Evaluation of the Efficacy of an Autologous Microbiome Transplant in Adult Atopic Dermatitis Patients VERSION 2 /May 19, 2016

NCT03158012

IND# 15786

INVESTIGATOR SIGNATURE PAGE	
Protocol:	**Version/Date:** 2/ May 19, 2016
Site Principal Investigator: *Richard Gallo, MD, PhD*	
Title: **Establishing a Skin Microbiome Transplant**	
Study Sponsor: The National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS)	
INSTRUCTIONS: *The site Principal Investigator 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:* **_Richard Gallo, MD, PhD_** **_9500 Gilman Drive_** **_Mail Code 0869_** **_La Jolla, CA 92093-0869_**	

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 International Conference on Harmonization (ICH) document *Guidance for Industry: E6 Good Clinical Practice: Consolidated Guidance* dated April 1996. 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 IRB and NIAMS.

_____

Site Principal Investigator (Print)

_____   _____

Site Principal Investigator (Signature)   **Date**

Protocol Synopsis

Title	Establishing a Skin Microbiome Transplant
Short Title	Establishing a Skin Microbiome Transplant
Clinical Phase	1
Number of Sites	1
IND Sponsor/Number	IND 15786
Study Objectives	Evaluate the capacity of AMT cream using live bacteria to decrease S. aureus colonization in patients with atopic dermatitis (AD).
Study Design	This is a single center phase 1, double-blind placebo controlled trial
Primary Endpoint(s)	The change in S. aureus abundance after 7 days of AMT cream using live bacteria by using skin swabs to determine the quantity of S. aureus by culture and qPCR.
Secondary Endpoint(s)	Secondary Lesional AD skin: - The abundance of the AMT bacterial strains on lesional skin at baseline and after 1 week BID application of AMT. - The quantity of AMT bacterial strains on lesional skin after stopping AMT cream 1 day, 2 days, and 4 days after last application. - The change in abundance of *Staph aureus* on lesional skin from baseline to 1 day, 2 days, and 4 days after stopping AMT. - Change in clinical assessments, including SCORAD, modified EASI, and EASI after 1 week BID application of AMT. - Identify the diversity of the lesional skin microbiome by DNA sequencing from baseline and after 1 week BID application of AMT. - Evaluate the lesional skin microbiome transcriptome by RNA-sequencing analysis from baseline and after 1 week BID application of AMT. Secondary Non-lesional skin: - The abundance of *Staph aureus* at baseline and after 1 week BID application of AMT on non-lesional skin. - The abundance of AMT bacterial strains on non-lesional skin at baseline and after 1 week BID application of AMT. - The quantity of AMT bacterial strains on non-lesional skin after stopping AMT cream 1 day, 2 days, and 4 days after last application. - The change in abundance of *Staph aureus* on non-lesional skin from baseline to 1 day, 2 days, and 4 days after stopping AMT. - Identify the diversity of the non-lesional skin microbiome by DNA sequencing from baseline and after 1 week BID application

Investigator Name: Richard Gallo, MD, PhD
Name of study: Establishing a Skin Microbiome Transplant
Grant number: 1 R21 AR067547-01A1
Version Date: May 19, 2016   Confidential   Page 5 of 53

	of AMT. 6. Evaluate the non-lesional skin microbiome transcriptome by RNA-sequencing analysis from baseline and after 1 week BID application of AMT. 1.
Accrual Objective	27 subjects
Study Duration	2 years
Treatment Description	Autologous microbiome transplant containing commensal *Staph* species to normalize dysbiosis of atopic dermatitis.
Inclusion Criteria	1. Male or female subjects who are not pregnant or lactating 2. 18-60 years of age 3. Diagnosis of atopic dermatitis for at least 6 months using the Hanifin and Rajka Diagnostic Criteria for atopic dermatitis 4. Presence of lesional atopic dermatitis skin in both antecubital fossae 5. Positive S. aureus colonization based on results of a skin culture taken from one of their AD- affected antecubital fossae during the screening visit 6. Positive for antimicrobial *CoNS* species from non-lesional AD skin
Exclusion Criteria	1. Use of any topical AD treatments (including topical steroids, topical calcineurin inhibitors) to either arm within one week of the Treatment visit 2. Use of any antihistamines 7 days within one week of the Treatment visit 3. Use of any oral/systemic AD therapies (steroids) within 28 days of the Treatment visit 4. Severe AD that would worsen significantly from holding a participant's usual topical/oral AD medications for the time periods required in the inclusion/exclusion criteria (one week prior to the Treatment visit for topical medications and antihistamines and 28 days prior to Treatment visits for oral medications) 5. Subjects who have taken a bleach bath within a week prior to the Treatment visit, or who take bleach baths during the study 6. Patients with severe medical condition(s) that in the view of the investigator prohibits participation in the study 7. Subjects with Netherton's syndrome or other genodermatoses that result in a defective epidermal barrier 8. Any subject who is immunocompromised (e.g. provides

	researchers with a history lymphoma, HIV/AIDS, Wiskott-Aldrich Syndrome) or has a history of malignant disease (with the exception of non-melanomatous skin cancer). This information will be gathered verbally from the patient while taking a medical history from the patient, and will not involve further testing such as an HIV test. 9. Subjects 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. Active bacterial, viral or fungal skin infections 11. Any noticeable breaks or cracks in the skin on either arm, including severely excoriated skin or skin with open or weeping wounds suggestive of an active infection or increased susceptibility to infection. 12. Ongoing participation in another investigational trial 13. Use of any oral or topical antibiotic for up to four weeks prior to screening 14. Use of any systemic immunosuppressive therapy (e.g. CsA, MTX, etc.) within four weeks of screening. 15. Sensitivity to or difficulty tolerating Dove fragrance-free bar soap, Cetaphil lotion 16. Subjects with prosthetic heart valves, pacemakers, intravascular catheters, or other foreign or prosthetic devices.
Study Stopping Rules	The following criteria will be used to determine whether or not the study should be stopped to protect the interest of all participants: 1. Any serious adverse event for which there is a reasonable possibility that the study product caused the serious adverse event. 2. The development of a serious adverse event for which attribution cannot be assessed as definitely unrelated to the study product in 10% or more of the study population. 3. The development of any severe (Grade 3) adverse events in 10% or more of the study population.

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Investigator Name: Richard Gallo, MD, PhD
Name of study: Establishing a Skin Microbiome Transplant
Grant number: 1 R21 AR067547-01A1
Version Date: May 19, 2016   Confidential   Page 8 of 53

Table of Contents

Glossary of Abbreviations

CFR	Code of Federal Regulations
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
FDA	Food and Drug Administration
GCP	Good Clinical Practice
ICH	International Conference on Harmonization
IND	Investigational New Drug
IRB	Institutional Review Board
MOP	Manual of Procedures
PI	[Site] Principal Investigator
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Suspected Adverse Reaction
SOP	Standard Operating Procedure
SUSAR	Serious Unexpected Suspected Adverse Reaction

1. **Background and Rationale**

1.1. Background and Scientific Rationale

It is essential for microbial communities to reside on our epithelial surfaces to maintain several functions of human health (1-3).   This conclusion is based in part on observations that dysbiosis, a state of microbial imbalance, is associated with various diseases (1). Systemic antibiotics, bleach baths, or other antimicrobial interventions have been used in patients with Atopic Dermatitis (AD) to attempt to correct dysbiosis (4). Unfortunately, in addition to promoting the development of antibiotic resistance in pathogens, the nonspecific use of antimicrobials also further disrupts the microbial ecosystem by killing both beneficial bacteria and pathogens. Therefore, the non-specific use of antimicrobials should be avoided when possible.

In this proposal, we describe a novel approach to attempt to treat dysbiosis of the skin surface. We have discovered that many of the strains of coagulase negative Staphylococcus (CoNS) that colonize healthy skin, such as Staphylococcus epidermidis (S. epidermidis), Staphylococcus hominis, Staphylococcus warneri and Staphylococcus capitis, produce antimicrobial activity against Staphylococcus aureus (SA). This activity is selective and kills many pathogens but does not inhibit the growth of the normal resident species. AD subjects have a deficiency in the abundance of bacteria that produce this activity. We hypothesize that reapplying the host's own antimicrobial bacteria will facilitate elimination of SA colonization.

We propose here to exploit the antimicrobial function of the microbiome as therapy of skin disease. We will apply skin commensal microbes that have been pre-identified to have antibacterial (anti-S. aureus) activity. These beneficial bacteria will be selected from the subject's own resident skin flora (autologous) and expanded in vitro for reapplication. We wish to determine if this approach will eliminate or reduce S. aureus, and how long this will last. Thus, the immediate significance of our work will be to show how the microbiome can be used to inhibit SA colonization on the skin. However, the long-term significance of this work will be that it will set the groundwork to test if such a transplant of skin commensal bacteria can be of lasting clinical benefit to patients with AD. This work will therefore also examine key variables to enable later tests of the capacity to alter the microbiome over a longer time period. If successful, the current project will permit future test of functions of the skin microbiome such as immune regulation and barrier development (5-9).

1.2. Rationale for Selection of Investigational Product or Intervention

Unlike healthy control skin, the skin of patients with atopic dermatitis (AD) is frequently colonized by Staphylococcus aureus (S. aureus) (Leyden JJ et al. 1974), putting these patients at increased risk of S. aureus skin infections. In addition, research in our lab has shown that these patients have fewer protective Staphylococcal species such as Staphylococcal epidermidis (S. epidermidis) that are known to produce antimicrobial peptides that play a role in protecting the skin from invading pathogens. In this study, we will attempt to decrease S. aureus colonization and increase colonization by protective Staph species in AD patients by first culturing the bacteria on subjects' lesional AD skin. We will selectively grow the subject's S. epidermidis colonies and place them into Cetaphil moisturizer. This will be a single site, double-blind, randomized phase 1 clinical trial evaluating a novel therapy for atopic dermatitis, an autologous microbiome transplant (AMT).

1.3. Preclinical Experience

This proposal introduces the innovative concept of autologous microbial transplant (AMT) therapy. The principle of skin AMT therapy is to isolate a subset of the subject's own microbes with beneficial functions, expand them in vitro, and then to transplant these specific microbes back onto diseased sites of the same subject. We have developed a novel high-throughput screening technique to select these microbes from skin flora. This therapy can be an attractive alternative to antimicrobial therapy because we use bacteria already on the subject and these microbes have therefore already demonstrated the capacity to be tolerated by the subject and not to cause disease. This theoretically reduces the risk compared to non-native species/strains. Thus, we believe the concept of AMT is attractive and innovative.

Our group's prior research has focused on the underlying molecular mechanisms for the microbial symbiosis that benefits our cutaneous immune system and homeostasis. In a 2009 publication in Nature Medicine, our group first demonstrated an anti-inflammatory benefit of S. epidermidis, a predominant CoNS species on normal human skin(6). We have also demonstrated that a small molecule of <10 kDa secreted from S. epidermidis increased expression of ß-defensins in murine skin and human keratinocytes, enhancing resistance to pathogens of mice at a steady state (5). Other groups also have shown that a product secreted from S. epidermidis strongly sensitizes the host's innate immune system to pathogens (7). In cultured human keratinocytes, activation of TLR2 by peptidoglycan, the major constituent of gram-positive cell walls, strengthens cellular tight junctions (9). More recently, it was demonstrated that skin commensal bacteria enhanced cutaneous T-cell functions via activation of IL-1 signaling (8). Indeed, mono-colonization of germ-free mouse skin by S. epidermidis protected these mice against pathogen infection (8).

Some strains of S. epidermidis, often the dominant bacteria found in the skin microflora, produce various types of antimicrobials (10). Because of their potential to kill pathogens in vitro, these antimicrobials can provide antimicrobial protection against pathogens on the skin surface. We have published several examples of this (5, 11), and are characterizing more by using our innovative screening technique. These findings indicate that multiple CoNS species inhabiting the surface of normal human skin can be a source of antimicrobial activity and contribute directly to the skin's antimicrobial defenses. Also, this shows an additional benefit that may be derived from this study. By isolating diverse clones of antimicrobial bacteria we may discover several new antimicrobials

Figure 1 shows the high-throughput technique for screening CoNS strains that produce antimicrobial activity against SA. This technique enables us to determine the frequency of antimicrobial CoNS strains on the skin and then select them for transplant. Preliminary data shown in our introduction to the revised protocol has illustrated how AD patients colonized by SA have a lower frequency of antimicrobial CoNS species than normal healthy skin or S. aureus-negative AD patients. The profile of these antimicrobial CoNS was determined to be different from that of non-antimicrobial species by sequencing of the full-length 16S rRNA gene of each clone. These findings show that the human skin harbors diverse Staphylococcal strains providing antimicrobial activity, and that a low frequency of antimicrobial CoNS may partially account for the susceptibility of AD skin to infection by SA. All subjects studied to date harbor CoNS species that will inhibit SA. Thus, a source of microbes for AMT is available on most subjects even though AD subjects have fewer available with this activity than normal subjects.

Figure. 1. High-throughput screening for antimicrobial activity produced by skin commensal *staphylococcus* species. Coagulase-negative *Staphylococcus* species are isolated on mannitol salt agar from skin by swabbing. Antimicrobial activity in conditioned media from each *Staphylococcus* clone is tested against *S. aureus* 113 strain.
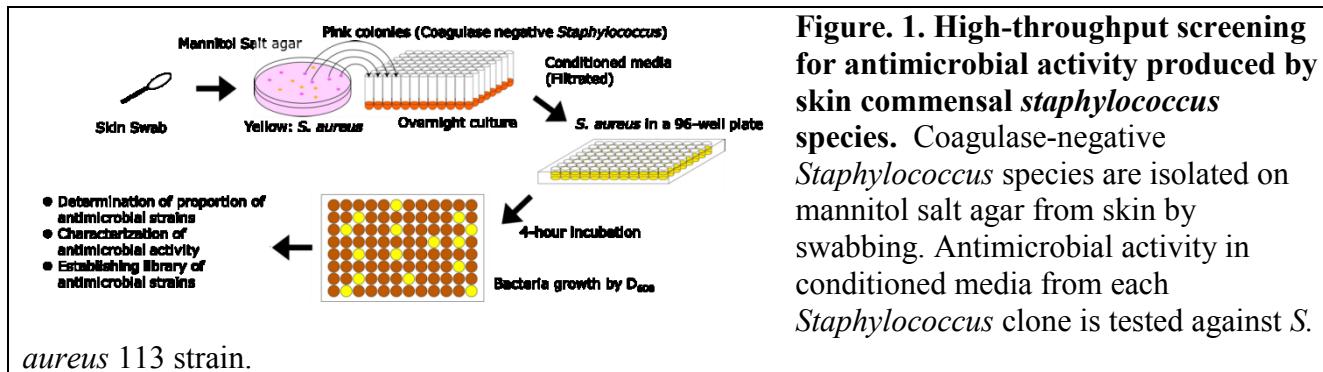
Preliminary work has identified an excellent vehicle for AMT. Clinical isolates of antimicrobial strains of S. hominis or known antimicrobial strains of S. epidermidis (ATCC12228) (12) were mixed with several different over-the-counter skin moisturizer creams at 1×10^7 CFU/gm. Cetaphil® Moisturizing Lotion (Galderma, TX) was found to be an excellent bacteriostatic vehicle. This formulation retained the viability of antimicrobial Staphylococcus for over 5 days at room temperature, but did not allow bacteria to grow (100% at day-2 and 50% at day-5). This formulation was active. A single topical application of this cream formulation containing a known antimicrobial strain of S. epidermidis (ATCC12228), or newly discovered antimicrobial strain of S. hominis, were found to successfully decrease S. aureus colonization on the skin of mice within 29 hrs in comparison to vehicle or similar formulations of known non-antimicrobial S. epidermidis strain (ATCC1457) (Fig.2). These data show that AMT on humans is likely to inhibit S. aureus colonization.

Figure 2. Effect of a bacterial transplant on the survival of *S. aureus* on mouse skin. Shaved back skin was inoculated with 1×10^5 CFU *S. aureus* 113 on a 2×2cm area. After 3 hours, Cetaphil® cream containing indicated dose of an antimicrobial strain of *S. hominis* (SH) (50mg) or a known antimicrobial strain of *S. epidermidis* (ATCC12228) (SE12228) was topically applied. Cetaphil® without bacteria was used as a vehicle control, and a non-antimicrobial strain of *S. epidermidis* (ATCC1457) (SE1457) was used as a negative control. *S. aureus* colonies measured 29 hrs later and by counting on a mannitol salt agar plate.

Clinical Studies

N/A

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 subjects, we conducted a preliminary double-blind, placebo-controlled autologous microbiome transplant study (AMT) under IND# 15786. An antimicrobial CoNS strain was isolated from the non-lesional

skin of each of 5 AD subjects who were *S. aureus* culture positive. To transiently increase their abundance on each subject, the active strains were expanded and then reapplied to the lesional forearm skin of the same subject, from which the active colonies were isolated, 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 this 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 (Fig. 4a-b). However, CoNS survival on the arm 24 hours after AMT 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. Therefore, we propose to apply the AMT cream for multiple applications (BID for seven days) for better survival of transplanted CoNS.
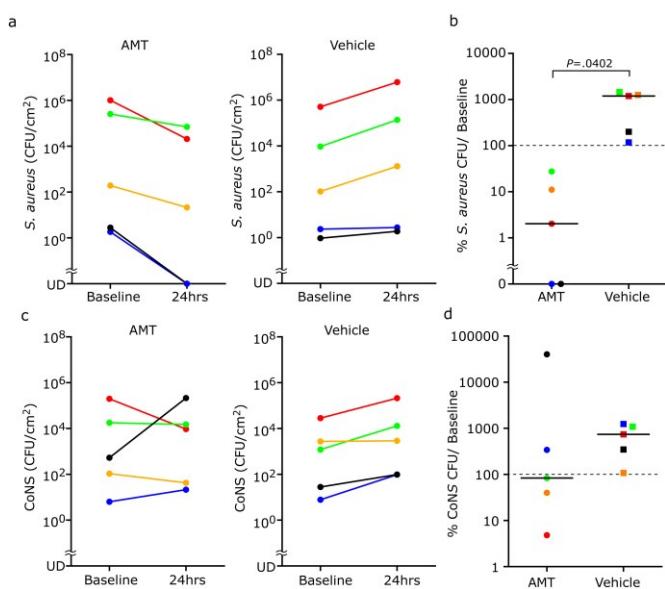
Figure 4. Transplantation of antimicrobial CoNS reduces survival of *S. aureus* colonized on the skin surface.

Figure 4 panel (a) demonstrates the effect of transplantation of antimicrobial CoNS strain(s) on survival of *S. aureus* on the skin surface of subjects with AD. *S. aureus* survival was measured on the lesional sites in both antecubital fossa of the AD subject before and 24 hours after either treatment with antimicrobial CoNS strain(s) autologously screened from the same subject 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 of *S. aureus* is shown as percent of baseline and different color symbols indicates data from each individual subject. In panel (c) CFUs of CoNS on the skin surface of subjects 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 our pilot study of 5 subjects evaluating an AMT cream (IND#15786), we noted no serious adverse events (SAEs). Two subjects noted mild worsening of AD, which were deemed unrelated to the AMT cream given AMT cream was not applied on the area with worsening AD. One subject 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 cream given the bilateral worsening of AD on the upper extremities. Overall, the AMT cream was very well tolerated and had no significant adverse events.

2. Study Hypotheses/Objectives

Our preliminary data strongly suggest that some strains of resident CoNS act to defend the skin surface against colonization by SA. The goal of this proposal is to restore this function of the microbiome on atopic skin by replacing these beneficial bacteria. Our approach will be very safe since we will select the beneficial microbes that are normal residents of healthy skin. These bacteria will be harvested from the subject, expanded and reapplied to the same subject. An FDA IND has been approved and institutional IRB obtained for this approach (IND #15786). After application of the bacteria the survival of SA will be measured. Thus, this autologous microbiome transplant (AMT) is a first step before a generalized "probiotic" therapeutic approach.

We propose here a randomized, double-blind, placebo-controlled trial that will apply beneficial bacteria from the AD host onto lesional skin of AD patients, thus performing an autologous microbiome transplant. The transplanted bacteria have been selected based on their capacity to inhibit *S. aureus* colonization. Our central hypothesis is that this intervention will decrease colonization of *S. aureus* and normalize immune functions of AD skin. This hypothesis will allow us to evaluate the function of the microbiome in AD, and normalize host defense properties in AD, and to develop an intervention that is highly likely to decrease *S. aureus* colonization and may also improve skin inflammation. During the course of this trial we will also obtain vital information to further explain how the microbiome functions and what variables control its composition.

Aim Evaluate the capacity of AMT cream to decrease S. aureus colonization in patients with atopic dermatitis (AD).
Hypothesis: Transplant of AMT will decrease *S. aureus* on AD skin
> **Study objectives**:
>> **Primary**: To determine the change in abundance of *Staph aureus* from baseline after 1 week BID application of AMT.
>>
>> **Secondary Lesional AD skin:**
>> 1. To measure the abundance of the AMT bacterial strains on lesional skin after 1 week BID application of transplant.
>> 2. To measure the rate of elimination of the microbiome transplant on lesional skin after stopping application of transplant cream
>> 3. To determine the rate of reappearance of *Staphylococcus aureus* on lesional skin after discontinuing 1 week application of AMT cream.
>> 4. To determine the change in clinical assessments, including SCORAD, modified EASI, and EASI after 1 week BID application of AMT.
>> 5. Evaluate the diversity of lesional skin microbiome by DNA sequencing from baseline and after 1 week BID application of AMT.
>> 6. Measure the difference in microbiome transcriptome on lesional skin by RNA-sequencing analysis from baseline and after 1 week BID application of AMT.
>>
>> **Secondary Non-lesional skin**:
>> 1. To determine the change in abundance of *Staph aureus* from baseline after 1 week BID application of AMT on non-lesional skin.

2. To measure the abundance of the AMT bacterial strains on non-lesional skin after 1 week BID application of transplant.
3. To measure the rate of elimination of the microbiome transplant on non-lesional skin after stopping application of AMT
4. To determine the rate of reappearance of *Staphylococcus aureus* on non-lesional skin after discontinuing 1 week application of AMT cream.
5. Evaluate the diversity of lesional skin microbiome by DNA sequencing from baseline and after 1 week BID application of AMT on non-lesional skin.
6. Measure the difference in microbiome transcriptome on non-lesional skin by RNA-sequencing analysis from baseline and after 1 week BID application of AMT.

3. Study Design

3.1. Description of Study Design

This is a single site, double blind placebo controlled study, which will assess the efficacy, safety, and steady state of Autologous Microbiome Transplant (AMT) cream in adults with AD. After providing informed consent, patients will be assessed for study eligibility at the screening visit, in which participants will consent to all of the study experiments separately. Subjects that withdraw from the study will be replaced. Eligible subjects will be randomized to receive either AMT cream or placebo in a 2:1 allocation ratio (AMT cream: placebo). Subjects will be instructed to apply either the AMT or vehicle cream to both arms twice a day for seven days. Both subjects and clinical investigators will be blinded. Optional follow-up visits to assess clinical effect and steady state may occur at 1 and 4 hours after initial cream application if the subjects are willing to return at these time points. 4 days after the treatment visit, subjects will return to the clinic for fresh aliquots of the AMT and vehicle creams as well as to assess safety and clinical effect. Subjects will return to clinic after the seven day course of AMT or vehicle creams for clinical and safety assessments. Subjects will then be asked to return to the clinic 1 day after completion of the AMT or vehicle cream course for additional safety and clinical effect assessments. Subsequently, subjects will return for additional follow-up visits only if their lesional atopic dermatitis sites have not yet returned to baseline (*S. aureus* is no longer inhibited and *Staph species* from the AMT cream is zero). Up to 27 subjects will be recruited for this entire study. The duration of this study is approximately 2 months. We anticipate the entire study duration will be approximately 2 years total, and that it will take approximately 1 year to reach recruitment and enrollment goals for this study. The study flow diagram is provided in Figure 1.

Intervention(s) and comparators: A coded cream consisting of Cetaphil® Moisturizing Lotion alone, or the microbiome transplant consisting of Cetaphil® Moisturizing Lotion plus 10^{5-8} CFU/gram Cetaphil® of coagulase negative *Staphylococci* clones expressing antimicrobial products isolated from the subject's own non-lesional skin. Since the two creams are indistinguishable in terms of color and texture, both the study coordinator and the subject will remain blinded to the identity of the cream. Our goal is to achieve a density of 1

X 10^5 CFU/10mg/cm^2 at the time of application. This abundance is known to be safe as it is the density of bacteria typically found on normal skin.

[Insert a schematic diagram of the trial here (include study cohorts and sample size, treatment allocation, time points of study intervention and subject follow-ups])

Table format

Cohort	27 subjects	ARM 1	18 subjects: AMT cream
		ARM 2	9 subjects: Vehicle cream only

3.2. Stratification, Randomization, and Blinding/Masking

This is a randomized, double-blind, placebo controlled study. After informed consent is obtained, eligibility information will be relayed to an unblinded laboratory staff member who will serve as the unblinded pharmacist. This staff member will then randomize subjects in a 2:1 allocation ratio (AMT cream: placebo). This unblinded pharmacist will use an online tool to generate a simple randomization list: http://www.randomization.com/. This lab member will notify the clinical team once participant has been randomized and is responsible for securely storing all randomization files.

3.2.1. Procedure for Unblinding/Unmasking

In order to minimize potential bias from Investigators, investigational staff, and patients, the study will be conducted in a double-blind manner, except for the unblinded laboratory staff for study drug preparation, who will be the only unblinded study site personnel at the site.

Unblinding will occur in cases of emergency situations requiring the knowledge of a given treatment. As per regulatory reporting requirements, the blind will be broken for all unexpected serious adverse events that are considered by the investigator to be related to the study drug. Details of patients who are unblinded during the study will be included in the Clinical Study Report. The blind will be broken only if specific emergency treatment would be dictated by knowing the treatment status of the patient. Unblinding will be approved by the study Safety Officer unless an immediate life threatening condition has developed and the Safety Officer is not accessible. The site investigator will also notify KAI and NIAMS regarding the emergency unblinding event on the next business day.

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 Medical Monitor and others who were notified. The reasons for unblinding of a participant's treatment will be included in the final study report.

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

4. Selection of Participants and Clinical Sites/Laboratories

4.1. Rationale for Study Population

In atopic dermatitis, 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.

4.2. Inclusion Criteria

Up to 54 subjects with atopic dermatitis will be enrolled in this study. Enrollment will not take race or gender into consideration. Pregnant women, children (under age 18), and unhealthy subjects with significant co-morbid conditions as determined by the PI will be excluded.

1. Male or female subjects who are not pregnant or lactating. Female subjects of child-bearing potential must have a negative urine pregnancy test on the day of the screening visit in order to be eligible for the study.
2. 18-60 years of age
3. Diagnosis of atopic dermatitis for at least 6 months using the Hanifin and Rajka Diagnostic Criteria for atopic dermatitis
4. Presence of lesional atopic dermatitis skin in both antecubital fossae
5. Positive *S. aureus* colonization based on results of a skin culture taken from one of their AD-affected antecubital fossae during the screening visit
6. Positive for antimicrobial CoNS species from non-lesional AD skin

4.3. Exclusion Criteria

Individuals who meet any of these criteria are not eligible for enrollment as study participants:
1. Use of any topical AD treatments (including topical steroids, topical calcineurin inhibitors) to either arm within one week of the Treatment visit
2. Use of any antihistamines 7 days within one week of the Treatment visit
3. Use of any oral/systemic AD therapies (steroids) within 28 days of the Treatment visit
4. Severe AD that would worsen significantly from holding a participant's usual topical/oral AD medications for the time periods required in the inclusion/exclusion criteria (one week prior to the

Treatment visit for topical medications and antihistamines and 28 days prior to Treatment visits for oral medications)

5. Subjects who have taken a bleach bath within a week prior to the Treatment visit, or who take bleach baths during the study
6. Patients with severe medical condition(s) that in the view of the investigator prohibits participation in the study
7. Subjects with Netherton's syndrome or other genodermatoses that result in a defective epidermal barrier
8. Any subject who is immunocompromised (e.g. provides researchers with a history lymphoma, HIV/AIDS, Wiskott-Aldrich Syndrome) or has a history of malignant disease (with the exception of non-melanomatous skin cancer). This information will be gathered verbally from the patient while taking a medical history from the patient, and will not involve further testing such as an HIV test.
9. Subjects 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. Active bacterial, viral or fungal skin infections
11. Any noticeable breaks or cracks in the skin on either arm, including severely excoriated skin or skin with open or weeping wounds suggestive of an active infection or increased susceptibility to infection.
12. Ongoing participation in another investigational trial
13. Use of any oral or topical antibiotic for up to four weeks prior to the Treatment visit
14. Use of any systemic immunosuppressive therapy (e.g. CsA, MTX, etc.) within four weeks of the Treatment visit.
15. Sensitivity to or difficulty tolerating Dove fragrance-free bar soap or Cetaphil lotion
16. Subjects with prosthetic heart valves, pacemakers, intravascular catheters, or other foreign or prosthetic devices.

5. Known and Potential Risks and Benefits to Participants

5.1. Risks of Investigational Product

Risk of applying microbial cream (Cetaphil® containing Staphylococcal bacteria)
Skin infection

The likelihood of subjects developing a skin infection is low given that we will be transplanting their non-pathogenic autologous bacteria that have antimicrobial peptide-producing abilities. Since we are transplanting the subject's own endogenous bacteria back onto them, we know that these bacteria are present on the subject's skin at baseline without causing an infection. In addition, the bacteria strains to be transplanted onto the patient will be screened prior to putting it into the AMT cream to ensure that it is not a skin pathogen. The known pathogen *S. aureus* that frequently colonizes AD skin will not even be considered as a potential bacteria to transplant because of its yellow color on mannitol-salt-agar plates, which isolated bacteria will be grown on. Only red colonies (*S. epidermidis*) growing on mannitol-salt-agar plates will be chosen and screened as potential bacteria to be transplanted onto the subject. If anything, we expect the population of *S. aureus* to be reduced with the use of the AMT cream, since the AMT cream will contain only bacteria that are known to inhibit *S. aureus*. Furthermore, we will be applying these bacteria topically and will exam all patients first to ensure that they do not have any cracks or excoriated skin on their arms before we apply it. We will also avoid applying the microbial cream to the hand since the hand would be a likely source of spreading these bacteria onto other surfaces that other people may contact. Should an infection occur (or should the subject be concerned about an infection), the subject should call Dr. Tissa Hata or Dr. Richard Gallo. The subject would be treated according to the standard treatment after examination by Drs. Tissa Hata or Richard Gallo. The treatment will include topical and/or oral antibiotics

(for infection) depending on the severity of the infection clinically, and Cetaphil® moisturizing lotion (for irritation and dryness), provided by Drs. Tissa Hata or Richard Gallo. The topical antibiotic of choice will be mupirocin. The oral antibiotic of choice will depend on the individual subject's medical history, including any possible contra-indications to certain medications due to allergies or other concomitant medication use, but doxycycline will be the first line oral antibiotic, followed by a cephalosporin or penicillinase-resistant penicillin.

If the subject calls to report a possible infection during a time when the clinic is closed, the subject will be directed to the closest urgent care facility for further management.

5.2. Risks of Study Procedures

Risk of loss of confidentiality: Every reasonable effort will be made to keep records confidential. Research records will be stored in a secure building under lock and key of the study personnel. Data stored in our computers are password protected. Unless required by law, only the Study Doctors, the Study Team, the UCSD Institutional Review Board, the Food and Drug Administration, and the National Institutes of Health (NIH) will have access to confidential data which identifies you by name. Because of the need to give these parties access to this information, absolute confidentiality cannot be guaranteed.

Withdrawal from Medications

Adverse events associated with stopping the use of protocol-prohibited medications may result in worsening of the condition being treated and reported as such. As listed in the inclusion/exclusion criteria, subjects with severe AD who may have difficulty tolerating periods without medication use will be excluded from participating.

Skin Swabs

There are no significant risks associated with taking swabs of the skin.

5.3. Potential Benefits

There are no direct benefits for the subjects who elect to enroll in this study. The results may provide identification and/or validation of new targets for the future development of therapeutics for subjects with atopic dermatitis, 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 /Device/Intervention

6.1. Investigational Agents/Devices/Interventions

6.1.1. Investigational Agent

6.1.1.1. Dosage, Preparation, and Administration

After isolation of each subject's antimicrobial *Staphylococcus* species from their skin swabs, a stock will be created that contains equal parts of each of these antimicrobial bacteria. This stock will be assigned a unique batch number, and the contents of this batch are what will be mixed with Cetaphil® at a

concentration between 1×10^5 and 1×10^8 CFU/gram Cetaphil® to create the subject's final AMT cream. This stock will be stored at -80C until the time when that subject's AMT cream is to be formulated. Prior to formulating the final AMT cream product that will be applied on the skin of that subject, though, this stock will undergo Microbial Limits Testing in accordance with USP <62>, as well as Microbial Limits Testing for the detection of *Streptococcus pyogenes*, *Acinetobacter baumanni*, and the skin fungus trichophyton in our laboratory both before and after formulation of the AMT cream with Cetaphil®.

The stock will be stored at -80C to prevent bacterial growth in the stock solution itself. If the stock is free from contaminants as shown by USP <62> testing, the stock will be formulated with Cetaphil® under sterile conditions in a sterile hood to prevent contamination. The stock will then be returned to -80C. If the stock is NOT free from contaminants as shown by USP <62> testing, the stock will be discarded appropriately. A sample of formulated AMT cream will then undergo UPS <62> tests for the organisms noted previously. Formulation of the subject's AMT cream will occur under sterile conditions in a sterile laminar flow hood to prevent contamination of the stock and AMT cream. A sample of Cetaphil® from each container of Cetaphil® used for the formulation of AMT cream will also undergo USP <62> testing within a week of using that Cetaphil® container for AMT cream formulation.

Bacterial concentration of the AMT cream will then be checked by plating a known quantity of the AMT cream on an agar plate and incubating the plate overnight at 37˚C. The number of viable colonies will be counted the following day. Only AMT that has a final concentration of bacteria that ranges from 1×10^5 CFU of bacteria/ gram Cetaphil® to 1×10^8 CFU of bacteria/ gram Cetaphil® will be used in the clinical trial.

The AMT cream will be provided in single-dose sealed containers. The cream should be stored at room temperature per USP definition of "controlled room temperature" for up to one week. The AMT cream is meant to be applied topically two times a day at a dose of 3×10^7 CFU/ gram Cetaphil®.

6.2. Study Design – dose schedule

The dosing schedule for the AMT cream or the Vehicle (Cetaphil® lotion) is described in Sections 8.3 and 8.5.

The first dose of the AMT cream or Vehicle cream will be administered to the subjects at the clinical site on Day 0 (Treatment Initiation Visit). At that time, they will be guided and given a handout instructing them to apply the AMT or Vehicle cream uniformly across both arms (this area will include S. aureus colonized lesions as well as non-lesional skin) twice daily for seven days. The participants will also be provided a subject diary and 18 single-use containers for dosing at home. An additional 14 single-use packets of AMT Product or Vehicle Product will be dispensed at the next clinic visit on Day 4 to use until Day 7. Subjects will be instructed that 2 additional single-use containers will be provided to them at Day 0 and Day 4 in the event that the subject should misplace a container of study cream. Accountability of the study creams will be performed, and subjects will be asked to return the jars of used and unused cream at the next in-clinic visit.

At the clinic visits on Day 4 and Day 7, the subjects will provide skin swab samples and be assessed for adverse events (AEs). Three additional clinic visits will be scheduled to assess safety and stability of the AMT cream. There will be a 30 day follow-up of all participants after the last treatment to assess safety and disease status. This will be brief phone call, and subjects will be asked to report any new adverse events and to answer questions regarding the status of their AD. Any subject 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.

6.3. Drug 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 agent, including the date and quantity of the drug received, to whom the drug was dispensed (participant-by-participant accounting), and a detailed accounting of any drug accidentally or deliberately destroyed.

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

All records regarding the disposition of the investigational product will be available for inspection. Unused product will be destroyed as per UCSD's hazardous waste protocol.

6.4. Assessment of Participant Compliance with Investigational Agent

Patients will be required to return all aliquots of medications given, used or unused, and each vial will be weighed and weight recorded.

6.5. Toxicity Prevention and Management

All steps will be taken to minimize potential risks of the study. Patients will be excluded from study with known sensitivities/allergies to any of the products used during this study. Patients with any cracks or breaks in their skin, including severely excoriated or bleeding skin suggesting that the patient may be susceptible to an infection will be excluded. Similarly, subjects with prosthetic or implanted devices will also be excluded. Any adverse event, defined as any undesirable sign, symptom, or medical condition occurring after the subject's written consent to participate is completed, will be recorded and reported to the PI as well as the Institutional Review Board 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 patient to continue in the study. To protect patient confidentiality, all samples will be encoded and anonymous. Strict adherence to current HIPPA guidelines will minimize the risk of loss of confidentiality. If the subject is injured as a result of participation in this research, treatment will be available. In the case of a skin infection, the subject would be treated according to standard treatment practices. The treatment will include antibiotics (for infection) and ointment (for irritation and dryness).

6.6. Premature Discontinuation of Investigational Agent

Study therapy 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 medication.

3. Evidence of contact allergy to investigative medication.

4. Evidence of non-compliance to study protocol that in the opinion of the investigator requires discontinuation of investigational agent.

5. Study therapy may also be prematurely discontinued for any participant if the investigator believes that the study treatment is no longer in the best interest of the participant.

7. Other Medications

7.1. Concomitant Medications

7.1.1. Protocol-mandated

At the start of the study, eligible subjects will receive Dove soap as well as Cetaphil moisturizing lotion to use throughout the duration of the study.

7.2. Prohibited Medications

1. Use of any topical AD treatments (including topical steroids and topical calcineurin inhibitors) to either arm within one week of the Treatment visit
2. Use of antihistamines within 7 days of the Treatment Visit
3. Use of any systemic AD therapies or any systemic immunosuppressive therapy (steroids, CsA, MTX, etc.) within 28 days of the Treatment visit
4. Use of any oral or topical antibiotics for up to four weks prior to the Treatment Visit
5. Use of bleach baths within 7 days of the Treatment Visit

7.3. Rescue Medications

If infection occurs, the 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 topical antibiotic of choice will be mupirocin. The oral antibiotic of choice will depend on the individual subject's medical history, including any possible contraindications to certain medications due to allergies or other concomitant medication use, but doxycycline will be the first line oral antibiotic, followed by a cephalosporin or penicillinase-resistant penicillin.

8. Study Procedures

8.1. Enrollment

The research study will be explained in lay terms to each potential research participant. The potential participant will sign an informed consent form before undergoing any study procedures. Once the informed consent has been signed, and meets all the inclusion and exclusion criteria, the participant is considered enrolled in the study and will be assigned a unique participant number.

8.2. Screening/Baseline Visit

The purpose of the screening period is to confirm eligibility to continue in the study.

1. The following procedures, assessments, and laboratory measures will be conducted to determine participant eligibility: Prior to beginning any study procedures, subjects will first be asked to review and sign the informed consent form.
2. After obtaining informed consent, the inclusion/exclusion criteria will be reviewed.
3. A brief medical history and physical exam/vital signs will be performed to assess whether the subject meets the required criteria for the study.
4. Female subjects of child-bearing potential will complete a urine pregnancy test while in the office to ensure that they are not pregnant.

5. Patients who meet the inclusion and exclusion criteria will then have their lesional AD skin scored according to the localized SCORAD grading scale, and the extent of AD lesional skin on their bilateral arms will be recorded.

6. A skin swab performed on the lesional atopic dermatitis skin of their antecubital fossae, will be performed to detect the presence of S. aureus.

Subjects who do not meet the inclusion/exclusion criteria due to the application or use of prohibited medications within the timeframe noted will be considered screen-failures. Skin swabs of these patients will not be performed. These subjects may return for another screening visit after completing the necessary wash-out period (7 days for topical medications, 28 days for oral medications) if they are interested in doing so. Subjects eligible for this study will be randomized at the Treatment visit 1 to either AMT cream or placebo in a 2:1 allocation ratio (AMT cream: placebo).

8.3. Study Visits or Study Assessments

Aim: To determine the change in abundance of *Staph aureus* from baseline after 1 week BID application of AMT.

The study flowchart is provided below:
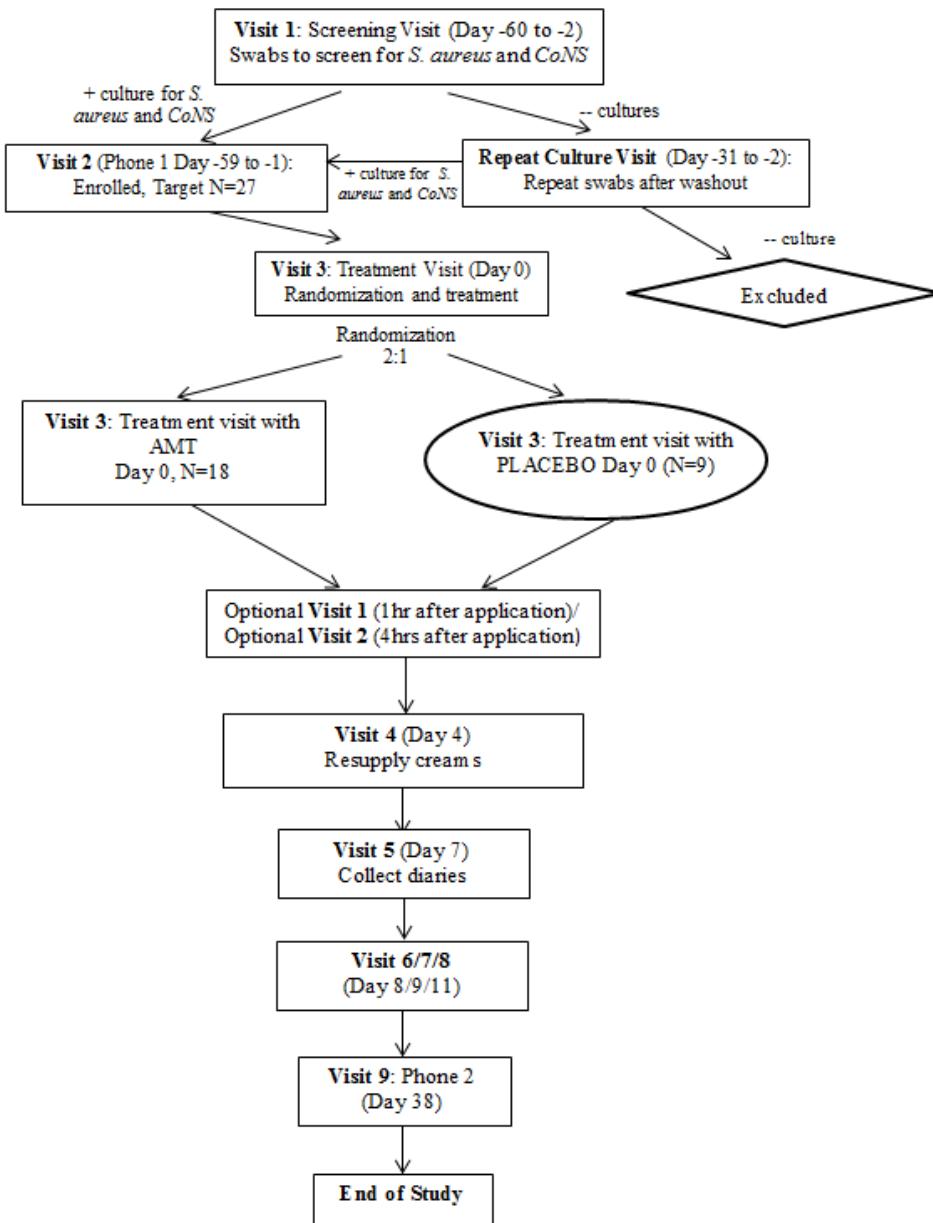
This experiment will consist of up to 9 office visits (Screening, Treatment, 2 optional visits, and 5 Follow-up visits) and two phone calls. All office visits will occur at the UCSD Dermatology Clinic. Up to twenty seven (27) subjects with AD will be recruited for this study. The exact study procedures are noted below:

Screening Visit 1 (Day -60 to Day -2)

Prior to beginning any study procedures, subjects will first be asked to review and sign the informed consent form. After obtaining informed consent, the inclusion/exclusion criteria will be reviewed. A brief medical history, vital signs, and physical exam will be performed in helping to assess whether the subject meets the required criteria for the study. Female subjects of child-bearing potential will complete a urine pregnancy test while in our office to ensure that they are not pregnant. Patients who meet the inclusion and exclusion criteria

will then have their lesional AD skin scored according to the SCORAD grading scale, EASI, and modified EASI, and localized SCORAD, and the extent of AD lesional skin on their bilateral arms will be recorded. A skin swab will be performed on the lesional atopic dermatitis skin of their antecubital fossae bilaterally to detect the presence of *S. aureus*. Additionally, we will take four (4) culture swabs from the subject's non-lesional (no AD) skin on their upper arms. These culture swabs will be placed in tryptic soy broth (TSB) until processed in the lab, where they will be used to grow the subject's antimicrobial peptide-producing *Staph* species to use for the autologous microbiome transplant. Subjects will then receive a bar of Dove moisturizing soap, which they will be instructed to use whenever they shower until after the follow-up visit is complete. This will conclude the screening visit. The total time for this visit will be approximately 40 minutes.

Subjects who do not meet inclusion and exclusion criteria due to their concomitant medications will be asked whether they would be willing to washout of their medications prior to Visit 3 should they have both *S. aureus* and *CoNS* colonization or for a repeat culture visit (if negative for *S. aureus* or *CoNS)*. Subjects who agree to the washout will have swabs collected for assessment of *S. aureus* and *CoNS* colonization. Subjects who do not wish to washout out of the prohibited medications will be identified as screen failures and will not continue in the study.

Visit 2, Phone 1 (Day -59 to Day -1)

Subjects will be notified via telephone whether or not their skin swab grew *S. aureus* and whether they have antimicrobial *Staphylococcus* species present. Subjects with a skin culture positive for *S. aureus* and antimicrobial coagulase-negative *Staph (CoNS)* will be enrolled in the study and visit 3 (Treatment visit) will be scheduled. For these subjects, they will be reminded to withhold their medications prior to Visit 3 according to protocol. Subjects with a negative *S. aureus* skin culture and/or are negative for *CoNS,* will be asked to withhold their medications according to the protocol (7 days for AD topical medication and antihistamines, and 28 days for oral AD medications), and a repeat screening visit will be scheduled for additional skin culture swabs .The total phone conversation should take approximately 5 minutes.

Repeat culture visit, as necessary (Day -31 to -2)

The purpose of the Repeat Culture Visit is to repeat skin swabbing to assess for the presence of *S. aureus* and *CoNS* following their medication washout (28 days for oral AD medications, 7 days for topicals and antihistamines). This visit will only be required for subjects who had negative cultures for *S. aureus* and/or *CoNS* and agree to washout of their medications for repeat swabbing.

Inclusion and exclusion criteria will be reviewed. Interim medical history and a physical exam will be performed. Female subjects of child-bearing potential will complete a urine pregnancy test while in our office to

ensure that they are not pregnant. Vital signs will be taken. Two (2) skin culture swabs total will be taken of their lesional antecubital fossae to assess for *S. aureus*. Additionally, four (4) skin culture swabs will be taken of their non-lesional skin to assess for antimicrobial *CoNS*. These culture swabs will be placed in tryptic soy broth (TSB) until processed in the lab, where they will be used to grow the subject's antimicrobial peptide-producing *Staph* species to use for the autologous microbiome transplant. This will conclude the repeat culture visit. The total time for this visit will be approximately 20 minutes.

Repeat phone call, as necessary (Day -30 to -1)

Subjects will be notified via telephone whether or not their skin swabs grew *S. aureus* and whether they have antimicrobial *Staphylococcus* species present. Subjects with a skin culture positive for *S. aureus* and antimicrobial coagulase-negative *Staph (CoNS)* will be enrolled in the study. For these subjects, Visit 3 (Treatment visit) will be scheduled. Subjects with a negative *S. aureus* skin culture or are negative for *CoNS* will be identified as screen failures.

Visit 3, Treatment Visit (Day 0)

Eligible subjects with + *S. aureus* and *CoNS* screening cultures will be enrolled in the study and instructed to return to the clinic. This waiting period will allow laboratory staff to grow and prepare the subject's endogenous AMP-producing bacteria to be mixed with Cetaphil for application at the treatment visit. A laboratory staff member will serve as an unblinded pharmacist and once eligibility information is relayed to the unblinded pharmacist, the subject will be randomized to an allocation ratio of 2:1 (AMT cream: vehicle cream). The subject will be asked not to bathe, shower, or exercise on the day of the treatment visit, and also that the application of any topical products to their arms on this day is prohibited. Hand washing is allowed, but subjects will be instructed to take care not to apply soap or water above their wrists.

This visit will begin by reviewing the subject's interim medical history, including medication history to ensure that he/she did not use any topical AD treatments on their arms or antihistamines within 7 days or oral AD treatments within 28 days of this visit. For all subjects, a focused physical exam will then be performed of the subject's bilateral arms to ensure that there is no evidence of cracks, breaks or infections of the skin on the arms. Vital signs will be taken. The localized SCORAD, modified EASI, global EASI and SCORAD grading scale will be used to rate the severity of the lesional AD skin on the subject's bilateral arms, and the extent of lesional AD skin on each arm will be recorded. Digital photographs of the lesional skin on both arms will be taken. Each photograph will also include a ruler so the scale of the lesions can be determined, as well as a card with the subject's identification number, visit date, and photograph location so that the photographs can be

identified when uploaded. Next, up to 3 skin swabs will be performed on each arm in a region of eczematous skin and non-lesional skin, for a total of up to 12 swabs. For the skin swabs, one culture swab and one to two DNA swabs will be taken from the eczematous skin from each arm, and one culture swab and one to two DNA swabs will be taken from the non-lesional skin on each upper inner arm for a total of up to twelve (12) swabs. After swabbing, the swabs are placed into either an empty Eppendorf tube (if analyzing the DNA of the bacteria present on the skin surface) or into a liquid medium such as tryptic soy broth (if analyzing the amount of bacteria present on the swabbed skin surface).   These swabs will be analyzed in the lab to determine how many and what type of bacteria are present. The AMT cream or vehicle cream will then be applied to both arms by the gloved hands of a study coordinator. The moisturizers will be provided by Dr. Gallo's laboratory and will be in identical containers labeled either A or B. Neither the study coordinator nor the subject will know which cream is in which container. Container A or B will contain either Cetaphil alone or Cetaphil plus colonies of the subject's endogenous AMP-producing Staph grown in the lab from the subject's screening swab. A thin coat of cream A or B will be applied to both arms (excluding hand) in order to demonstrate proper application. Up to twelve (12) skin swabs will then be performed on both arms in the areas of cream application on lesional and non-lesional skin, between 5 minutes to an hour after cream application, depending on the discretion of the investigator. Following the skin swabs, the Cetaphil alone or Cetaphil with AMP-producing Staph will be reapplied onto the subjects' arms as previously described. The subject will be instructed to withhold use of any topical or oral AD medications until after their last in-clinic follow-up visit (Visit 8). The subject will be provided with aliquots of either the transplant or vehicle creams, enough for at least 4 days of application. Subjects will be instructed to apply the cream twice a day for the next 4 days, and will be instructed to apply the provided cream from one aliquot per arm. To minimize cross contamination, we will instruct subjects to wear long sleeve clothes and change into clean clothes every day. All creams should be stored at room temperature. Subjects will be reminded to use Dove soap provided to them, whenever they shower, but to avoid the upper extremities, until after their last in-clinic visit on Day 11 is complete. Subjects will be instructed to continue their daily skin care routine, and discouraged from using antibacterial soaps and swimming in the ocean or pool until their last in-clinic follow-up visit. They will also be instructed that if these events occur, to please notify the study staff at their next visit. At the end of the visit, subjects will be given an instruction sheet and a subject diary to assess for cream application appliance. They will be asked to bring the diary pages to their Follow-up Visit 4. This will conclude the treatment visit. We expect this visit to take approximately 20 minutes.

Optional Visit 1 (1 hour after cream application +/- 2 hours): Subjects will have the option to return to clinic for an optional visit approximately 1 hour after AMT cream application. At this optional visit, up to 12 skin swabs will be taken of the lesional and non-lesional skin on the arms (one culture swab and one to two DNA swabs for lesional and non-lesional skin of each arm). This visit will take approximately 15 minutes.

Optional Visit 2 (4 hours after cream application +/- 2 hours): Subjects will have the option to return to clinic for an optional visit approximately 4 hours after AMT cream application. At this optional visit, up to 12 skin swabs will be taken of the lesional arms and non-lesional skin on the arms (one culture swab and one to two DNA swabs for lesional and non-lesional skin of each arm). This visit will take approximately 15 minutes.

Visit 4 (Day 4 +/- 5): Subjects will return to the clinic to pick up fresh aliquots of the AMT or vehicle creams, and to return approximately 4 hours after last cream application. Any adverse events will be recorded. Subjects will be asked to refrain from bathing, exercising, or showering starting at 12:01am of Visit 4. Vital signs will be taken. Subject diaries will be collected at this time and a new subject diary will be dispensed to assess for cream application appliance. Used aliquots of cream will be collected at this visit. The localized SCORAD, modified EASI, global EASI and SCORAD grading scale will be used to rate the severity of the lesional AD skin on the subject's bilateral arms, and the extent of lesional AD skin on each arm will be recorded. Digital photographs of the lesional skin on both arms will be taken. Each photograph will also include a ruler so the scale of the lesions can be determined, as well as a card with the subject's identification number, visit date, and photograph location so that the photographs can be identified when uploaded. Next, one culture swab and one to two DNA swabs will be taken from the eczematous skin from each arm, and one culture swab and one to two DNA swabs will be taken from the non-lesional skin on each upper inner arm for a total of up to twelve (12) swabs. We expect this visit to take approximately 15 minutes.

Visit 5 (Day 7 +/- 7): Subjects will be asked to return to the clinic for Visit 5 approximately 4 hours after last cream application. Subject diaries will be collected at this time.   Any used and unused aliquots of cream will be collected at this visit. Subjects will be asked to refrain from bathing, exercising, or showering starting at 12:01am of Visit 5. Vital signs will be taken. Any adverse events since the last visit will be recorded. A brief exam of the skin on subjects' arms will be performed. The severity of AD on each arm will be graded using the localized SCORAD, modified EASI and global SCORAD and EASI grading. Subjects will also be asked to grade their pruritus on a scale of 1-10, with 10 being the worst pruritus of their life. Digital photographs of the lesional skin on both arms will be taken. Next, one culture swab and one to two DNA swabs will be taken from the eczematous skin from each arm, and one culture swab and one to two DNA swabs will be taken from the non-lesional skin on each upper inner arm for a total of up to twelve (12) swabs to determine the microbial diversity and abundance. We expect this visit to take approximately 15 minutes.

Visit 6 (Day 8 +/- 7): Subjects will be asked to return to the clinic for follow-up. Any adverse events since the last visit will be recorded. A brief exam of the skin on subjects' arms will be performed. Subjects will be asked to refrain from bathing, exercising, or showering starting at 12:01am of Visit 6. Vital signs will be taken. The severity of AD on each arm will be graded using the localized SCORAD, modified EASI, and global SCORAD and EASI grading. Subjects will also be asked to grade their pruritus on a scale of 1-10, with 10 being the worst pruritus of their life. Digital photographs of the lesional skin on both arms will be taken. Next, one culture swab and one to two DNA swabs will be taken from the eczematous skin from each arm, and one culture swab and one to two DNA swabs will be taken from the non-lesional skin on each upper inner arm for a total of up to twelve (12) swabs. Any used cream will be collected. We expect this visit to take approximately 15 minutes.

Visit 7 (Day 9 +/- 7): Subjects will be asked to return to the clinic for Visit 7 for follow-up. Subjects will be asked to refrain from bathing, exercising, or showering starting at 12:01am of Visit 7. Vital signs will be taken. Any adverse events since the last visit will be recorded. A brief exam of the skin on subjects' arms will be performed. For subjects with AD, the severity of AD on each arm will be graded using the localized SCORAD, modified EASI, and global SCORAD and EASI grading. Subjects will also be asked to grade their pruritus on a scale of 1-10, with 10 being the worst pruritus of their life. Digital photographs of the lesional skin on both arms will be taken. Next, one culture swab and one to two DNA swabs will be taken from the eczematous skin from each arm, and one culture swab and one to two DNA swabs will be taken from the non-lesional skin on the upper inner arm for a total of up to twelve (12) swabs. We expect this visit to take approximately 15 minutes.

Visit 8 (Day 11 +/- 7): Subjects will be asked to return to the clinic for Visit 8 for follow-up. Subjects will be asked to refrain from bathing, exercising, or showering starting at 12:01am of Visit 8. Vital signs will be taken. Any adverse events since the last visit will be recorded. A brief exam of the skin on subjects' arms will be performed. For subjects with AD, the severity of AD on each arm will be graded using the modified EASI, and global SCORAD and EASI grading. Subjects will also be asked to grade their pruritus on a scale of 1-10, with 10 being the worst pruritus of their life. Digital photographs of the lesional skin on both arms will be taken. Next, one culture swab and one to two DNA swabs will be taken from the eczematous skin from each arm, and one culture swab and one to two DNA swabs will be taken from the non-lesional skin on the upper inner arm for a total of up to twelve (12) swabs. At the conclusion of this visit, subjects may resume prohibited medications, as needed, and may use their choice of soap and emollient. We expect this visit to take approximately 15 minutes.

Visit 9, Phone 2 (Day 38 +/- 7 days)

Subjects will be called approximately one month after their Visit 6. During this phone call, subjects will be asked about any adverse events (including serious adverse events) since their last follow-up visit, as well as how their eczema is currently doing. Although this phone contact will be the last scheduled contact for subjects with respect to this study, any subject with an ongoing adverse event (including serious adverse event) at the time of this phone contact will continue to be followed by study personnel until resolution of the event.

8.4. Unscheduled Visits

If disease activity increases or other concerns arise between regularly scheduled visits, participants should be instructed to contact study personnel and may be asked to return to the study site for an "unscheduled" visit.

8.5. Visit Windows
Study visits should take place within the time limits specified below: the designated visit windows *(i.e. +/- n days)* for each scheduled visit are also indicated on the Table of Events

Table 1: Schedule of Events 2:1 randomization of AMT to placebo cream for 1 week of BID application

Study Procedures Visit (V)	V1	V2 (Phone 1)^a,b	Repeat Culture Visit^c	Repeat Phone Call^d	V3 (Treatment)	Optional visit 1^e	Optional visit 2^e	V4 (Resupply)	V5 (End of Treatment)	V6	V7	V8	V9 (phone 2)
Day (D), visit window (d= day, h= hours)	D -60 to -2	D -59 to -1	D -31 to -2	D -30 to -1	D0	D0, 1 h after V4 +/- 2 h	D0, 4 h after V4 +/- 2h	D 4+/- 5d	D7+/- 7d	D8 +/- 7d	D9 +/- 7d	D11+/- 7d	D38+/- 7d
Screening/Baseline:													
Informed consent	X												
Inclusion/ Exclusion	X	X	X	X	X								
Medical history/ demographics	X		X		X								
Physical exam	X		X		X								
Con meds/procedures	X		X		X			X	X	X	X	X	
Screening skin culture swabs	X		X										
Treatment:													
Apply AMT cream or vehicle cream					X								
Dispense IP					X			X					
Dispense Subject Diary					X			X					
Returned unused creams								X	X				
Collect Subject Diary								X	X				
Efficacy:													
Skin swabs					X	X	X	X	X	X	X	X	
SCORAD	X				X			X	X	X	X	X	
EASI	X				X			X	X	X	X	X	
Photos	X		X		X			X	X	X	X	X	
Safety:													
Weight	X												
Height	X												
Vital Signs	X		X		X			X	X	X	X	X	
Skin exam	X		X		X	X	X	X	X	X	X	X	
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X
Testing													
Pregnancy test (urine)	X		X										

a. Screened subjects who are + for *Staph aureus* and *CoNS* will be contacted via telephone to schedule their treatment initiation Day 0 visit.
b. Subjects who are negative for *Staph aureus* and/or *CoNS* and agree to medication washout will be contacted via telephone to schedule a repeat culture visit.
c. Only subjects who are negative for *Staph aureus* and/or *CoNS* and agree to washout will return for a repeat culture visit.
d. Only subjects who complete the repeat culture visit will have a repeat phone call.
e. Only subjects who agree to participate in optional visits, as indicated on their informed consent form, will complete this visit.

9.1 Skin swabs Samples

9.1.1. Skin Swab collection

The antecubital fossae bilaterally will be defined as the lesional swab location. The non-lesional swab location will be defined as the upper inner arm without AD skin. The designated site for swabbing will be marked with a pen and non-identifying digital photographs will be taken of the swabbing site for future visits. The designated site for swabbing will be measured for each subject, and the total area (cm x cm) recorded. Using a skin 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. Up to 6 swabs may be obtained from each site.

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 be resuspended in 1.5 mL tryptic soy broth (TSB) containing 15% (v/v) glycerol. Dilutions of culture medium will then be used to inoculate on mannitol salt agar plates with egg yolk for selective growth of Staphylococcus species and differentiation of coagulase-positive and coagulase-negative strains. Plates will be incubated for 24 hours in a 5% CO2 incubator at 37∘ C. The plates will be evaluated for the presence of S. aureus colonies, which appear yellow with a surrounding clear zone.

9.1.3.   9.1.2. CFU Quantification of Live Staphylococcus aureus

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 tryptic soy broth (TSB) containing 15% (v/v) glycerol and stored frozen at -80°C. Live CFUs of coagulase positive *S. aureus* will be determined by 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 coagulase negative Staphylococcus 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.

9.1.4. Quantification of Staphylococcus aureus, S. hominis, S. epidermidis, and total Staphylococcus DNA by q PCR

To collect bacterial DNA, pre-measured areas similar 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 quantitative PCR with the UltraClean Microbial DNA Isolation Kit (Mo Bio Laboratories:12224). DNA will be eluted with 50uL DNAse free water. 0.5 µL of the elution will be used for quantitative real-time PCR (qPCR) using universal 16SrRNA primers and species-specific primers. For quantification of *S. aureus*, *S. epidermidis* and*S. hominis* DNA, DNA extracted from an authentic number of each bacteria (ATCC35556, ATCC12228 and ATCC27845, respectively) will be used for standard curve. The standard curve will be used for determination of absolute number of bacteria (CFU/uL). This known amount of bacteria for *S. aureus* and *CoNS* will range from $1x\ 10^1$ to $1x10^6$ CFU/microliter. The absolute number of bacteria will then be converted to relative CFU (rCFU)/cm^2 based on pre-measured swab area.

We have confirmed that each primer set does not cross-react to other species of bacteria. In addition, we have confirmed specificity of primer sequence by BLAST search.

For total 16SrRNA quantification, relative abundance will be calculated with the $\Delta\Delta$Ct method. The following universal 16S primer set will be used to determine relative abundance of 16S rRNA gene according to the reference [J Clin Microbiol 43, 5332-5337 (2005)]. This universal primer set covers 79% of Proteobacteria, 85% of Actinobacteria, 72% of Firmicutes, and 61% of Bacteroidetes. Thus, the reactivity of this primer set differs depending on bacteria genus level. Therefore, we cannot determine absolute abundance of 16S rRNA gene from bacterial mixture using a standard curve of bacterial DNA extracted from single species. Accordingly, we will determine relative abundance of the total 16S rRNA gene. We will determine relative abundance of 16S rRNA gene by comparing it to baseline abundance. Baseline will be set to 1 and relative abundance will be determined by the Delta-Delta-Ct method.

10.   Biospecimen Storage

Instructions for sample preparation, handling, storage, and shipping are included in the MOOP. The Principal Investigator (PI) 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.

Isolated and speciated *CoNS* strains that have been chosen for use in the AMT cream will be stored at -80 degrees C in 15% TSB-glycerol medium until ready to be used for formulating each subject's AMT cream.

Each subject will be assigned a unique numerical identifier. All bacterial stocks will be stored at -80 degrees C in Eppendorf tubes. Each tube will be labeled with subject's unique numerical identifier as well as a separate bacteria identifier linked to the bacteria stock in the tube. The key explaining the contents of each of the bacteria identifiers to what they are will be stored in the laboratory notebook for the AMT study.

Any bacteria stock that is unused after the completion of this protocol will be disposed of as per UCSD's hazardous waste protocol.

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

11.1. Participant Completion

This study will be defined as complete when all study procedures are completed, including the follow-up phone call to assess any adverse events.

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).

3. The participant dies.

4. The Investigator no longer believes participation is in the best interest of the participant.

11.3. Participant Replacement

Participants who withdraw or are withdrawn will be replaced.

11.4. Follow-up after Early Study Withdrawal

If a participant is withdrawn from the study for any reason, the participant may be asked to complete a final visit by phone to assess any adverse events after AMT application. During this phone call, subjects will be asked about any adverse events (including serious adverse events) since their last visit, as well as how their eczema is currently doing. Any subject with an ongoing adverse event (including serious adverse event) at the time of this phone contact will continue to be followed by study personnel until resolution of the event. Should the subject withdraw prior to completion of 7 days of cream application, subject will be asked to return to the clinic in order to return aliquots of unused and used cream as well as to assess any adverse events after AMT application. Should subjects experience an adverse event, subject will continued to be followed until resolution of the event as in above.

11.5.   Study Stopping Rules

The study may be prematurely terminated for the following reasons:

Rules for stopping the entire study will include:

•   Any serious adverse event for which there is a reasonable possibility that the study product caused the serious adverse event.

•   The development of a serious adverse event for which attribution cannot be assessed as definitely unrelated to the study product in 10% or more of the study population.

•   The development of any severe (Grade 3) adverse events in 10% or more of the study population.

If any of these criteria for halting the study are met, the study will not be resumed until the relevant information has been discussed with the FDA and the FDA concurs with resumption of the study.

12.   Safety Monitoring and Reporting

12.2 Definitions

An adverse event (AE) is any untoward medical occurrence in a subject during participation in the clinical study or with use of the experimental agent being studied. An adverse finding can include a sign, symptom, abnormal assessment (laboratory test value, vital signs, electrocardiogram finding, etc.), or any combination of these.

A serious adverse event is defined by regulatory agencies as one that suggests a significant hazard or adverse event, regardless of the investigator or sponsor's opinion on the relationship to investigational product. This includes, but may not be limited to, any event that (at any dose):

- is fatal

- is life threatening (places the subject at immediate risk of death)

- requires in-patient hospitalization or prolongation of existing hospitalization

- results in persistent or significant disability/incapacity

- constitutes a congenital anomaly/birth defect

- is medically significant, in that it may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

Any event that does not exactly meet this definition, but in the investigator's opinion represents a significant hazard (eg, emergency room visit or outpatient surgery), can be assigned the "other significant hazard" in regards to reporting serious criteria.

Additionally, important medical events that may not be immediately life threatening or result in death or hospitalization but that may jeopardize the subject or require intervention to prevent one of the outcomes listed above, or result in urgent investigation, may be considered serious. Examples include allergic bronchospasm, convulsions, and blood dyscrasias.

All subjects will receive verbal and written instructions with site contact information for whom to contact if they experience an AE associated with study procedures performed for this protocol or if the subject resumes their protocol-prohibited medication during the washout period.

EXPECTED RISKS

Risks of applying microbial cream (Cetaphil® Moisturizing Lotion mixed with the subject's endogenous bacteria):

1.   Skin infection

The likelihood of subjects developing a skin infection is low given that we will be transplanting their non-pathogenic autologous bacteria that have antimicrobial peptide-producing abilities. Since we are transplanting the subject's own endogenous bacteria back onto them, we know that these bacteria are present on the subject's skin at baseline without causing an infection. In addition, the bacteria strains to be transplanted onto the patient will be screened prior to putting it into the AMT cream to ensure that it is not a skin pathogen. The known pathogen S. aureus that frequently colonizes AD skin will not even be considered as a potential bacteria to transplant because of its yellow color on mannitol-salt-agar plates, which isolated bacteria will be grown on. Only red colonies (S. epidermidis) growing on mannitol-salt-agar plates will be chosen and screened as potential bacteria to be transplanted onto the subject. If anything, we expect the population of S. aureus to be reduced with the use of the AMT cream, since the AMT cream will contain only bacteria that are known to inhibit S. aureus. Furthermore, we will be applying these bacteria topically and will exam all patients first to ensure that they do not have any cracks or excoriated skin on their arms before we apply it. We will also avoid applying the microbial cream to the hand since the hand would be a likely source of spreading these bacteria onto other surfaces that other people may contact. Should an infection occur (or should the subject be concerned about an infection), the subject should call Drs. Richard Gallo or Tissa Hata. The subject would be treated according to the standard treatment after examination by Drs. Richard Gallo or Tissa Hata. The treatment will include topical and/or oral antibiotics (for infection) depending on the severity of the infection clinically, and Cetaphil® moisturizing lotion (for irritation and dryness), provided by Drs. Richard Gallo or Tissa Hata. The topical antibiotic of choice will be mupirocin. The oral antibiotic of choice will depend on the individual subject's medical history, including any possible contra-indications to certain medications due to allergies or other concomitant medication use, but doxycycline will be the first line oral antibiotic, followed by a cephalosporin or penicillinase-resistant penicillin.

If the subject calls to report a possible infection during a time when the clinic is closed, the subject will be directed to the resident on call or closest urgent care facility for further management.

2. Risk of medication withdrawal: Discontinuation of a person's AD medication prior to participating in this study and also the prohibition of using AD medications for periods of time during this study may result in worsening or temporary flaring of the patient's AD.

As listed in the inclusion/exclusion criteria (section 10), subjects with severe AD who may have difficulty tolerating periods without medication use will be excluded from participating. Subjects enrolled in the study will be able to use over-the-counter moisturizers as needed. Moisturizers play a role in helping to prevent and control AD flares, and therefore may be of use to subjects whose AD is worsening. The time during which subjects are unable to treat their AD during the study will be limited to the 7-days prior to each screening and treatment/follow-up visit for topical medications, and 28-days prior to each screening and treatment/follow-up visit for oral medications. If for some reason severe worsening of AD occurs during a time in the study that subjects are not permitted to use their medication, the subject will be permitted to use their medication, however the upcoming visit must be rescheduled so that the required 7-day and 28-day wash-out periods before each screening and treatment/follow-up visits are observed. Since the visit dates for the study are relatively flexible (the only real time-dependent visit is the Follow-up Visit, which must occur approximately 24 hours after the Treatment Visit), rescheduling subject visits should not affect study results. AD itself is a chronic condition characterized by periods of flaring and remission. Any worsening of the subject's AD would constitute a disease flare that should be correctable with the use of medications. Flares of AD do not result in permanent deterioration of the subject's

condition. Finally, topical medication discontinuation must only be observed on the subject's bilateral arms since this is the site being studied during the trial. Subjects may treat the AD on all other body surfaces with topical medications throughout the study.

3. Risk of loss of confidentiality: Every reasonable effort will be made to keep records confidential. Research records will be stored in a secure building under lock and key of the study personnel. Data stored in our computers are password protected.   Unless required by law, only the Study Doctors, the Study Team, and the UCSD Institutional Review Board will have access to confidential data which identifies subject by name. Because of the need to give these parties access to this information, absolute confidentiality cannot be guaranteed.

12.2.1 Adverse Event (AE)

Reporting Procedures for All Adverse Events

The investigator is responsible for ensuring that all adverse events are properly recorded in the subjects' records. After signing of the informed consent form, all adverse events observed by the investigator or reported by the subject (whether or not attributed to investigational product), will be recorded in the case report form.

The following attributes must be assigned by the investigator: description; dates of onset and resolution; severity; assessment of relatedness to investigational product, and action taken. The investigator may need to provide follow-up information, medical records, and extracts from medical records.

UCSD IRB

Unanticipated problems involving risk to participants or others (UPRs) are defined as any problem or event, which in the opinion of the Principal Investigator was: 1) unanticipated, 2) serious, AND 3) at least possibly related to the research procedures. If the event meets all 3 criteria for a UPR, it should be reported to the UCSD IRB within 10 working days. The following are events that meet the definition of UPR:

1. Any serious event (including injuries, side effects, deaths or other problems) that in the opinion of the Principal Investigator was unanticipated, involved risk to subjects or others, and was at least possibly related to the research procedures.
2. Any serious accidental or unintentional change to the IRB-approved protocol that alters the level of risk.
3. Any deviation from the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research subject.
4. Any new information (e.g. publication, safety monitoring report, updated safety report), interim result or other finding that indicates an unexpected change to the risk/benefit ratio of the research.
5. Any breach in confidentiality that may involve risk to the subject or others.

6. Any complaint of a subject that indicates an unanticipated risk or that cannot be resolved by the Principal Investigator.

A brief summary of all UPRs as well as a brief summary of all adverse events will be submitted with the continuing review.

Food and Drug Administration

The Principal Investigator will notify the FDA directly in a written IND safety report of any adverse experience associated with the use of the drug that is both serious and unexpected or any finding from tests in laboratory animals that suggests a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity. Each notification will be made as soon as possible and in no event later than 15 calendar days after the initial receipt of the information. Each written notification will be submitted on FDA Form 3500A. The Principal Investigator will also notify FDA by telephone or by facsimile transmission of any unexpected fatal or life-threatening experience associated with the use of the drug as soon as possible but in no event later than 7 calendar days after initial receipt of the information.

On an annual basis as part of the update to the study IND, the Principal Investigator will submit to the FDA directly a narrative or tabular summary showing the following:

•The most frequent and most serious adverse experiences by body system

•A summary of all IND safety reports submitted during the past year

•A list of subjects who died during participation in the investigation, with the cause of death for each subject

•A list of subjects who dropped out during the course of the investigation in association with any adverse experience, whether or not thought to be drug related

12.3 Grading and Attribution of Adverse Events

12.3.1 Grading Criteria

Clinical investigators will "grade" the severity of all AEs using the following scale; the results will be reported on the SAE form, if necessary.

1 = MILD – aware of sign or symptom, but easily tolerated

2 = MODERATE – discomfort enough to cause interference with usual activity

3 = SEVERE – incapacitating with inability to work or do usual activity

4 = LIFE-THREATENING – refers to an event in which the patient was, in the view of the investigator, at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe.

5 = FATAL

12.3.2 Attribution Definitions

The relationship, or attribution, of an adverse event to the study therapy regimen or study procedure(s) will initially be determined by the site investigator and recorded on the appropriate AE/SAE CRF. Final determination of attribution for safety reporting will be determined by NIAMS. The relationship of an adverse event 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 primary investigational product and/or other concurrent mandated study therapy or study procedure)
UNRELATED CATEGORY		
1	Not related	The adverse event is clearly not related: there is insufficient evidence to suggest a causal relationship.
RELATED CATEGORIES		
2	Possibly/ Probably	The adverse event has a <u>reasonable possibility</u> to be related; there is evidence to suggest a causal relationship.
3	Definitely related	The adverse event is clearly related.

12.5 Reporting of Serious Adverse Events and Adverse Events
Recording and Reporting Obligations for SAEs

The investigator will provide the IRB appropriate information concerning any findings that suggest there has been an SAE related to the study.

The investigator will notify the UCSD Human Research Protections Program of any unexpected, fatal or life-threatening experiences in writing no later than 10 working days of the event, according to standard IRB procedures. All serious and medically significant adverse events considered related to the product by the investigator will be followed until resolved or considered stable.

All SAE's will undergo expedited reporting to the NIAMs and the Independent Safety Officer through KAI within 48 hours of the investigator becoming aware of the event.

SAEs that are unanticipated, serious, and possibly related to the study intervention will be reported to the IRB, FDA, and Independent Safety Officer in accordance with requirements. For the IND/IDE:

- 7-day IND Safety Report (unexpected fatal or life-threatening AEs related to the intervention); a copy of the report sent to the FDA will be submitted to the Independent Safety Officer within 24 hours of FDA notification.

- 15-day IND Safety Report (any other serious and unexpected AE related to the intervention); a copy of the report submitted to the FDA will be submitted to the Independent Safety Officer within 24 hours of FDA notification.

- Other AEs documented during the course of the trial will be included in the annual IND report. In the annual AE summary, the Independent Safety Officer Report will state that they have reviewed all AE reports.

12.6   Pregnancy Reporting

The investigator shall be informed immediately of any pregnancy in all study subjects or a partner of a study subject. A pregnant subject shall be instructed to stop taking study medication. The investigator shall counsel the subject and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the pregnant subject shall continue until the conclusion of the pregnancy. The investigator will report to NIAMS within 1 business day of notification of the event.

12.8 Review of Safety Information

12.8.1 Medical Monitor Review
The Safety Officer shall receive bi-annual reports from the investigator compiling new and accumulating information on AEs, SAEs, and pregnancies recorded by the study site.

In addition, the Medical Monitor (Tissa Hata M.D.) shall review and make decisions on the disposition of the SAE and pregnancy reports.

12.8.2 Safety Officer Review

12.8.2.1 Planned Safety Officer Reviews

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

The SO will be informed of an Expedited Safety Report in a timely manner

12.8.2.2 *Ad hoc* Safety Reviews

Triggers for ad hoc review:

- Any serious adverse event for which there is a reasonable possibility that the study product caused the serious adverse event.

- The development of a serious adverse event for which attribution cannot be assessed as definitely unrelated to the study product in 10% or more of the study population.

- The development of any severe (Grade 3) adverse events in 10% or more of the study population.

If any of these criteria for halting the study are met, the study will not be resumed until the relevant information has been discussed with the IRB, Safety Officer, NIAMS, and the FDA and these bodies concur with resumption of the study.

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

12.8.2.2.1 Temporary Suspension of enrollment and drug dosing for ad hoc Safety Review

The following are events that could trigger a safety review, resulting in temporary suspension of enrollment and drug dosing

- Any serious adverse event for which there is a reasonable possibility that the study product caused the serious adverse event.

- The development of a serious adverse event for which attribution cannot be assessed as definitely unrelated to the study product in 10% or more of the study population.

- The development of any severe (Grade 3) adverse events in 10% or more of the study population.

A temporary halt *in enrollment and drug dosing will* be implemented if an *ad hoc* SO safety review is required. No new participants will be consented during this time and subjects will be instructed to stop application of the cream until the IRB, FDA, and Safety Officer concur that study procedures may be resumed.

13.   Statistical Considerations and Analytical Plan

13.1   Overview
We propose here a randomized, double-blind, placebo-controlled trial that will apply beneficial bacteria from the AD host onto lesional skin of AD patients, thus performing an autologous microbiome transplant. Up to 54 subjects with AD will be recruited.

13.2   Endpoints/Outcomes

13.2.1 Primary Endpoint(s)/Outcome(s)

- The change in *S. aureus* abundance after 7 days of AMT cream by using skin swabs to determine the quantity of *S. aureus* using culture and qPCR.

13.2.2 Secondary Endpoint(s)/Outcome(s)

Secondary Lesional AD skin:
1. The abundance of the AMT bacterial strains on lesional skin at baseline and after 1 week BID application of AMT.
2. The quantity of transplanted bacteria on lesional skin after stopping AMT cream 1 day, 2 days, and 4 days after last application.
3. The change in abundance of *Staph aureus* on lesional skin from baseline to 1 day, 2 days, and 4 days after stopping AMT.
4. Change in clinical assessments, including SCORAD, modified EASI, and EASI after 1 week BID application of AMT.
5. Identify lesional skin microbiome by DNA sequencing from baseline and after 1 week BID application of AMT.
6. Evaluate the lesional skin microbiome transcriptome by RNA-sequencing analysis from baseline and after 1 week BID application of AMT.

Secondary Non-lesional skin:
1. The abundance of *Staph aureus* at baseline and after 1 week BID application of AMT on non-lesional skin.
2. The abundance of AMT bacterial strains on non-lesional skin at baseline and after 1 week BID application of AMT.
3. The quantity of transplanted bacteria on non-lesional skin after stopping AMT cream 1 day, 2 days, and 4 days after last application.
4. The change in abundance of *Staph aureus* on non-lesional skin from baseline to 1 day, 2 days, and 4 days after stopping AMT.
5. Identify non-lesional skin microbiome by DNA sequencing from baseline and after 1 week BID application of AMT.
6. Evaluate the non-lesional skin microbiome transcriptome by RNA-sequencing analysis from baseline and after 1 week BID application of AMT.

13.3   Measures to Minimize Bias

Subjects will be randomized into a 2:1 allocation ratio of AMT cream to placebo. Both subjects and investigators making clinical assessments will be blinded.

13.4   Analysis Plan

13.4.1 Analysis Populations.

Screening Population: The Screening Population will consist of all subjects who sign informed consent. Subject disposition, demographics, and safety measures will be summarized for this

population. Additionally, differences between screened and randomized subjects and between screened and not randomized subjects will be examined.

Safety Population: All randomized subjects who provide any safety data will be included in the Safety Population. A subject will not be required to complete all safety assessments to be included in this population. Analyses of this population will be classified according to the treatment actually received.

Efficacy Population: An intention-to-treat analysis will be conducted that includes all subjects who had at least one follow-up visit after intervention. A per-protocol analysis will also be conducted that includes all subjects who were compliant with the full intervention regimen with no major protocol violations.

13.4.2 Primary Analysis of Primary Endpoint(s)/Outcome(s)

Aim: The change in S. aureus abundance after 7 days of AMT cream by using skin swabs to determine the quantity of S. aureus using culture and qPCR.

For each AD subject, the abundance of *S. aureus* will be calculated using CFU counts from baseline (day 28) to Day 35 after 7 days of AMT cream application or placebo application. Comparisons of *S. aureus* reduction will be made between AMT cream and placebo subjects using a two-tailed t-test for independent samples with type II error rate of 0.2 and type I error rate of 0.05. The sample size will include 18 subjects in the AMT cream arm and 9 subjects in the placebo arm. Comparisons of AMT cream versus placebo cream will be reported as means, standard deviations, medians, and minimum and maximum. An intention-to-treat analysis will be conducted that includes all subjects who had at least one follow-up visit after intervention. A per-protocol analysis will also be conducted that includes all subjects who were compliant with the full intervention regimen with no major protocol violations.

13.4.4 Analyses of Secondary and Other Endpoint(s)/Outcome(s)

Secondary Endpoints, Lesional AD skin:
1. The abundance of the AMT bacterial strains on lesional skin at baseline and after 1 week BID application of AMT.
    a. The abundance of live *CoNS* species on lesional skin will be compared from baseline (day 28) to Day 35 after 1 week BID application of AMT cream versus placebo. This will be calculated using a two-tailed t-test for independent samples with type II error rate of 0.2 and type I error rate of 0.05.
2. The quantity of transplanted bacteria on lesional skin after stopping AMT cream 1 day, 2 days, and 4 days after last application.
    a. Live antimicrobial *Staph* species will be measured on lesional skin at baseline (day 28), Day 35 (after 7 days of cream application), Day 36, Day 37, and Day 39 in order to evaluate the abundance of the antimicrobial *CoNS* species that remains after

stopping cream application. In order to take into account the variance of the abundance of antimicrobial *CoNS* species found on each subject's skin at baseline, we will perform CFU counts at each time point (Day 28, 35, 36, 37, and 38) and evaluate the percentage of antimicrobial species present at each time point. The percentage of antimicrobial species present at each time point will be compared between the AMT cream arm and placebo arm using a two-tailed t-test for independent samples with type II error rate of 0.2 and type I error rate of 0.05.

3. The change in abundance of *Staph aureus* on lesional skin from baseline to 1 day, 2 days, and 4 days after stopping AMT.
    a. The abundance of live *Staph aureus* on lesional skin will be evaluated using CFU counting at baseline, 1 hour, and 4 hours after initial application of AMT or placebo cream, and after 4 and 7 days of cream application. At each time point, we will compare the AMT cream and placebo arms by using a two-tailed t-test for independent samples with a type I error rate of 0.05 and type II error rate of 0.2.

4. Change in clinical assessments, including SCORAD, modified EASI, and EASI after 1 week BID application of AMT.
    a. A modified EASI, global SCORAD and EASI score will be assessed at baseline 1 week BID application of AMT cream or vehicle cream. Using percentage of improvement of these scores, we will compare percent reduction of AD severity between AMT cream and placebo cream using a two-tailed t-test with type I error rate of 0.05.

5. Identify lesional skin microbiome by DNA sequencing from baseline and after 1 week BID application of AMT.
    a. Using DNA sequencing, the microbiome of lesional skin will be identified at baseline and after 1 week BID application of AMT.

6. Evaluate the lesional skin microbiome transcriptome by RNA-sequencing analysis from baseline and after 1 week BID application of AMT.
    a. Using RNA-sequencing analysis, the microbiome of lesional skin will be evaluated at baseline and after 1 week BID application of AMT.

Secondary Endpoints, Non-lesional skin:

1. The abundance of *Staph aureus* at baseline and after 1 week BID application of AMT on non-lesional skin.
    a. The abundance of *Staph aureus* on non-lesional skin will be compared from baseline (day 28) to Day 35 after 1 week BID application of AMT cream versus placebo. This will be calculated using a two-tailed t-test for independent samples with type II error rate of 0.2 and type I error rate of 0.05.

2. The abundance of the AMT bacterial strains on non-lesional skin after 1 week BID application of AMT.
    a. The abundance of live *CoNS* species on non-lesional skin will be compared from baseline (day 28) to Day 35 after 1 week BID application of AMT cream versus placebo. This will be calculated using a two-tailed t-test for independent samples with type II error rate of 0.2 and type I error rate of 0.05.

3. The quantity of transplanted bacteria on non-lesional skin after stopping AMT cream 1 day, 2 days, and 4 days after last application.

    a. Live antimicrobial *Staph* species will be measured on non-lesional skin at baseline (day 28), Day 35 (after 7 days of cream application), Day 36, Day 37, and Day 39 in order to evaluate the abundance of the antimicrobial *CoNS* species that remains after stopping cream application. In order to take into account the variance of the abundance of antimicrobial *CoNS* species found on each subject's skin at baseline, we will perform CFU counts at each time point (Day 28, 35, 36, 37, and 38) and evaluate the percentage of antimicrobial species present at each time point. The percentage of antimicrobial species present at each time point will be compared between the AMT cream arm and placebo arm using a two-tailed t-test for independent samples with type II error rate of 0.2 and type I error rate of 0.05.

4. The change in abundance of *Staph aureus* on non-lesional skin from baseline to 1 day, 2 days, and 4 days after stopping AMT.
   a. The abundance of live *Staph aureus* on non- lesional skin will be evaluated using CFU counting at baseline, 1 hour, and 4 hours after initial application of AMT or placebo cream, and after 4 and 7 days of cream application. At each time point, we will compare the AMT cream and placebo arms by using a two-tailed t-test for independent samples with a type I error rate of 0.05 and type II error rate of 0.2.
5. Identify lesional skin microbiome by DNA sequencing from baseline and after 1 week BID application of AMT.
   a. Using DNA sequencing, the microbiome of lesional skin will be identified at baseline and after 1 week BID application of AMT.
6. Evaluate the lesional skin microbiome transcriptome by RNA-sequencing analysis from baseline and after 1 week BID application of AMT.
   a. Using RNA-sequencing analysis, the microbiome of lesional skin will be evaluated at baseline and after 1 week BID application of AMT.

13.4.5 Analyses of Exploratory Endpoint(s)/Outcome(s)

13.4.6 Descriptive Analyses

Background and baseline demographic, physical examination, and subject disposition data will be tabulated and presented for all enrolled subjects by randomized treatment group. Disposition will be tabulated according to the number and percentage of subjects who have: signed informed consent, provided baseline/screening information, were randomized to receive AMT cream or placebo, and completed at least one follow-up visit after intervention. Continuous data (e.g., age, body weight, height) will be summarized descriptively by mean, standard deviation, median, and range. Categorical data (e.g., gender, race, severity of disease) will be presented as enumerations and percentages. An intention-to-treat analysis will be conducted that includes all subjects who had at least one follow-up visit after intervention. A per-protocol analysis will also be conducted that includes all subjects who were compliant with the full intervention regimen with no major protocol violations.

13.5   Interim Analyses

Interim analyses will include distributions of AEs by subject population. No efficacy analyses will be performed unless specifically requested by the SO. Additional analyses may be requested by the NIAMS SO.

13.7   Sample Size Considerations

Number of S. aureus colonies will be a continuous variable, and change in S. aureus abundance will be measured as the absolute difference in number of colony-forming units at specified time points.

The sample size was calculated using G-Power Data Analysis software for a two-tailed t-test for independent samples, type II error rate of 0.2, and type I error rate of 0.05. The values used in the analysis were determined from our pilot study in which we quantified the reduction in *S. aureus* on the forearms of 5 atopic dermatitis patients twenty-four hours after treatment with a live microbiome transplant. Since the sample size was small in this study, we used the median and median average deviation as measures of central tendency. The median *S. aureus* present on the forearms of atopic dermatitis patients at baseline was 192.86 CFU/cm2 with deviation of 191.86. We observed that a log-base 10 reduction by 1 of the number of bacteria present on the forearms to be a clinically significant reduction at 24 hours, with deviation of 21.42. Using $\mu_1 = 192.86$, $\mu_2 = 19.186$, and $\sigma_1 = 191.86$, and $\sigma_2 = 21.42$, the total sample size necessary to see this difference would be n=24 for an unequal allocation of subjects 2:1 in the treatment to placebo groups.

Assuming a drop-out rate of 20%, we propose a total n=27, with n= 18 in the treatment group and n= 9 in the placebo group. Assignment to treatment or placebo groups will be determined using simple randomization with an online random number generator.

This sample size is reasonable and practical within the scope of this funding mechanism and recruitment capabilities.

An intention-to-treat analysis will be conducted that includes all subjects who had at least one follow-up visit after intervention. A per-protocol analysis will also be conducted that includes all subjects who were compliant with the full intervention regimen with no major protocol violations.

14.   Identification and Access to Source Data

14.1. Source Data

Source documents and source data are considered to be the original documentation where subject information, visits 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 KAI, NIAMS, 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 subject'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.

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 subject'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 principal investigator has the responsibility to identify, document and report protocol deviations as directed by the study Sponsor. 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 notify the site Principal Investigator and complete a Protocol Deviation form. Protocol deviations, as determined by the PI, will be reported to the appropriate review bodies including the IRB, SO, and FDA, who will review and approve the action plan that will be implemented as a result of the protocol deviation.

16.   Ethical Considerations and Compliance with Good Clinical Practice

16.1. Statement of Compliance

This clinical study will be conducted using good clinical practice (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 IRB and FDA. Any amendments to the protocol or to the consent materials will also be approved by the IRB and FDA before they are implemented.

16.2. 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 principal investigator or designee listed on the *FDA 1572* will review the consent and answer questions. 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. All participants (or their legally acceptable representative) will read, sign, and date a consent form before undergoing any study procedures. Consent materials will be presented in participants' primary language. A copy of the signed consent form will be given to the participant.

The 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.

16.3. Privacy and Confidentiality

A participant's privacy and confidentiality will be respected 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. Site personnel will not transmit documents containing personal health identifiers (PHI) to the study sponsor or their representatives.

17.   Publication Policy

The NIAMS policy on the publication of study results will apply to this trial.

18.   References

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