

6.1.1.2.2. Preparation

Boxed TMT packets, 22 packets per box, will be shipped to clinical sites on dry ice and stored at -80°C until dispensation to study participants. TMT will be thawed to 4˚C prior to the first IP administration in clinic at the Treatment Initiation (Day 0) Visit. Four packets of TMT will be applied in clinic. The remaining TMT, 18 packets, will be dispensed to participants with instructions that the packets should be stored at 4˚C. An additional supply of TMT product, 22 packets, will be dispensed to participants at their Mid-Treatment (Day 4) Visit.

6.1.1.2.3. Administration

The TMT will be provided in single-dose sealed packets. The lotion should be stored at 4°C. Participants randomized to active TMT will apply 2 g of TMT to each ventral upper extremity (wrist to upper arm) twice a day for one week.

6.1.2. Placebo

6.1.2.1. Formulation, Packaging, and Labeling

Formulation of matching placebo will be identical to TMT, but without the bacteria and will be supplied in identical boxes of sterile single-use packets. Each single-use packet will contain 2 g of placebo containing 50% Vegetable glycerin and 50% Cetaphil® moisturizing lotion. Prior to shipping, each batch of placebo will undergo quality control testing, and additionally one percent of the aliquots will be randomly selected for release testing after packaging into single-use packets. Each aliquot will undergo Microbial Limits Testing in accordance with USP <61> and <62> testing. If the product is not free from contaminants in accordance with USP <61> and <62> testing, the entire lot will be discarded appropriately, and will not be provided to clinical sites.

6.1.2.2. Dosage, Preparation, and Administration

6.1.2.2.1. Dosage

Each single-use packet will contain 2 g of placebo containing 50% Vegetable glycerin and 50% Cetaphil® moisturizing lotion.

6.1.2.2.2. Preparation

Boxed placebo packets, 22 packets per box, will be shipped to clinical sites on dry ice, and stored at -80°C until dispensation to study participants. Placebo will be thawed to 4˚C prior to the first IP administration in clinic at the Treatment Initiation (Day 0) Visit. Four packets of placebo will be applied

in clinic. The remaining placebo, 18 packets, will be dispensed to participants with instructions that the packets should be stored at 4°C. An additional supply of placebo, 22 packets, will be dispensed to participants at their Mid-Treatment (Day 4) visit.

6.1.2.2.3. Administration

Placebo will be provided in single-dose sealed packets. The lotion should be stored at 4˚C. Participants randomized to placebo will apply 2 g of placebo to each ventral upper extremity (wrist to upper arm) twice a day for one week.

6.2. Investigational Products Accountability

Under Title 21 of the Code of Federal Regulations (21CFR §312.62), the investigator will maintain adequate records of the disposition of the investigational products, including the date and quantity of the product received, to whom the product was dispensed (participant-by-participant accounting), and a detailed accounting of any product accidentally or deliberately destroyed. Details of investigational product dispensation to each participating site will be maintained by the manufacturing lab.

Records for receipt, storage, use, and disposition will be maintained by the study site. A product-dispensing log will be kept current for each participant. This log will contain the study identification number of each participant and the date and quantity of product dispensed.

All records regarding the disposition of the investigational product will be available for inspection. At the termination of the study, all unused product will be destroyed. For more information on handling of the investigational product please refer to the manual of procedures (MOP).

6.3. Assessment of Participant Compliance with Investigational Agent

Each clinical site will keep an inventory of TMT and placebo packets with information on date of original preparation, and date received on site. Participants will be required to return all packets of product dispensed, used or unused. Study staff will record the count of opened, unopened, and missing packets. A reconciliation of recorded data per box, prior to dispensation and after, will be used to assess compliance. In addition, participants will record completed and missed applications in their participant diary.

6.4. Toxicity Prevention and Management

All steps will be taken to minimize potential risks of the study. Participants with known sensitivities/allergies to any of the products used during this study will be excluded from enrollment and study participation. Participants with any cracks or breaks in their skin, including severely excoriated or bleeding skin suggesting that the participant may be susceptible to an infection will be excluded. Similarly, participants with prosthetic or implanted devices will also be excluded. Any AE, defined as any undesirable sign, symptom, or medical condition occurring after the participant's written consent to participate is completed, will be recorded and reported to the Principal Investigator (PI), DAIT/NIAID, FDA, and the IRB as required. If the PI believes an AE is possibly related to the study, the PI will determine whether or not it is in the best interest of the participant to continue in the study. If the participant is injured as a result of participation in this research, treatment will be available at the clinical center where they are completing the study. Further details of the services, including costs and coverage will be explained in the informed consent, signed by the participant. In the case of a skin infection, the participant would be treated according to standard treatment practices. The treatment will include antibiotics (for infection) and Cetaphil® moisturizing lotion (for irritation and dryness). Refer to Section 7.4 for more information on rescue medications.

6.5. Premature Discontinuation of Investigational Products

Investigational products may be prematurely discontinued for any participant for any of the following reasons:

1. Evidence of infection either cutaneously or systemically.
2. Severe worsening of AD which in the opinion of the investigator requires stopping of the investigational product.
3. Evidence of contact allergy to investigational product.
4. Evidence of non-compliance to study protocol or eligibility criteria that in the opinion of the investigator requires discontinuation of investigational product.
5. Any serious or unexpected AE that is possibly or definitely related to the investigational product.
6. Pregnancy in a female participant or in a partner of a male participant.

Investigational product may also be prematurely discontinued for any participant if the investigator believes that continuing use of the investigational product is no longer in the best interest of the participant.

If a participant is prematurely discontinued from investigational product, the participant will be asked to complete any remaining study visits, per protocol, through Day 38.

7. Other Medications

7.1. Concomitant Medications

7.1.1. Protocol-mandated

Not Applicable

7.1.2. Other permitted concomitant medications

During study participation, participants are permitted to use topical steroids per discretion of the investigator on areas of their body outside of the investigational product treatment areas.

Use of antihistamines will not be exclusionary for protocol enrollment. However, participants using antihistamines at the time of the Treatment Initiation Visit will be asked to maintain a stable dose until their completion of the 96 Hour (Day 11) Follow-Up Visit.

7.2. Prophylactic Medications

Female participants of child-bearing potential must use an effective method of contraception (e.g. total abstinence, oral contraceptives, IUDs, barrier method with spermicide, surgical sterilization or surgically sterilized partner, Depo-Provera, Norplant, NuvaRing, or hormonal implants) for the duration of study participation. Male participants must also use an acceptable method of contraception (e.g. barrier methods with spermicide, surgical sterilization, or surgically sterilized partner) or abstain until participation in the study is complete.

Pregnancy tests will be performed on female participants of child-bearing potential who do not self-report as pregnant at the Screening, Repeat Culture, Treatment Initiation (Day 0), Mid-Treatment (Day 4), and End of Treatment (Day 7) Visits, and a final pregnancy test will be conducted at the 96 Hour Follow-Up Visit (Day 11). These participants will also be asked during their End of Study (Day 38) Phone Visit whether they have tested positive to a pregnancy test since their last study visit.

Male participants will be asked if a partner has tested positive to a pregnancy test at the Screening, Repeat Culture, Treatment Initiation (Day 0), Mid-Treatment (Day 4), End of Treatment (Day 7), 96 Hour Follow-Up Visit (Day 11), and End of Study (Day 38) Visits.

All reported pregnancies, in female participants and the partners of male participants will be followed as described in Section 12.6.

7.3. Prohibited Medications/Therapies

Until participant completion of the 96 Hour (Day 11) Follow-Up Visit, participants are prohibited to use the following medications/therapies:

- Topical AD treatments (including steroids and topical calcineurin inhibitors); Use of topical steroids on areas outside of where investigational product is applied is permitted per investigator discretion, as described in Section 7.1.2.
- Any lotions or creams other than Cetaphil ® moisturizing lotion (provided by the research clinic) including, prescription moisturizers classified as a medical device (e.g., Atopiclair®, MimyX®, Epiceram®, Cerave®, etc.)
- Oral or topical antibiotics
- Bleach baths
- Oral AD therapies (steroids, immunosuppressive therapies)
- Phototherapy (such as NBUVB, UVB, UVA1, PUVA) or tanning beds

7.4. Rescue Medications

If infection occurs, treatment will include topical and/or oral antibiotics depending on the severity of the infection clinically, and Cetaphil® moisturizing lotion (for irritation and dryness). The antibiotic sensitivity profile of *Staphylococcus hominis* A9, includes sensitivity to Mupirocin, Clindamycin, Linezolid, Rifampin, Trimethoprim, Sulfamethoxazole, Vancomycin, Cefazolin, Daptomycin, and Oxacillin; intermediate sensitivity to Erythromycin, and Ciprofloxacin; and resistant to Penicillin G and Tetracycline. Thus the topical antibiotic of choice will be Mupirocin. The oral antibiotic of choice will depend on the individual participant's medical history, including any possible contraindications to certain medications due to allergies or other concomitant medication use, but Dicloxacillin will be the first line oral antibiotic, followed by Trimethoprim/Sulfamethoxazole or Clindamycin. Contact allergic reactions to Cetaphil® lotion or Vegetable glycerin or palm kernels will be treated with mid-potency topical steroids such as fluocinonide ointment 0.05%.

8. Study Procedures

A summary of complete study procedures is included in Appendix B Schedule of Events.

8.1. Recruitment Visit

Potential participants will be recruited using standardized questionnaires that collect contact information and disease status related to inclusion and exclusion criteria. Participants may be recruited by phone or in person. Those who have no obvious characteristics making them ineligible for the study and who are interested in participating will be invited to clinic to complete the Screening Visit.

8.2. Screening Visit (Day -38 ± to -2)

The purpose of the Screening Visit is to confirm eligibility to continue in the study. During the visit, written informed consent will be obtained from the participant prior to performing any other study procedures.

The following procedures, assessments, and laboratory measures will be conducted to determine participant eligibility:

- Collection of demographics

- Medical History and physical examination, including assessment of AD severity (IGA of ventral arms, EASI of ventral arms, SCORAD and Pruritus VAS of ventral arms) by a study physician or other qualified medical professional
- Urine pregnancy test for female participants of child-bearing potential who do not self-report as pregnant
- Assessment of concomitant medications
- Vital signs, plus height and weight
- Skin Swab Collection to assess for *S. aureus* colonization
- Assessment of AEs

Participants who meet inclusion and exclusion criteria including the required washout periods for medications/therapies will have a skin swab collected. Participants who do not meet inclusion and exclusion criteria due to assessment of their concomitant medications/therapies will be asked whether they would be willing to washout of their medications/therapies if they are eligible based on their preliminary swab. Participants who agree to the washout will have a skin swab collected for a preliminary assessment of *S. aureus* colonization. Participants who do not wish to washout out of the prohibited medications/therapies will be identified as screen failures and will not continue in the study.

Participants who are eligible at this point will be given Dove moisturizing soap and will be instructed to use it whenever they shower, but to avoid the upper extremities. Participants will be given an instructional hand card with restrictions on bathing/showering, exercise, and use of emollient. Participants will be instructed to refrain from swimming in chlorinated pools and hot tubs for the duration of the study. Participants will be provided emollient for use outside of the treatment area. At the conclusion of this visit, participants will be instructed to refrain from the use of prohibited medications/therapies as described in Section 7.3. Female participants of child-bearing potential and male participants will be instructed to use an acceptable method of contraception as defined in Section 7.2.

8.3. Post-Screening Phone Visit (Day -37 to -1)

The purpose of the Post-Screening Phone Visit is to inform the participant of his/her eligibility based on whether the lesional swab culture was positive for *S. aureus* and to assess for AEs.

Participants who tested positive for *S. aureus* and require a medication/ therapy washout of 14 days or less will be eligible and will be asked to schedule their Day 0 Treatment Initiation Visit. These participants must be randomized within 14 days of their Screening Visit.

Participants who tested positive for *S. aureus* and require a medication / therapy washout of more than 14 days to meet the full inclusion and exclusion criteria prior to receiving study treatment will be required to complete a Repeat Culture Visit following their washout. The washout periods will be defined such that the participant's Day 0 Treatment Initiation Visit can occur within the visit Window of the Day -14 Post-Culture Phone Visit.

Participants who tested negative for *S. aureus* and those who tested positive but do not wish to complete the medication/therapy washout will be identified as screen failures. These participants may resume the use of prohibited medications/therapies, bathing/showering, exercising, and swimming without restrictions, and the use of the soap and emollient of their choice.

Participants who are continuing in the study at this point will be reminded to use Dove moisturizing soap, whenever they shower, but to avoid the upper extremities. Participants will be given instructions regarding restrictions on

bathing/showering, exercise, and use of emollient. Participants will be reminded to refrain from swimming in chlorinated pools and hot tubs and to refrain from the use of prohibited medications/therapies as described in Section 7.3. Female participants of child-bearing potential and male participants will be instructed to use an acceptable method of contraception as defined in Section 7.2.

8.4. Repeat Culture Visit, as needed (Day -15 to -2)

The purpose of the Repeat Culture Visit is to confirm if the participant is still *S. aureus* culture positive, following the protocol mandated medication/therapy washout. This visit will only be required for participants who were *S. aureus* positive at their Screening Visit and required a medication/therapy washout of more than 14 days to meet full inclusion and exclusion criteria.

The following procedures, assessments, and laboratory measures will be conducted to determine participant eligibility:

- Interim medical history and physical examination, including assessment of AD severity (IGA of ventral arms, EASI of ventral arms, SCORAD, and Pruritus VAS of ventral arms) by a study physician or other qualified medical professional
- Urine pregnancy test for female participants of child-bearing potential who do not self-report as pregnant
- Assessment of concomitant medications
- Vital signs
- Skin Swab Collection for assessment of *S. aureus* colonization
- Assessment of AEs

Participants who are still eligible at this point will have a skin swab collected and will be instructed to use Dove moisturizing soap whenever they shower, but to avoid the upper extremities. Participants will be given instructions regarding restrictions on bathing/showering, exercise, and use of emollient. Participants will be reminded to refrain from swimming in chlorinated pools and hot tubs and to refrain from the use of prohibited medications/therapies as described in Section 7.3. Female participants of child-bearing potential and male participants will be instructed to use an acceptable method of contraception as described in Section 7.2.

8.5. Post-Culture Phone Visit, as needed (Day -14 to-1)

The purpose of the Post-Culture Phone Visit is to inform the participant of his/her eligibility based on whether his/her repeat lesional swab culture was positive for *S. aureus* and to assess for AEs. Participants who tested positive for *S. aureus* will be eligible and will be asked to schedule their Day 0 Treatment Initiation Visit. These participants will be reminded to use Dove moisturizing soap whenever they shower, but to avoid the upper extremities. Participants will be given instructions regarding restrictions on bathing/showering, exercise, and use of emollient. Participants will be reminded to refrain from swimming in chlorinated pools and hot tubs and to refrain from the use of prohibited medications/therapies as described in Section 7.3. Female participants of child-bearing potential and male participants will be instructed to use an acceptable method of contraception as described in Section 7.2.

Participants who tested negative for *S. aureus* and those who tested positive but do not wish to continue the medication/therapy washout will be identified as screen failures. These participants may resume the use of prohibited medications/therapies, bathing/showering, exercising, and swimming without restrictions, and the use of the soap and emollient of their choice. Screen failures may rescreen in 7 days.

8.6. Treatment Initiation Visit (Day 0)

The purpose of the Day 0 Treatment Initiation Visit is to initiate study treatment. The following procedures, assessments, and laboratory measures will be conducted at this initial treatment visit:

- Interim medical history and physical examination, including assessment of AD severity (EASI of ventral arms, RL, SCORAD, and Pruritus VAS of ventral arms) by study physician or other qualified medical professional
- Urine pregnancy test for female participants of child-bearing potential who do not self-report as pregnant
- Assessment of concomitant medications
- Vital Signs
- Randomization
- AD Lesion Assessment, including photographs of the measured lesional and non-lesional sites
- Lesional and non-lesional skin swab collection for assessment of live *S. aureus* and CoNS colonization by CFU quantification and the assessment of bacterial genomic DNA by qPCR and 16S ribosomal ribonucleic acid (rRNA) sequencing
- TMT or placebo application
- Participants will remain in clinic for up to 1 hour following the application of investigational product. Additional lesional and non-lesional skin swabs for *S. aureus* and CoNS colonization and the assessment of bacterial genomic DNA by qPCR and 16S rRNA sequencing will be collected at 1 hour post-application.
- TMT or placebo reapplication after 1 hour swab
- Assessment of AEs
- TMT or placebo dispensation with participant instructions to apply twice daily as detailed in the MOP
- Completion of baseline diary entry and review of participant diary. Participants will be instructed to complete one diary entry per day until their final clinic visit on Day 8. Participants will be asked to record any symptoms and track their compliance for each lotion application in an electronic diary. Paper diaries will be provided to track symptoms and compliance in the event participants do not have access to a device with internet access.

At the conclusion of this visit, participants will be reminded to complete their daily diary entry, use Dove moisturizing soap, whenever they shower, but to avoid the upper extremities, until after their last in-clinic visit on Day 11 is complete. Participants will be given instructions regarding restrictions on bathing/showering, exercise, and use of emollient. Participants will be reminded to refrain from swimming in chlorinated pools and hot tubs and to refrain from the use of prohibited medications/therapies as described in Section 7.3. Female participants of child-bearing potential and male participants will be instructed to use an acceptable method of contraception as described in Section 7.2.

Participants will be given an instructional hand card describing the symptoms of skin and systemic infections, such as pain or swelling at the site of treatment; fever; chills; or night sweats. Participants will be provided instructions on when to contact the research clinic if they experience any of the listed symptoms. Contact information for the study physician and the 24 hour on call physician will also be listed on the hand card. The physician receiving the call will assess if the participant will need to be seen in clinic or if any further treatment is necessary. Alternatively, the participants will be instructed to take an emergency contact hand card with them should they seek medical care at another facility. The emergency contact hand card will state that the participant is currently in a clinical trial involving the application of non-pathogenic bacteria to the skin, and will also include a list of the antibiotic sensitivities of *S. hominis*, and instructions to call the research clinic if an infection of the skin or blood is suspected. The hand card will recommend that the acute

care physician collect at minimum two skin swabs using a standard sterile swab for culture and speciation, a blood culture, and a CBC for local processing, in addition to any other tests they deem medically necessary.

8.7. Mid-Treatment Visit (Day 4 ± 1 Day)

The purpose of the Mid-Treatment Day 4 Visit is to return for clinical and AE assessments and dispense a new supply of investigational product. Participants will be asked to return to the clinic on Day 4 approximately 4 hours after their last investigational product application.

The following procedures, assessments, and laboratory measures will be conducted at this visit:

- Collection of dispensed product, including empty packets
- Review of participant diary
- Assessment of AEs
- Assessment of AD severity (EASI of ventral arms, SCORAD, and Pruritus VAS of ventral arms) by study physician or other qualified medical professional
- Urine pregnancy test for female participants of child-bearing potential who do not self-report as pregnant
- Assessment of concomitant medications
- Vital Signs
- AD Lesion Assessment, including photographs of the measured lesional and non-lesional sites
- Lesional and non-lesional skin swab collection for assessment of *S. aureus* and CoNS colonization by CFU quantification and the assessment of bacterial DNA by qPCR and 16S rRNA sequencing
- TMT or placebo dispensation with participant instructions as detailed in the MOP
- Dispensation of paper participant diary

At the conclusion of this visit, participants will be reminded to complete their daily diary entry, use Dove moisturizing soap, whenever they shower, but to avoid the upper extremities, until after their last in-clinic visit on Day 11 is complete. Participants will be given instructions regarding restrictions on bathing/showering, exercise, and use of emollient. Participants will be reminded to refrain from swimming in chlorinated pools and hot tubs and to refrain from the use of prohibited medications/therapies as described in Section 7.3. Female participants of child-bearing potential and male participants will be instructed to use an acceptable method of contraception as described in Section 7.2.

Replacement instructional and emergency contact hand cards, as described in Section 8.6, describing the symptoms of skin and systemic infections, sensitivities of *S. hominis*, and whom to contact at the research clinic if they experience any of the listed symptoms will be redistributed to participants, as needed.

8.8. End of Treatment Visit (Day 7 ±1 Day)

The purpose of the End of Treatment Visit is to conduct clinical and AE assessments following a one week application of TMT or placebo. Participants will be asked to return to the clinic on Day 7 approximately 4 hours after their last lotion application.

The following procedures, assessments, and laboratory measures will be conducted at this visit:

- Collection of dispensed product, including empty packets
- Review of participant diary
- Assessment of AEs

- Assessment of AD severity (EASI of ventral arms, RL, SCORAD, and Pruritus VAS of ventral arms) by study physician or other qualified medical professional.
- Urine pregnancy test for female participants of child-bearing potential who do not self-report as pregnant
- Assessment of concomitant medications
- Vital Signs
- AD Lesion Assessment, including photographs of the measured lesional and non-lesional sites
- Lesional and non-lesional skin swab collection for assessment of *S. aureus* and CoNS colonization by CFU quantification and the assessment of bacterial genomic DNA by qPCR and 16S rRNA sequencing
- Dispensation of paper participant diary

At the conclusion of this visit, participants will be reminded to complete their daily diary entry, use Dove moisturizing soap, whenever they shower, but to avoid the upper extremities, until after their last in-clinic visit on Day 11 is complete. Participants will be given instructions regarding restrictions on bathing/showering, exercise, and use of emollient. Participants will be reminded to refrain from swimming in chlorinated pools and hot tubs and to refrain from the use of prohibited medications/therapies as described in Section 7.3. Female participants of child-bearing potential and male participants will be instructed to use an acceptable method of contraception as described in Section 7.2.

8.9. 24 Hour Follow-Up Visit (Day 8)

The purpose of the 24 Hour Follow-Up Visit is to assess *S. aureus* and CoNS, including *S. hominis* A9, bacteria abundance, and AEs after the completion of TMT or placebo application.

The following procedures, assessments, and laboratory measures will be conducted at this visit:

- Review of participant diary
- Assessment of AEs
- Assessment of AD severity (EASI of ventral arms, SCORAD, and Pruritus VAS of ventral arms) by study physician or other qualified medical professional.
- Assessment of concomitant medications
- Vital Signs
- AD Lesion Assessment, including photographs of the measured lesional and non-lesional sites
- Lesional and non-lesional skin swab collection for assessment of *S. aureus* and CoNS colonization by CFU quantification and the assessment of bacterial genomic DNA by qPCR and 16S rRNA sequencing

At the conclusion of this visit, participants will be reminded to use Dove moisturizing soap, whenever they shower, but to avoid the upper extremities, until after their last in-clinic visit on Day 11 is complete. Participants will be given instructions regarding restrictions on bathing/showering, exercise, and use of emollient. Participants will be reminded to refrain from swimming in chlorinated pools and hot tubs and to refrain from the use of prohibited medications/therapies as described in Section 7.3. Female participants of child-bearing potential and male participants will be instructed to use an acceptable method of contraception as described in Section 7.2.

8.10. 96 Hour Follow-Up Visit (Day 11 ±1 Day)

The purpose of the 96 Hour Follow-Up Visit is to assess *S. aureus* and CoNS, including *S. hominis* A9, bacteria abundance after the completion of TMT or placebo application.

The following procedures, assessments, and laboratory measures will be conducted at this visit:

- Assessment of AEs
- Assessment of AD severity (EASI of ventral arms, SCORAD, and Pruritus VAS of ventral arms) by study physician or other qualified medical professional
- Urine pregnancy test for female participants of child-bearing potential who do not self-report as pregnant
- Assessment of concomitant medications
- Vital Signs
- AD Lesion Assessment, including photographs of the measured lesional and non-lesional sites
- Lesional and non-lesional skin swab collection for assessment of *S. aureus* and CoNS colonization by CFU quantification and the assessment of bacterial genomic DNA by qPCR and 16S rRNA sequencing

At the conclusion of this visit, participants may resume prohibited medications/therapies as described in Section 7.3, as needed, bathing/showering, exercising, and swimming without restrictions, and may use their choice of soap and emollient. Female participants of child-bearing potential and male participants will be instructed to use an acceptable method of contraception as described in Section 7.2.

8.11. End of Study Phone Visit (Day 38 ±7 Days)

The purpose of the Day 38 End of Study Phone Visit is to complete participant follow-up. The visit will occur approximately one month following the participant's scheduled 24 Hour Follow-Up Visit. This visit will be brief, and participants will be asked to report any new AEs and to answer questions regarding the status of their AD and concomitant medication use. Female participants of child-bearing potential will be asked whether they have tested positive to a pregnancy test since their last visit. Male participants will be asked whether their partner has tested positive to a pregnancy test since their last visit. Any participant with an ongoing AE/SAE at the time of this phone contact will continue to be followed until the event is resolved with or without sequelae or the AE/SAE stabilizes.

8.12. Unscheduled Visits

If disease activity increases, participants experience signs and symptoms as described on the study hand card, or other concerns arise between regularly scheduled visits, participants will be instructed to contact study personnel and may be asked to return to the study site for an "unscheduled" visit.

The following procedures, assessments, and laboratory measures will be conducted at the unscheduled visit:

- Assessment of AEs
- Physical examination, including assessment of AD severity (EASI of the ventral arms, SCORAD, and Pruritus VAS of ventral arms) by study physician or other qualified medical professional
- Assessment of concomitant medications
- Vital Signs
- AD Lesion Assessment, including photographs of the measured lesional and non-lesional sites
- Skin Swab collection, per investigator discretion
- Blood collection, per investigator discretion

If a participant presents to the research clinic with a suspected skin or systemic infection, skin swabs and/or blood samples may be collected. If collected, skin swab samples will be cultured and speciated to determine whether the transplant Staph strain caused the infection. A complete blood count (CBC) with differential and blood culture will be performed on the blood sample, if collected, to assess for infection.

Participants will be given a hand card to present to clinical staff should they seek care for an infection at another facility. The hand card, as described in Section 8.6, will state that the participant is currently in a clinical trial involving the application of non-pathogenic bacteria to the skin, list the sensitivities of *S. hominis,* and include instructions to call the research site if an infection of the skin or blood is suspected.

8.13. Visit Windows

Study visits should take place within the time limits specified above: the designated visit windows *(i.e. +/- n days)* for each scheduled visit are also indicated in Appendix B Schedule of Events.

9. Sample Collection and Assays

This section describes the proposed methodologies for this study. The techniques are state-of-the-art at the time of the writing of this protocol. Even so, the techniques will be updated and/or changed should there be additional technical breakthroughs in this area of research. Details of the laboratory processes are described in Standard Operating Procedures (SOPs) maintained by each laboratory.

9.1. Skin Swab Samples

9.1.1. Skin Swab Collection Procedure

The most severe lesion measuring at least 15 cm^2 on the ventral upper extremities will be defined as the lesional swab location. Lesions closer to the antecubital region will take precedence in defining this area. One lesional swab location will be defined at Screening and the Repeat Culture Visits. A lesional and non-lesional site will be defined at Day 0, for assessment through Day 11. The non-lesional swab location will be defined as an area of normal skin on the ventral upper extremities measuring at least 15 cm^2. The designated lesional and non-lesional sites for swabbing will be marked with a pen and non-identifying digital photographs will be taken for reference during future visits. The designated sites for swabbing will be measured for each participant and the total lesional and non-lesional area (cm x cm) recorded. A minimum of 6 swabs, 3 lesional swabs and 3 non-lesional swabs will be obtained from each participant. Each swab will cover a 5 cm^2 area. Using a swab, the lateral edge of the swab will be rubbed across the measured area in a cross-wise manner while rotating the swab handle between the thumb and forefinger. Swabbing will be performed for at least 30 seconds. If sampling area allows, up to 6 additional swabs may be obtained from each participant, 3 additional swabs from the lesional and non-lesional each. A maximum of 12 swabs may be obtained from each participant.

9.1.2. *Staphylococcus aureus* Screening Assay

Skin swabs will be collected from pre-measured areas of lesional and non-lesional skin per Section 9.1.1. Swabs will then be used to inoculate blood agar plates (5% sheep blood; No. R01202; Remel Inc.; Lenexa, Kansas). A Spectra MRSA agar plate (Remel Spectra MRSA Screen plate; No. R01821; Remel Inc.) will also be swabbed for qualitative evaluation only. Blood agar plates will be incubated up to 48 hours in a 5% CO_2 incubator at 37◦ C. Colonies appearing to be *Staphylococcus aureus* on the blood agar will be tested for coagulase and catalase. If the tests are positive, the colonies will be identified as *Staphylococcus aureus* species. Positive plates will be scored from 1-4 based on the number of quadrants with *S. aureus* growth. The MRSA plates will be incubated up to 24 hours in a non-CO_2 incubator at 35°C. Any colonies growing on the Spectra MRSA plate after 24 hours will be identified as MRSA.

9.1.3. CFU Quantification of Live *Staphylococcus aureus* and CoNS

Skin swabs will be collected from pre-measured areas of lesional and non-lesional skin per Section 9.1.1. Swabs will be suspended in 1.5 mL TSB containing 15% (v/v) USP-grade glycerin and stored frozen at -80°C. Live CFUs of coagulase positive *S. aureus* will be determined by rapid thawing of frozen swab samples and then immediate inoculation on a bacterial culture plate containing mannitol salt agar with egg yolk for selective growth of Staphylococcus spp. *S. aureus* will be conventionally distinguished from CoNS according to mannitol metabolism and the egg yolk reaction, and coagulase positive and negative Staphylococcus will be quantified by manual counting of limiting dilutions of the stock solution.

Lesional and non-lesional skin swabs will be separately analyzed in batches consisting of all applicable swabs available for a pre-selected minimum number of participants after all selected participants have either completed the Day 38 Visit or terminated early from the study. In order to help maintain the blind throughout the process of sample analysis, swabs in each batch will be relabeled and processed in random order so that swabs from a single participant are not analyzed in sequence. To help ensure the random order and to maintain the blind within the lab, a separate technician will arrange the samples for processing and assay the samples.

9.1.4. Quantification of *S. aureus*, *S. hominis*, Combined Staphylococci, and Combined Bacteria DNA by qPCR

To collect bacterial DNA, pre-measured areas adjacent to those used for bacterial culture will be rubbed with a swab pre-moistened with Tris-EDTA buffer containing 0.1% TritonX-100 and 0.05% Tween-20 (w/v). Bacterial-genomic DNA will be extracted for qPCR with the QIAamp DNA Micro kit (QIAGEN: 56304). DNA will be eluted with 25 µL elution buffer. One µL of the elution will be used for qPCR using universal 16S rRNA primers and species- or genus-specific primers. For quantification of *S. aureus* and *S. hominis* DNA, DNA extracted from an authentic number of each bacterium (*S. aureus* ATCC35556 and *S. hominis* ATCC27845, respectively) will be used for standard curves. For quantification of combined staphylococci DNA, a standard curve from an authentic number of *S. epidermidis* ATCC12228 will be used. The standard curve will be used for determination of absolute number of bacteria (as rCFU/µL). This known amount of bacteria for *S. aureus* and *S. hominis* will range from 1x 10^1 to 1x10^6 CFU/µL. For total 16S rRNA quantification, relative abundance will be calculated with the Delta-Delta-Ct method.

Lesional and non-lesional skin swabs will be analyzed in batches consisting of all applicable swabs available for a pre-selected minimum number of participants after all selected participants have either completed the Day 38 Visit or terminated early from the study. In order to help maintain the blind throughout the process of sample analysis, swabs in each batch will be relabeled and processed in random order so that swabs from a single participant are not analyzed in sequence. To help ensure the random order and to maintain the blind within the lab, a separate technician will arrange the samples for processing and assay the samples.

9.1.5. Analysis of Microbial Populations by 16S rRNA Sequencing

V1-V3 16S rRNA gene will be amplified with Phusion High-Fidelity polymerase (Thermo Scientific, Waltham, MA) using dual-indexed coded primers, normalized, pooled and paired-end sequenced (2 × 300bp) on an Illumina MiSeq (Illumina, San Diego, CA). The 16S rRNA bacterial sequence reads will be assessed for quality and analyzed using phylogenetic and Operational Taxonomic Unit (OTU) methods in the Quantitative Insights into Microbial Ecology (QIIME) software, version 1.9.1. Read pairs will be assembled using fastq-join from the ea-utils package (http://code.google.com/p/ea-utils), requiring at least 20 bases of overlap and allowing a maximum of 10% mismatched bases. OTUs will be picked using the reference-based USEARCH (version 5.2) pipeline in QIIME, using the May 2013 release of the GreenGenes 99% OTU database. OTU clusters with less than four sequences

will be removed, and representative sequences used to make taxonomic assignments for each cluster will be selected on the basis of abundance. The RDP Naïve Bayesian Classifier will be used for taxonomic classification with the GreenGenes reference database.

9.2. Blood Samples

9.2.1. Blood Collection Procedure

If disease activity increases, participants experience signs and symptoms as described on the study hand card, or other concerns arise between regularly scheduled visits, participants may return to clinic for an Unscheduled Visit. If a participant presents to the research clinic with a suspected skin or systemic infection, whole blood samples may be collected by venipuncture.

9.2.2. Blood Tests

In order to assess for infection, venous blood will be collected for culture and a CBC with differential.

10. Biospecimen Storage

Lesional and non-lesional skin swabs will be obtained from all study participants. These skin swabs and any derivative samples will be obtained for potential future analysis of additional parameters that describe the composition and function of the skin microbiome. Such analysis may include deep sequencing of bacterial DNA for more precise identification of the metagenome or analysis of the metabalome by advanced mass spectrometry techniques. Participants will be asked to give permission for long-term storage and future use during the consent process. All samples stored for future use will be kept in a central repository at UCSD.

Instructions for sample preparation, handling, storage, and shipping are included in the MOP. The PIs will be responsible for acknowledging and implementing all the regulations for classification, sample handling, packaging and labeling, permits or authorizations, and personnel training for shipment of biological and hazardous materials required for the conduct of this study.

11. Criteria for Participant and Study Completion and Premature Study Termination

11.1. Participant Completion

Participant participation will be defined as complete at the conclusion of the Day 38 End of Study Phone Visit.

11.2. Participant Stopping Rules and Withdrawal Criteria

Participants may be prematurely terminated from the study for the following reasons:

1. The participant elects to withdraw consent from all future study activities, including follow-up.
2. The participant is “lost to follow-up” (i.e., no further follow-up is possible because attempts to reestablish contact with the participant have failed). The ADRN standard definition of lost to follow-up, as defined in the MOP will be used.
3. The participant dies.
4. The investigator no longer believes participation is in the best interest of the participant.
5. Individual safety stopping rules
    a. Evidence of infection either cutaneously or systemically.

b. Severe worsening of AD which in the opinion of the investigator requires stopping of the investigational product.
c. Evidence of contact allergy to investigational product.
d. Evidence of non-compliance to study protocol or eligibility criteria that in the opinion of the investigator requires discontinuation of investigational product.
e. Any serious or unexpected AE that is possibly or definitely related to the investigational product.
f. Pregnancy in a female participant or in a partner of a male participant.

11.3. Participant Replacement

Participants who were randomized and who withdraw or are withdrawn prior to the completion of the End of Treatment Visit on Day 7 or who fail to meet the criteria of the Modified-Intent-to-Treat (MITT) sample may be replaced as necessary to obtain an MITT sample as defined in Section 13.4.1.

11.4. Follow-up after Early Study Withdrawal

If a participant is withdrawn from the study for any reason, the participant will be asked to complete a final visit by phone to assess any AEs since his/her last visit and to answer questions about the status of his/her AD. Any participant with an ongoing AE/SAE at the time of this phone contact will continue to be followed until the event is resolved with or without sequelae or until the AE stabilizes or until 30 days after the participant prematurely withdraws (without withdrawing consent)/or is withdrawn from the study, whichever occurs first.

11.5. Study Stopping Rules

The trial will be stopped pending immediate DSMB review for the following reasons:

- A single participant experiences any SAE for which there is a reasonable possibility that the investigational product caused the SAE.
- The development of any severe (Grade 3) AE for which attribution is defined as related or possibly related in:
    - 1 out of the first 10 participants enrolled,
    - 2 out of the first 20 participants enrolled,
    - 3 out of the first 30 participants enrolled,
    - 4 out of the first 40 participants enrolled,
    - 5 out of the first 50 participants enrolled.

If any of these criteria are met, enrollment will be suspended and participants currently receiving study treatment will be instructed to stop applying investigational product. The study will not be resumed until the relevant information has been discussed with the DSMB and the DSMB concurs with resumption of the study.

12. Safety Monitoring and Reporting

12.1 Overview

This section defines the types of safety data that will be collected under this protocol and outlines the procedures for appropriately collecting, grading, recording, and reporting those data. AEs that are classified as serious according to the definition of health authorities must be reported promptly (per Section 12.5) to the sponsor DAIT/NIAID. Appropriate notifications will also be made to site PIs, IRBs, and health authorities.

Information in this section complies with *International Conference on Harmonization* (*ICH) Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, ICH Guideline E-6: Guideline for Good Clinical Practice (GCP)*, 21CFR Parts 312 and 320, and applies the standards set forth in the National Cancer Institute (NCI), Common Terminology Criteria for Adverse Events (CTCAE), Version 4.03: http://ctep.cancer.gov/reporting/ctc.html.

12.2 Definitions

12.2.1 Adverse Event (AE)

Any untoward or unfavorable medical occurrence associated with the participant's participation in the research, whether or not considered related to the participant's participation in the research (modified from the definition of AEs in the 2018 ICH E-6 Guidelines for Good Clinical Practice) (from Office of Human Research Protections (OHRP) "Guidance on Reviewing and Reporting Unanticipated Problems Involving Risks to Subjects or Others and Adverse Events (1/15/07)" http://www.hhs.gov/ohrp/policy/advevntguid.html#Q2)

For this study, an AE will include any untoward or unfavorable medical occurrence associated with:

- **TMT or Placebo regimen:** An AE occurring from the time of consent and within one month after the last study-mandated TMT or placebo application
- **Study mandated procedures**:
    - **Blood Draw**

      The following events related to the blood draw procedure will be considered an AE if they occur within 48 hours of the blood draw:

    - Fainting / Vasovagal Events
    - Bruising at the puncture site larger than 2 cm in diameter
    - Bleeding from the puncture site lasting more than 30 minutes
    - Swelling at puncture site larger than 2 cm

12.2.1.1 Suspected Adverse Reaction (SAR)

Any AE for which there is a reasonable possibility that the investigational study product regimen caused the AE. For the purposes of safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the product and the AE. A SAR implies a lesser degree of certainty about causality than adverse reaction, which means any AE caused by a drug (21 CFR 312.32(a)).

12.2.2 Unexpected Adverse Event

An AE or SAR is considered "unexpected" if it is not listed in the Investigator Brochure or is not listed at the specificity, severity, or rate of occurrence that has been observed.

12.2.3 Serious Adverse Event (SAE)

An AE or SAR is considered "serious" if, in the view of either the investigator or the DAIT/NIAID Medical Monitor, it results in any of the following outcomes (21 CFR 312.32(a)):

1. Death.
2. A life-threatening event: An AE or SAR is considered "life-threatening" if, in the view of either the investigator or the DAIT/NIAID Medical Monitor, its occurrence places the participant at immediate risk of death. It does not include an AE or SAR that, had it occurred in a more severe form, might have caused death.
3. Inpatient hospitalization or prolongation of existing hospitalization.

4. Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
5. Congenital anomaly or birth defect.
6. Important medical events that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

Elective hospitalizations are not to be reported as an SAE unless hospitalization is prolonged due to complications.

12.3 Grading and Attribution of Adverse Events

12.3.1 Grading Criteria

The study site will grade the severity of AEs experienced by the study participants according to the criteria set forth in the NCI-CTCAE Version 4.03. This document (referred to herein as the NCI-CTCAE manual) provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all AEs. The NCI-CTCAE has been reviewed by the study investigators and sponsor and has been deemed appropriate for the participant population to be studied in this protocol.

AEs will be graded on a scale from 1 to 5 according to the following standards in the NCI-CTCAE manual:

Grade 1 = mild AE

Grade 2 = moderate AE

Grade 3 = severe and undesirable AE

Grade 4 = life-threatening or disabling AE

Grade 5 = death

Events grade 1 or higher will be recorded on the appropriate AE electronic case report form (eCRF) for this study.

For grading an abnormal value or result of a clinical or laboratory evaluation (including, but not limited to, a radiograph, an ultrasound, an electrocardiogram etc.), a treatment-emergent AE is defined as an increase in grade from baseline or from the last post-baseline value that doesn't meet grading criteria. Changes in grade from screening to pre-treatment and enrollment at Day 0 will also be recorded as AEs, but are not treatment-emergent. If a specific event or result from a given clinical or laboratory evaluation is not included in the NCI-CTCAE manual, then an abnormal result would be considered an AE if changes in therapy or monitoring are implemented as a result of the event/result.

For additional information and a printable version of the NCI-CTCAE manual, consult the NCI-CTCAE web site: http://ctep.cancer.gov/reporting/ctc.html.

12.3.2 Attribution Definitions

The relationship, or attribution, of an AE to the investigational product regimen or study procedure(s) will initially be determined by the site investigator and recorded on the appropriate AE eCRF. Final determination of attribution for safety reporting will be determined by the DAIT/NIAID Medical Monitor. The relationship of an AE to study therapy regimen or procedures will be determined using the descriptors and definitions provided in Table 12.3.2.

Table 12.3.2 Attribution of Adverse Events

Code	Descriptor	Relationship (to investigational products or study procedure: blood draw)
NOT RELATED CATEGORY		
1	Not Related	The AE is clearly not related: there is insufficient evidence to suggest a causal relationship.
RELATED CATEGORIES		
2	Possible	The AE has a reasonable possibility to be related; there is evidence to suggest a causal relationship.
3	Related	The AE is clearly related.

12.4 Collection and Recording of Adverse Events

12.4.1 Collection Period

AEs will be collected from the time of consent until a participant completes study participation or until 30 days after he/she prematurely withdraws (without withdrawing consent) or is withdrawn from the study.

12.4.2 Collecting Adverse Events

AEs (including SAEs) may be discovered through any of these methods:

- Observing the participant.
- Interviewing the participant in an objective manner [e.g., using structured questioning].
- Reviewing the participant diary.
- Receiving an unsolicited complaint from the participant.
- Receiving a call from the participant outside of their regular study visits; instructions for contacting the clinic will be provided on an instructional hand card at the Treatment Initiation Visit, as well as in the participant diary.
- In addition, an abnormal value or result from a clinical or laboratory evaluation can also indicate an AE, as defined in Section 12.3.

12.4.3 Recording Adverse Events

Throughout the study, the investigator will record AEs and SAEs as described previously (Section 12.2*)* on the appropriate AE/SAE eCRF regardless of the relationship to study therapy regimen or study procedure.

Once recorded, an AE/SAE will be followed until it resolves with or without sequelae, or the AE/SAE stabilizes, or until the end of study participation, or until 30 days after the participant prematurely withdraws (without withdrawing consent)/or is withdrawn from the study, whichever occurs first.

12.5 Reporting of Serious Adverse Events and Adverse Events

12.5.1 Reporting of Serious Adverse Events to DAIT/NIAID

This section describes the responsibilities of the site investigator to report SAEs to the sponsor and the SACCC via the SAE eCRF. Timely reporting of AEs is required by 21 CFR and ICH E6 guidelines.

Site investigators will report all SAEs (see Section 12.2.3), regardless of relationship or expectedness within 24 hours of discovering the event.

For SAEs, all requested information on the SAE eCRF will be provided. However, unavailable details of the event will not delay submission of the known information. Initial SAE eCRF should include as much information as possible, but at a minimum must include the following:

- AE term.
- Relationship to investigational product.
- Relationship to stopping protocol prohibited medications/therapy.
- Relationship to study procedure.
- Reason why the event is serious.
- Supplementary case report form (CRF) pages that are current at the time of SAE reporting: medical history, concomitant medications, demographics, investigational product administration.

As additional details become available, the SAE eCRF should be updated and submitted. Every time the SAE eCRF is submitted, it should be electronically signed by the investigator or sub-investigator.

For additional information regarding SAE reporting, contact Rho Product Safety:

Rho Product Safety
6330 Quadrangle Drive, Suite 500
Chapel Hill, NC 27517
Toll-free: 1-888-746-7231
SAE Fax Line: 1-888-746-3293
Email: rho_productsafety@rhoworld.com

12.5.2 Reporting to Health Authority

After an AE, requiring 24 hour reporting (per Section 12.5.1), is submitted by the site investigator and assessed by DAIT/NIAID, there are two options for DAIT/NIAID to report the AE to the appropriate health authorities:

12.5.2.1 Annual Reporting

DAIT/NIAID will include in the IND Annual Report to the FDA all reported AEs classified as:

- Serious, expected, suspected adverse reactions (see Section 12.2.1.1 and Section 12.2.2).
- Serious and not a suspected adverse reaction (see Section 12.2.2).
- Pregnancies.

Note that all AEs (not just those requiring 24-hour reporting) will be reported in the IND Annual Report

12.5.2.2 Expedited Safety Reporting

This option, with 2 possible categories, applies if the AE is classified as one of the following:

Category 1: **Serious and unexpected suspected adverse reaction** [**SUSAR]** (see Section 12.2.1.1, Section 12.2, and 21 CFR 312.32(c) (1) i).

The sponsor shall report any SAR that is both serious and unexpected. The sponsor shall report an AE as a SAR only if there is evidence to suggest a causal relationship between the investigational product and the AE, such as:

1. A single occurrence of an event that is uncommon and known to be strongly associated with investigational product exposure (e.g. sepsis, cellulitis or deep tissue infections);
2. One or more occurrences of an event that is not commonly associated with investigational product exposure, but is otherwise uncommon in the population exposed to the drug (e.g., tendon rupture);
3. An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of investigational product regimen) that indicates those events occur more frequently in the investigational product treatment group than in a concurrent or historical control group.

Category 2: **Any findings from studies that suggest a significant human risk**

The sponsor shall report any findings from other epidemiological studies, analyses of AEs within the current study or pooled analysis across clinical studies or animal or *in vitro* testing (e.g. mutagenicity, teratogenicity, carcinogenicity) that suggest a significant risk in humans exposed to the investigational product that would result in a safety-related change in the protocol, informed consent, investigator brochure or package insert or other aspects of the overall conduct of the study.

DAIT/NIAID shall notify the FDA and all participating investigators of expedited Safety Reports within 15 calendar days; unexpected fatal or immediately life-threatening suspected adverse reaction(s) shall be reported as soon as possible or within 7 calendar days.

12.5.3 Reporting of Adverse Events to the Central IRB

All investigators shall report AEs, including expedited reports, in a timely fashion to their local and central IRB in accordance with applicable regulations and guidelines. All Safety Reports to the FDA shall be distributed by DAIT/NIAID or designee to all participating institutions for site IRB/ Ethics Committee (EC) submission.

12.6 Pregnancy Reporting

The investigator shall be informed of any pregnancy in a female study participant or the partner of a male study participant immediately upon becoming aware of the event. A pregnant participant, or a male participant with a pregnant partner, shall be instructed immediately to stop application of investigational product and will be withdrawn from the study, as described in Section 11.2. The investigator shall counsel the participant and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the pregnant participant or partner of the male participant shall continue until the conclusion of the pregnancy.

The investigator shall report to the SACCC all pregnancies within 1 business day of becoming aware of the event using the Pregnancy eCRF. The SACCC will report all pregnancies to DAIT/NIAID. All pregnancies identified during the study shall be followed to conclusion and the outcome of each must be reported. The Pregnancy eCRF shall be updated and submitted to the SACCC when details about the outcome are available.

Information requested about the delivery shall include:

- Gestational age at delivery
- Birth weight, length, and head circumference
- Gender

- Appearance, pulse, grimace, activity, and respiration (APGAR) score at 1 minute, 5 minutes, and 24 hours after birth, if available
- Any abnormalities.

Should pregnancy complications result in a congenital abnormality, birth defect, miscarriage, or medically indicated abortion - an SAE must be submitted to the SACCC using the SAE reporting procedures described above.

12.7 Reporting of Other Safety Information

An investigator shall promptly notify their local and central IRB, in accordance with applicable regulations and guidelines, as well as the SACCC and DAIT/NIAID via email when an "unanticipated problem involving risks to participants or others" is identified, which is not otherwise reportable as an AE.

12.8 Review of Safety Information

12.8.1 Medical Monitor Review

The DAIT/NIAID Medical Monitor shall receive monthly reports from the SACCC compiling new and accumulating information on AEs, SAEs, and pregnancies recorded by the study sites on appropriate eCRFs.

In addition, the DAIT/NIAID Medical Monitor shall review and make decisions on the disposition of the SAE and pregnancy reports received by the SACCC (See Sections 12.5.1and 12.6).

12.8.2 DSMB Review

The SACCC will provide the NIAID Allergy-Asthma [Alpha] DSMB with a listing of all AEs and SAEs on an ongoing basis, including quarterly reports of all SAEs. Furthermore, the DSMB will be informed of expedited reports of SAEs.

12.8.2.1 Planned DSMB Reviews

The DSMB shall review safety data at least yearly during planned DSMB Data Review Meetings. Data for the planned safety reviews will include, at a minimum, a listing of all reported AEs and SAEs.

12.8.2.2 *Ad hoc* DSMB Reviews

In addition to the pre-scheduled data reviews and planned safety monitoring, the DSMB may be called upon for *ad hoc* reviews when an event occurs that is of sufficient concern to the DAIT/NIAID Medical Monitor and/or the protocol chair(s) to warrant DSMB review, this includes all Expedited Safety Reports. The DSMB will be notified within 24 to 48 hours by the NIAID Medical Monitor and will promptly review any event that potentially impacts safety at the request of the protocol chair or DAIT/NIAID or any occurrence that meets the definition of the *Participant Stopping Rules or Study Stopping Rules* defined in Sections 11.2 and 11.5.

After review of the data, the DSMB will make recommendations regarding study conduct and/or continuation.

12.8.2.2.1 Temporary Suspension of Enrollment and Study Treatment for *ad hoc* DSMB Safety Review

A temporary halt in enrollment and investigational product application will be implemented if an *ad hoc* DSMB safety review is required. New participants will not be consented for study

participation during the enrollment and treatment halt. Participants already on therapy will not continue TMT or placebo application. Participants screened but not yet randomized will not be allowed to continue with the Day 0 Treatment Initiation Visit. All participants not randomized within 14 days of the Screening Visit must rescreen.

13. Statistical Considerations and Analytical Plan

13.1 Overview

This is a phase I, first in man, randomized, double-blind placebo controlled multi-site trial designed to assess the safety, efficacy, and steady-state of allogeneic TMT in adults with moderate-to-severe AD. Approximately 54 adult AD participants, 18 to 80 years of age, will complete TMT or placebo applications to non-lesional and *S. aureus* colonized lesional skin over the course of 1 week.

13.2 Endpoints

13.2.1 Primary Endpoint

The count of serious and non-serious treatment-emergent AEs per participant during the time period of Day 0 to Day 8

13.2.2 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

13.2.3 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

13.3 Measures to Minimize Bias

Stratified block randomization will be performed centrally at the SACCC, and will balance baseline *S. aureus* abundance (low vs high) between treatment arms by clinical site. Clinical staff, laboratory personnel analyzing samples, and study participants will be blinded to treatment arm. Laboratory personnel analyzing samples will remain blinded as described in Section 3.5.

13.4 Analysis Plan

13.4.1 Analysis Samples

- Safety Sample
    - The safety sample will include all participants who are randomized and receive any amount of TMT/placebo.
- 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 75% of doses of TMT/placebo. The MITT sample will not exclude participants with lesional CFU=0 at the Treatment Initiation Visit.
- 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=0 at the Treatment Initiation Visit.
    - Additional PP samples will be defined in the statistical analysis plan (SAP).
- 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
        - Provide skin swabs with analyzable results at each of the Days 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.

13.4.2 Primary Analyses of Primary Endpoint

The primary analysis will be conducted using a negative binomial or Poisson model with robust standard error, as appropriate, adjusting for site and *S. aureus* growth stratum at Screening. Zero-inflated negative binomial or Poisson models will be considered if a large number of participants experience no AEs throughout the study. Analyses will be performed on the Safety Sample within the TMT treatment arm and the placebo arm separately.

As this is a Phase 1 study, the primary endpoint is descriptive, and comparisons between TMT and placebo arms will be done as secondary analyses. All analyses will be used to inform future studies.

13.4.3 Supportive Analyses of the Primary Endpoint

Similar analyses as those described above will be performed as sensitivity analyses, adjusting for covariates found to be relevant and unbalanced between treatment arms (e.g. severity measures, age, gender, etc.).

Further sensitivity analyses will be performed similarly on the MITT and PP samples and on additional PP samples defined in the SAP.

13.4.4 Analyses of Secondary and Exploratory Endpoints

Comparisons between treatment arms of the counts of serious or non-serious adverse events per participant will be conducted using a similar model as described in the primary analysis and corresponding supportive analyses, adjusting for time in days of follow-up. Comparisons between treatment arms of the proportions of participants experiencing at least one serious or non-serious adverse event will be performed using a Pearson Chi-square test or Fisher's exact test, as appropriate.

Analyses of disease severity (e.g. SCORAD, RL, EASI of ventral arms, and Pruritus VAS of ventral arms) will be conducted using analysis of covariance (ANCOVA) comparing treatment arms at each applicable visit, adjusting for Day 0 severity measure and site. Random effects of participant and time will be included in the model. Covariates similar to those used in support of the primary endpoint will be considered for inclusion in severity analyses.

Secondary analyses comparing bacterial abundance (e.g. CoNS, *S. hominis* A9 and *S. aureus)* on lesional and non-lesional skin between treatment arms will be performed using ANCOVA on the $\log_{10}$ transformed abundance of bacteria over all applicable timepoints, adjusting for the $\log_{10}$ (pre-dosing Day 0 bacteria abundance) and site. Random effects of participant and time will be included in the model. Comparisons will be made on the MITT sample between the TMT treatment arm and the placebo arm.

Change from baseline in *S. aureus*, CoNS or *S. hominis* A9 bacteria abundance comparisons between lesional and non-lesional skin in the applicable treatment arm will be analyzed using ANCOVA on the difference in $\log_{10}$ transformed abundance over baseline, adjusting for the $\log_{10}$ (pre-dosing Day 0 bacteria abundance) and site. Random effects of participant and time will be included in the model. Additional analyses adjusting for the abundance at other visits in lieu of pre-dosing Day 0 abundance will be considered and defined in the SAP.

Analyses of microbiome diversity (e.g. % relative abundance by Phylum: Class and Shannon Diversity Index) will be conducted using ANCOVA comparing treatment arms at Day 7, adjusting for 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.

13.4.5 Descriptive Analyses

Descriptive analyses will be reported separately for each treatment arm. Continuous baseline measures will be reported as 1) means (or geometric means) with 95% confidence intervals, or 2) median with 1^{st} and 3^{rd} quartile, as appropriate. Categorical baseline and demographic characteristics and study disposition will be reported as proportions with 95% exact confidence intervals.

13.5 Interim Analyses

13.5.1 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 13.4.1).

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 or if there is an increase in clinical disease severity. The interim analysis will also inform secondary endpoints regarding the ability of the microbiome transplant to decrease *S. aureus* survival and total abundance. This information is necessary for design of future studies. There will be no decisions made regarding 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. Analyses will be performed as specified in Section 13.4.4.

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. Furthermore, AE data will not be analyzed in any fashion for the interim analysis. Blinding will be maintained for all fully blinded individuals, according to the guidelines defined in the Randomization Plan. By-participant data listings will not be included. Results will be reported 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, there will be no penalty in type I error adjustment of the primary analysis for an early look at the data.

Further details for the interim analysis will be specified in the SAP.

13.5.2 Interim Analysis of Safety Data

The DSMB will receive periodic safety reports on enrolled participants along with all investigational product discontinuation cases. The DSMB may request modifications to the protocol based on its review of the findings.

13.5.3 Futility Analysis

No formal interim analyses are planned for futility.

13.6 Statistical Hypotheses

For the analysis of the primary endpoint, we hypothesize that the TMT application taken over the course of 1 week is safe for AD participants with *S. aureus* skin colonization. Safety will be assessed by the count of serious and non-serious treatment-emergent AEs per participant during the time period of Day 0 to Day 8. As this is a Phase 1 study, the primary endpoint is descriptive, and comparisons between TMT and placebo arms will be done as secondary analyses. All analyses will be used to inform future studies.

Null hypotheses of secondary analyses are described below. All corresponding alternative hypotheses are that there are differences between both groups being compared (i.e. 2-sided comparisons).

1. There is no difference in 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 TMT and placebo arm.
2. There is no difference in the count of serious and non-serious AEs per participant between the TMT and placebo arm during study participation.
3. There is no difference in the proportion of participants experiencing at least one serious or non-serious AE between the TMT and placebo arm during study participation.
4. There is no difference in the EASI score of the ventral arms between the TMT and placebo arm at each applicable time point (individually).
5. There is no difference in the SCORAD score between the TMT and placebo arm at each applicable time point (individually).
6. There is no difference in the Pruritus VAS of the ventral arms between the TMT and placebo arm at each applicable time point (individually).
7. There is no difference in the RL score between the TMT and placebo arm at each applicable time point (individually).
8. There is no difference in CoNS bacteria abundance on lesional and non-lesional skin between the TMT and placebo arm at each applicable time point (individually).
9. There is no difference in the change from baseline of CoNS bacteria abundance between lesional and non-lesional skin at each applicable timepoint (individually).
10. There is no difference in the change from baseline of *S. hominis* A9 bacteria abundance between lesional and non-lesional skin at each applicable timepoint (individually).
11. There is no difference in *S. aureus* abundance on lesional and non-lesional skin between the TMT arm and the placebo arm at each applicable time point (individually).
12. There is no difference in the change from baseline of *S. aureus* abundance between lesional and non-lesional skin at each applicable time point (individually).
13. There is no difference in the bacterial DNA abundance on lesional and non-lesional skin between the TMT arm and the placebo arm at each applicable time point (individually).

13.7 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 13.7 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 13.7 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

14. Identification and Access to Source Data

14.1. Source Data

Source documents and source data are considered to be the original documentation where participant information, visit consultations, examinations and other information are recorded. Documentation of source data is necessary for the reconstruction, evaluation, and validation of clinical findings, observations and other activities during a clinical trial.

14.2. Access to Source Data

The site investigators and site staff will make all source data available to the DAIT/NIAID, authorized representatives of DAIT/NIAID, as well as to relevant health authorities. Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that may be linked to identified individuals.

15. Protocol Deviations

15.1. Protocol Deviation Definitions

Protocol Deviation – The investigators and site staff will conduct the study in accordance to the protocol; no deviations from the protocol are permitted. Any change, divergence, or departure from the study design or procedures constitutes a protocol deviation. As a result of any deviation, corrective actions will be developed by the site and implemented promptly.

Major Protocol Deviation (Protocol Violation) - A Protocol Violation is a deviation from the IRB approved protocol that may affect the participant's rights, safety, or well-being and/or the completeness, accuracy and reliability of the study data. In addition, protocol violations include willful or knowing breaches of human subject protection regulations, or policies, any action that is inconsistent with the NIH Human Research Protection Program's research, medical, and ethical principles, and a serious or continuing noncompliance with federal, state, local or institutional human subject protection regulations, policies, or procedures. Examples of Major Protocol Deviations are described in the MOP.

Non-Major Protocol Deviation - A non-major protocol deviation is any change, divergence, or departure from the study design or procedures of a research protocol that does not have a major impact on the participant's rights, safety, or well-being, or the completeness, accuracy and reliability of the study data.

15.2. Reporting and Managing Protocol Deviations

The study site PI has the responsibility to identify, document and report protocol deviations as directed by DAIT/NIAID. However, protocol deviations may also be identified during site monitoring visits or during other forms of study conduct review.

Upon determination that a protocol deviation has occurred, the study staff will a) notify the site PI, b) notify the SACCC and c) will complete a Protocol Deviation form. The protocol deviation form will document at minimum the date the deviation occurred, the date it was identified, a description of the event, whether the deviation resulted in an SAE/AE, PI signature, IRB report requirement, and documentation of a corrective action plan. DAIT/NIAID may request discussion with the site PI to determine the effect of the protocol deviation on the study participant and his/her further study participation, the effect of the protocol deviation on the overall study, and corrective actions. The PI will sign the paper source Protocol Deviation CRF, electronically sign Major Deviations in the electronic data capture (EDC) system, and submit the deviation to the central IRB, and local IRB/EC per IRB regulations. Major protocol deviations will be reported to the DSMB by the DAIT/NIAID Medical Monitor at the medical monitor's discretion.

16. Ethical Considerations and Compliance with Good Clinical Practice

16.1. Quality Control and Quality Assurance

The investigator is required to keep accurate records to ensure that the conduct of the study is fully documented. The investigator is required to ensure that all CRFs are completed for every participant entered in the trial.

The sponsor is responsible for regular inspection of the conduct of the trial, for verifying adherence to the protocol, and for confirming the completeness, consistency, and accuracy of all documented data.

The CRFs will be completed online via a web-based EDC system that has been validated and is compliant with Part 11 Title 21 of the Code of Federal Regulations. Study staff at the site will enter information into the eCRFs, and the data will be stored remotely at a central database. Data quality will be ensured through the EDC system's continuous monitoring of data and real-time detection and correction of errors. All elements of data entry (i.e., time, date, verbatim text, and the name of the person performing the data entry) will be recorded in an electronic audit trail to allow all changes in the database to be monitored and maintained in accordance with federal regulations.

16.2. Statement of Compliance

This clinical study will be conducted using GCP, as delineated in *Guidance for Industry: E6 Good Clinical Practice Consolidated Guidance*, and according to the criteria specified in this study protocol. Before study initiation, the protocol and the informed consent documents will be reviewed and approved by the central IRB. Any amendments to the protocol or to the consent materials will also be approved by the central IRB before they are implemented.

16.3. Informed Consent Process

The consent process will provide information about the study to a prospective participant and will allow adequate time for review and discussion prior to his/her decision. The PI listed on the FDA 1572 or a designee will review the consent and answer questions. Consent designees must be listed on the site delegation of responsibilities log, complete consenting certification, and have demonstrated knowledge of the protocol and study procedures. The prospective

participant will be told that being in the trial is voluntary and that he or she may withdraw from the study at any time, for any reason. The participant will read, sign, and date a consent form before undergoing any study procedures. Consent materials will be presented in the participant's primary language. A copy of the signed informed consent form will be given to the participant.

The consent process will be ongoing. The informed consent form will be revised when important new safety information is available, the protocol is amended, and/or new information becomes available that may affect participation in the study.

16.4. Privacy and Confidentiality

Following the Health Insurance Portability and Accountability Act (HIPAA) guidelines a participant's privacy and confidentiality will be maintained throughout the study. Each participant will be assigned a unique identification number and these numbers rather than names will be used to collect, store, and report participant information. All biological samples will be labeled with the identification number. Data reported in medical journals or scientific meetings will be presented in aggregate for participants as a whole. No individual participant will be identified in any way. Site personnel will not transmit documents containing personal health identifiers (PHI) to the study sponsor or their representatives.

17. Publication Policy

The ADRN Publications Policy will apply to presentations and publications of the results of this trial.

18. References

Cogen AL, Yamasaki K, Muto J, Sanchez KM, Crotty Alexander L, Tanios J, Lai Y, Kim JE, Nizet V, and Gallo RL. Staphylococcus epidermidis antimicrobial delta-toxin (phenol-soluble modulin-gamma) cooperates with host antimicrobial peptides to kill group A Streptococcus. *PLoS One.* 2010;5(1):e8557.

Cogen AL, Yamasaki K, Sanchez KM, Dorschner RA, Lai Y, MacLeod DT, Torpey JW, Otto M, Nizet V, Kim JE, et al. Selective antimicrobial action is provided by phenol-soluble modulins derived from Staphylococcus epidermidis, a normal resident of the skin. *J Invest Dermatol.* 2010;130(1):192-200.

De Benedetto A, Agnihothri R, McGirt LY, Bankova LG, Beck LA. Atopic dermatitis: a disease caused by innate immune defects? *J Invest Dermatology.* 129(1):14-30, 2009.

Hata T and Gallo R. Antimicrobial peptides, skin infections, and atopic dermatitis. *Seminars In Cutaneous Medicine and Surgery.* 2008;27(2):144-50.

Higaki S ,Morohashi M, Yamagishi T, Hasegawa Y. Comparative study of *staphylococci* from the skin of atopic dermatitis patients and from healthy participants. *Int J Dermatol*. 38:265-9, 1999.

Irvine AD. Fleshing out filaggrin phenotypes. *J Invest Dermatol.* 2007;127(3):504-7.

Iwase T, Uehara Y, Shinji H, Tajima A, Seo H, Takada K, Agata T, and Mizunoe Y. Staphylococcus epidermidis Esp inhibits *Staphylococcus aureus* biofilm formation and nasal colonization. *Nature.* 2010;465(7296):346-9.

Kong HH, Oh J, Deming C, Conlan S, Grice EA, Beatson MA, Nomicos E, Polley EC, Komarow HD, Murray PR, et al. Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. *Genome Res.* 2012;22(5):850-9.

Kong HH and Segre JA. Skin microbiome: looking back to move forward. *J Invest Dermatol.* 2012;132(3 Pt 2):933-9.

Kuo IH, Carpenter-Mendini A, Yoshida T, McGirt LY, Ivanov AI, Barnes KC, Gallo RL, Borkowski AW, Yamasaki K, Leung DY, et al. Activation of epidermal toll-like receptor 2 enhances tight junction function: implications for atopic dermatitis and skin barrier repair. *J Invest Dermatol.* 2013;133(4):988-98.

Lai Y, Cogen AL, Radek KA, Park HJ, Macleod DT, Leichtle A, Ryan AF, Di Nardo A, and Gallo RL. Activation of TLR2 by a small molecule produced by Staphylococcus epidermidis increases antimicrobial defense against bacterial skin infections. *J Invest Dermatol.* 2010;130(9):2211-21.

Lai Y, Di Nardo A, Nakatsuji T, Leichtle A, Yang Y, Cogen AL, Wu ZR, Hooper LV, Schmidt RR, von Aulock S, et al. Commensal bacteria regulate Toll-like receptor 3-dependent inflammation after skin injury. *Nat Med.* 2009;15(12):1377-82.

Mosteller RD. Simplified Calculation of Body-Surface Area. *The New England Journal of Medicine*. 317(17): 1098, 1987

Nakamura Y, Oscherwitz J, Cease KB, Chan SM, Munoz-Planillo R, Hasegawa M, Villaruz AE, Cheung GY, McGavin MJ, Travers JB, et al. Staphylococcus delta-toxin induces allergic skin disease by activating mast cells. *Nature.* 2013;503(7476):397-401.

Nakatsuji T, Chen TH, Narala S, Chun KA, Two AM, Yun T, Shafiq F, Kotol PF, Bouslimani A, Melnik AV, Latif H, Kim JN, Lockhart A, Artis K, David G, Taylor P, Streib J, Dorrestein PC, Grier A, Gill SR, Zengler K, Hata TR, Leung DY, Gallo RL. Antimicrobials from human skin commensal bacteria protect against Staphylococcus aureus and are deficient in atopic dermatitis. *Sci Transl Med.* 2017 Feb 22;9(378).

Ong PY, Ohtake T, Brandt C, Strickland I, Boguniewicz M, Ganz T, Gallo RL, and Leung DY. Endogenous antimicrobial peptides and skin infections in atopic dermatitis. *N Engl J Med.*2002;347(15):1151-60.

Ricci G, Patrizi A, Neri I, Bendandi B, Masi M. Frequency and clinical role of *Staphylococcus aureus* over infection in atopic dermatitis in children. *Pediatr Dermatol,* 20:389-92, 2003.

Schnopp C, Ring J, and Mempel M. The role of antibacterial therapy in atopic eczema. *Expert Opin Pharmacother.* 2010;11(6):929-36.

Sugimoto K, Ishikawa N, Terano T, Kitukawa Y, Kubosawa H, Ito S, Hattori T. The importance of bacterial superantigens produced by *Staphylococcus aureus* in the treatment of atopic dermatitis using povidone-iodine. *Dermatology*. 212 Suppl 1:26-34, 2006.

Travers JB, Norris DA, Leung DY. The keratinocyte as a target for *staphylococcal* bacterial toxins. *J Investig Dermatol Symp Proc*. 6:225-30, 2001.

Appendix A: ADRN Standard Diagnostic Criteria (Version 3.0 09May2014)

The following definitions will be used consistently throughout ADRN protocols. In children less than 4 years of age, the disease must be present for at least six months to minimize the likelihood of recruiting children with other eczematous disorders that mimic atopic dermatitis.

I. Atopic Dermatitis (AD)[1]

A. Active Atopic Dermatitis (AD)[1]

Participants must have Atopic Dermatitis (as defined below) within the last 3 months.

B. Inactive Atopic Dermatitis (AD)[1]

Participants must have an absence of Atopic Dermatitis (as defined below) within the last 12 months.

C. Definition

Participants must have, according to medical records, or based on a careful and credible history (provided by the participant, caregiver, parent, or guardian) or by physical exam by an ADRN investigator:

1. Pruritus
2. Eczema (acute, subacute, chronic)
    a. Typical morphology and age-specific patterns which include:
        - Facial, neck, and extensor involvement in infants and children
        - Current or prior flexural lesions in any age group
        - Sparing groin and axillary regions
    b. Chronic or relapsing history
    c. Most participants will have the following clinical associations that add support to the diagnosis:
        - Early age at onset
        - Atopy
            a. Personal and/or family history
            b. Immunoglobulin (IgE) reactivity
        - Xerosis

Participants may have the following clinical associations which help to suggest the diagnosis of AD but are too non-specific for defining or detecting AD for research or epidemiological studies:

1. Atypical vascular responses (e.g., facial pallor, white dermographism, delayed blanch response)
2. Keratosis pilaris/hyperlinear palms/ichthyosis
3. Ocular/peri-orbital changes
4. Other regional findings (e.g., peri-oral changes/peri-auricular lesions)
5. Peri-follicular accentuation/lichenification/prurigo lesions

II. References

1. Eichenfield F, Hanifin J, Luger T, Stevens S, Pride H. Consensus Conference on Pediatric Atopic Dermatitis. J Am Acad Dermatology 2003;49:1088-95.

Appendix B: 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]

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