PROTOCOL ADRN-13

A PILOT STUDY TO EVALUATE THE SURVIVAL OF TRANSPLANTED *STAPHYLOCOCCUS HOMINIS* A9 ON THE SKIN OF ADULTS WITH MODERATE-TO-SEVERE ATOPIC DERMATITIS

TARGETED INVESTIGATION OF MICROBIOME ELIMINATION (TIME-1)

3.0 /28 JUL 2023

INVESTIGATIONAL NEW DRUG (IND)# 17286

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Return Signed Form to: *[The original signature page must be kept for your records. Return an electronic PDF copy of the signed signature page (*as described below) to the* [redacted]	
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, 312, and 812 and in the International Conference for Harmonisation (ICH) document entitled *Integrated Addendum to ICH E6(R1): Guideline for Good Clinical Practice E6(R2)*. Further, I will conduct the study in keeping with local legal and regulatory requirements. As the site Principal Investigator, I agree to carry out the study by the criteria written in the protocol and understand that no changes can be made to this protocol without the written permission of the Institutional Review Board (IRB) and NIAID. *[*The site Principal Investigator should print, sign, and date at the indicated location below. A written signature is acceptable, and an electronic signature is acceptable in a pdf version of the form.]* _____ **Site Principal Investigator (Print)** _____ _____ **Site Principal Investigator (Signature)** **Date**	

Protocol Synopsis

Title	A Pilot Study to Evaluate the Survival of Transplanted *Staphylococcus Hominis* A9 on the Skin of Adults with Moderate-to-Severe Atopic Dermatitis
Short Title	Targeted Investigation of Microbiome Elimination (TIME-1)
Clinical Phase	Phase 1
Number of Sites	1 Clinical Site in the United States
IND Sponsor/Number	NIAID / IND # 17286

Study Objectives	**Primary Objective** To assess the duration of survival of coagulase negative staphylococcal species (CoNS) as measured by colony-forming unit (CFU) during a maximum of 24 days after the application of *Staphylococcus hominis* (*S. hominis*) A9 on the lesional ventral arm skin of Atopic Dermatitis (AD) participants positive for *Staphylococcus aureus* (*S. aureus*) (AD SA+) **Secondary Objectives** 1. To assess the duration of survival of CoNS as measured by CFU during a maximum of 24 days after the application of *S. hominis* A9 on nonlesional ventral arm skin of AD participants positive for *S. aureus* (AD SA+) 2. To assess the safety of *S. hominis* A9 or placebo application, as determined by the count of serious and non-serious treatment-emergent adverse events (AEs) during the time period of Day 0 to Day 31 per participant within each group **Exploratory Objectives** 1. To assess the association of *S. hominis* A9 estimated CFU on the skin of the ventral arm contralateral to the arm of application and face 2. To assess the duration of survival of CoNS as measured by CFU during a maximum of 24 days after the application of *S. hominis* A9 on lesional ventral arm skin of AD participants negative for *S. aureus* (AD SA-) 3. To assess the duration of survival of CoNS as measured by CFU during a maximum of 24 days after the application of *S. hominis* A9 on nonlesional ventral arm skin of AD participants negative for *S. aureus* (AD SA-) 4. To compare the duration of survival of CoNS as measured by CFU on lesional skin between AD SA+ vs AD SA- participants 5. To compare the duration of survival of CoNS as measured by CFU on non-lesional skin between AD SA+ vs AD SA- participants 6. To compare the duration of survival of CoNS as measured by CFU between lesional and non-lesional skin on AD SA+ participants

	7. To compare the duration of survival of CoNS as measured by CFU between lesional and non-lesional skin on AD SA- participants 8. To compare disease severity measures (local Eczema Area and Severity Index [L-EASI], SCORing Atopic Dermatitis [SCORAD] and local Pruritus Numerical Rating Scale [NRS]) between Day 0 and the last in-clinic visit among AD SA+ participants, and independently, among AD SA- 9. To determine the antibiotic sensitivity of the skin CoNS microbiome to penicillin G, tetracycline, and erythromycin before and after treatment with ShA9

Study Design	This is a Phase 1 pilot, open label, single center trial designed to assess the kinetics of *S. hominis* A9 survival on the skin of adults with moderate-to-severe atopic dermatitis on the ventral arms who are culture positive or negative for *S. aureus* colonization.
Primary Endpoint	The duration of survival, measured as the time needed for CoNS CFU to drop below baseline density measured before application of ShA9 + 100 CFU/cm^2 on the lesional ventral arm skin of AD participants positive for *S. aureus* (AD SA+)
Secondary Endpoints	1. The duration of survival, measured as the time needed for CoNS CFU to drop below baseline density measured before application of ShA9 + 100 CFU/cm^2 on non-lesional ventral arm skin of AD participants positive for *S. aureus* (AD SA+) 2. The count of serious and non-serious treatment-emergent AEs per participant during the time period of Day 0 to Day 31
Exploratory Endpoints	1. Association of *S. hominis* A9 bacteria abundance estimated CFU as measured by % *S. hominis* A9 positive colonies by PCR x CoNS CFU while CoNS CFU is above baseline density measured before application of ShA9 + 100 CFU/cm^2 on the lesional and non-lesional, separately, ventral arm skin of AD participants between the arm treated with *S. hominis* A9 and the contralateral arm treated with placebo, and independently, with the participant's face 2. The duration of survival, measured as the time needed for CoNS CFU to drop below baseline density measured before application of ShA9 + 100 CFU/cm^2 on the lesional ventral arm skin of AD participants negative for *S. aureus* (AD SA-) 3. The duration of survival, measured as the time needed for CoNS CFU to drop below baseline density measured before application of ShA9 + 100 CFU/cm^2 on the non-lesional ventral arm skin of AD participants negative for *S. aureus* (AD SA-) 4. Comparison of time needed for CoNS CFU to drop below baseline density measured before application of ShA9 + 100 CFU/cm^2 on the lesional and non-lesional, separately, ventral arm skin between AD participants positive (AD SA+) vs negative (AD SA-) for *S. aureus* 5. Comparison of time needed for CoNS CFU to drop below baseline density measured before application of ShA9 + 100 CFU/cm^2 on lesional

	vs non-lesional skin of AD participants positive (AD SA+) and negative (AD SA-) for *S. aureus,* separately 6. Comparison of the L-EASI score of the ventral arms at Day 0 vs the last in-clinic visit among AD SA+, and independently, among AD SA- 7. Comparison of the SCORAD score at Day 0 vs the last in-clinic visit among AD SA+, and independently, among AD SA- 8. Comparison of the local Pruritus NRS of the ventral arms at Day 0 vs the last in-clinic visit among AD SA+, and independently, among AD SA- 9. Comparison of the proportion of antibiotic-sensitive CoNS to penicillin G, tetracycline, and erythromycin isolated from skin swabs at baseline and after ShA9 treatment, at the last swab collection from lesional skin
Accrual Objective	This study will enroll a minimum of 20 adult participants (a minimum of 13 *S. aureus* positive and a minimum of 7 *S. aureus* negative), 18-80 years of age, with moderate-to-severe atopic dermatitis on the ventral arms.
Study Duration	This study will take approximately 18 months to complete participant recruitment, enrollment, and follow-up. An individual participant's participation in this study will consist of up to 2 weeks of Screening, a 1-week pre-treatment phase, 1 day of treatment, and a maximum of 31 days for follow-up. Screened participants who meet all inclusion and exclusion criteria, including medication and therapy washouts, may complete Screening and Pre-treatment on the same day.
Treatment Description (Investigational Products)	Active (ShA9): 85% Phosphate-buffered saline solution (PBS) and 15% Glycerol containing healthy donor-derived (allogeneic) commensal Staph species, *S. hominis* A9 (Manufactured and packaged by University of California – San Diego [UCSD]) applied once to the right or left ventral arm (wrist to upper arm) Placebo: 85% PBS and 15% Glycerol (Manufactured and packaged by UCSD) applied once to the contralateral ventral arm

Inclusion Criteria	Individuals who meet all of the following criteria are eligible for enrollment as study participants: 1. Participant must be able to understand and provide informed consent 2. Male or female participants 18 to 80 years of age, inclusive at time of the Screening Visit 3. Meet ADRN Standard Diagnostic Criteria (Appendix A) for active AD 4. At least 21 cm^2 of lesional and 21 cm^2 of non-lesional skin on both the right and left ventral arms. The required area (lesional or non-lesional) may be one contiguous area or may encompass multiple areas with a total cumulative area of 21 cm^2. 5. An Investigator Global Assessment (IGA) score, on the ventral arms of at least moderate severity 6. Body surface area (BSA), as measured by Mostellar BSA Calculator, between 1.26 m^2 and 2.25 m^2 7. If female of child bearing potential, must agree to remain abstinent (refrain from heterosexual intercourse) or use acceptable contraception
	(e.g., oral contraceptives, intrauterine device (IUD), barrier method with spermicide, surgically sterilized partner, Depo-Provera, Norplant, NuvaRing, or hormonal implants) for the duration of study participation

Exclusion Criteria	Individuals who meet any of these criteria are not eligible for enrollment as study participants: 1. Inability or unwillingness of a participant to give written informed consent or comply with study protocol 2. Pregnant or lactating females 3. Active bacterial, viral, or fungal skin infections 4. Any noticeable breaks or cracks in the skin on the target areas of investigational product application, including severely excoriated skin or skin with open or weeping wounds suggestive of an active infection or increased susceptibility to infection 5. Sensitivity to or difficulty tolerating Dove fragrance-free bar soap, Cetaphil® lotion, alcohol-based cleaners, glycerol, or soy products 6. Participants with Netherton's syndrome or other genodermatoses that result in a defective epidermal barrier 7. Any participant who is immunocompromised (e.g., history of lymphoma, Human Immunodeficiency Virus (HIV)/Acquired Immunodeficiency Syndrome (AIDS), Wiskott-Aldrich Syndrome), has an immune system disorder (e.g., autoimmune disease), or is using a systemic immunosuppressant (e.g., systemic corticosteroids, cyclosporine, methotrexate) 8. Any participant with current malignant disease (with the exception of non-melanoma skin cancer in an area not affected by treatment) 9. Participants with a history of psychiatric disease or history of alcohol or drug abuse that would interfere with the ability to comply with the study protocol 10. Past or current medical problems or findings from physical examination or laboratory testing that are not listed above, which, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the participant's ability to comply with study requirements or that may impact the quality or interpretation of the data obtained from the study 11. Ongoing participation in another investigational trial or use of investigational drugs within 8 weeks, or 5 half-lives (if known), whichever is longer, of the Screening Visit 12. Treatment with non-steroid systemic immunosuppressant within 6 months of the Screening Visit 13. Treatment with Dupilumab within 16 weeks of the Screening Visit 14. Treatment with oral or injectable therapy for AD (excluding oral steroids) within 5 half-lives (if known) or 16 weeks before the Screening Visit, whichever is longer 15. Participants with close contacts (e.g., spouse, children, or members in the same household) that have severe barrier defects or are immunocompromised

	16. Use of topical (including steroids and calcineurin inhibitors) AD treatments on the ventral arms or face within 7 days of the Treatment Visit; Use of topical steroids on areas outside of where investigational product is to be applied or swabbing is to be performed may be permitted, per investigator discretion. 17. Treatment with prescription moisturizers classified as medical device (e.g., Atopiclair®, MimyX®, Epiceram®, etc.) on the ventral arms or face within 7 days of the Treatment Visit; Use on areas outside of where investigational product is to be applied or swabbing is to be performed is permitted 18. Use of any oral or topical antibiotic within 7 days of the Treatment Visit 19. Participants who have taken a bleach bath within 7 days of the Treatment Visit 20. Use of any oral steroid therapies within 28 days of the Treatment Visit 21. Any phototherapy for skin disease (such as narrow band ultraviolet B [NBUVB], ultraviolet B [UVB], ultraviolet A1 [UVA1], psoralen + UVA [PUVA]) or regular use (more than 2 visits per week) of a tanning bed within 28 days of the Treatment Visit
Study Stopping Rules	Study enrollment will be suspended pending Data and Safety Monitoring Board (DSMB) expedited review of all pertinent data if any of the following occur: 1. A single participant experiences any Serious Adverse Event (SAE) for which there is a reasonable possibility that the investigational product caused the SAE 2. The development of any severe (Grade 3) AE for which attribution is defined as related or possibly related

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Glossary of Abbreviations

AD	Atopic Dermatitis
ADRN	Atopic Dermatitis Research Network
AE	Adverse Event
AIP	Autoinducing Peptide
AMP	Antimicrobial peptide
BSA	Body Surface Area
CoNS	Coagulase Negative Staphylococcal Species

CFR	Code of Federal Regulations
CFU	Colony-Forming Unit
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
DAIT	Division of Allergy, Immunology, and Transplantation
DSMB	Data and Safety Monitoring Board
eCRF	Electronic Case Report Form
FDA	Food and Drug Administration
GCP	Good Clinical Practice
ICH	International Conference on Harmonisation
IGA	Investigator Global Assessment
IRB	Institutional Review Board
ITT	Intent To Treat
L-EASI	Local Eczema Area and Severity Index
MCB	Master Cell Bank
MOP	Manual of Procedures
MRSA	Methicillin-resistant *Staphylococcus aureus*
NBUVB	Narrow Band Ultraviolet B
NCI	National Cancer Institute
NIAID	National Institute of Allergy and Infectious Diseases
NRS	Numerical Rating Scale
OVA	Ovalbumin
PBS	Phosphate-Buffered Saline Solution
PI	Principal Investigator
PUVA	Psoralen Ultraviolet A
S. aureus	*Staphylococcus aureus*
S. hominis	*Staphylococcus hominis*
SACCC	Statistical and Clinical Coordinating Center

SAE	Serious Adverse Event
SAR	Suspected Adverse Reaction
SCORAD	Scoring Atopic Dermatitis
SOP	Standard Operating Procedure
SUSAR	Serious and Unexpected Suspected Adverse Reaction
TSB	Tryptic Soy Broth
USP	United States Pharmacopeia
UVA	Ultraviolet A
UVB	Ultraviolet B
WCB	Working Cell Bank

1. Background and Rationale

1.1. Background and Scientific Rationale

Atopic Dermatitis (AD) is a common, chronic, inflammatory skin disease complicated by recurrent bacterial as well as viral skin infections (Gao, et al., 2012). The skin of AD patients is highly susceptible to infections by viruses, bacteria, and fungi. These microorganisms interact with the human immune system to trigger the onset or exacerbate the disease. Studies suggest that 7-10% of AD patients have difficulty containing infections caused by viruses, including herpes simplex virus, vaccinia virus (causing eczema vaccinatum), and molluscum contagiosum virus (Howell, et al., 2006). Preliminary data in the Atopic Dermatitis Research Network (ADRN) Registry study indicate 40% of AD subjects, and only 2% of non-atopics are *Staphylococcus aureus* (*S. aureus*) colonized on lesional or non-lesional skin. These observations are consistent with the understanding that patients with AD have defects in innate and adaptive immune responses and thus have a general defect in the control of skin infections.

Several lines of experimental evidence support the concept that the multiple defects in epithelial barrier function in AD promote dysbiosis, a state of microbial imbalance, and that this dysbiosis then promotes the immunological disorder characteristic of AD. Dysbiosis in AD has been shown to be closely associated with disease severity (Leyden, et al., 1974). Antibiotic therapy is a mainstay for treatment of bacterial infections; however, antibiotic treatment nonspecifically kills bacteria and may induce additional dysbiosis. In addition, long-term antibiotic treatments generate resistant bacteria. Human antimicrobial peptides (AMPs) such as defensins and cathelicidins are important mediators to maintain the balance of microflora on the skin. Our studies indicate that the innate immune defense of the skin is mediated not only by AMPs produced by an individual's own cells, but also by antimicrobial molecules contributed by commensal coagulase negative staphylococcus species (CoNS), such as *S. epidermidis* and *S. hominis* (Nakatsuji, et al., 2017; Dorschner, et al., 2001; Murakami, et al., 2002; DiNardo, et al., 2003; Zaiou, et al., 2003). More recently, we have found that autoinducing peptide (AIP) produced by skin commensal CoNS suppresses production of virulence factors, including Phenol-soluble modulin-alpha, in *S. aureus* (Williams, et al., 2019). Our clinical research also suggests that AD skin colonized by *S. aureus* has a lower frequency of these antimicrobial Staph strains (CoNS AM+) and CoNS strains with capacity to produce AIP compared to normal skin (Nakatsuji, et al., 2017). Studies have also shown that AD patients colonized by *S. aureus* have more severe disease states and are prone to more frequent flares of their AD (Leyden, et al., 1974; Kong, et al., 2012). We hypothesize that the skin of patients with AD selectively inhibits the survival of beneficial bacteria (CoNS AM+) that inhibit *S. aureus*, and that an increase in the amount of CoNS AM+ to the level found on most healthy skin would normalize the balance of microflora in AD patients, thereby improving abnormal immune reactions by AD skin. The goal of this study is to understand why beneficial bacteria are lost in AD, and to better understand the factors involved in survival of beneficial bacterial strains (CoNS AM+).

1.2. Rationale for Selection of Investigational Product

The functions described in Section 1.1 for the microbiome have focused on *S. epidermidis* and *S. aureus* bacterial species. However, we have made a highly innovative discovery of newly recognized species such as *S. hominis*, as well as newly identified strains of *S. epidermidis* from the human skin microbiome that produce previously unknown antimicrobials that will selectively kill pathogens such as *S. aureus* while not harming the resident commensals. Because of this, these commensal bacteria can provide selective antimicrobial protection against pathogens on the skin surface yet maintain diversity.

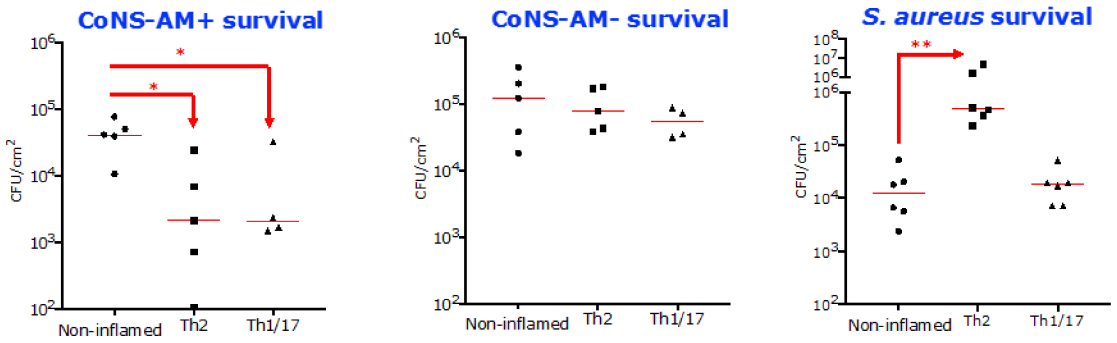
One example of such protective bacteria is a strain of *S. epidermidis* that produces Phenol Soluble Modulins gamma and delta (Cogen, et al., 2010a; Cogen, et al., 2010b). We have shown that these helical peptides are potent against *S. aureus* in vitro and on human skin. More recently, with the prior support of the National Institute of Allergy and

Infectious Diseases (NIAID), we have discovered a novel *S. epidermidis*-derived antibiotic we have named 6-nhydroxyaminopurine that selectively kills *S. aureus* (including methicillin-resistant *S. aureus* [MRSA] strains), group A Streptococcus , group B Streptococcus and Pseudomonas aeruginosa (Nakatsuji, et al., 2018). Another commensal strain of *S. epidermidis* produces a specific protease (esp) that disrupts *S. aureus* biofilms and has been used successfully in a human clinical trial to decrease nasal colonization (Archer, et al., 2011). We have also discovered that *S. hominis*, which is also a major commensal on the human skin, produces a novel AMP we have named Sh-lantibiotic-α that kills *S. aureus* by a different mechanism (Nakatsuji, et al., 2017). These findings indicate that multiple bacteria inhabiting the surface of normal human skin contribute directly to the skin's antimicrobial defenses. Importantly, we have now identified 4 specific strains in the microbiome with beneficial antimicrobial function that can be used in a new paradigm for host defense whereby the commensal bacteria defend their host by producing AMPs. In our recent, vehicle controlled double blind, 7 day study supported by NIAID, we observed that the commensal Staph species *S. hominis* A9 (ShA9) was well tolerated by AD participants, killed *S. aureus,* and improved disease severity in participants that demonstrated a decrease in *S. aureus* colonization. ShA9 was also observed to decrease *S. aureus* on inflamed skin within 4 hours of application (Nakatsuji, et al., 2021). In this study, ShA9 was formulated in 50% Glycerol/ Cetaphil® lotion and required freezing at <-70°C to maintain survival of the ShA9 for greater than 2 years. In the current proposed trial, ShA9 will be reformulated in a vehicle that enables bacterial survival for up to 2 weeks at room temperature, and the rate of elimination and spread of ShA9 on AD skin will be studied in greater detail. These observations will inform dosing and design of a future Phase 2 trial of ShA9.

1.3. Preclinical Experience

To understand the mechanism responsible for selective loss of CoNS-AM+ strains of bacteria, we tested CoNS-AM+ survival on two opposing experimental inflammation models in mice. In the first model, a Th2 inflammatory model was induced by 3 cycles of 1-week cutaneous exposure to ovalbumin (OVA) on filaggrin mutant Balb/c mice (Nakatsuji, et al., 2016). In the second model, a Th1/17 immune response was induced on other groups of mice by daily application of 5% Imiquimod for a week (van der Fits, et al., 2009). Expression of AMPs, such as cathelicidin and β-defensins, was elevated in both inflammatory models in comparison to non-inflamed mock-treated skin. CoNS-AM+ or AM- strains of *S. epidermidis* or *S. hominis* were then applied on the inflamed or non-inflamed skin. Colonization by CoNS-AM+ strains was selectively inhibited on inflamed skin of both models in comparison to non-inflamed skin (Figure 1.3a). CoNS-AM- strains equally colonized inflamed and non-inflamed skin independent of Th2 or Th1/17 predominance.

Figure 1.3a. CoNS-AM- and *S. aureus* survive on inflamed mouse skin but CoNS-AM+ are inhibited.

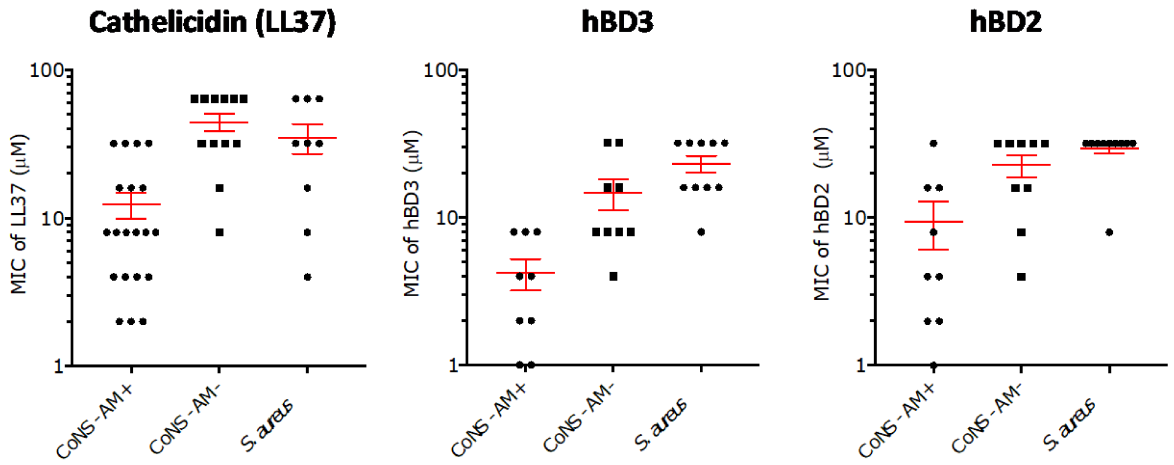


Survival of CoNS-AM+, CoNS-AM- strains (*S. hominis* and *S. epidermidis*) or *S. aureus* on the skin of OVA-sensitized (Th2 inflammation), Imiquimod (IMQ)- treated mice (Th1/17 inflammation) or control (non-inflamed). Analysis was performed 72 hours after bacterial application ($1x10^6$ CFU). Each dot represents data from a distinct strain of CoNS on each mouse. (Unpublished data, Gallo lab-UCSD)

We next sought to examine what role human AMPs play in selectively inhibiting skin colonization by CoNS-AM+ on inflamed skin. The human AMPs, cathelicidin and β-defensins typically exist at very low levels on healthy skin. Induction of these AMPs is triggered by inflammation or injury (Zhang and Gallo, 2016; Gallo and Hooper, 2012). These AMPs are present in both AD and psoriasis, but in AD, the AMP increase is suppressed by Th2 cytokines (Howell, et al., 2006) such that the relative expression of AMPs is much less than in psoriasis or wound repair and insufficient to effectively resist infections (Howell, et al., 2006; Mallbris, et al., 2010; Ong, et al., 2002; Hata, et al., 2010; Howell, et al., 2006; Finlay, et al., 1989). It is important to recognize that AMP levels are elevated in AD skin compared with normal skin. We now have observed that an increase in human AMPs is associated with the loss of CoNS-AM+ strains in both AD and psoriasis (Unpublished observations, Gallo Lab UCSD).

To examine if the increase in host AMPs might be responsible for selectively killing CoNS-AM+ strains, we measured minimal inhibitory concentrations (MIC) of human cathelicidin LL37, human β-defensin 2 (hBD2), and hBD3 against clinical isolate strains of CoNS-AM+, CoNS-AM-, and *S. aureus* (Figure 1.3b). These are AMPs significantly elevated in AD lesional skin compared to non-atopic healthy skin or unaffected skin from AD patients (Hata, et al., 2010; Ong, et al., 2002). The human AMPs demonstrated greater antibacterial activity against CoNS-AM+ compared with CoNS-AM- or *S. aureus*. To determine if the susceptibility of CoNS-AM+ is attributed to the capacity to produce AMs itself, we deleted the gene encoding the lantibiotics from *S. hominis* A9 by allelic exchange mutagenesis (Figure 1.3c Panel A). This deletion mutant lost its ability to kill *S. aureus* (Figure 1.3c Panel B), but then became much more resistant to killing by the human cathelicidin LL-37 (Figure 1.3c Panel C). These data suggest that the capacity of bacteria to make their own antibiotic (lantibiotic) renders them more susceptible to being killed by the human AMPs.

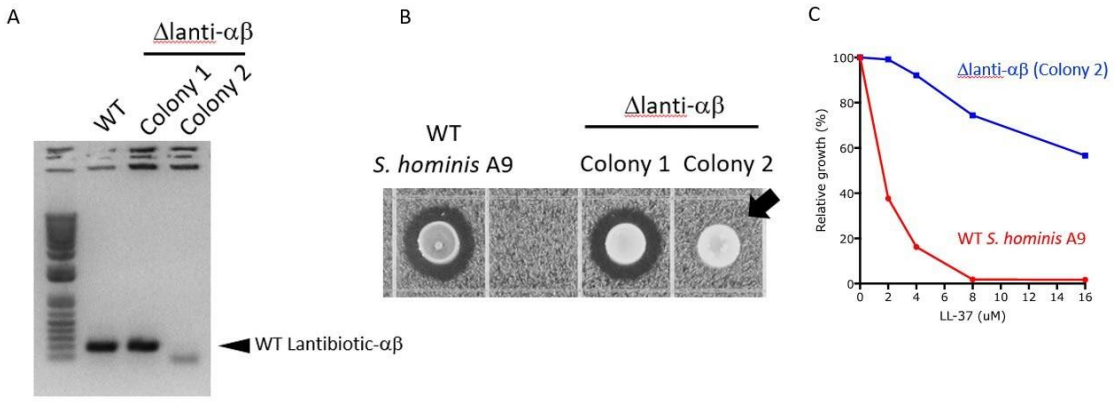
Figure 1.3b. Sensitivity to host innate AMPs is greatest in CoNS-AM+



Minimal inhibitory concentration (MICs) of human AMPs, cathelicidin LL-37, hBD3 and hBD2, was measured against clinical isolates of CoNS-AM+, CoNS-AM-, and *S. aureus* in Muller Hinton Broth. * p<0.05, **p<0.01, ***p<0.001. (Unpublished data, Gallo lab-UCSD)

Thus, the preliminary data from our recent 7 day Phase 1 trial suggest an explanation for *S. hominis* A9 being eliminated more quickly on inflamed skin than non-inflamed skin, but left the question regarding the rate of elimination. Therefore, the goal of the current proposed study is to better understand the rate of elimination of ShA9 in order to inform dosing frequency and to understand factors that affect survival of the bacteria once applied to the skin of AD participants.

Figure 1.3c. Deletion of lantibiotic from a CoNS-AM+ *S. hominis* strain converts it to CoNS-AM- and improves its resistance to human AMP



(A) lantibiotics-alpha-hogocidin and beta-hogocidin genes were deleted by allelic reaction with pKOR1 plasmid in colony 2 (arrow head). (B) Colony 2 did not produce antimicrobial activity against *S. aureus*. Antimicrobial activity is visualized by zone of inhibition around (arrow) bacteria colony (white dot). (C) Shows that expression of the lantibiotics makes *S. hominis* A9 more susceptible to killing by LL-37. The alpha and beta lantibiotic mutant, or the parent WT *S. hominis* A9, was cultured in the presence of indicated concentrations of the human AMP LL-37 at 30°C for 24 hours. Relative growth was calculated from OD600.(Unpublished data, Gallo lab-UCSD)

The skin pathogen *Staphylococcus aureus* has been reported to have highly efficient mechanisms for horizontal gene transfer between strains (Chen, et al., 2018; Humphrey, et al., 2021). Similar mechanisms of transfer have not been reported for *Staphylococcus hominis*. Additionally, in our analysis of ShA9, there has been no evidence of *S. aureus* lateral gene transduction elements in ShA9 and no evidence ShA9 has acquired virulence or antibiotic resistance genes via conjugative transposon from other species. Plasmids for antibiotic resistance genes or virulence factors also have not been found in our analysis of *S. hominis*. However, we did identify the presence of two prophages containing a betalactamase homolog in the ShA9 DNA sequence (unpublished data, Gallo lab-UCSD). The antibiotic resistance profile present in ShA9 to beta-lactam antibiotics, tetracycline, and erythromycin is also already ubiquitously present in the healthy human skin microbiome (Oh, et al., 2014; Oh, et al., 2016; and Baron, et al., 2018); therefore, the effect of this occurrence is unclear. Due to the theoretical possibility of transfer of antibiotic resistance from ShA9 to *Staphylococci* present on the skin of study participants, we will investigate the antibiotic sensitivity to penicillin G, tetracycline, and erythromycin in CoNS isolated from skin swabs at baseline and at the last swab collection from lesional skin.

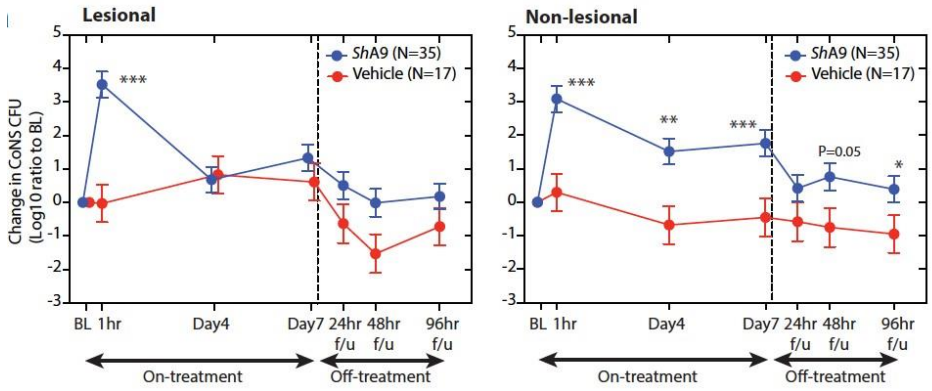
1.4. Clinical Studies

In a previous study conducted under this IND (Protocol ADRN-08), we were able to establish that dermal application of ShA9 was safe to use on humans. ADRN-08 was a phase 1 study of 54 *S. aureus* positive AD subjects randomized 2:1 active to vehicle (29 female and 25 male, ages 18-57; ClinicalTrials.gov, NCT03151148). We found application of *S. hominis* A9 to be safe in comparison to vehicle, with no difference in the per participant adverse event (AE) rate between active drug and vehicle (p=0.075). In addition, there was a statistically significant decrease in live *S. aureus* (p<0.001), on participants treated with ShA9 compared to vehicle (Nakatsuji, et al., 2021).

In our previous Phase 1 trial, we obtained data that led us to test the hypothesis that the elimination of ShA9 from lesional skin of AD participants was due to susceptibility of this strain to killing by antimicrobials produced by inflamed skin (Nakatsuji, et al., 2021). We further hypothesized that this susceptibility is a consequence of the expression of the lantibiotics produced by ShA9 but not by other strains of *S. hominis* that do not produce lantibiotics. This hypothesis was supported by the pre-clinical observations shown in Figure 1.3 a-c that CoNS-AM+ strains survive less well on inflamed

mouse skin, are more susceptible to killing by the human AMPs, such as cathelicidin and β -defensins -2 and-3 present on inflamed skin of patients with AD, and ShA9 increases survival upon deletion of its lantibiotics. To further test this hypothesis on human skin in the clinical setting, we measured abundance of ShA9 on the skin of participants from the Phase 1 trial under Protocol ADRN-08. We measured abundance of ShA9 on inflamed and non-inflamed skin. ShA9 abundance was measured by multiple approaches, including direct counting of total CoNS CFUs of live bacteria grown on selective agar plates, abundance of ShA9 DNA by qPCR, and measurement of mRNA by RT-qPCR for candidate ShA9 gene expression. Total CoNS CFUs were used as a surrogate measurement of live ShA9 CFU, since ShA9 is not specifically measurable by colony counting methods. However, since ShA9 is a type of CoNS, and the abundance of ShA9 applied greatly exceeded other CoNS recoverable from AD skin, this estimation was helpful to define live compared to dead bacteria. Furthermore, measurements by qPCR of ShA9 DNA and mRNA provide a more specific method to validate this assumption. On Day 0, an initial swab was collected one hour after application of ShA9 or vehicle. Both lesional and non-lesional skin showed a 3- to 4-log increase in live CoNS on the skin one hour after the first application of ShA9 but no change after application of the vehicle alone (Figure 1.4a). However, a difference in CoNS survival was observed between lesional and non-lesional sites at Days 4 and 7. At these time points, skin swabs were collected approximately four hours after the morning application. Swabs from lesional skin collected at these later times on Days 4 and 7 showed that live CoNS had decreased to a level similar to baseline, and no significant difference was detected in comparison to that from vehicle-treated subjects. This suggested the longer period between the morning application of ShA9 and the skin sampling resulted in a decrease in the live ShA9 recoverable by swab from lesional skin. In contrast, CoNS recovered from non-lesional skin was greater on both Day 4 and Day 7 on participants treated with ShA9 compared to vehicle (Figure 1.4a). These data support our preclinical observations that CoNS-AM+ bacteria survive less well in an inflamed skin environment that will have expression of human AMPs.

Figure 1.4a. CoNS-AM+ persist on non-lesional skin but not on inflamed skin of AD adult participants.

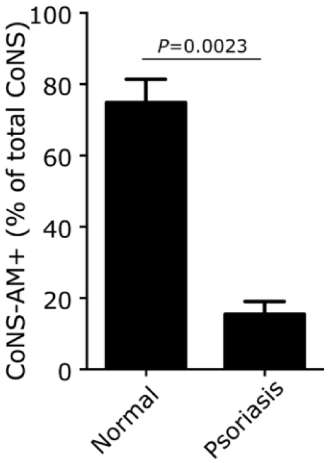


Change in live coagulase-negative Staphylococci (CoNS) recovered by skin swab from patient's skin compared to baseline at indicated time points after application of *Sh*A9 or vehicle. *Sh*A9 (n=35), vehicle (n=17). Data represent Mean ± SEM. * p< 0.05, ** p< 0.01, *** p< 0.001, by t-test. (Nakatsuji, et al., 2021)

To determine if other forms of skin inflammation also alter the CoNS-AM+ population, we have also recently measured CoNS-AM+ on patients with psoriasis. We reasoned that since psoriasis patients have even higher amounts of AMPs on their skin than AD subjects, the host innate immune system of psoriasis would select against CoNS-AM+. This would occur despite the contrast in T-cell repertoire between AD and psoriasis (Guttman-Yassky, et al., 2011a; Guttman-Yassky, et al., 2011b). Our preliminary data suggest patients with psoriasis also have a deficiency of CoNS-AM+ (Figure 1.4b). Thus, although AD and psoriasis are driven by distinct immune responses, the loss of CoNS-AM+ strains was observed on the skin of both diseases. These data strongly support our hypothesis that AMPs select against CoNS-AM+. We further hypothesize that since psoriasis has high mammalian AMPs, this level is sufficient to resist dysbiosis driven by *S. aureus*.

In contrast, AD which has lower AMPs and low CoNS-AM+, then sets up a situation that enables *S. aureus* to survive and damage the skin barrier.

Figure 1.4b. Psoriasis lesional skin lacks CoNS-AM+ strains.



Frequency of isolated CoNS strains that can inhibit *S. aureus* growth was determined from lesional skin swab samples of psoriasis subjects, and age and site matched normal control subjects. (Unpublished data, Gallo lab-UCSD)

2. Study Hypotheses/Objectives

2.1. Hypotheses

We propose an interventional clinical trial that will apply specific beneficial bacteria *S. hominis* A9 from the normal skin microbiome to AD patients, thus performing a microbiome transplant of beneficial bacteria (CoNS AM+) abundant in healthy skin, but deficient in AD. The transplant bacteria have been selected based on their capacity to inhibit *S. aureus* colonization (Nakatsuji, et al., 2017). Our central hypothesis is that inflamed AD skin selectively inhibits beneficial members of the AD microbiome that defend against *S. aureus,* and the skin environment prior to the microbiome transplant (positive for *S. aureus* [AD SA+] or without *S. aureus* [AD SA-]) will affect both the survival of the transplant and the decrease in colonization with *S. aureus*. This study will allow us to evaluate why beneficial bacteria are lost in AD and the environmental conditions of the skin surface which best promote survival of the beneficial bacteria in lesional and non-lesional skin. Data obtained from this study will allow us to better optimize the dosing frequency and better understand factors that affect survival of the bacteria once applied to AD skin.

2.2. Primary Objective

To assess the duration of survival of CoNS as measured by CFU during a maximum of 24 days after the application of *S. hominis* A9 on the lesional ventral arm skin of AD participants positive for *S. aureus* (AD SA+)

2.3. Secondary Objectives

1. To assess the duration of survival of CoNS as measured by CFU during a maximum of 24 days after the application of *S. hominis* A9 on non-lesional ventral arm skin of AD participants positive for *S. aureus* (AD SA+)
2. To assess the safety of *S. hominis* A9 or placebo application, as determined by the count of serious and nonserious treatment-emergent AEs during the time period of Day 0 to Day 31 per participant within each group

2.4. Exploratory Objectives

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

3. Study Design

3.1. Description of Study Design

Protocol ADRN-13 is a Phase 1 pilot, open label, single center trial designed to assess the kinetics of *S. hominis* A9 survival in adults with moderate-to-severe atopic dermatitis on the ventral arms who are culture positive or negative for S*taphylococcus aureus* colonization. Total CoNS CFU will be used to assess *S. hominis* A9 survival since this specific species cannot be distinguished by culture and it is expected to be the predominant CoNS species after the treatment application.

A minimum of 20 AD subjects (13 *S. aureus* positive and 7 *S. aureus* negative), 18 to 80 years of age, will have a single application of *S. hominis* A9 applied to their right or left ventral arm (wrist to upper arm); a single application of placebo will be applied to the contralateral arm. If any participants withdraw or are withdrawn prior to achieving CoNS elimination (CoNS CFU below baseline density measured before application of ShA9 + 100 CFU/cm^2), additional participants may be enrolled as necessary to obtain a modified intent to treat (ITT) sample as defined in Section 13.4.1.

Potential participants will be recruited in person or over the phone and assessed for eligibility. Potentially eligible participants, who have no obvious characteristics making them ineligible and who are interested in study participation, will be invited to complete an in-clinic Screening Visit. During the Screening Visit, participants will provide informed consent for study participation. Consented participants will then be further assessed for full study eligibility through the collection of medical history, including medication/therapy use, a physical exam, and assessment of AD severity. Participants who meet all eligibility requirements, including medication and therapy washouts (no topical AD treatments

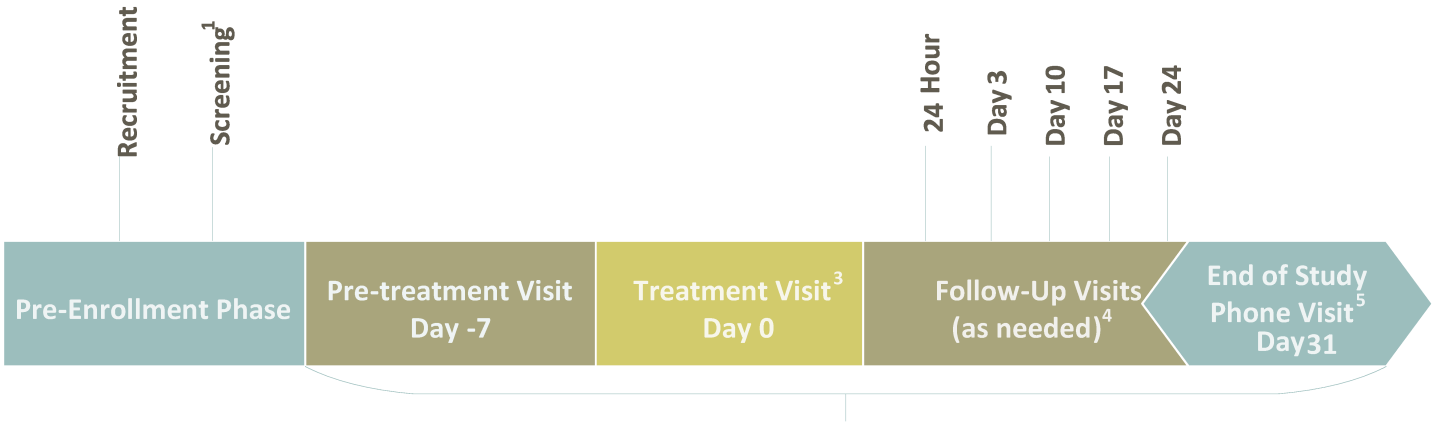
or prescription moisturizers on the ventral arms or face, and no oral or topical antibiotics, or bleach baths in the last 7 days, and no oral steroid therapy, phototherapy, or regular tanning bed use [more than 2 visits per week] in the last 28 days), may complete the Pre-treatment Visit on the same day of the Screening Visit. Participants who require a medication/therapy washout of more than 14 days will be required to rescreen.

Screened and eligible participants will complete a Pre-treatment Visit (Day -7 ± 1 Day), approximately 7 days prior to their Day 0 Visit. During the visit, target lesional and non-lesional sites, each measuring at least 21 cm^2 total, will be identified on the participant's right and left ventral arms. The sites will be photographed for reference at the participant's future visits, and swabs for *S. aureus* screening by culture, *S. aureus* quantification by PCR, CoNS CFU, and *S. hominis* A9 estimated CFU will be collected from the identified sites. Participants will also be provided Cetaphil lotion and Dove soap during this visit, for use during the week prior to their Treatment Visit (Day 0).

Participants who complete the pre-treatment phase (7 ± 1 days total) and meet enrollment criteria for AD SA+ or AD SA-, will come back to clinic for their Treatment Visit (Day 0). Participants must be cultured to determine *S. aureus* colonization status. Given the rates of *S. aureus* colonization, it is likely that enrollment will be complete for the AD SA- group before the AD SA+ group. Once the enrollment goals for AD SA+ or AD SA- are met, additional participants will be identified as screen failures. During the Treatment Visit, the participant will have ShA9 applied to their right or left ventral arm and placebo applied to their contralateral ventral arm. The assignment of ShA9 and placebo to the dominant and non-dominant arms will be randomized. Prior to the application of the ShA9 and placebo treatments, skin swabs will be collected from the lesional and non-lesional sites on the ventral arms and one non-lesional site on the participant's face. A blinded clinical team member will apply the designated treatment to each arm, and additional swabs will be collected from the arms 15 minutes and 1, 2, 4, and 6 hours after application. Participants will return to the clinic 24 hours after application (24 Hour Visit) and on Days 3, 10, 17, and 24 for the assessment of AEs and the collection of skin swabs from the identified target sites, as needed. After completion of the Day 3 Visit, a participant will not be required to complete the remaining follow up clinic visits (Day 10 through Day 24) if their lesional swabs from both arms (right and left) are CoNS negative, defined as CoNS below baseline density measured before application of ShA9 + 100 CFU/cm^2. All randomized participants will complete a final End of Study Phone Visit on Day 31 to assess safety and disease status.

A minimum of 13 *S. aureus* positive (defined as *S. aureus* CFU ≥ 100 CFU/cm^2 for lesional swabs from both arms) and a minimum of 7 *S. aureus* negative (defined as *S. aureus* CFU < 100 CFU/cm^2 for lesional swabs from both arms) participants with moderate-to-severe AD will be enrolled to receive study treatment in this trial. Based on preliminary data from the ADRN Registry study (unpublished data), we estimate that approximately 40% of AD participants will be *S. aureus* positive on their lesional skin, and we may need to screen (including pretreatment) approximately 33 participants to enroll 13 AD *S. aureus* positive participants, as colonization is determined once skin swabs are collected at the Pretreatment Visit. We anticipate it will take approximately 18 months to reach the recruitment and enrollment goals and to complete participant follow up for this trial. The study flow diagram is provided in Figure 3.1.

Figure 3.1 Study Design Schematic



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

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

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

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

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

3.2. Primary Endpoint

The primary endpoint for this pilot study is the duration of survival, measured as the time needed for the CoNS CFU to drop below baseline density measured before application of ShA9 + 100 CFU/cm^2 on the lesional ventral arm skin of AD participants positive for *S. aureus* (AD SA+).

3.3. Secondary Endpoints

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

3.4. Exploratory Endpoints

1. Association of *S. hominis* A9 bacteria abundance estimated CFU as measured by % *S. hominis* A9 positive colonies by PCR x CoNS CFU while CoNS CFU is above baseline density measured before application of ShA9 + 100 CFU/cm^2 on the lesional and non-lesional, separately, ventral arm skin of AD participants between the arm treated with *S. hominis* A9 and the contralateral arm treated with placebo, and independently, with the participant's face

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

3.5. Stratification, Randomization, and Blinding/Masking

Arm dominance may affect the spread of ShA9 to the contralateral arm; therefore, the design will stratify by arm dominance, with the dominant arm being the right arm for right-handed participants, and the left arm for left-handed participants. A minimum of 13 participants with *S. aureus* colonized lesions on their ventral arms and a minimum of 7 participants with *S. aureus* negative lesions on their ventral arms will be randomized to one of two treatment groups: 1) ShA9 dominant and placebo non-dominant or 2) ShA9 non-dominant and placebo dominant. It is anticipated that enrollment will be higher for right-handed participants as that is the more dominant arm in the general population; enrollment will not target equal numbers of right-handed and left-handed participants. Randomization will be performed centrally at the Statistical and Clinical Coordinating Center (SACCC) using a stratified randomization design.

This is an open-label study, as all participants will receive active and placebo treatment. However, investigators, all investigational site staff, including those responsible for preparing/administering the investigational product, and all participants in this study will be blinded as to which arm received which treatment. Laboratory staff performing the mechanistic assays will be unblinded to which arm received which treatment, to ensure the correct dilutions can be applied to each sample prior to completing assays for quantification of live bacteria by culture (*S. aureus* and CoNS) and bacterial DNA (*S. hominis* A9) by qPCR (Refer to Section 9).

3.5.1. Procedure for Unblinding/Unmasking

If a clinically significant event occurs and knowledge of the treatment assignment for a given arm is required, the study treatment may be unblinded. Unblinding must be approved by the study Medical Monitor unless an immediate life-threatening condition has developed and the Medical Monitor is not accessible. The site investigator will notify the Medical Monitor of the unblinding event within 1 business day, following the emergency unblinding. The emergency unblinding will also be reported to the Data and Safety Monitoring Board (DSMB).

A full account of the event will be recorded, including the date and time of the unblinding, the reason for the decision to unblind, 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 will also occur for Investigational New Drug Safety Reports that will be reported to the Food and Drug Administration (FDA), DSMB, and Institutional Review Board (IRB) as specified in the current FDA IND Safety Reporting Guidance.

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

4. Selection of Participants and Clinical Sites/Laboratories

4.1. Rationale for Study Population

In 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 (Paller, et al., 2019). Previous results have further suggested that *S. hominis* A9 produces bacterocins that kill *S. aureus* (Nakatsuji, et al., 2017). These characteristics, dysbiosis, and immunological disorder make AD patients most suitable for the transplant of beneficial skin microbiome species, such *as S. hominis* A9, to help regulate the imbalance. In this study, we will examine the survival of *S. hominis* A9 transplanted bacteria on lesional and non-lesional skin of AD subjects with and without *S. aureus* colonization. The inclusion of AD subjects without *S. aureus* colonization will permit assessment of the potential of *S. aureus* to produce factors that limit the survival of *S. hominis* A9. Each participant will receive *S. hominis* A9 on one arm and placebo on the contralateral arm which will serve as a control. This study will be limited to adult AD patients, ages 18-80 years.

4.2. Inclusion Criteria

Individuals who meet all of the following criteria are eligible for enrollment as study participants:

1. Participant must be able to understand and provide informed consent.
2. Male or female participants 18 to 80 years of age, inclusive at time of the Screening Visit.
3. Meet ADRN Standard Diagnostic Criteria (Appendix A) for active AD.
4. At least 21 cm^2 of lesional and 21 cm^2 of non-lesional skin on both the right and left ventral arms. The required area (lesional or non-lesional) may be one contiguous area or may encompass multiple areas with a total cumulative area of 21 cm^2.
5. An Investigator Global Assessment (IGA) score, on the ventral arms of at least moderate severity.
6. Body surface area (BSA), as measured by Mostellar BSA Calculator, between 1.26 m^2 and 2.25 m^2.
7. If female of child bearing potential, must agree to remain abstinent (refrain from heterosexual intercourse) or use acceptable contraception (e.g. oral contraceptives, intrauterine device [IUD], barrier method with spermicide, surgically sterilized partner, Depo-Provera, Norplant, NuvaRing, or hormonal implants) for the duration of study participation.

4.3. Exclusion Criteria

Individuals who meet any of these criteria are not eligible for enrollment as study participants:

1. Inability or unwillingness of a participant to give written informed consent or comply with study protocol
2. Pregnant or lactating females.
3. Active bacterial, viral, or fungal skin infections.
4. Any noticeable breaks or cracks in the skin on the target areas of investigational product application, including severely excoriated skin or skin with open or weeping wounds suggestive of an active infection or increased susceptibility to infection.
5. Sensitivity to or difficulty tolerating Dove fragrance-free bar soap, Cetaphil® lotion, alcohol-based cleaners, glycerol, or soy products.
6. Participants with Netherton's syndrome or other genodermatoses that result in a defective epidermal barrier
7. Any participant who is immunocompromised (e.g. history of lymphoma, Human Immunodeficiency Virus [HIV]/Acquired Immunodeficiency Syndrome [AIDS], Wiskott-Aldrich Syndrome), has an immune system disorder (e.g. autoimmune disease), or is using a systemic immunosuppressant (e.g. systemic corticosteroids, cyclosporine, methotrexate).
8. Any participant with current malignant disease (with the exception of non-melanoma skin cancer in an area not affected by treatment).
9. Participants with a history of psychiatric disease or history of alcohol or drug abuse that would interfere with the ability to comply with the study protocol.
10. Past or current medical problems or findings from physical examination or laboratory testing that are not listed above, which, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the participant's ability to comply with study requirements or that may impact the quality or interpretation of the data obtained from the study.
11. Ongoing participation in another investigational trial or use of investigational drugs within 8 weeks, or 5 halflives (if known), whichever is longer, of the Screening Visit.
12. Treatment with non-steroid systemic immunosuppressant within 6 months of the Screening Visit.
13. Treatment with Dupilumab within 16 weeks of the Screening Visit.
14. Treatment with oral or injectable therapy for AD (excluding oral steroids) within 5 half-lives (if known) or 16 weeks before the Screening Visit, whichever is longer.
15. Participants with close contacts (e.g. spouse, children, or members in the same household) that have severe barrier defects or are immunocompromised.
16. Use of topical (including steroids and calcineurin inhibitors) AD treatments on the ventral arms or face within 7 days of the Treatment Visit; Use of topical steroids on areas outside of where investigational product is to be applied or swabbing is to be performed may be permitted, per investigator discretion.
17. Treatment with prescription moisturizers classified as medical device (e.g., Atopiclair®, MimyX®, Epiceram®, etc.) on the ventral arms or face within 7 days of the Treatment Visit; Use on areas outside of where investigational product is to be applied or swabbing is to be performed is permitted.
18. Use of any oral or topical antibiotic within 7 days of the Treatment Visit.
19. Participants who have taken a bleach bath within 7 days of the Treatment Visit.
20. Use of any oral steroid therapies within 28 days of the Treatment Visit.
21. Any phototherapy for skin disease (such as narrow band ultraviolet B [NBUVB], ultraviolet B [UVB], ultraviolet A1 [UVA1], psoralen + UVA [PUVA]) or regular use (more than 2 visits per week) of a tanning bed within 28 days of the Treatment Visit.

4.4. Selection of Clinical Sites/Labs

This study is being conducted at the University of California, San Diego (UCSD), which was a clinical site for the previous Phase I Targeted Microbiome Transplant in Atopic Dermatitis study, ADRN-08, conducted under this IND

(ClinicalTrials.gov, NCT03151148). In addition, UCSD is the manufacturer of the *S. hominis A9* being used for this study and will perform assays for quantification of live bacteria by culture (*S. aureus* and CoNS) and bacterial DNA (*S. hominis* A9) by qPCR (Refer to Section 9). UCSD has optimized these assays and has successfully deployed them for previous studies.

5. Known and Potential Risks and Benefits to Participants

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

There is a theoretical risk of skin infection associated with the application of *S. hominis* A9, although the likelihood of participants developing a skin infection is low given the applied product will only include nonpathogenic bacteria that have AMP-producing abilities. Furthermore, we will be applying these bacteria topically and will examine all participants prior to application to ensure that they do not have any cracks or excoriated skin on their arms. Theoretical risk of contact dermatitis to glycerol is also possible, both with the *S. hominis* A9 and the placebo. We will avoid application of the product to the hand since the hand would be a likely source of spreading these bacteria onto other surfaces that other people may contact. Participants will be excluded from participating if they have close contacts (e.g. spouse, children, or members in the same household) that have severe skin barrier defects or are immunocompromised, per study exclusion criteria.

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

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

Not applicable

5.3. Risks of Other Protocol Specified Medications

Theoretical risk of contact dermatitis to Cetaphil is possible and will be treated with mid-potency topical steroids, such as fluocinonide ointment 0.05%. Refer to Section 7.4 for more information on rescue medications.

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

5.4. Risks of Study Procedures

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

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

5.4.2. Risks Associated with Skin Swab Collection

There are no significant risks associated with skin swab collection.

5.4.3. Risks Associated with Physical Exam

There are no known risks associated with the physical exam.

5.4.4. Risks associated with Questionnaires

There is a possibility that participants may find questions too personal. Participants may refuse to answer any questions that make them feel uncomfortable. There is also a possibility that a participant's answers may be read by others; however, participants' records are carefully protected so this is very unlikely. See Section 16.4 for more information on confidentiality.

5.4.5. Risks associated with Blood Collection

There is no scheduled blood draw, but blood may be collected during an Unscheduled Visit per investigator discretion. Risks associated with drawing blood include possible pain when the needle is inserted, as well as bleeding, bruising and/or infection at the puncture site. Some people may experience lightheadedness, nausea, or fainting. There is a potential for slight psychological stress from the procedure. If psychological stress is too much in the opinion of the participant or the physician/nurse, the procedures will be halted. NIH guidelines for blood collection (amount and frequency based on age) will be followed.

5.5. Risks Associated with Electronic Data Systems

It is possible that computer/data management systems could be maliciously attacked and personal information as well as other study documents compromised. There is also the potential of accidental loss of privacy during the consenting and enrollment process. To minimize or prevent this from happening, standard procedures such as using coded forms, locked files, and encryption at rest and in transit for all data will be employed. Investigators and the appropriate study team members will complete training courses in secure data management/privacy. Finally, the Rho SACCC data systems are fully compliant with Federal regulations and have up-to-date virus and malware protection.

5.6. Potential Benefits

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

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

6. Investigational Agent

6.1. Investigational Agent

6.1.1. *S. hominis* A9 Product

6.1.1.1. Formulation, Packaging, and Labeling

The *S. hominis* A9 product will be manufactured and packaged at UCSD. The UCSD Manufacturing Lab has isolated *Staphylococcus hominis* strain A9 from the skin surface of a healthy donor. This *S. hominis* A9 strain produces potent antimicrobial activity against *S. aureus*. UCSD has characterized antimicrobial peptides produced by this strain with protein purification, protein sequencing, and genomic sequencing approaches. The UCSD Manufacturing Lab has established the Master Cell Bank (MCB) of the *S. hominis*

A9 strain, and it is stored in a locked -80°C freezer at UCSD. The Working Cell Bank (WCB) has been prepared from a single colony of the MCB and stored in a locked -80°C freezer at UCSD.

Each batch of the S*. hominis* A9 product will be prepared from a single-use vial from the WCB. A vial of the *S. hominis* A9 from the WCB will be thawed and cultured in United States Pharmacopeia (USP) grade animal-free tryptic soy broth (TSB) media at 37°C to expand the number of *S. hominis* A9. Bacterial concentration will be assessed, after culturing, by OD_{600}. One unit of OD_{600} is comparable to $5x10^7$ CFU/ml. The cultured bacteria will then be washed twice with 100 mL of USP grade normal saline to remove the animal-free TSB. Based on pre-clinical data, proteins are undetectable in the supernatant after the second wash. Bacterial density of the resuspension will be measured by OD_{600}.

Washed bacteria will be mixed under sterile conditions in 85% phosphate-buffered saline solution (PBS) containing 15% glycerol to achieve a density similar to the concentration of microbes on normal skin of 1×10^5 CFU/cm^2. Each batch of formulated product will be assigned a unique lot number and will undergo a visual test for appearance, potency testing by bacterial concentration testing and radial diffusion testing, and identity testing by PCR for the hogocidin gene. Aliquots of final *S. hominis* A9 product will be transferred into a sterile dispenser with a spray pump (10 mL/dispenser) and sealed in a sterile laminar-flow hood. The dispensers will be grouped in boxes and labeled with the lot number and box ID. Packaged boxes will be stored in a -80°C freezer at UCSD until transfer to the clinical site. After packaging, 2 dispensers from each lot will be randomly selected, and potential pathogens will be screened for Microbial Limits Testing in accordance with USP <61> and <62> testing, visual appearance testing, potency testing by bacterial concentration testing and radial diffusion testing, and identity testing by PCR for the hogocidin gene. The final *S. hominis* A9 product will be formulated at a *S. hominis* A9 concentration range from 1×10^8 CFU of bacteria/mL to 4×10^9 CFU of bacteria/mL in PBS-Glycerol.

Potency testing will be performed by both the radial diffusion assay and bacterial concentration testing. Potency of *S. hominis* A9 product is acceptable if 4 out of 5 *S. hominis* A9 colonies grown from the product show *S. aureus* inhibition zones greater than 1 mm in diameter. Bacterial concentration of the product will be checked by plating a known quantity of the *S. hominis* A9 product on an agar plate and incubating the plate overnight at 37°C. The number of viable colonies will be counted the following day. All colonies will be checked to ensure they show the same morphology. Only *S. hominis* A9 that has a final concentration of bacteria that ranges from 1×10^8 CFU of bacteria/mL to 4×10^9 CFU of bacteria/mL in PBS-Glycerol will be used in the clinical trial.

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

6.1.1.2. Dosage, Preparation, and Administration

6.1.1.2.1. Dosage - Targeted Density of Transplanted CoNS

There is considerable variability in the abundance of CoNS bacteria on human skin. A recent unpublished study by the UCSD laboratory has found CoNS bacteria with antimicrobial activity can be detected at a range of abundance between 1×10^2 CFU/cm^2 and 1×10^7 CFU/cm^2. Therefore, the application of CoNS to a final density between 1×10^2 CFU/cm^2 and 1×10^7 CFU/cm^2 is the target goal that will be within the density typically observed on healthy human skin and can be considered safe. This target density for topically applied CoNS bacteria has also been found safe and effective at

inhibiting *S. aureus* in prior studies of both autologous microbiome transplant (Nakatsuji, et al.,2017) and after topical application of *S. hominis* A9 (Nakatsuji, et al., 2021).

Using the body surface area calculations of Mosteller (Mosteller, 1987), we estimate that the smallest potential participant of 4'10" and 85 pounds has a BSA of 1.26 m^2. The largest participant of 6'3" and 210 pounds has a BSA of 2.25 m^2. Using the Rule of Nines, each arm is estimated as 9% of the total BSA. Therefore, the ventral surface would be 4.5% of the total BSA. Since one hand is 1% of BSA, the ventral surface of one arm not including the hand would be 3.5% of BSA. Thus, the smallest participant has an area on their ventral arm of 441 cm^2, and the largest participant 787 cm^2**.**

The investigational product will be formulated at a final concentration of bacteria that ranges from 1 x 10^8 CFU of bacteria/mL to 4 x 10^9 CFU of bacteria/mL, and a total volume of approximately 1 mL of product will be applied to each participant's arm once. To dispense approximately 1 mL, 6 pumps will be applied per dose (160 µL/pump). The acceptable dosage range will be between 2 x 10^3 CFU/cm^2 and 1 x 10^7 CFU/cm^2 to account for variability in participant surface area, bacteria survival, and loss of product during application. The calculated concentration for the arms will be 1.27 x 10^5 CFU/cm^2 and 9.07 x 10^6 CFU/cm^2 for BSAs of 1.26 m^2 and 2.25 m^2, respectively. This range is within the predicted effective and safe concentration of 10^2 CFU/cm^2 to 10^7 CFU/cm^2.

6.1.1.2.2. Preparation

Boxed *S. hominis* A9 single-dose dispensers will be stored at -80°C until dispensation to the clinical staff. *S. hominis* A9 will be thawed to 4°C prior to administration in clinic at the Treatment Visit (Day 0). Further details regarding thawing and administration will be included in the Pharmacy Manual.

6.1.1.2.3. Administration

Participants will have a single dose of *S. hominis* A9 applied to one of their ventral arm (wrist to upper arm) by clinic staff. The clinical staff member administering the product will record the associated dispenser ID and applicable arm.

6.1.2. Placebo

6.1.2.1. Formulation, Packaging, and Labeling

Formulation of matching placebo will be identical to *S. hominis* A9, but without the bacteria and will be supplied in an identical sterile dispenser with spray head. Each dispenser will contain 10 mL of placebo containing 85% USP grade PBS containing 15% USP grade glycerol. Prior to shipping, each batch of placebo will undergo quality control testing, and additionally two aliquots from each lot will be randomly selected for release testing after packaging into sterile dispensers. One aliquot will undergo Microbial Limits Testing in accordance with USP <61> and <62> testing, and the other will undergo visual testing. If the product is not free from contaminants in accordance with USP <61> and <62> testing, the entire lot will be discarded appropriately, and will not be provided to the clinical site.

6.1.2.2. Dosage, Preparation, and Administration

6.1.2.2.1. Dosage

Each single-use dispenser will be used to dispense approximately 1 mL of placebo containing 85% PBS containing 15% glycerol. To dispense approximately 1 mL, 6 pumps will be applied per dose (160 µL/pump).

6.1.2.2.2. Preparation

Boxed dispensers will be stored at -80°C until dispensation to the clinical staff. Placebo will be thawed to 4°C prior to administration in clinic at the Treatment Visit (Day 0). Further details regarding thawing and administration will be included in the Pharmacy Manual.

6.1.2.2.3. Administration

Participants will have a single dose of placebo applied to their contralateral ventral arm (wrist to upper arm) by clinic staff. The clinical staff member administering the product will record the associated dispenser ID and applicable arm.

6.2. Investigational Product 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 product, including the date and quantity of the product received, to whom the product was dispensed (participant-by-participant accounting), and a detailed accounting of any product accidentally or deliberately destroyed. Details of investigational product dispensation to the clinical site will be maintained by the manufacturing lab.

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

All records regarding the disposition of the investigational product will be available for inspection. At the termination of the study, all dispensed, unused product will be destroyed. For more information on handling of the investigational product, please refer to the Pharmacy Manual.

6.3. Assessment of Participant Compliance with Investigational Agent

Investigational product will be administered by a blinded study staff member and thus is an observed compliance. Participant compliance will be determined by the number and percentage of participants who receive a dose of ShA9 on their right or left arm and placebo on their contralateral arm at the Treatment Visit (Day 0). Any deviations from the dosing schedule outside the defined visit windows will be recorded on the appropriate electronic case report form (eCRF).

6.4. Toxicity Prevention and Management

All steps will be taken to minimize potential risks of the study. Participants with known sensitivities/allergies to any of the products used during this study will be excluded from study. Participants 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. Any AE, defined as any undesirable sign, symptom, or medical condition occurring after the participant's written consent to participate is completed, will be recorded and reported to the Principal Investigator (PI), Division of Allergy, Immunology, and Transplantation (DAIT)/NIAID, FDA, and the IRB as required. If the PI believes an AE is possibly related to the study product, the PI will determine whether or not it is in the best interest of the subject to continue in

the study. If the participant is injured as a result of participation in this research, treatment will be available at the clinical center. Further details of the services, including costs, and coverage will be explained in the informed consent, signed by the participant. In the case of a skin infection, the participant would be treated according to standard treatment practices. The treatment will include antibiotics (for infection) and Cetaphil® lotion (for irritation and dryness). Refer to Section 7.4 for more information on rescue medications.

6.5. Premature Discontinuation of Investigational Agent

Study therapy may be prematurely discontinued, not applied fully to both the right and left ventral arms, for any participant who shows evidence of contact allergy to investigational product.

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

If a subject is prematurely discontinued from study therapy, the subject will be asked to complete any remaining study visits, per protocol, through end of study (Day 31).

7. Other Medications

7.1. Concomitant Medications

7.1.1 Protocol-mandated

Participants will be required to apply Cetaphil® lotion to their ventral arms twice daily for approximately seven days (Pre-treatment Visit until Treatment Visit).

7.1.2 Other permitted concomitant medications

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

Use of antihistamines will not be exclusionary for protocol enrollment. However, participants using antihistamines at the time of the Screening Visit will be asked to maintain a stable dose until completion of their last in-clinic visit.

7.2. Prophylactic Medications

Female participants of child-bearing potential must use an effective method of contraception (e.g. total abstinence, oral contraceptive, IUD, barrier method with spermicide, surgically sterilized partner, Depo-Provera, Norplant, NuvaRing, or hormonal implant) for the duration of study participation.

Pregnancy tests will be performed on female participants of child-bearing potential who do not self-report as pregnant at the Screening and Treatment (Day 0) Visits. At all other study visits, female participants of child-bearing potential will be asked if they are pregnant.

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

7.3. Prohibited Medications/Therapies

Participants are prohibited from using the following medications/therapies:

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

After the date of their CoNS falling below baseline density measured before application of ShA9 + 100 CFU/cm^2, the participant will be allowed to use prohibited medications/therapies, as needed. Use of medications/therapies will be recorded on the concomitant medications case report form (CRF).

7.4. Rescue Medications

If infection occurs, treatment will include topical and/or oral antibiotics depending on the severity of the infection clinically, and Cetaphil® lotion (for irritation and dryness). The antibiotic sensitivity profile of *Staphylococcus hominis* A9, includes sensitivity to ampicillin-sulbactam, cefazolin, cefoxitin, clindamycin, daptomycin, levofloxacin, linezolid, minocycline, moxifloxacin, mupirocin, nitrofurantoin, oxacillin, rifampin, trimethoprim-sulfamethoxazole, and vancomycin; intermediate sensitivity to doxycycline; and resistance to erythromycin, penicillin G and tetracycline. The antibiotic of choice will depend on the individual participant's medical history, including any possible contraindications to certain medications due to allergies or other concomitant medication use, but mupirocin will be the first line topical antibiotic of choice, and minocycline will be the first line oral antibiotic, followed by first generation cephalosporins, trimethoprim-sulfamethoxazole or rifampin. Contact allergic reactions to Cetaphil lotion or glycerol will be treated with mid-potency topical steroids, such as fluocinonide ointment 0.05%.

8. Study Procedures

A summary of complete study procedures is included in Appendix B Schedule of Events. To ensure participant safety, participants will be asked to comply with UCSD's current institutional policies and procedures related to COVID-19, which are based on recommendations from the Centers for Disease Control and Prevention when scheduling and attending their appointments.

8.1. Recruitment Visit

Potential participants will be recruited using standardized questionnaires that collect contact information and disease status related to inclusion and exclusion criteria. Participants may be recruited by phone or in person. Potential participants from UCSD departments outside of Dermatology will be identified using EPIC slicer dicer tool, then confirmed with their primary care provider to see if their patients can be approached for recruitment. Once confirmed by their primary care provider, patients will be contacted through a message on MyChart to assess their interest in participating. Those interested will then be followed up by a phone call to assess their eligibility. Those who have no obvious characteristics making them ineligible for the study and who are interested in participating will be invited to clinic to complete the Screening Visit.

8.2. Screening Visit (Day -14 to -7)

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

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

- Collection of demographics
- Medical History and physical examination, including assessment of AD severity (IGA, L-EASI on the ventral arms, SCORAD, and local pruritus NRS) by a study physician or other qualified medical professional
- Pregnancy status and urine pregnancy test: Female participants will be asked if they are pregnant. A urine pregnancy test will be completed for all female participants of child bearing potential who do not self-report as pregnant.
- Assessment of concomitant medications
- Vital signs and growth parameters (height and weight)
- Assessment of Adverse Events (AEs)

Participants who meet inclusion and exclusion criteria including the required washout periods for medications/therapies will be considered screened and eligible. Participants who do not meet inclusion and exclusion criteria due to assessment of their concomitant medications and require a washout of 14 days or more will be required to rescreen, prior to enrolling in the study. Participants who do not wish to washout of the prohibited medications or do not meet full inclusion and exclusion criteria will be identified as screen failures and will not continue in the study.

8.3. Pre-treatment Visit (Day -7 ± 1 Day)

Screened and eligible participants will be invited to complete the Pre-treatment Visit. If a participant meets all required washout periods for medications/therapies during their Screening Visit, they may complete the Pre-treatment Visit on the same day of the Screening Visit. The Pre-treatment Visit must be completed 7 days (± 1 Day) prior to the Day 0 Visit. During this visit, participants will have their initial skin swabs collected for the assessment of live bacteria by culture (*S. aureus* and CoNS) and bacterial DNA (*S. hominis* A9) by qPCR.

The following procedures, assessments, and laboratory measures will be conducted at this Pre-treatment Visit:

- Interim medical history and physical exam, including assessment of AD severity (L-EASI, SCORAD, and local Pruritus NRS) by study physician or other qualified medical professional (if conducted on a different day than the Screening Visit)
- Pregnancy status of participant and partner of participant, as applicable (if conducted on a different day than the Screening Visit)
- Assessment of concomitant medications (if conducted on a different day than the Screening Visit)
- Vital signs (if conducted on a different day than the Screening Visit)
- Identification of measured lesional and non-lesional sites on the participant's ventral arms, including photographs, and skin swab collection, to assess live bacteria by CFU and bacterial DNA by qPCR. Two lesional and two non-lesional swabs will be collected from each arm (8 swabs total).
- Assessment of AEs

During the Pre-treatment Visit, participants will be given Cetaphil® lotion and Dove moisturizing soap. Participants will be instructed to apply a thin layer of Cetaphil® lotion to each arm, twice a day for approximately 1 week (Pre-treatment

Visit until Treatment Visit) and to use the soap when they shower, avoiding the arms. At the conclusion of the visit, participants will be given instructions regarding restrictions on bathing/showering, exercise, and use of emollient. Participants will be instructed to refrain from swimming in chlorinated pools and hot tubs and to refrain from the use of prohibited medications, for the duration of their study participation. Female subjects of child-bearing potential will be instructed to use an effective method of birth control or abstinence.

8.4. Treatment Visit (Day 0)

Prior to the Treatment Visit, the clinical team will contact study participants to confirm if their pre-treatment swab results meet criteria for enrolling in the AD SA+ or AD SA- group. If the enrollment goal(s) for AD SA+ or AD SA- have been met, the participant will be identified as a screen failure.

The purpose of the Day 0 Treatment Visit is to complete study treatment, application of ShA9 and placebo. The following procedures, assessments, and laboratory measures will be conducted at the Treatment Visit:

- Interim medical history and physical exam, including assessment of AD severity (L-EASI, SCORAD, and local Pruritus NRS) by study physician or other qualified medical professional
- Pregnancy status of participant and partner of participant, as applicable, and urine pregnancy test (female participants only), per Section 8.2
- Assessment of concomitant medications
- Vital Signs
- Randomization
- Identification of measured non-lesional site on the participant's face, including photograph, and skin swab collection for the assessment of ShA9 spread. Two non-lesional swabs will be collected from the participant's face.
- Skin Swab Collections from the ventral arms, to assess live bacteria by CFU, bacterial DNA by qPCR, and antibiotic sensitivity to penicillin G, tetracycline, and erythromycin. Two lesional and two non-lesional swabs will be collected from each ventral arm (8 swabs total).
- *S. hominis* A9 and placebo application
- Participants will remain in clinic for up to 6 hours following the application of investigational product. Additional skin swabs will be collected from the participant's arms at 15 minutes, 1, 2, 4, and 6 hours post-application. Two lesional and two non-lesional swabs will be collected from each ventral arm for a total of eight swabs at each time point.
- Assessment of AEs

At the conclusion of this visit, participants will be reminded to use Dove moisturizing soap, whenever they shower, but to avoid the arms, until after their last in-clinic visit is complete. Participants will be allowed to use Cetaphil® lotion to moisturize if necessary. Participants will be given instructions regarding restrictions on bathing/showering, exercise, and use of emollient. Participants will be reminded to refrain from swimming in chlorinated pools and hot tubs and to refrain from the use of prohibited medications, for the duration of their study participation. Participants will be asked to try to restrict pets from licking the treated areas. Female subjects of child-bearing potential will be instructed to use an effective method of birth control or abstinence.

Participants will be given a handcard describing the symptoms of skin and systemic infections and will be told to contact the research clinic if they experience any of the listed symptoms. The participants will be instructed to take the handcard with them should they seek medical care at another facility. The handcard will state that the patient is

currently in a study involving the application of non-pathogenic bacteria to the skin and will instruct them to call the research site if an infection of the skin or blood is suspected.

8.5. Follow-Up Visits (24 Hour and Days 3, 10, 17 and 24)

After the Treatment Visit, participants will be asked to return to the clinic for a follow up 24 Hours after the Day 0 Visit and on Days 3 (± 1 day), 10 (± 1 day), 17 (± 2 days), and 24 (± 2 days). The purpose of the follow-up visits is to assess *S. aureus* and CoNS bacteria abundance and AEs.

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

- Assessment of AEs
- Assessment of AD severity (L-EASI, SCORAD, and local Pruritus NRS) by study physician or other qualified medical professional
- Pregnancy status of participant and partner of participant, as applicable
- Assessment of concomitant medications
- Vital Signs
- Skin Swab Collections, (lesional and non-lesional) from the ventral arms, to assess live bacteria by CFU and bacterial DNA by qPCR and non-lesional skin swab collection from the participant's face for the assessment of ShA9 spread. Two lesional and two non-lesional swabs will be collected from each ventral arm, and two additional non-lesional swabs will be collected from the face (10 swabs total).
- Lesional skin swabs taken at the last Follow-Up Visit will also be analyzed for antibiotic sensitivity to penicillin G, tetracycline, and erythromycin.

At the conclusion of each visit, participants will be reminded to use Dove moisturizing soap, whenever they shower, but to avoid the arms, until after their last in-clinic visit is complete. Participants will be given instructions regarding restrictions on bathing/showering, exercise, and use of emollient. Participants will be reminded to refrain from swimming in chlorinated pools and to refrain from the use of prohibited medications. Female participants of childbearing potential will be instructed to use an effective method of birth control or abstinence.

Upon completion of the Day 3 Visit, a participant will be notified prior to their next scheduled follow-up visit if they need to return to the clinic. A participant will not be required to complete the remaining follow up clinic visits (Day 10 through Day 24) if the lesional swabs from both arms collected at their most recent prior visit are CoNS negative, defined as below baseline density measured before application of ShA9 + 100 CFU/cm^2. The lesional skin swabs taken at the last completed Follow-Up Visit will be used in analysis for antibiotic sensitivity to penicillin G, tetracycline, and erythromycin. Once a participant completes his/her required follow up clinic visits, he/she may resume prohibited medications as described in Section 7.3, as needed, and may use his/her choice of soap and emollient.

8.6. End of Study Phone Visit (Day 31 ± 7 Days)

The purpose of the Day 31 End of Study Phone Visit is to complete participant follow-up. This visit will be brief, and participants will be asked to report any new AEs and to answer questions regarding the status of their AD and concomitant medication use. Female participants of child-bearing potential will be asked whether they have tested positive to a pregnancy test since their last visit. Participants will be asked whether their partner has tested positive to a pregnancy test since their last visit, as applicable.

Any participant with an ongoing AE/Serious Adverse Event (SAE) at the time of this visit will continue to be followed until the event is resolved with or without sequelae or the AE/SAE stabilizes.

8.7. Unscheduled Visits

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

The following procedures, assessments, and laboratory measures will be conducted at the Unscheduled Visit:

- Assessment of AEs
- Physical examination, including assessment of AD severity (L-EASI, SCORAD, and local Pruritus NRS) by study physician or other qualified medical professional
- Pregnancy status of participant and partner of participant, as applicable
- Vital Signs
- Skin Swab collection, per investigator discretion
- Blood collection, per investigator discretion

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

Participants will be given a handcard to present to clinical staff should they seek care for an infection at another facility. The handcard, as described in Section 8.4, will state that the participant is currently in a clinical trial involving the application of non-pathogenic bacteria to the skin, list the sensitivities of *S. hominis,* and include instructions to call the research site if an infection of the skin or blood is suspected.

8.8. Visit Windows

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

9. Mechanistic Assays

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

9.1. Skin Swab Collection Procedure

Lesional and non-lesional swabbing areas (21 cm^2 total for each; 7 areas of 3 cm x 1 cm of lesional and non-lesional skin each) will be defined on each ventral arm at the Pre-treatment Visit. The lesional swab locations will be selected from the most severe lesional areas and may not be contiguous. Lesions closer to the antecubital region will take precedence in defining these areas. The non-lesional swab locations will be defined as areas of normal skin on the ventral arms and also may not be contiguous. In addition, a non-lesional swabbing area of 3 cm x 1 cm will be defined on the face at the Treatment Visit. The designated lesional and non-lesional sites for swabbing will be marked with a pen and nonidentifying digital photographs will be taken for reference during future visits. The number and location of swabs to be collected at each visit and time point are delineated in Section 8 and the Schedule of Events. Each swab will cover a 3 cm^2 area. 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. Refer to the MOP for additional details regarding the collection of skin swabs.

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

Skin swabs will be collected from pre-measured areas of lesional and non-lesional skin per Section 9.1 using swabs premoistened with TSB. Collected swab tips for CFU quantification will be stored in 1 mL of 85% TSB/15% Glycerol and stored at -80°C until analysis. Live CoNS will be measured by counting CFU on mannitol salt agar with egg yolk, which is a selective media for staphylococcus species. CoNS and *S. aureus* can be distinguished by the colony morphology (CoNS: pink color without egg yolk reaction; *S. aureus*: yellow color with egg yolk reaction). Live *S. aureus* will be measured on Bear-Parker agar, which is a selective media for *S. aureus*. CFU quantification will give us quantification of live *S. aureus* and CoNS, as each will be grown in selective media and are important for our Primary Endpoint, Secondary Endpoint 1, and Exploratory Endpoints 2-5.

9.3. Quantification of *S. hominis* A9 CFU

To specifically identify colonies of *S. hominis* A9, 24-48 CoNS colonies will be picked from each culture plate and subjected to colony PCR using strain specific primers for the lantibiotic gene. CFUs of *S. hominis* A9 will be estimated from total CoNS CFU × % of *S. hominis* A9 positive colonies. Quantification of *S. hominis* A9 estimated CFU will allow us to quantify *S. hominis* A9 specifically as outlined in Exploratory Endpoint 1.

9.4. Quantification of Bacterial DNA

Skin swabs will be collected from pre-measured areas of lesional and non-lesional skin per Section 9.1, using swabs premoistened with Tris-EDTA buffer containing 0.1% TritonX-100 and 0.05% Tween-20 (w/v). Microbial DNA will be extracted with the PureLink Microbiome DNA Purification kit (Invitrogen). Abundance of DNA from total staphylococcus (G-staph), *S. aureus*, *S. hominis* and Sh-lantibiotic will be quantified by qPCR using genus-, species- and gene- specific primer sets, respectively. Quantification of bacterial DNA will allow for the assessment of specific shifts in the microbiome pre and post application of *S. hominis* A9, correlation between *S. hominis* A9 and Sh-lantibiotic, and correlation between *S. aureus* and Sh-lantibiotic, supporting the primary objective to assess the factors that overall influence survival.

9.5 Analysis of Antibiotic Sensitivity

Lesional skin swabs from the Baseline Visit and last Follow-Up Visit will be tested for antimicrobial sensitivity to erythromycin, tetracycline, and penicillin G. The same volume of bacterial swab samples from lesional skin at Baseline and last Follow-Up Visit time points will be spread on culture plates containing Mannitol-salt agar, a staphylococcus selective agar, without or with erythromycin (4 µg/mL), or tetracycline (8 µg/mL), or penicillin G (1 µg/mL). The concentration of each antibiotic was determined based on the minimal inhibitory concentration used to define ShA9 as resistant to these antibiotics. The proportion of CoNS with antibiotic sensitivity will be determined by comparing the CFU measured on duplicate agar plates without antibiotic to the CFU measured on agar plates with each antibiotic by the equation [(CFU without antibiotic containing plate - CFU with antibiotic containing plate) / CFU without antibiotic containing plate]. The proportion of antibiotic-sensitive CoNS detected at baseline will be compared to the proportion from swabs collected at the last Follow-Up Visit. If an increase in the proportion of antibiotic resistant CoNS is measured to be greater than 10%, resistant colonies will be evaluated to determine if they are ShA9. If these colonies are not ShA9, DNA sequencing will be performed to determine if they contain antibiotic resistance genes that were originally present in ShA9, thus indicating the likelihood for horizontal gene transfer.

10. Biospecimen Storage

Lesional and non-lesional skin swabs will be obtained from all study participants. These skin swabs and any derivative samples will be obtained for potential future analysis of additional parameters that describe the composition and function of the skin microbiome. Participants will be asked to give permission for long-term storage and future use during the consent process. All samples stored for future use will be kept in a central repository at UCSD.

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

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

11.1. Participant Completion

Participant participation will be defined as complete at the conclusion of the Day 31 End of Study Phone Visit.

11.2. Participant Stopping Rules and Withdrawal Criteria

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

1. The participant elects to withdraw consent from all future study activities, including follow-up.
2. The participant is "lost to follow-up" (i.e., no further follow-up is possible because attempts to reestablish contact with the participant have failed).
3. The participant dies.
4. The Investigator no longer believes participation is in the best interest of the participant.
5. Individual safety stopping rules
    a. Evidence of infection either cutaneously or systemically which in the opinion of the investigator makes the participant a poor candidate for receipt of the investigational product or requires treatment with a prohibited medication/therapy that would interfere with assessment of the study endpoints.
    b. Severe worsening of AD which in the opinion of the investigator makes the participant a poor candidate for receipt of the investigational product or requires treatment with a prohibited medication/therapy that would interfere with assessment of the study endpoints.
    c. Pregnancy in a female participant prior to the Treatment Visit (Refer to Section 12.6).

11.3. Participant Replacement

If any participants withdraw or are withdrawn prior to achieving CoNS elimination (CoNS CFU below baseline density measured before application of ShA9 + 100 CFU/cm^2), additional participants may be enrolled as necessary to obtain a modified ITT sample as defined in Section 13.4.1.

11.4. Follow-up after Early Study Withdrawal

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

11.5. Study Stopping Rules

Screening, enrollment, and application of treatment for this trial will be suspended pending immediate DSMB review for the following reasons:

- A single participant experiences any SAE for which there is a reasonable possibility that the investigational product caused the SAE
- The development of any severe (Grade 3) AE for which attribution is defined as related or possibly related

Follow up visits will continue to be conducted per protocol while under DSMB review. After review of the data, the DSMB will make recommendations regarding study conduct and/or continuation.

12. Safety Monitoring and Reporting

12.1 Overview

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

Information in this section complies with *International Conference on Harmonisation* (*ICH) Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, ICH Guideline E-6: Guideline for Good Clinical Practice (GCP)*, 21CFR Parts 312 and 320, and applies the standards set forth in the National Cancer Institute (NCI), Common Terminology Criteria for Adverse Events (CTCAE), Version 5.0: http://ctep.cancer.gov/reporting/ctc.html.

12.2 Definitions

12.2.1 Adverse Event (AE)

Any untoward or unfavorable medical occurrence associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research (modified from the definition of adverse events in 21 CFR 312.32(a), the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) E-6*(R2)* Guidelines for Good Clinical Practice and OHRP "Guidance on Reviewing and Reporting Unanticipated Problems Involving Risks to Subjects or Others and Adverse Events (1/15/07)" http://www.hhs.gov/ohrp/policy/advevntguid.html#Q2).

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

- **Study Treatment regimen:** An AE occurring from the time of consent and within one month of *S. hominis* A9 product administration
- **Study mandated procedures**:
  - **Blood Draw** (to only occur at Unscheduled Visit at the discretion of the study physician or other qualified medical professional)

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

  - Fainting / Vasovagal Events

- Bruising at the puncture site larger than 2 cm in diameter ○ Bleeding from the puncture site lasting more than 30 minutes ○ Swelling at puncture site larger than 2 cm

12.2.1.1 Suspected Adverse Reaction (SAR)

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

12.2.2 Unexpected Adverse Event or Unexpected Suspected Adverse Reaction

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the Investigator Brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the investigator brochure listed only cerebral vascular accidents. "Unexpected," as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation 21 CFR 312.32(a)).

12.2.3 Serious Adverse Event (SAE) or Serious Suspected Adverse Reaction

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or the Sponsor, it results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse (21 CFR 312.32(a)).

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

12.3 Grading and Attribution of Adverse Events

12.3.1 Grading Criteria

The study site will grade the severity of AEs experienced by the study participants according to the criteria set forth in the NCI-CTCAE Version 5.0. This document (referred to herein as the NCI-CTCAE manual) provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all AEs. The NCI-CTCAE has been reviewed by the study investigators and sponsor and has been deemed appropriate for the participant population to be studied in this protocol.

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

Grade 1 = mild AE

Grade 2 = moderate AE

Grade 3 = severe AE

Grade 4 = life-threatening AE or urgent intervention indicated

Grade 5 = death

Events grade 1 or higher will be recorded on the appropriate AE eCRF for this study.

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

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

12.3.2 Attribution Definitions

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

Table 12.3.2 Attribution of Adverse Events

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

12.4 Collection and Recording of Adverse Events

12.4.1 Collection Period

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

12.4.2 Collecting Adverse Events

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

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

12.4.3 Recording Adverse Events

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

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

12.5 Reporting of Serious Adverse Events and Adverse Events

12.5.1 Reporting of Serious Adverse Events to DAIT/NIAID

This section describes the responsibilities of the site investigator to report SAEs to the sponsor and the SACCC via the SAE eCRF. Timely reporting of AEs is required by 21 CFR 312.32 and ICH E6 guidelines.

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

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

- AE term
- Relationship to investigational product
- Relationship to stopping protocol prohibited medications/therapy
- Relationship to study procedure
- Reason why the event is serious

- Supplementary CRF pages that are current at the time of SAE reporting: medical history, concomitant medications, demographics, and investigational product administration

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

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

Rho Product Safety
2635 E. NC Hwy 54
Durham, NC 27713
Toll-free: 1-888-746-7231
SAE Fax Line: 1-888-746-3293
Email: rho_productsafety@rhoworld.com

12.5.2 Reporting to Health Authority

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

12.5.2.1 Annual Reporting

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

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

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

12.5.2.2 Expedited Safety Reporting

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

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

The sponsor shall report any suspected adverse reaction that is both serious and unexpected. The sponsor shall report an adverse event as a suspected adverse reaction only if there is

evidence to suggest a causal relationship between the investigational product and the adverse event, such as:

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

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

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

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

12.5.3 Reporting of Adverse Events to IRB/IEC

All investigators shall report AEs, including expedited reports, in a timely fashion to their local IRB in accordance with applicable regulations and guidelines. All Safety Reports to the FDA shall be distributed by DAIT/NIAID or designee to UCSD for site IRB submission.

12.6 Pregnancy Reporting

The investigator shall be informed of any pregnancy in a female study participant immediately upon becoming aware of the event. If a female participant becomes pregnant prior to the Treatment Visit, the participant will no longer be eligible to participate in the study and will be withdrawn as described in Section 11.2. If a pregnancy occurs following the Treatment Visit, the pregnant participant will continue to be followed per the Schedule of Events. The investigator shall counsel the participant and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the pregnant participant shall continue until the conclusion of the pregnancy.

The investigator shall report to the SACCC all pregnancies within 1 business day of becoming aware of the event using the Pregnancy eCRF. The SACCC will report all pregnancies to DAIT/NIAID using the procedures as outlined in the Safety Management Plan. All pregnancies identified during the study shall be followed to conclusion, and the outcome of each must be reported. The Pregnancy eCRF shall be updated and submitted to the SACCC

when details about the outcome are available. When possible, similar information shall be obtained for a pregnancy occurring in a partner of a study participant. Information on this pregnancy will be collected on the Pregnancy eCRF. In order for Sponsor or designee to collect any pregnancy surveillance information from the female partner, the female partner must sign an informed consent form for disclosure of this information.

Information requested about the delivery shall include:

- Gestational age at delivery
- Birth weight, length, and head circumference
- Gender
- Appearance, pulse, grimace, activity, and respiration (APGAR) score at 1 minute, 5 minutes, and 24 hours after birth, if available
- Any abnormalities.

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

12.7 Reporting of Other Safety Information

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

12.8 Review of Safety Information

12.8.1 Medical Monitor Review

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

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

12.8.2 DSMB Review

The SACCC will provide the NIAID Allergy-Asthma [Alpha] DSMB with a listing of all AEs and SAEs at least annually. Furthermore, the DSMB will be informed of expedited reports of SAEs.

12.8.2.1 Planned DSMB Reviews

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

12.8.2.2 *Ad hoc* DSMB Reviews

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

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

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

A temporary halt in screening, enrollment, and investigational product application will be implemented if an *ad hoc* DSMB safety review is required. New participants will not be consented for study participation during the enrollment and treatment halt. Participants who were screened or who completed the Pre-treatment Visit but who are not yet randomized will not be allowed to continue with the Day 0 Treatment Visit. All participants not randomized within 14 days of the Screening Visit must rescreen. Follow up visits will continue per the Schedule of Events.

13. Statistical Considerations and Analytical Plan

13.1. Overview

This is a pilot, open-label, single-center trial designed to assess the survival of transplanted *S. hominis* A9 on the skin of adults with moderate-to-severe atopic dermatitis on the ventral arms who are culture positive or negative for *S. aureus* colonization.

13.2. Endpoints

13.2.1 Primary Endpoint

The primary endpoint for this pilot study is the duration of survival, measured as the time needed for the CoNS CFU to drop below baseline density measured before application of ShA9 + 100 CFU/cm^2 on the lesional ventral arm skin of AD participants positive for *S. aureus* (AD SA+)

13.2.2 Secondary Endpoints

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

13.2.3 Exploratory Endpoints

1. Association of *S. hominis* A9 bacteria abundance estimated CFU as measured by % *S. hominis* A9 positive colonies by PCR x CoNS CFU while CoNS CFU is above baseline density measured before application of ShA9 + 100 CFU/cm^2 on the lesional and non-lesional, separately, ventral arm skin of AD participants between the arm treated with *S. hominis* A9 and the contralateral arm treated with placebo, and independently, with the participant's face
2. The duration of survival, measured as the time needed for CoNS CFU to drop below baseline density measured before application of ShA9 + 100 CFU/cm^2 on the lesional ventral arm skin of AD participants negative for *S. aureus* (AD SA-)
3. The duration of survival, measured as the time needed for CoNS CFU to drop below baseline density measured before application of ShA9 + 100 CFU/cm^2 on the non-lesional ventral arm skin of AD participants negative for *S. aureus* (AD SA-)
4. Comparison of time needed for CoNS CFU to drop below baseline density measured before application of ShA9 + 100 CFU/cm^2 on the lesional and non-lesional, separately, ventral arm skin between AD participants positive (AD SA+) vs negative (AD SA-) for *S. aureus*
5. Comparison of time needed for CoNS CFU to drop below baseline density measured before application of ShA9 + 100 CFU/cm^2 on lesional vs non-lesional skin of AD participants positive (AD SA+) and negative (AD SA-) for *S. aureus*, separately
6. Comparison of the L-EASI score of the ventral arms at Day 0 vs the last in-clinic visit among AD SA+, and independently, among AD SA-
7. Comparison of the SCORAD score at Day 0 vs the last in-clinic visit among AD SA+, and independently, among AD SA-
8. Comparison of the local Pruritus NRS of the ventral arms at Day 0 vs the last in-clinic visit among AD SA+, and independently, among AD SA-

9. Comparison of the proportion of antibiotic-sensitive CoNS to penicillin G, tetracycline, and erythromycin isolated from skin swabs at baseline and after ShA9 treatment, at the last swab collection from lesional skin

13.3. Measures to Minimize Bias

Bias will be minimized by stratifying by arm-dominance and then randomizing active-dominant, placebo-non-dominant vs placebo-dominant, active-non-dominant, where active is considered *S. hominis* A9.

13.4. Analysis Plan

13.4.1 Analysis Populations

Safety Sample and modified ITT sample will include all participants who are enrolled and receive any amount of *S. hominis* A9. For analysis, *S. aureus* CFU results from swabs obtained prior to treatment application during the Treatment Visit will be used to define SA+ vs SA- status of participants.

13.4.2 Primary Analysis of Primary Endpoint

The primary endpoint for this pilot study is the time needed for CoNS CFU to drop below baseline density measured before application of ShA9 + 100 CFU/cm^2 (time-to-CoNS elimination) in AD participants positive for *S. aureus* colonization. Time-to-CoNS elimination is defined as the time from Day 0 to the first occurrence of a value below baseline density measured before application of ShA9 + 100 CFU/cm^2. The time to event for participants who did not reach CoNS CFU below baseline density measured before application of ShA9 + 100 CFU/cm^2 during the study will be right-censored.

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

13.4.3 Supportive Analyses of the Primary Endpoint

A nonlinear time-response regression analysis will be fitted between CoNS CFU/cm^2 and time, using either the two or three-parameter log-logistic model. Each of the models will be fitted to the data, and their fit compared using the Akaike information criteria (AIC), where the model with the lowest AIC will be the primary model for the derivation of the ED50 (or T50, time at which half of the maximum CoNS CFU/cm^2 is reached) (Ritz, et al., 2015).

13.4.4 Analyses of Secondary Endpoints

Overall survival comparisons between culture-positive and culture-negative for *S. aureus* colonization will be performed using a two-sided log-rank test with a significance level of 10%. Additionally, if the log-rank test is significant, the hazard ratio (HR) along with the 95% CI will be reported from a Cox proportional hazards regression model (discrete/exact method).

The summary of all treatment-emergent adverse events and treatment-emergent serious adverse events will be provided by system organ class, high-level term, and preferred term in descending order of frequency, and by preferred term in descending order of frequency. All analyses for adverse events will be conducted for the safety population by *S. hominis* A9 and Placebo.

13.4.5 Analyses of Exploratory Endpoints

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

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

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

13.4.6 Descriptive Analyses

Patient baseline characteristics will be tabulated. Continuous measures will be reported using the mean, standard deviation, minimum, maximum, median, first and third quartiles, and sample size. Categorical baseline and demographic characteristics and study disposition will be reported as frequencies and proportions.

13.5. Interim Analyses

13.5.1 Interim Analysis of Primary Endpoint

The primary analysis, that is, the time needed for CoNS CFU to drop below baseline density measured before application of ShA9 + 100 CFU/cm^2 (time-to-CoNS elimination) in AD participants positive for *S. aureus* colonization, will be generated when complete data for 50% of participants becomes available. The purpose of this interim analysis is to inform the development of upcoming trials, especially with regards to their design and power/sample size considerations.

13.5.2 Interim Analysis of Safety Data

The DSMB will receive periodic safety reports on enrolled participants. However, no formal interim analysis of safety data will be conducted. The DSMB may request modifications to the protocol based on its review of the findings.

13.5.3 Futility Analysis

No formal interim analyses are planned for futility (Not Applicable).

13.6. Statistical Hypotheses

The main goal of this pilot study is the estimation of the time needed for the CoNS CFU to drop below baseline density measured before application of ShA9 + 100 CFU/cm^2 (time-to-CoNS elimination). There will be no formal statistical hypothesis test for the primary analysis of the primary endpoint.

13.7. Sample Size Considerations

The proposed sample size for this study is 20 participants with 13 *S. aureus* positive and 7 *S. aureus* negative. The sample size was derived from ADRN-08: Targeted Microbiome Transplant in Atopic Dermatitis where, using a parametric survival model with exponential distribution, we estimated a hazard rate (λ) of approximately 0.09. This estimate was derived from a follow-up time of 11 days (shorter study) with 35 participants where nine (25%) were right-censored at trial end. The goal for this trial is to estimate the hazard rate and its 95% confidence interval with precision for the width of the confidence interval as 0.20. The 13 participants (9 events) proposed for the primary analysis were derived using these previous data as a starting point but considering more conservative estimates with a hazard rate of 0.15 and 30% right censoring, which produces a two-sided 95% confidence interval width equal to 0.194.

14. Identification and Access to Source Data

14.1. Source Data

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

14.2. Access to Source Data

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

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

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

15.2. Reporting and Managing Protocol Deviations

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

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

16. Ethical Considerations and Compliance with Good Clinical Practice

16.1. Quality Control and Quality Assurance

The sponsor will review site processes for quality management of the study prior to enrollment, to include processes for data and biological specimen collection. Expectations will be communicated to the site regarding study conduct. In addition, all study staff are required to have GCP, Human Subject Protection, and ICH training.

The investigator is expected to perform internal quality management of study conduct and documentation and is required to keep accurate records to ensure that the conduct of the study is fully documented and to ensure that all CRFs are completed for every participant entered in the trial.

Plans for quality control of electronic data capture and data management will be detailed in the data management and data validation plans and will be reviewed by the sponsor prior to study onset. The CRFs will be completed online via a web-based EDC system that has been validated and is compliant with Part 11 Title 21 of the Code of Federal Regulations (CFR). Study staff at the site will enter information into the eCRFs, and the data will be stored remotely at a central database. Quality control (QC) procedures will be implemented by the EDC system's continuous monitoring of data and real-time detection and correction of errors. All elements of data entry (i.e., time, date, verbatim text, and the name of the person performing the data entry) will be recorded in an electronic audit trail to allow all changes in the database to be monitored and maintained in accordance with federal regulations. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

The Sponsor will develop a risk-based monitoring plan to direct study monitoring. Sponsor monitors will follow written Standard Operating Procedures (SOPs) to verify that the clinical trial is conducted, data are generated, and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonization Good Clinical Practice (ICH E6(R2)), and applicable regulatory requirements. The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

16.2. 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 local IRB. Any amendments to the protocol or to the consent materials will also be approved by the local IRB before they are implemented.

16.3. Informed Consent Process

The consent process will provide information about the study to a prospective participant and will allow adequate time for review and discussion prior to his/her decision. The principal investigator listed on the FDA 1572 or designee 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. The participant (or their legally acceptable representative) will read, sign, and date a consent form before undergoing any study procedures. Consent materials will be presented in the participant's primary language. A copy of the signed informed consent form will be given to the participant.

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

16.4. Privacy and Confidentiality

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

17. Publication Policy

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

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

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

I. Atopic Dermatitis (AD)[1] A. Active Atopic Dermatitis (AD)[1]

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

B. Inactive Atopic Dermatitis (AD)[1]

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

C. Definition

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

1. Pruritus
2. Eczema (acute, subacute, chronic)

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

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

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

II. References

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Appendix B: Schedule of Events

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

1. Participants may be recruited by phone or during an in-person visit.

2. Assessment of full inclusion and exclusion criteria will occur during the Screening Visit, after participants have consented to study participation.
3. Participants who meet full inclusion and exclusion criteria including medication and therapy washouts, may complete the Screening and Pre-treatment Visits on the same day. The following assessments will not be repeated, if the Pre-treatment Visit is conducted on the same day as Screening: medical history, physical exam, AD severity assessment, concomitant medications, pregnancy test, and vitals.
4. Upon completion of the Day 3 Follow-Up Visit, a participant will be notified prior to their next scheduled follow-up visit if they need to return to the clinic. A participant will not be required to complete the remaining follow up clinic visits (Day 10 through Day 24) if the lesional swabs from both arms (right and left) collected at their most recent prior visit, have CoNS CFU below baseline density measured before application of ShA9 + 100 CFU/cm^2.

5. If disease activity increases, participants experience signs and symptoms as described on the instructional handcard, or other concerns arise between regularly scheduled visits, participants may be asked to return to the study site for an Unscheduled Visit.
6. An abbreviated Medical History will be collected.
7. AD disease severity will be assessed using the Investigator Global Assessment (Screening only), local Eczema Area and Severity Index (L-EASI) on the arms, SCORing Atopic Dermatitis (SCORAD), and local Pruritus Numerical Rating Scale (NRS).
8. A urine pregnancy test will be completed for all female participants of child bearing potential who do not self-report as pregnant at the Screening and Treatment Visits.
9. Vital signs will include temperature, heart rate, respiration rate, systolic blood pressure, and diastolic blood pressure. Growth parameters (height and weight) will only be collected at the Screening Visit.
10. Skin swab collection from the participant's ventral arms will occur during each visit, and skin swab collection from the participant's face will begin at the Treatment Visit.
11. Lesional and non-lesional sites will be identified on the participant's ventral arms for skin swab collection. Digital photographs of the lesional and non-lesional swab sites will be taken prior to swab collection. Each photograph will include a ruler so the scale of the sites can be determined. These sites will be swabbed at subsequent visits.
12. A non-lesional site will be identified on the participant's face for skin swab collection. A digital photograph of the non-lesional swab site will be taken prior to swab collection. The photograph will include a ruler so the scale of the site can be determined. This site will be swabbed at subsequent visits.
13. Participants will remain in clinic for up to 6 hours following the application of investigational product during the Treatment Visit. Additional skin swabs will be collected from the ventral arms at 15 minutes, 1, 2, 4, and 6 hours post application.
14. Skin swabs and/or blood may be collected during an Unscheduled Visit per investigator discretion.