Beginning Assessment of Cutaneous Treatment Efficacy of Roseomonas In Atopic Dermatitis Phase I/II

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Study Staff Roster

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List of Abbreviations

AD	Atopic dermatitis
AE	Adverse event
AR	Adverse reaction
BIW	Twice weekly
BTRIS	Biomedical Translational Research Information System
CBC	Complete blood count
CC	Clinical Center
CDLQI	Children's Dermatology Life Quality Index
CFR	United States Code of Federal Regulations
CGN	Commensal Gram-negative bacteria
CRIMSON	Clinical Research Information Management System of the NIAID
CRP	C-reactive protein
CSO	Clinical Safety Office
D10	Dextrose 10 solution
DAIDS	Division of AIDS
DCR	Division of Clinical Research
EASI	Eczema Area and Severity Index
ELISA	Enzyme-linked immunosorbent assay
ESR	Erythrocyte sedimentation rate
FDA	United States Food and Drug Administration
FDLQI	Family Dermatology Life Quality Index
GCP	Good Clinical Practice
HRPP	Human Research Protections Program
HV	Healthy volunteer
ICH	International Council on Harmonisation
IND	Investigational New Drug
IRB	Institutional Review Board
ISM	Independent safety monitor
LCIM	Laboratory of Clinical Immunology and Microbiology
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
OCRPRO	Office of Clinical Research Policy and Regulatory Operations
OHRP	Office for Human Research Protections
OHSRP	Office of Human Subjects Research Protections
PCR	Polymerase chain reaction
PI	Principal investigator
SAE	Serious adverse event
SAR	Suspected adverse reaction
SCORAD	SCORing Atopic Dermatitis
SERF	Safety expedited report form
SOP	Standard operating procedure
SRCP	Safety review and communication plan
SUSAR	Serious and unexpected suspected adverse reaction
TEWL	Transepidermal water loss
TLC	Thin layer chromatography
TNF	Tumor necrosis factor
UP	Unanticipated problem
UPnonAE	Unanticipated problem that is not an adverse event

Protocol Summary

Short Title: BACTERiAD I/II

Clinical Phase: I/II

Sample Size: N = 49 (10 young adults and adults [aged 18+ years] and 39 children [aged 3-17 years] diagnosed with atopic dermatitis [AD])

Part 1: 10 adults and young adults, aged 18+ years
Part 2: 5 children, aged 7-17 years
34 children, aged 3-17 years

Accrual Ceiling: N = 70

Study Population: Adults with AD (aged 18+ years) who are otherwise healthy will be recruited for the initial dose escalation/safety portion of this study (part 1). Children (aged 3-17 years) with previously diagnosed AD who are otherwise healthy will be recruited for the activity/safety portion of this study (part 2). Children aged 3-6 years will not be enrolled until 5 children aged 7-17 have completed at least 1 month of high-dose therapy (3 months of total treatment) without safety signals.

Accrual Period: 15 months

Study Duration: Start Date: December 2016
End Date: December 2021
Total length of individual subject participation: 6 weeks for part 1 (aged 18+ years), up to 1.5 years for part 2 (aged 3-17 years).

Study Design: This is an open-label, dose-escalation study to evaluate the activity and safety of *Roseomonas mucosa*, a Gram-negative commensal bacterium, to treat AD in children. Adults with AD (aged 18+ years) will also be recruited for the purpose of evaluating safety before minors are enrolled. After 5 children (aged 7-17 years) have been enrolled (Part 2A), 34 children 3-17 years old (Part 2B) will be enrolled. For part 2A, treatment will be for up to 4 months at the effective target dose (10^3, 10^4, or 10^5 CFU). For part 2B, treatment will be for up to 4 months at 10^5 CFU. Participants will be enrolled into stratified categories for mild, moderate, and severe AD at a target of 11-14 participants for each group.

Study Agent/ Intervention Description:	A solution of *R mucosa* to be applied directly to the affected antecubital area on the skin twice per week. For part 1, treatment lasts for 6 weeks (2-week intervals of escalating doses 10^3, 10^4, and then 10^5 CFU). For part 2A, treatment will be for up to 4 months at the effective target dose (10^3, 10^4, or 10^5 CFU). For part 2B, treatment will be for up to 4 months at 10^5 CFU.
Primary Objective:	To evaluate the safety and activity of *R mucosa* as a live biotherapeutic product for treatment of AD.
Secondary Objective:	To evaluate the effect of *R mucosa* live biotherapy on quality of life of participants with AD.
Primary Endpoint:	A 50% reduction in antecubital-specific SCORing Atopic Dermatitis (SCORAD) with no adverse events related to product use. Frequency of solicited adverse events, unsolicited adverse events, serious adverse events, and death.
Secondary Endpoints:	1. 50% reduction in total-body SCORAD 2. A 30% improvement in quality of life as measured by the validated Children's Dermatology Life Quality Index (CDLQI) and the Family Dermatology Life Quality Index (FDLQI). 3. 50% reduction in Eczema Area and Severity Index (EASI) score.

Précis

The underlying pathology of atopic dermatitis (AD) consists of defective skin barrier function, susceptibility to *Staphylococcus aureus* skin infection, and immune imbalance. There is currently no cure for AD. Preclinical data in a mouse model of AD suggest that commensal Gram-negative bacteria (CGN), such as *Roseomonas mucosa*, from a healthy source can relieve symptoms of AD and have antimicrobial effects. In this study, we will first evaluate the safety of *R mucosa*-based biotherapy in adults with AD (age 18+ years; part 1), and then evaluate the safety and activity of *R mucosa*-based biotherapy in children (ages 3-17 years) with AD (parts 2A and 2B). In part 1, participants will receive twice-weekly doses of CGN biotherapy for 6 weeks, with dose escalations at 2 and 4 weeks. In part 2, participants will receive twice-weekly doses of CGN biotherapy for 4 months, with possible dose escalations at 4 and 8 weeks (part 2A only). Starting at 12 weeks for both parts 2A and 2B, dosing frequency may be increased to every other day. Participants in part 1 will be contacted 30 ± 10 days after end of treatment for assessment of safety. Participants in parts 2A and 2B will also be followed for up to 1 year after the end of treatment for evaluation of long-term activity and safety. This will be the first study to test cutaneous live biotherapeutic products for AD. We hypothesize that altering the strains of CGN on the skin of people with AD will improve the patient's clinical outcome. We do not expect serious toxicities because *R mucosa* is rarely pathogenic; reported cases of bacteremia have typically been associated with percutaneous catheters in immunocompromised patients, who are excluded from this study.

1 Background Information and Scientific Rationale

1.1 Background Information

Atopic dermatitis (AD) is characterized by itching, a scaly rash, dry skin, and cutaneous sensitization to allergens. The underlying pathology of AD consists of a triad of defective skin barrier function, susceptibility to *Staphylococcus aureus* skin infection, and immune imbalance (overactive adaptive immunity in lieu of innate immunity).[1]

1.1.1 Features of Atopic Dermatitis Pathology

Atopic dermatitis is marked by a loss of skin barrier function, leading to increased transepidermal water loss (TEWL) and subsequent dry and itchy skin.[1] In addition to failing to keep in water, the skin in AD patients fails to keep antigens out. While AD typically resolves on its own in children,[2] the years of barrier deficiency results in cutaneous sensitization that may predispose them to other allergic diseases (a process known as the atopic march). The incidence of asthma in patients with AD is more than twice the general population.[2] Two of three AD patients will develop allergic rhinitis[3] while early-onset AD markedly increases the risk of food allergies.[4] Therefore, treatments that could truly affect barrier function may not only improve AD but also prevent the related allergic sequelae. These barrier defects have been linked to genetic pathways, such as mutations in FILAGGRIN[3] as well as lipid metabolic pathways involving ceramides.[5-7]

In addition to the barrier defects, AD is associated with intrinsic immune dysregulation. In general, skin in AD patients displays a reduction in innate immunity with an overactive adaptive response.[8] AD skin is deficient in innate antimicrobial peptides such as cathelicidin, the beta defensins,[8] and the newly discovered Reg3γ.[9] AD skin has an overabundance of T-cell infiltration, particularly Th2 cells that produce allergic cytokines.[10] The current model of AD places vitamin D upstream of the immune imbalance.[11] Vitamin D enhances antimicrobial production while suppressing T cells and other cells in adaptive immune pathways.[11,12] This is the reasoning behind ongoing trials using topical vitamin D as a therapy in AD.[13]

Lastly, overgrowth and infection with *S aureus* are both contributors to, and consequences of the immune imbalance and poor barrier function. The innate immune system is as vital to controlling *S aureus* as the adaptive system is dispensable.[14,15] *S aureus* can directly activate allergic mast cells[16,17] and T cells.[18] Treatment antibiotics can reduce *S aureus* colonization and improve symptoms, but do not normalize the underlying pathology.[13]

1.1.2 Limitations of Current Therapies

There is currently no cure for AD. Barrier dysfunction can be improved with emollients; antibiotics can limit *S aureus*; and immune balance can be partly restored by topical steroids or calcineurin inhibitors.[13]

While the combinations of these therapies can provide visual normalization of the skin, the amount of effort required for full treatment takes a substantial emotional toll on patients and their families. The symptoms of AD substantially reduce multiple quality-of-life metrics while the multiple-times-per-day treatment demands can drain families and generate guilt for missed doses.[19]

Recent work has revealed that the skin microbiome is significantly different between healthy controls and patients with AD and that symptoms are associated with a loss of commensal diversity.[20] While host and staphylococcal factors must be at play in the pathogenesis of AD, little is known as to the potential causal role of the microbiome in AD. We hypothesize that the dysbiosis seen in AD contributes to the disease state and thus the bacterial flora can serve as a viable therapeutic target to improve symptoms. Consistent with this hypothesis, adjunct use of a lysate from the Gram-negative bacteria *Vitreoscilla filiformis* significantly improved atopic dermatitis symptoms during the study's 1 month of treatment.[21]

1.2 Description of the Study Agent

1.2.1 Commensal Gram-negative Flora Differ Between AD and Healthy Controls

Genetic-based microbiome identification revealed significant differences in the Gram-negative skin biome between AD patients and healthy controls.[20] While these findings were profound, they were limited because of the requirement of cultured isolates to obtain species-level identification or evaluate host-bacterial interaction. Our group was able to develop the first consistent method for culturing Gram-negative skin flora through a novel selection of media, antibiotics, and temperature.[22] We found substantial differences in the bacteria present on the skin of AD patients and healthy volunteers (HV, Figure 1).[23] We found the predominant species of skin commensal Gram-negative bacteria (CGN) in HV to be *Roseomonas mucosa*. Over 50% of AD patients did not have any culturable Gram-negative flora, consistent with DNA-based analysis.[20]

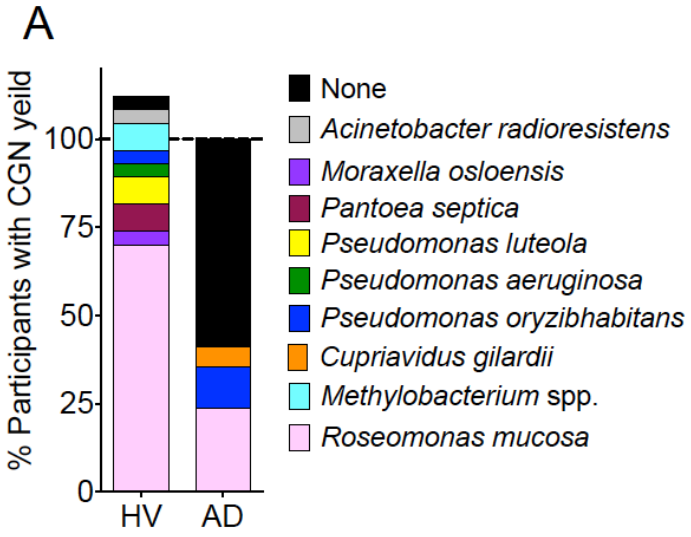

Figure 1 Commensal Gram Negative Isolates Differ Between Healthy Volunteers and Patients with Atopic Dermatitis

Commensal Gram-negative (CGN) bacteria differ between patients with atopic dermatitis (AD) and healthy volunteers (HV). Percentage of CGN results for patients with indicated CGN grown from skin swab cultures are shown for 23 HVs and 42 patients with AD. Participants with more than 1 isolate were counted separately so that the total percentage for HV was greater than 100.

1.2.2 CGN From Healthy Controls Enhances Innate Immunity and Barrier Function

The immune profile of patients with AD tends to have a relative reduction in innate immunity markers such as beta-defensins, cathelidcidin,[8] the newly discovered Reg3γ[9] as well as a relative increase in adaptive T-cell signals,[8] particularly the allergy-associated Th2 cells.[10] To some extent, vitamin D may serve as an upstream modulator of cutaneous innate pathways.[11,12] We stimulated a foreskin-derived, primary human keratinocyte line with equal CFU of CGN taken from HV versus patients with AD. We found that keratinocytes stimulated with HV-CGN had enhanced transcription of CAMP (the cathelicidin precursor), DEFβ4A, and the potential upstream modulators CYP27b1 (a vitamin D–converting enzyme) and the vitamin D receptor.[24]

Furthermore, we found in vivo differences in human responses to the different strains of CGN. We obtained a United States Food and Drug Administration (FDA) Investigational New Drug (IND) application (#16024) to allow challenge of the suction blisters with lethally irradiated bacteria and directly measure human cutaneous responses to 7 different strains of clinically relevant bacteria.[25] After approximately 20-24 hours of stimulation, blister cytokine levels for IL-6 were substantially higher in response to representative *R mucosa* from HV compared to a selected *R mucosa* strain from a patient with AD (Figure 2A-B). There were no differences in traditional Th cytokines such as IL-17, IFNγ, or IL-4 (

Figure 2A-B), nor were there substantial differences in other immune markers.

The loss of barrier function in AD causes dry, itchy skin through failure to keep moisture in,[1] and may lead to TEWL and cutaneous sensitization (a process known as the atopic march) through failure to keep antigens out.[2] For at least a subset of patients, this barrier defect is associated with dysfunction in the tight-junction protein FILAGGRIN.[3] Inoculating healthy mouse ears with our same representative isolates of CGN enhanced transcript levels of FILAGGRIN (

Figure 2C) without any noticeable change in redness or ear thickness (not shown). Application of AD-CGN increased TEWL, while HV-CGN was inert (

Figure 2D).[24] Taken together, these data suggest that strains of CGN associated with an AD status induce potentially harmful immune outcomes related to activation of vitamin D, stimulate innate immunity, and affect barrier function.

1.2.3 CGN Bacteria From Healthy Controls Inhibit *S aureus*

Next, we evaluated the effect of CGN on the growth of *S aureus*. Supernatants from HV CGN inhibited *S aureus* growth irrespective of the species of CGN or the source of the *S aureus* against which it was pitted (AD patients, HV, or autologous) (Figure 3A-B). In contrast, CGN from AD patients had a more variable effect, with the majority of isolates failing to inhibit *S aureus* (Figure 3A-B). Similar results were seen when mouse ears were co-inoculated with both *S aureus* and CGN (Figure 3C-D).

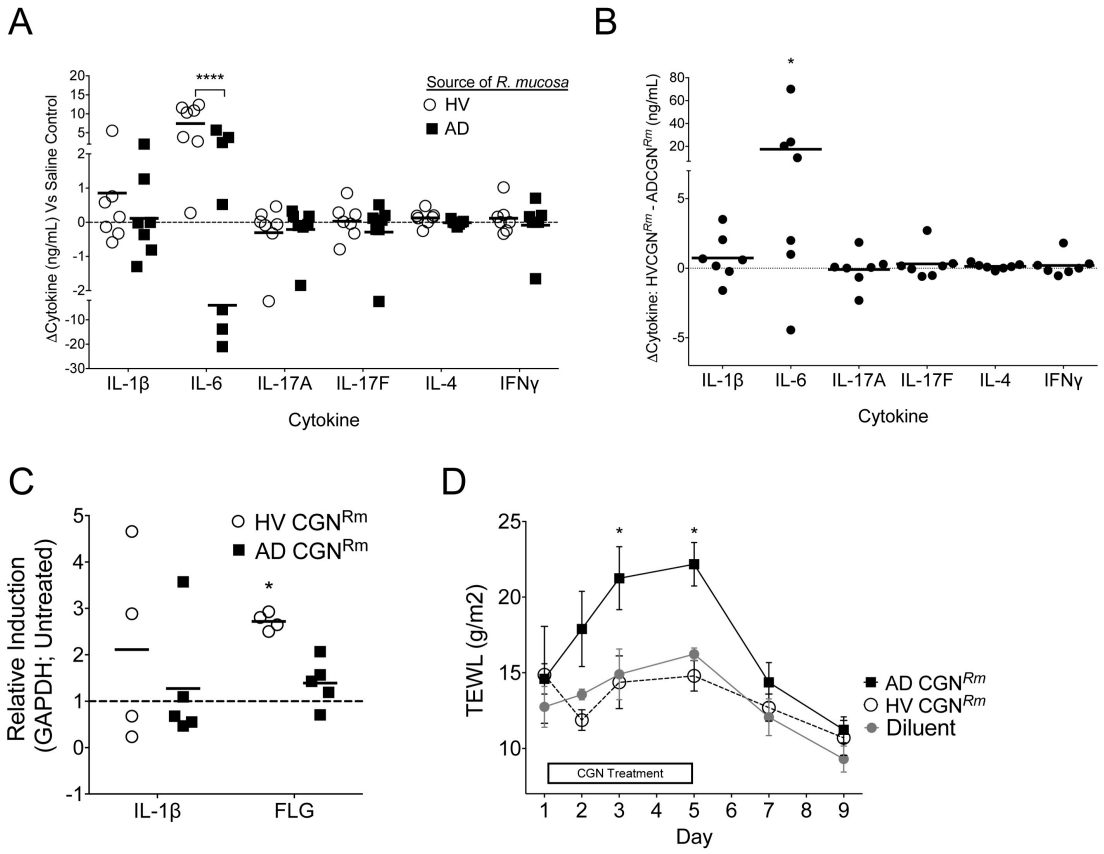

Figure 2 Commensal Gram Negative Bacteria Affect Innate Immune Markers and Transepidermal Water Loss

Commensal Gram-negative bacteria (CGN) from healthy volunteers (HV) enhance pathways that are defective in atopic dermatitis (AD). (A, B) Total cytokine analysis (A) and paired analysis showing difference in response between the response to the HV strain of *R mucosa* minus the response to the AD strain (B) for in vivo human blister challenge, N = 7. (C) Mouse ears inoculated for 3 days with 10^7 CFU *R mucosa* (Rm) from either HV or AD, day 5 mRNA for IL-1β and Filaggrin (FLG) standardized to GAPDH and compared to untreated mice shown, N = 4-5 mice per group. (D) Mouse backs were shaved and hair chemically removed on day 0. Trans-epidermal water loss (TEWL) was measured, then 10^7 CFU Rm from either HV or AD were applied daily for 5 days, N = 3-5 mice per group. Modified from reference.[24]

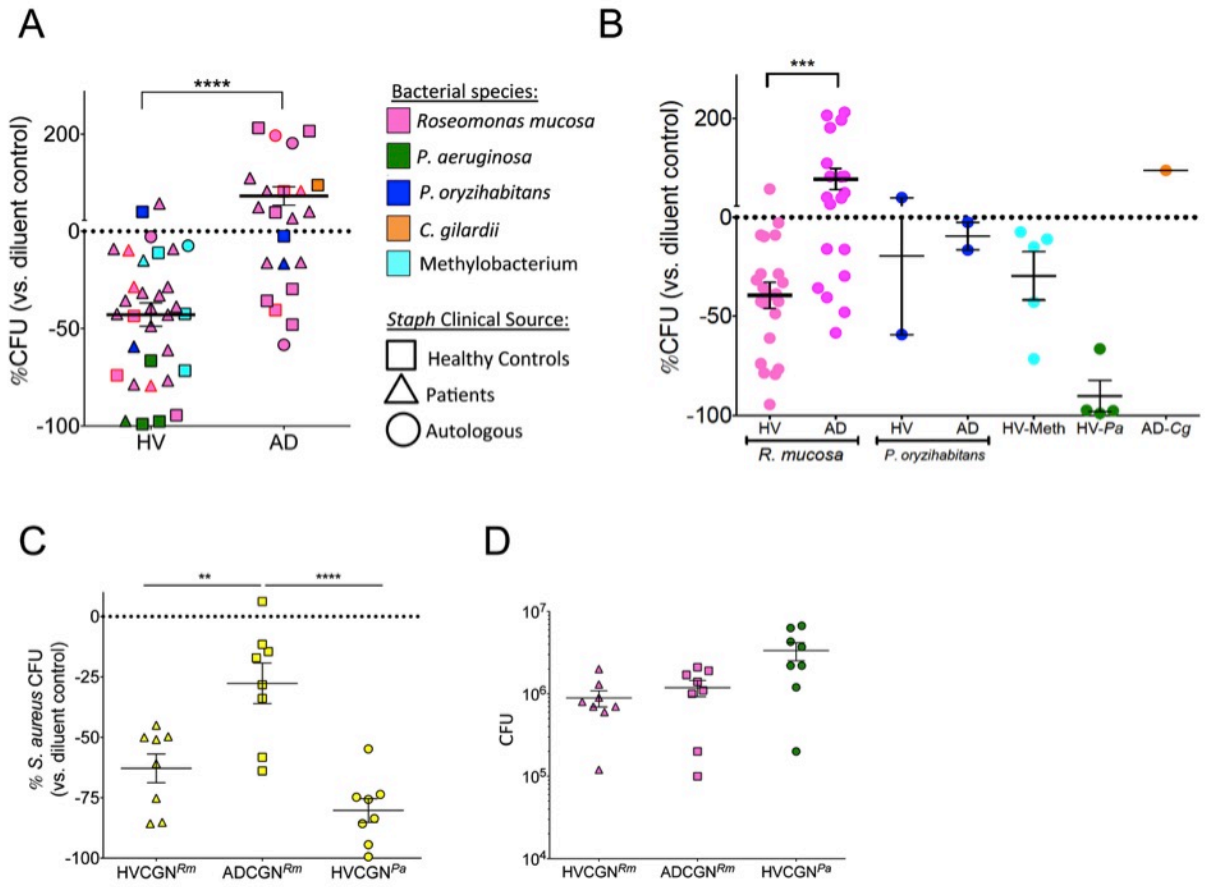

Figure 3 Commensal Gram Negative Bacteria Inhibit Growth of *S aureus*

Commensal Gram negative (CGN) inhibition of *Staphylococcus aureus* growth. (A-B) *S aureus* isolates cultured from healthy volunteers (HV) and atopic dermatitis (AD) patients were grown in the presence of either CGN supernatant or CGN growth media. Each dot represents the difference for one isolate CGN supernatant versus media growth control; shapes represent sourcing of *S aureus* as either the participant's autologous *S aureus*, or 1 isolate from HV or AD patients. Displayed as collective results by group (A) or separated into species and group (B). (C-D) MC903-treated mice were co-inoculated with *S aureus* and *Roseomonas mucosa* (Rm) from HV or AD patients, or *Pseudomonas aeruginosa* (Pa) from a HV for 10 days. Day 12 ears were homogenized and plated by serial dilution for CFU for *S aureus* (C) and CGN (D). Modified from reference.[24]

1.2.4 CGN Improves Outcomes In Mouse Models of AD

Given our findings that CGN from HV improved barrier function, immune balance, and *S aureus* control, we assessed the effect of our CGN strains in a preclinical mouse model. We used the established AD model of MC903, a low-calcemic vitamin D analogue, placed on the ear of mice to induce a similar phenotype to human AD.[26] We enhanced this model with co-application of the SAAS9 strain of *S aureus*; NIH collaborators have recently described this strain as a strong inducer of AD symptoms in mouse models.[27] Application of HV-sourced *R mucosa* protected

against onset of MC903-induced dermatitis as measured by visual redness (not shown) and ear thickness (Figure 4A). In contrast, AD-sourced *R mucosa* failed to protect against disease onset as did the HV-sourced *Pseudomonas aeruginosa* (Figure 4A), despite its impact on keratinocyte activation and *S aureus* inhibition. Application of an AD-sourced *R mucosa* enhanced IgE induction, while the HV-sourced *R mucosa* showed no substantial impact on IgE (Figure 4B). Consistent with inoculation of healthy mouse ears, transcript levels of *Filaggrin* were substantially lower in MC903-treated mice exposed to AD-sourced *R mucosa.*[24]

Treatment, rather than prevention, would likely be the more clinically relevant model. Therefore, we induced AD-like dermatitis with MC903, then inoculated the ears with CGN daily for 3 days. Treatment with HV-CGN was associated with a reduction in visible redness and ear thickness (Figure 4C, D). To evaluate if a live biotherapeutic was necessary, or if surface and secreted factors alone could provide similar results, we applied an equivalent CFU concentration of lethally irradiated HV-CGN suspended in the supernatant. Neither this "dead mix" nor the supernatant alone provided any benefit, and by thickness may have actually worsened symptoms (Figure 4C, D). There was no detectable CGN bacterial translocation into the bloodstream.

Taken together, these data indicate that *R mucosa* from healthy controls can improve AD symptoms through the central pathways of AD pathology: improving barrier function, enhancing immune balance, and inhibiting *S aureus* growth. However, the failed treatment approach of secreted and surface bacterial products without live commensals suggest that the standard therapeutic development approach of isolating bioactive compounds may not provide assistance in AD. Rather, the dynamic interaction of host-commensal may be required for clinical utility. However, use of a live therapeutic may carry the additional benefit of limited dosing requirements through the potential for colonization.

1.2.5 *R mucosa* is Sensitive to Aminoglycosides and Fluoroquinolones

We conducted antibiotic resistance/susceptibility testing on several strains of *R mucosa* (4 from HV, 1 from a patient with AD). All sensitivity patterns were the same (Table 1). Therefore in this study, participants that take either aminoglycosides or fluoroquinolones during the 4-month treatment window will be excluded (see Section 4.3).

Table 1 Sensitivity of *R mucosa* to Antibiotics

Antibiotic	Minimum Inhibitory Concentration	Interpretation
Amikacin	≤ 8	Sensitive
Aztreonam	> 16	Resistant
Cefepime	16	Intermediate
Ceftazidine	> 16	Resistant
Ciprofloxacin	≤ 1	Sensitive
Gentamicin	≤ 1	Sensitive
Levofloxacin	≤ 1	Sensitive
Meropenem	> 8	Resistant
Piperacillin/tazobactam	> 64/4	Resistant
Tobramycin	≤ 1	Sensitive
Trimth/Sulfa	> 2/38	Resistant

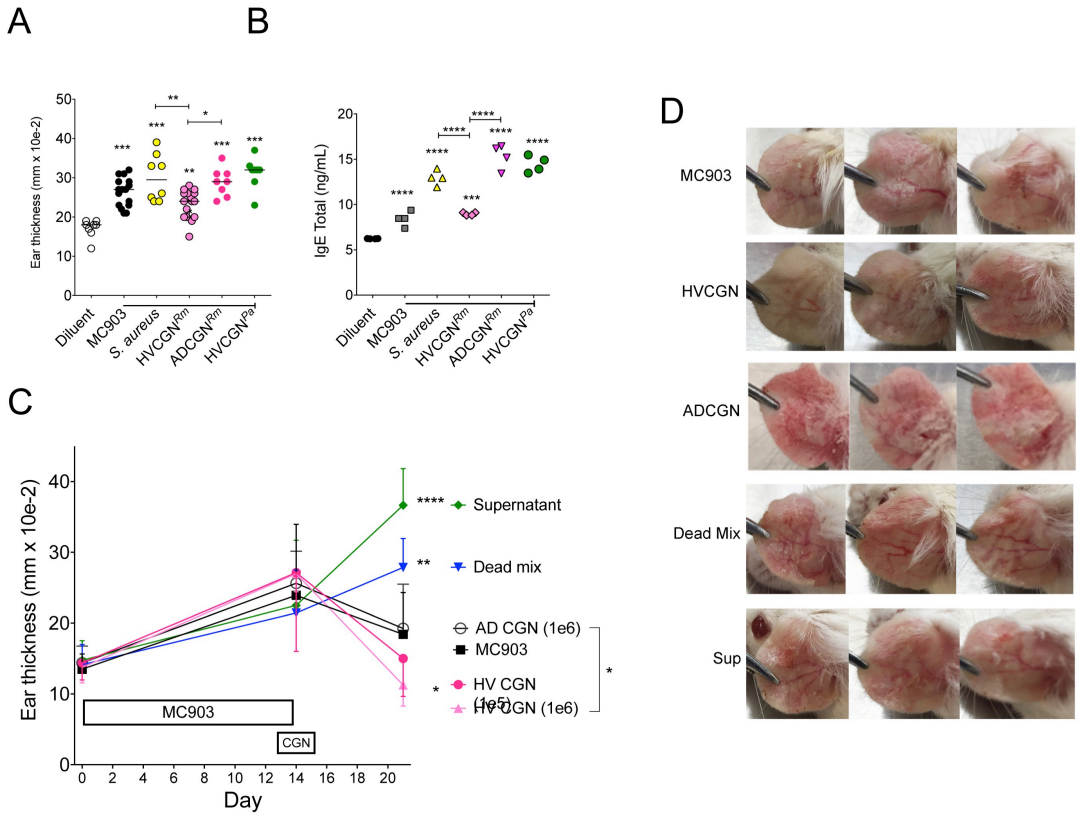

Figure 4 Commensal Gram Negative Bacteria from Healthy Volunteers Improve Outcomes in the MC903 Mouse Model of AD-like Dermatitis

Healthy volunteer (HV) commensal Gram-negative (CGN) bacteria improve outcomes in mouse atopic dermatitis (AD) models. (A, B) Mice were treated *Roseomonas mucosa* (Rm) from HV or AD patients, or *Pseudomonas aeruginosa* (Pa) from a HV for 2 days prior to MC903 application. Then CGN were co-applied with MC903 daily for 13 days. Day 14 ear thickness (A) for each ear and serum total IgE levels (B) are shown; N= 4-8 mice per group. (C, D) Mice were treated with MC903 for 14 days to induce AD-like dermatitis. Starting on day 13, mice were treated daily for 3 day with 10^6 CFU of live Rm from HV or AD patients (HVCGN and ADCGN), 10^6 CFU of killed Rm from the same HV resuspended in the supernatant from 3×10^6 CFU of autologous Rm (Dead mix), or supernatant from 10^7 CFU of the HV Rm (Sup). Day 21 visual redness (C) and ear thickness (D) are shown; N = 3-5 mice per group. Modified from reference.[24]

1.2.6 Summary of Prior Infections with *R mucosa*

R mucosa does not routinely cause clinical infections. However, there have been reports of 22 catheter infections in patients with neoplastic disease and chemo-induced neutropenia within 1 week of presentation.[28] Additionally, there is 1 case report of catheter-associated bacteremia linked with acute leukemia and associated neutropenia and 1 report of septic arthritis in a patient with a history of rheumatoid arthritis on active anti–tumor necrosis factor (TNF) treatment.[29,30]

All cases resolved with antibiotic therapy of fluoroquinolones, beta-lactams, and/or aminoglycosides. Exclusion criteria will include neutropenia and anti-TNF treatment (see Section 4.3).

1.3 Hypothesis and Scientific Rationale

Preclinical data in a mouse model of AD suggest that CGN from a healthy source can relieve symptoms of AD and have antimicrobial effects.[24] Because of the molecular and immunologic differences between mice and humans, the relevance of these findings to humans is unknown. We therefore propose to investigate CGN therapy in humans with AD. This study will be the first to test cutaneous live biotherapeutic products for AD. We hypothesize that altering the strains of CGN on the skin of people with AD will improve the patient's clinical outcome.

Our animal studies suggest that a live biotherapeutic product is necessary for clinical benefit, as opposed to a bacterial lysate and/or metabolome. We anticipate that our product may have some residual dead bacterial cells. These are not expected to interfere with the results of benefit given preparations used in the mouse experiments also are expected to contain some dead bacterial cells. Furthermore, our blister challenge results (

Figure 2A) suggest that responses to the strains of *R mucosa* taken from healthy controls are distinct from those taken from patients with AD. Therefore, normal physiologic bacterial turnover on the skin will present some dead bacterial cells to the immune system. If these bacteria are of a beneficial strain then the interaction is expected to provide benefit rather than interfere with the main mechanisms of living bacterial products.

We will first evaluate the safety of *R mucosa* in people with AD aged 18+ years. We do not expect serious toxicities because *R mucosa* is rarely pathogenic; bacteremia has typically been observed only in immunocompromised patients.[28] We will then enroll children (aged 3-17 years) clinically diagnosed with AD. After 5 children (aged 7-17 years) complete 1 month of high-dose treatment (3 months of total treatment), enrollment will expand for the remaining 34 participants to children aged 3-17 years, who will start at the high dose. Participants will apply treatment to the infected area and we will evaluate them for safety and activity of treatment.

All parents are instructed to apply the treatment immediately before bed on treatment days. To help protect treated areas from interference in children age 3-6, we will instruct parents that children 3-6 years sleep in long-sleeve shirts.

We plan to enroll children because they are one of the intended populations for this biotherapy. Because most patients outgrow the disease before age 6 years,[31-33] inclusion of younger participants will be vital to provide a true reflection of the AD population. However, in order to maximize safety in participants younger than the age of assent, we will not enroll children aged

3-6 years until after 5 participants aged 7-17 years have completed at least once month of high-dose therapy (3 months of total treatment) without any safety signal concerns.

Given no case reports exist of an otherwise healthy child or adult with *R mucosa* infection, we do not perceive this as a significant risk. Fewer than 30 cases associated with chemotherapy, leukemia, and/or central lines exist in the literature. With zero cases in the AIDS population, primary immune deficiency population, or immune suppressed population without central lines, it is our assessment that *R mucosa* (a commensal organism found on > 75% of healthy controls thus far) does not present pathogenic capacity in the AD population. Furthermore, we have conducted safety testing in mice and found that intravenous injection of 10^8 CFU of *R mucosa* did not have any adverse effects. Monitored over 7-10 days and compared to saline-injected controls, mice injected with *R mucosa* had no weight change, no change in activity, no moribundity, no morbidity, and had no signs of infection or inflammation on histologic examination of their liver, spleen, and kidneys (Appendix G). This failure to generate pathology in mice means that *R mucosa* fails to fulfill Koch's postulates (the third being the requirement that an organism isolated from an infected individual be able to re-create disease in an otherwise healthy mammal). Therefore, statistically speaking, it is more likely that the reported line infections represent a false positive staphylococcal culture than a true infection with *R mucosa*.

Additionally, our evidence that dysbiosis is a significant contributor to AD pathology (as outlined in the animal model data showing impacts on barrier function, *S aureus* infection, and immune regulation) must be viewed along side new evidence on the atopic march.[34,35] Data surrounding the atopic march suggest that prolonged skin defects associated with AD contribute to cutaneous sensitizations that predispose the patients to significantly higher rates of asthma, food allergies, and allergic rhinitis. Thus, if prolonged AD contributes to the subsequent risk of atopic disorders, and prolonged dysbiosis contributes to AD, then the cost/benefit assessment is better in younger children compared to older. If children treated prior to the onset of associated atopic diseases (typically also after age 6) may be protected from these diseases,[34,35] use for children aged 3-6 years offers a greater chance of benefit than our current 7 and older population without increased risk of complications.

1.4 Selection of Bacterial Strains

We have performed full genetic sequencing of multiple *R mucosa* strains. Two healthy controls and 2 strains taken from patients with AD were evaluated for genetic differences. Per FDA protocols, the bacteria were assessed for antibiotic resistance genes. While several were present, these were limited to chromosomal DNA. Further analysis was performed to evaluate if the chromosomal resistance genes were flanked by DNA sequences that suggest propensity for translocation. Only 1 resistance gene possessed such splicing elements on both ends of the gene; for this gene the splicing elements were over 10,000 base pairs apart. Thus, use of this treatment is highly unlikely to spread any antibiotic resistance in the environment. There were

87 sequences shared by the healthy controls but missing in the AD strains, and conversely 127 sequences in the AD-associated strains that were not present in the healthy control strains (Figure 5; note not all differing sequences were considered genes). While the genomes (approximately 3900 genes) and large plasmid (approximately 430 genes) were similar between the healthy and AD-associated strains, there was a second large plasmid (306 genes) found only in the healthy controls. When performing direct genetic comparisons, the identifiable genes that differed included 11 chromosomal genes, 3 genes on plasmid 1, and 19 genes on plasmid 2. We will plan to use an equal CFU combination of 2-4 strains from healthy controls. Strains will be selected on the basis of the presence of the second large plasmid. Prior to incorporation into the therapeutic, all considered strains will undergo full sequencing to assure absence of plasmid bacterial resistance genes.

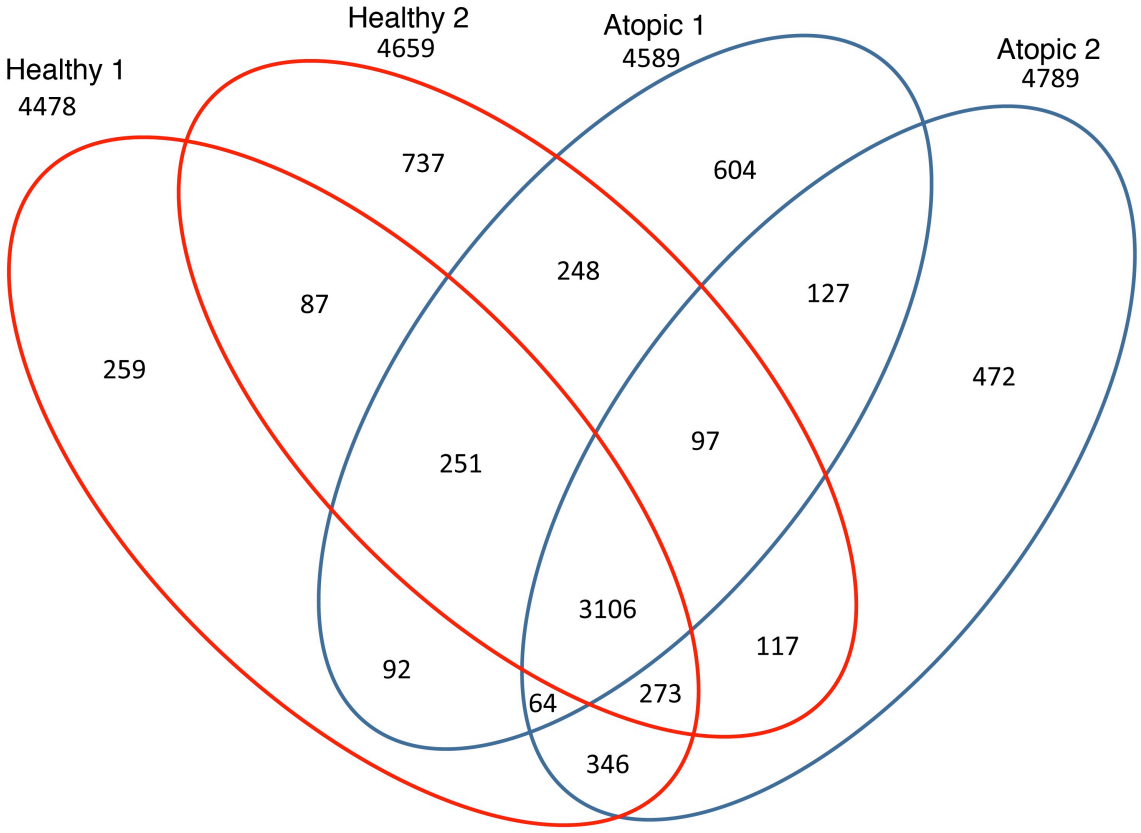

Figure 5 Strains of *R mucosa* From Healthy Volunteers and Patients with AD Have Differing Genetics

Total identifiable sequences for *R mucosa* strains from either healthy controls or patients with atopic dermatitis. Sequences do not necessarily indicate genes. Venn diagram indicating shared and differing sequences between the 4 isolates.

2 Study Objectives

2.1 Primary Objective

To evaluate the safety and activity of *R mucosa* as a live biotherapeutic product for treatment of AD.

2.2 Secondary Objective

To evaluate the effect of *R mucosa* live biotherapy on quality of life of participants with AD.

2.3 Exploratory Objectives

1. Measure TEWL
2. Characterize changes to total IgE
3. Evaluate potential changes to pre-diagnosed asthma and/or food allergies
4. Evaluate incidence of *S aureus* infections that require treatment
5. Persistence of *R mucosa* colonization after treatment

3 Study Design

3.1 Description of the Study Design

A tabulated summary of the study design is provided in Table 2 below.

We cannot enroll children as healthy volunteers in a phase I study for which there is no reasonable expectation of benefit. However, a phase I in healthy adults would not be valid due to the intrinsic difference in safety for adults with an intact skin barrier compared to a study in children with disrupted skin barrier function such as that seen in AD. As such, this study will be a combined phase I/II trial in which all subjects will have AD and will be assessed for treatment benefit and safety.

Therefore, as constructed, this study is divided into 2 portions (parts 1 and 2). In the first portion (part 1), we will evaluate the safety of *R mucosa* in adult volunteers with the diagnosis of AD. Ten patients aged 18 years and older will be treated in 2-week intervals with 10^3, 10^4, and then 10^5 CFU of *R mucosa* twice per week (BIW) for a total of 6 weeks. The BIW dosing schedule was selected to give the usually slow-growing *R mucosa* inoculum time to establish colonization prior to repeat exposure and to allow assessment for reaction before repeat dosing.[36] These participants will remain on their current treatment for AD throughout their participation.

Participants will come to the NIH Clinical Center (CC) at the beginning and end of dosing for clinical evaluation and assessment of whether the live *R mucosa* was recoverable. Safety monitoring will continue in this phase for 30 days after final treatment.

If there are no safety concerns noted after the full 30 (± 10)-day follow-up is completed for all participants in part 1, then we will proceed to the second portion of the study (part 2), to evaluate the activity and safety of *R mucosa* biotherapy on children diagnosed with AD. We will enroll 39 children aged 3 to 17 years. Participants will be enrolled into stratified categories for mild, moderate, and severe AD at a target of 11-14 participants for each group. As per accepted clinical standards, participants with SCORing Atopic Dermatitis (SCORAD) values under 25 will be considered mild, 25-49 considered moderate, and 50 or greater will be considered severe.[13,37] All inclusion and exclusion criteria will apply to all severity groups, and they will be analyzed primarily as a total group (ie, irrespective of enrollment SCORAD) with subgroup analysis by strata. Young participants who are unable to reliably provide a pruritus score (0-10) verbally will be shown a visual scale (Appendix E) to help assess their itching. Additionally, we will use the Eczema Area and Severity Index (EASI) scoring system (Appendix F), which is another validating tool for tracking AD symptoms.[38] The EASI may provide better assessment in younger participants that the SCORAD due to the EASI not having any subjective pruritus component. The EASI will be used as a secondary assessment.

Most patients with AD are younger than 6,[31-33] and therefore accurate reflection of the AD population will require inclusion of this age cohort. Furthermore, patients as young as 2 years old have been included in clinical trials for novel treatments including topical immune suppressants such as PDE-4 inhibitors and injectable immune modulators like anti-IL4Rα. However, for maximizing safety in participants younger than the age of assent, we will not enroll children aged 3-6 years until after 5 participants aged 7-17 years have completed at least 1 month of high-dose therapy (3 months of total treatment) without any safety signal concerns. The safety events that would halt recruitment of participants aged 3-6 years are as follows:

- One participant experienced any serious adverse event (SAE) that was unexpected and possibly, probably, or definitely related to the study agent;
  OR
- Two or more of the same or similar adverse events (AEs) in different participants that were grade 3 or above and were unexpected and possibly, probably, or definitely related to the study agent;
  OR
- Any safety issue that the principal investigator (PI) and/or the Clinical Safety Office (CSO) determines should halt the study.

The CSO will specifically assess safety before progression to expanded enrollment. Dr Shirley Jankelevich, Dr Hirsh Komarow, and Dr Jenna Bergerson will jointly assess the safety data prior to expansion into the younger cohort.

As in part 1, all participants in part 2 will remain on their current treatment for AD throughout this study. The first five participants aged 7-17 years (part 2A) will self-administer *R mucosa* at a

dose of 10^3 CFU BIW for 1 month. Activity and safety assessments will occur via phone, text, or email on a weekly to biweekly basis but the study team will be available for any unexpected concerns. After 1 month of treatment, we will evaluate the participant and either continue the same dosage for 3 more months or, if treatment was well tolerated but there is room for improvement, then the dosage will be increased to 10^4 CFU BIW for 1 month. If treatment is well tolerated and efficacious after this period, then the dosage will continue for 2 more months. If there is further room for improvement and the treatment is well tolerated, then the dosage will be increased to 10^5 CFU BIW for 4 weeks. If the participant tolerates this dosage and shows signs of improvement, then they will continue 10^5 CFU BIW for 4 more weeks. However, if this dosage is tolerable but the participant does not reach > 75% improvement from baseline antiecubital SCORAD values, then their dosage will be changed to 10^5 CFU once every other day for 4 more weeks. The remaining 34 participants aged 3-17 years (part 2B) will follow the same schedule of visits and evaluations, but will self-administer at a dose of 10^5 CFU BIW for 12 weeks. If this dosage is tolerable, but the participant does not reach > 75% improvement from baseline antiecubital SCORAD values, then their dosage will be changed to 10^5 CFU once every other day for 4 more weeks.

Participants who received therapy will be monitored for up to 1 year after the end of treatment to evaluate long-term activity and safety effects.

For all participants, all pre-study therapies will be continued. Participants will be instructed to document their daily therapies as well as verify the *R mucosa* treatments on the provided memory card and symptom tracker (Appendix B). Topical steroids and other treatments are unlikely to negatively affect our bacterial strains because treatment of AD is associated with an expansion of the proportion of Gram-negative bacteria on the skin.[20]

Table 2 Summary of Study Design

Study Part	Intervention	Duration	Population	Design
1	$10^{3,}$ $10^{4,}$ 10^{5} CFU *R mucosa* twice per week (BIW), applied topically.	Dose escalation every 2 weeks. 6 weeks treatment. Final in-person visit at week 6. Final remote follow-up at week 10.	Adults aged > 18 years N = 10	Open label
2A	10^{3}, 10^{4}, 10^{5} CFU BIW or 10^{5} CFU every other day.	Monthly dose escalation determined by clinical response. 4 months of treatment, monthly visits during treatment. Final in-person visit 4-10 months after treatment ends. Final remote follow-up at 1 year after treatment ends.	Pediatric patients aged 7-17 years N = 5	Open label
2B	10^{5} CFU BIW or every other day.	Dose escalation after 12 weeks determined by clinical response. 4 months of treatment, monthly visits during treatment. Final in-person visit 4-10 months after treatment ends. Final remote follow-up at 1 year after treatment ends.	Pediatric patients aged 3-17 years N = 34	Open label

3.2 Study Endpoints

3.2.1 Primary Endpoint

A 50% reduction in antecubital-specific SCORing Atopic Dermatitis (SCORAD) with no AEs related to product use. Frequency of solicited AEs, unsolicited AEs, SAEs, and death.

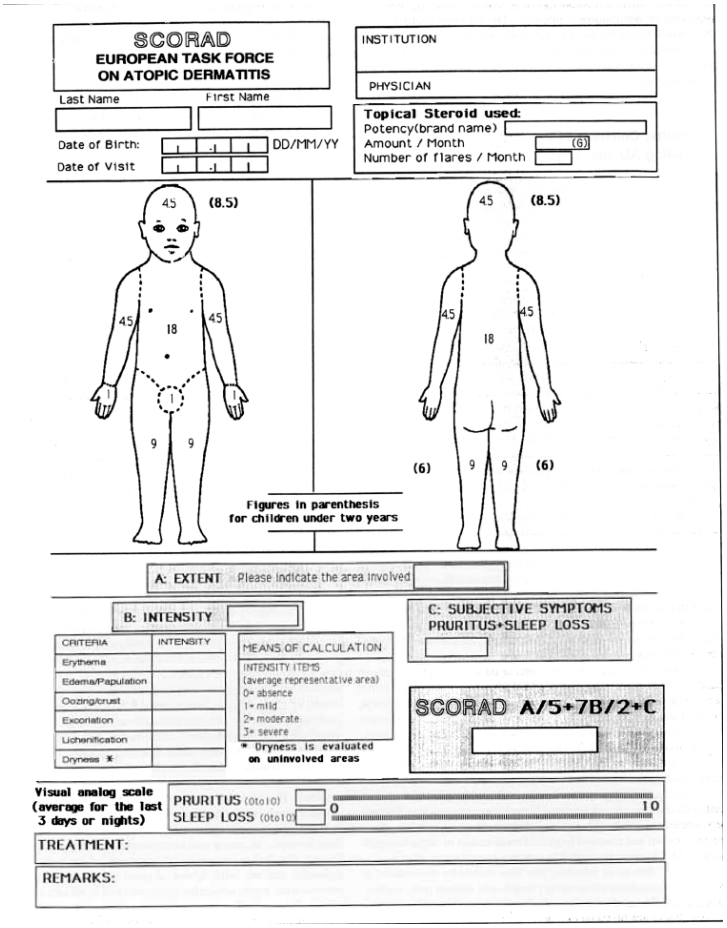

Figure 6 SCORAD Evaluation

SCORing atopic dermatitis (SCORAD), the standard clinical assessment for atopic dermatitis calculation sheet.

3.2.2 Secondary Endpoints

1. 50% reduction in total-body SCORAD from baseline
2. A 30% improvement in quality of life as measured by the validated Children's Dermatology Life Quality Index (CDLQI) and the Family Dermatology Life Quality Index (FDLQI) at treatment completion from baseline
3. 50% reduction in EASI score from baseline

3.2.3 Exploratory Endpoints

1. A 20% reduction in TEWL during the 12-week treatment period
2. A 20% reduction in total serum IgE during the 12-week treatment period
3. Any significant differences in the incidence of atopic disease such as allergy rhinitis or allergic asthma during the course of treatment and follow up from historic controls.
4. New diagnosis of skin infection and/or *S aureus* skin infection requiring antibiotics during the course of treatment and follow-up from historic controls.
5. Dose escalation or therapy expansion of pre-study treatment regimen.

4 Study Population

4.1 Recruitment Plan

Participants will be recruited from the NIH Clinical Center, the Walter Reed National Military Medical Center, or from referrals from outside physicians. Outside physicians will be asked to post fliers or provide hand-outs in their offices regarding our study to facilitate recruitment.

Recruitment of NIH employees: NIH employees and members of their immediate families may participate in this protocol. We will follow the Guidelines for the Inclusion of Employees in NIH Research Studies and will give each employee a copy of the "NIH Information Sheet on Employee Research Participation."

For NIH employees:

- NIH staff may be a vulnerable class of subjects.
- Neither participation nor refusal to participate will have an effect, either beneficial or adverse, on the participant's employment or work situation.
- The NIH information sheet regarding NIH employee research participation will be distributed to all potential subjects who are NIH employees.
- The employee subject's privacy and confidentiality will be preserved in accordance with NIH CC and NIAID policies, which define the scope and limitations of the protections.
- For NIH employee subjects, consent will be obtained by an individual independent of the employee's team. Those in a supervisory position to any employee and co-workers of the employee will not obtain consent.
- The importance of maintaining confidentiality when obtaining potentially sensitive and private information from co-workers or subordinates will be reviewed with the study staff at least annually and more often if warranted.

4.2 Participant Inclusion Criteria

4.2.1 Inclusion Criteria for Young Adults and Adults with AD (Part 1)

1. Age 18+ years
2. SCORAD of at least 10
3. Have a clinical diagnosis of AD with active involvement of the antecubital fossa
4. Willing to allow storage of blood for future research
5. No history of other skin disease
6. Initiated or attempted standard of care therapy at least 6 months prior to enrollment
7. Must agree to use adequate contraception (hormonal or barrier method of birth control or abstinence) when engaging in sexual activities that can result in pregnancy. The effects of CGN live biotherapy on the developing human fetus are unknown. Adequate contraception must be used consistently, beginning before the first dose and lasting for the duration of study participation. Participants of childbearing potential must have a negative pregnancy test result before they receive CGN live biotherapy. During the course of the study, if a participant becomes pregnant or suspects they are pregnant, then they should inform the study staff and their primary care physician immediately.

4.2.2 Inclusion Criteria for Children with AD (Part 2)

1. Age 3-17 years
2. SCORAD of at least 10
3. Have a clinical diagnosis of AD with active involvement of the antecubital fossa
4. Willing to allow storage of blood and bacterial swabs for future research
5. Initiated or attempted standard of care therapy at least 6 months prior to enrollment
6. Participants who have begun menstruating must agree to use adequate contraception (hormonal or barrier method of birth control or abstinence) when engaging in sexual activities that can result in pregnancy, as above (see Section 4.2.1)

4.3 Participant Exclusion Criteria

1. Presence of an indwelling venous or arterial catheter
2. Individuals living with anyone with a diagnosed immunodeficiency, cardiac valvular disease, and/or indwelling catheter
3. Presence of allergies to amikacin, ciprofloxacin, gentamicin, levofloxacin, and tobramycin (which would preclude treatment of any unexpected infection)
4. History of cardiac valvular disease
5. Any history of grade 2 or higher neutropenia or leukopenia
6. Clinical suspicion of immunodeficiency, liver disorder, kidney disorder, and/or HIV
7. Pregnant or breastfeeding

8. Any history of anti-TNF treatment
9. Inability to demonstrate proper bacteria administration procedure despite coaching and training
10. Use of fluoroquinolone or aminoglycoside antibiotics within 2 weeks of enrollment
11. Any condition that, in the opinion of the investigator, contraindicates participation in this study

Co-enrollment guidelines: Co-enrollment in other trials is restricted, other than enrollment on observational studies or those evaluating the use of a licensed medication. Study staff should be notified of co-enrollment as it may require the approval of the investigator.

4.4 Justification for Exclusion of Special Populations

Exclusion of women:

- Pregnancy: Pregnant participants are excluded from this study because the effects of CGN live biotherapy on the developing human fetus are unknown.
- Breastfeeding: Because there is an unknown risk for adverse events (AEs) in nursing infants secondary to treatment of the mother with CGN live biotherapy, breastfeeding should be discontinued.

5 Study Agent/Interventions

We will use *R mucosa*, a CGN, to investigate the effect of live biotherapy on AD. Bacteria will be cultured from 4 previously identified healthy controls whose bacterial isolates have shown preclinical benefit and will be rated at biosafety level 1 (ie, a strain that is well characterized and does not usually cause disease in immunocompetent adults).

R mucosa will be grown in Hank's balanced salt solution. Growth media will therefore not contain any animal products. Bacteria will then be washed, quantitated spectrophotometrically, suspended in 10%-15% sucrose, and lyophilized using a FreeZone Benchtop Freeze Dry System (Labconco [Kansas City, MO, USA]). Lyophilized samples (10^3-10^5 CFU) will be aliquoted to sterile vials. Allotments from each batch will be cultured on multiple bacterial and fungal agar plates. While FDA guidelines suggest cutoffs of 200 colonies of unexpected bacteria and 20 colonies of unexpected fungi, any unexpected bacterial or fungal contamination in our samples will prompt the disposal of the entire batch.

The work will be done in our BSL2 laboratory space in Building 11 of the NIH campus (Bethesda). All work will be done using sterile technique to avoid cross-contamination, including utilization of single-use supplies and wiping down of hoods and equipment with 70% ethanol before and after use. In addition, plating of organisms is done routinely in the course of

laboratory work to verify purity. All other equipment such as incubators and lyophilizers will be sterilized with 70% ethanol.

5.1 Disposition and Dispensation

Study agent will be distributed via the NIH pharmacy according to standard pharmacy procedures. Participants will be provided with a supply of the following to take home so they can self-administer:

1. Sterile spray bottles pre-filled with lyophilized *R mucosa* (10^3, 10^4, or 10^5 CFU)
2. Sterile eye dropper bottles filled with sterile water (1.5 mL)
3. Step-by-step picture booklet of correct administration procedures for reference

Each of these items except the instruction booklet is for a single use and can be discarded in the trash after administration. Each batch of lyophilized bacteria will be resuspended and cultured to verify the total number of live bacterial isolates present. CFU dose will be based on viable recovered colonies, and will thus be lower than starting inoculum, given some loss during the lyophilization process. Batches with less than 20% viability versus starting inoculum will be discarded.

5.1.1 Formulation, Packaging, and Labeling

Each spray bottle of *R mucosa* will be individually labeled with dosing instructions, recommended storage conditions, the name and address of the manufacturer, randomization number, Investigational Use Statement ("Limited by Federal [USA] Law to Investigational Use"), expiration date, and a statement that the agent should be kept out of reach of children. Spray bottles will be dispensed to the participants in packets of 8-24 depending upon their stage in the treatment. The packet of spray bottles will be in a plastic bag which will bear a sticker with the identical information as above reprinted in larger lettering.

Each bottle of sterile water will be packaged with the spray bottles. These packets will be labeled with recommended storage conditions.

5.2 Study Agent Storage and Stability

The spray bottles of lyophilized *R mucosa* should be stored in the refrigerator while in the participant's possession (approximately 4°C). The bottles of sterile water can be stored at room temperature (approximately 23°C). In one study, the lyophilized Gram-negative bacteria *Enterococcus faecalis* can maintained viability for 20 years at room temperature.[39] We will instruct the participants to store their product at 4°C until immediately before use. Our supply will be stored at −70°C or below. We will consider a single-use spray bottle expired if it has been at > 4°C for more than 4 weeks, at > −70°C for more than 4 months, or if more than 1 year has

elapsed since lyophilization. The 4-month expiration date will be written on the label when removed from −70°C as will instructions to "store in refrigerator."

All microbe strains contained in the product will be obtained from the microbiota of healthy volunteers under SOPs approved under IND 16024. Briefly, we swabbed the antecubital fossa of a subject with 2 sterile swabs moistened with sterile saline. To isolate *R mucosa*, swabs were immediately placed in a sterile 15-mL conical tube containing 1-mL R2A broth (Teknova, Hollister, CA) supplemented with vancomycin and amphotericin. The tubes were then sealed with an airtight cap and carried back to Building 11 in a sealed secondary container. In our BSL2 laboratory in Building 33 and subsequently Building 11, each tube containing the antecubital fossa swab was vortexed for 30 seconds under a bacterial safety hood. To isolate the slower growing *R mucosa*, the R2A tube was incubated with swab in place for 48 hours at 32°C in an incubator used only for growth of *R mucosa*. After 48 hours, the swab was removed under sterile conditions, and a sample of the culture was Gram stained to verify growth of Gram-negative organisms. Further propagation of individual colonies occurs in 10 mL of R2A broth, and aliquots are stored at −80°C in sterile Microbank bead cryopreservation vials. Species-level identification of bacteria isolated by the above procedures is done by the clinical microbiology lab at the NIH Clinical Center using standard microbiological techniques and matrix-assisted laser desorption/ionization-based mass spectrometry.

Strains intended for use in this study will have additionally undergone full genomic sequencing, verified free of transferable antibiotic resistance genes, and tested for antibiotic treatment options in standard clinical resistance profiling. All microbes will be lyophilized and vialed in Building 10 of the NIH, Bethesda, MD. All procedures will be conducted in a research-grade good manufacturing practices facility.

All chosen strains will be rated at or below biosafety level 2 (ie, a strain that holds moderate potential to cause disease in humans). Organisms will be cultured in a biosafety cabinet that has been cleaned thoroughly with 70% ethanol prior to use. The organisms will be grown in appropriate tissue-culture–grade solution (eg, Hank's balanced salt solution). Organisms will be cultured in incubators that have been cleaned thoroughly with 70% ethanol prior to use. Microbes will be washed in sterile, clinical-grade isotonic saline before use and quantitated spectrophotometrically. A sample of each suspension will be plated to permit exact enumeration of CFU/mL, to be used for dose standardization following bacterial lyophilization. The remaining suspension will then be suspended in clinical-grade (D10), frozen to −20°C, and lyophilized in a lyophilizer that has been cleaned thoroughly with 70% ethanol prior to use.

Control of Excipients

The microbes will be suspended in sterile, clinical grade D10, or sterile, clinical grade 15% sucrose. No additional testing will be performed on the D10 before or after vialing. No other culture media will be used.

Control of Drug Product

The dose of viable bacteria will be empirically determined for each batch of product by plating a sample of each suspension to permit exact enumeration and comparison of CFU values versus pre-lyophilization values. Batches with less than 30% viability will be discarded. Colony purity will also be determined by evaluating the pre- and post-lyophilization cultures. Any batch with ≥ 1 colony with morphology different from that of the expected bacteria will be discarded. Post-lyophilization plates will be secondarily evaluated in the CC microbiology lab. A CC microbiology staff member will serve as a second evaluator, confirm 0 unexpected morphology CFU, and then sign off on the internally kept batch records. Discarded batches will also prompt thorough cleaning of all production equipment. Equipment will be verified as sterile by touch-plate methods prior to initiating production of subsequent batches.

5.3 Preparation, Administration, and Dosage of Study Agent

Participants (or a parent/legal guardian of minor participants) will open the top portion of the sprayer, separating the spray mechanism from the base. They will then empty the entire contents of the eye dropper bottle of sterile water into the sprayers of *R mucosa*. They will gently shake the sprayer to ensure that the solute is fully dissolved, and then prime the sprayer by pumping until at least 3 full mist sprays are produced. Then they will spray 4 pumps onto the affected area of the skin. They should then repeat the procedure on the opposite arm. The affected area should remain uncovered and undisturbed until the liquid dries. Because only a small volume (approximately 240 µL per arm) will be administered, it will dry quickly.

The study team will demonstrate preparation, administration, and storage of the study agent to the participant and parent/legal guardian before the first dose.

For both parts of the study, participants will be instructed to apply doses BIW with 2-day treatment windows separated by 2 days. The BIW dosing has been chosen due to the roughly 72-hour growth curve of the bacteria.[22] This will allow the administered dose time to proliferate and effect change. Dosing at shorter intervals may cause stacking of CFU, which could provide greater-than-intended amounts. For example, the first dose could be given on Monday or Tuesday and the second Friday or Saturday. If the 2-day treatment window is missed entirely, then the participant should note the missed dose on the memory aid and apply at the next scheduled window (eg, they should dose once on Friday/Saturday, but not make up the first dose

on Wednesday/Thursday or take 2 doses on Friday/Saturday). For part 2, the study team will tabulate total bacterial CFU given during the 4 months and will calculate to reflect missed doses.

Starting dose was determined by preclinical treatment models. Activity in mice was seen with an applied dose of 10^3-10^6 CFU, in a dose-dependent manner. This dose was applied to a mouse ear, which has a much smaller area than a human antecubital fossa. We anticipate the CGN will be better adept at proliferating on the skin of their natural human hosts and thus do not anticipate doses over 10^5 CFU will be necessary in humans.

5.4 Study Product Accountability Procedures

The NIH pharmacy will maintain accurate accountability records. Instructions and the required accountability documentation will be provided to the study pharmacist. When the study is completed, copies of the study drug accountability records will be returned to the sponsor, and the originals maintained at the study site. Copies of the drug accountability records must be maintained with the rest of the documentation for the study. All unused study agent must be disposed of upon authorization by the sponsor. All records regarding the disposition of study drug must be available for inspection by the study monitors and regulatory authorities.

5.5 Assessment of Participant Compliance with Study Agent

The study team will provide all participants with a memory aid with which to keep track of their doses. Unused and unopened materials will be returned. Unused materials with compromised sterility seals will be discarded.

5.6 Concomitant Medications and Procedures

All concomitant prescription and nonprescription (including over-the-counter) medications taken during study participation will be recorded. For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician.

5.7 Prohibited Medications and Procedures

Insertion of an indwelling catheter is prohibited during the duration of CGN therapy because of the small risk of infection.[28] Treatment with CGN will be terminated and not restarted if an indwelling catheter is inserted. Use of fluoroquinolone or aminoglycoside antibiotics is prohibited during the treatment phase. Use of these antibiotics can be resumed after treatment has been completed.

6 Study Schedule

All study visits will take place at the NIH CC. This study will be conducted in 2 parts. A table of the study schedules for both parts is presented in Appendix A.

6.1 Part 1: Safety Evaluation in Young Adults and Adults with AD

6.1.1 Screening

Informed consent will be obtained from all participants before any study procedure begins. After the informed consent document is signed, the study team will document the participant's medical history (including recent illness and medication use), conduct a physical exam with vital signs, and do a SCORAD assessment. The study team will draw blood for chemistry panels (acute, hepatic, and mineral), complete blood count (CBC) with differential, and enzyme-linked immunosorbent assay (ELISA) for HIV. Participants with a documented negative HIV test within the preceding 3 months will not need to undergo another test. Participants who can get pregnant will also undergo a pregnancy test. Screening visits will occur within 3 months of the baseline visit. Participants will be trained on proper administration of the medication before being asked to demonstrate the procedure for the study team. Any participant that fails to demonstrate correct procedures in 3 tries despite corrections will not be eligible for enrollment.

6.1.2 Baseline Visit/Beginning of Dosing

At the baseline visit, the study team will provide the participant with a 6-week supply of the *R mucosa* materials (see Section 5.1) with dosing instructions (see Section 5.3). Participants will be directed to apply the *R mucosa* solution BIW for 6 weeks; the initial dose is 10^3 CFU for the first 2 weeks, then 10^4 CFU for the next 2 weeks, and then 10^5 CFU for the final 2 weeks. Participants will communicate with the study team via text, email, or phone to assure there have been no AEs prior to each dose escalation. If any AEs are suggested, then the participant will be instructed to stop applying *R mucosa* and return to the clinic for evaluation. They may resume treatment if the study team determines that there was no AE, or the AE was unrelated to the study drug. They will resume where they left off in the dosing progression. Participants will be given a memory aid and symptom tracker with instructions for filling them out. A research blood sample will also be collected for storage of peripheral blood mononuclear cells. Participants will also have a SCORAD assessment and clinical photography.

Participants may contact the study team by phone, text, or email during the dosing period to discuss CGN-related AEs.

6.1.3 Final Study Visit

Participants will return for a final in-person study visit at 6 weeks ± 10 days after the beginning of dosing. Participants will undergo a physical exam, assessment of AEs, SCORAD assessment, clinical photography, and will turn in the completed memory aid and symptom tracker. We will perform 1 remote follow-up 30 days (± 10 days) after the week 6 visit by text, phone, or email for assessment of AEs. Participation in this study will be complete at the end of the week 10 remote visit.

6.2 Progression from Part 1 to Part 2

After all 10 participants in part 1 have completed their week 10 final follow-up, part 2 enrollment may begin unless:

- Two or more participants experienced the same or similar SAEs that were unexpected and possibly, probably, or definitely related to the study agent;
  OR
- Three or more of the same or similar AEs in different participants that were grade 3 or above and were unexpected and possibly, probably, or definitely related to the study agent;
  OR
- Any safety issue that the PI and/or the CSO determines should halt the study.

6.3 Part 2: Activity and Safety Evaluation in Children with AD

As described in Section 3.1, part 2 of this study is further subdivided into parts 2A and 2B. In 2A, 5 participants aged 7-17 years will be enrolled and start treatment at 10^3 CFU, with possible dose escalation to 10^4 CFU at week 4 and 10^5 CFU at week 8. If after 1 month of treatment at 10^5 CFU there are no safety signal concerns, then the study will proceed to part 2B, in which 34 participants aged 3-17 years will be enrolled and begin treatment at 10^5 CFU.

The study schedule described below applies to both parts 2A and 2B. The only differences between these parts are the minimum ages of participants and the doses and escalations.

6.3.1 Screening Visit

Informed consent from the minor participant's parent/legal guardian will be obtained before any study procedures begin. Participants will also provide their assent. The study team will collect and evaluate the participant's medical history, perform a physical exam with collection of vital signs, do a SCORAD assessment, and measure antecubital TEWL with a Velometers (Delfin Technologies Ltd [Kupio, Finland]). The same member of the study team who assesses the participant SCORAD will measure the TEWL. The study team will draw blood for chemistry

panels (acute, hepatic, and mineral) and CBC with differential. If the participant has any history of blood transfusion or if they are sexually active then blood will be collected for an ELISA for HIV. Participants with a documented negative HIV test within the preceding 6 months, or without history of transfusions or is not sexually active will not need to have an HIV test. Participants who have had their first period will also have a pregnancy test.

Screening visits will occur within 3 months of the baseline visit. Screening and baseline may be combined into a single visit for the convenience of participants' schedules.

6.3.2 Baseline Visit and First Dose

Once eligibility has been confirmed, the participants will undergo the following procedures at the baseline visit:

1. Medical history and physical exam with vital signs
2. Total body and antecubital-limited SCORAD
3. EASI scoring
4. Quality of life assessments with CDLQI and FDLQI
5. TEWL measurements of antecubital regions by VapoMeter
6. Swab of affected area to collect bacteria for species identification, DNA extraction for polymerase chain reaction (PCR) identification, microbiome analysis, and storage
7. Photography of affected area
8. Collection of venous blood for research and safety evaluations that include but are not limited to chemistry panels (acute, hepatic, and mineral), CBC with differential, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), serum vitamin D, total IgE
9. Collection of D-squame sampling. This is a non-invasive sampling method that removes the upper most layer of corneocytes. It has been validated in children with AD as young as 1 year of age[40]
10. Detailed environmental exposure questionnaire (see Appendix C)

The study team will then provide the participant with a 6-week supply of the *R mucosa* materials (see Section 5.1) with dosing instructions (see Section 5.3), a memory aid to facilitate proper dosing, and a scoring system for solicited AEs derived from the "Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events" (see Appendix B).

Treatment will begin within 1 week of the baseline visit. Participants and their parent/legal guardian will be instructed to apply the *R mucosa* solution (10^3 CFU for part 2A, 10^5 CFU for part 2B) BIW for 4 weeks (see Sections 5.1 and 5.3) to both antecubital fossae. The study team will contact the participant's parent/legal guardian once every 12 ± 4 days via phone/text or email to discuss the participant's response to treatment. Parents/legal guardians may send

photographs of the participant's affected area to the study team via mail, text, or email if they have questions or concerns.

6.3.3 Week 4 (± 10 days)

Participants will return to the NIH CC about 4 weeks after the beginning of treatment. They will bring any leftover dosing materials to return to the study team. At this visit, the participant will undergo the following assessments:

1. Medical history and physical exam with vital signs
2. Assessment of AEs
3. Review of memory aid
4. Detailed environmental exposure questionnaire (Appendix C)
5. Total body and antecubital-limited SCORAD
6. EASI scoring
7. Quality of life assessments with CDLQI and FDLQI
8. TEWL measurements of antecubital regions by VapoMeter
9. Swab of affected area to collect bacteria for species identification, DNA extraction for PCR identification, microbiome analysis, and storage
10. Photography of affected area
11. Research/safety blood draw

If the treatment is well tolerated and there is a noticeable improvement in the participant's AD, then the study team will provide them with another 6-week supply of *R mucosa* materials (4 weeks of dosing plus extra in case the next visit is delayed). Tolerance of therapy will be defined as a complete absence of SAEs and no Grade 2 or higher scores on the memory aide/symptom tracker. If the participant in part 2A tolerates treatment at 10^3 CFU BIW after 4 weeks but their AD has not substantially improved (ie, improvement in SCORAD and/or arm-specific SCORAD of less than 75% of baseline), then the dosage will be increased to 10^4 CFU BIW for 4 more weeks. Participants in part 2B will continue at 10^5 CFU BIW.

The study team will continue to contact the participant's parent/legal guardian on a weekly basis to discuss how the biotherapy affects the participant. Parents/legal guardians may send photographs of the participant's affected area to the study team via mail, text, or email.

6.3.4 Week 8 (± 10 days)

After about another 4 weeks of treatment (week 8), the participant will return to the NIH CC. The following assessments will be conducted:

1. Medical history and physical exam with vital signs
2. Assessment of AEs

3. Review of memory aid
4. Detailed environmental exposure questionnaire (Appendix C)
5. Total body and antecubital-limited SCORAD
6. EASI scoring
7. Quality of life assessments with CDLQI and FDLQI
8. TEWL measurements of antecubital regions by VapoMeter
9. Swab of affected area to collect bacteria for species identification, DNA extraction for PCR identification, microbiome analysis, and storage
10. Photography of affected area
11. Research/safety blood draw

If the treatment is well tolerated and there is a noticeable improvement in the participant's AD, then we will provide them with another 8-week supply of *R mucosa* materials at their current dose. If participants assigned to the dosage of 10^4 CFU BIW (part 2A) have tolerated the dosage after 4 weeks but their AD has not substantially improved, then the dosage will be increased to 10^5 CFU BIW for 4 weeks. Participants in part 2B will continue at 10^5 CFU BIW.

The study team will continue to contact the participant's parent/legal guardian on a weekly basis to discuss how the biotherapy affects the participant. Parents/legal guardians may send photographs of the participant's affected area to the study team via mail, text, or email.

6.3.5 Week 12 (± 10 days)

At around week 12, the participant will return to the NIH CC and undergo the following assessments:

1. Medical history and physical exam with vital signs
2. Assessment of AEs
3. Review of memory aid
4. Detailed environmental exposure questionnaire (Appendix C)
5. Total body and antecubital-limited SCORAD
6. EASI scoring
7. Quality of life assessments with CDLQI and FDLQI
8. TEWL measurements of antecubital regions by VapoMeter
9. Swab of affected area to collect bacteria for species identification, DNA extraction for PCR identification, microbiome analysis, and storage
10. Photography of affected area
11. Research/safety blood draw

If the participant tolerates the dosage of 10^5 CFU BIW (both parts 2A and 2B) at week 12 but does not show signs of improvement since they started this dosage, then the dosage will be

changed to 10^5 CFU once every other day for 4 weeks. If they are showing improvement, though, then they will continue at 10^5 CFU BIW for 4 more weeks. The study team will continue to contact the participant's parent/legal guardian on a weekly basis to discuss how the biotherapy affects the participant. Parents/legal guardians may send photographs of the participant's affected area to the study team via mail, text, or email.

6.3.6 Week 16 (± 10 days)

At around week 16, the participant will return to the NIH CC and undergo the following assessments:

1. Medical history and physical exam with vital signs
2. Assessment of AEs
3. Review of memory aid
4. Detailed environmental exposure questionnaire (Appendix C)
5. Total body and antecubital-limited SCORAD
6. EASI scoring
7. Quality of life assessments with CDLQI and FDLQI
8. TEWL measurements of antecubital regions by VapoMeter
9. Swab of affected area to collect bacteria for species identification, DNA extraction for PCR identification, microbiome analysis, and storage
10. Photography of affected area
11. Research/safety blood draw
12. Collection of D-squame sampling

Participants will not receive any more dosing materials after this visit.

6.3.7 Follow-up

The study team will remain in contact with participants and their parent/legal guardian for up to 1 year after treatment ends. Parents/legal guardians may take photographs of the participant's affected area and send them to the study team via mail, text, or email. Participants will visit the NIH CC once between 4-10 months after completion of treatment for SCORAD, EASI, QOL assessment, and bacterial swab collection. The purpose of this visit is to assess for bacterial colonization and any resultant/associated sustained benefit while off treatment.

A member of the study team will contact the participant and their parent/legal guardian by phone 1 year after completion of treatment to discuss the participant's long-term response. Study participation will end after this contact.

7 Potential Risks and Benefits

7.1 Potential Risks

***R mucosa* live biotherapy:** There is a risk of infection from *R mucosa*, including damage or discoloration of the skin and bacteremia. However, most reports in the literature are in patients with percutaneous catheters who are immunocompromised as a result of malignancies or other chronic conditions. Therefore, the risk of bacteremia in participants of this study is very low. Any participant with suspected infectious complication will be evaluated for the potential of *R mucosa* infection with blood cultures and local swabs. Any organism that is grown will undergo identification.

There is the possibility that this treatment will have no effect on AD so the condition may worsen. There may be additional risks of *R mucosa* live biotherapy that are currently unknown.

Genetic testing: Genetic testing, which may include whole genome, transcriptome, and/or exome sequencing, may be done on collected specimens in an effort to identify genetic components of these diseases and syndromes. Results of genetic testing may have psychological implications for participants such as revelations regarding future health risks, incurable conditions, and/or information contradictory to stated biological relationships. Genetics counseling and advice is available from the NIH to help participants with the implications of findings, where appropriate.

Further testing may be performed on gene variants that are discovered, principally those that are the focus of the main objective of this protocol that have unknown functions or that are believed to have health implications. Depending on how much information is known about a particular gene, such testing may take several years to complete. The results of this testing may require examination in other participants with immune disorders or may require additional tests to be performed on relatives, particularly parents and siblings. As new information becomes available, existing sequencing data may require reexamination. Genetic counseling and clinical genetics consultation is available at the NIH CC to help patients with the implications of findings, where appropriate.

Following genetic testing, the data will be shared in a controlled-access public database for other investigators to benefit from it (eg, dbGaP, the Database of Genotypes and Phenotypes). However no personal, identifiable information will be shared in this process as the results will be identified only by a code.

Blood draw: The risks of drawing blood include pain, bruising, bleeding, and, rarely, fainting or infection. The amount of blood drawn for research purposes will be within the limits allowed for pediatric research participants by the NIH CC (Medical Administrative Policy 95-9, Guidelines

for Limits of Blood Drawn for Research Purposes in the Clinical Center: http://cc-internal.cc.nih.gov/policies/PDF/M95-9.pdf). Participants will be offered a numbing cream to reduce the pain of the blood draw. Skin evaluations (ie, bacterial swabs, TEWL, photography, and visual assessments of AD) will be done before application of the cream.

D-squame tape-stripping: This process has been used in cohorts with AD as young as 1 year of age. There were no reported complications, although mild local irritation may be foreseeable.

Clinical photography: Taking pictures of the face and body may be embarrassing to some people. These photographs may be published in medical journals without identifying the participant. We will attempt to preserve the anonymity of the participant as much as possible, while providing the information needed to support the research being published. Participants may decline photographs or place any restrictions on their use. Participants will be given the opportunity to discuss this with the investigators.

7.2 Potential Benefits

Participants may not benefit from this study. There is the possibility that some participants may experience some degree of resolution of their condition. The results of this study may improve the investigators' understanding of live CGN biotherapy as treatment for AD and related conditions.

8 Research Use of Stored Human Samples, Specimens, or Data

Intended Use: Samples, specimens, and data collected under this protocol may be used to study the skin microbiome. Genetic testing will be performed. A separate signed informed consent document will be obtained for any other research not described in this protocol.

Storage: All of the stored study research samples are labeled by a code that only the investigators can link to the participant. Samples are stored in building 10, room 11S256, which is a secure, locked freezer with limited access. Data will be kept in password-protected computers. Only investigators or their designees will have access to the samples and data.

Tracking: Samples and data acquired under this protocol will be tracked on password-protected Microsoft Excel (Microsoft Corporation [Redmond, WA, USA]) files coded to matching sample boxes in laboratory freezers.

Disposition at the Completion of the Protocol:

- In the future, other investigators (both at NIH and outside) may wish to study these samples and/or data. Before any sharing of samples, data, or clinical information, either IRB approval must be obtained or the NIH Office of Human Subjects Research

Protections (OHSRP) must determine that the research is exempt from IRB oversight. OHSRP can make this determination for some research where the samples or data have no personal identifying information about the study participant and the researcher is not able to ascertain it.

- At the time of protocol termination, samples will either be destroyed, or after IRB approval, transferred to another existing protocol. Data will be archived by the study team in compliance with requirements for retention of research records; alternatively, after IRB approval, the data may be either destroyed or transferred to another repository.

Reporting the Loss or Destruction of Samples/Specimens/Data to the IRB:

- Any loss or unanticipated destruction of samples or data (for example, due to freezer malfunction) or data (for example, misplacing a printout of data with identifiers) that meets the definition of protocol deviation, unanticipated problem (UP), and/or compromises the scientific integrity of the data collected for the study will be reported to the NIAID IRB.
- Additionally, participants may decide at any point not to have their samples stored. In this case, the PI will destroy all known remaining samples and report what was done to both the participant and to the IRB. This decision will not affect the participant's participation in this protocol or any other protocols at NIH.

9 Data Sharing Plan

Human data generated in this study for future research will be shared as follows:

- Any genetic data generated in the study will be de-identified and deposited in an NIH-funded or approved public repository such as the NCI Genomic Data Commons.
- Identified data in the Biomedical Translational Research Information System (BTRIS, automatic for activities in the NIH CC).
- De-identified or identified data with approved outside collaborators under appropriate agreements.
- Data sharing may be complicated or limited in certain cases by contractual obligations or agreements with outside collaborators, such as Cooperative Research and Development Agreements, Clinical Trial Agreements, other restraints, etc.

Data will be shared before publication.

10 Remuneration Plan for Participants

Participants will be compensated for travel according to the NIAID/NIH travel policy. There will be no remuneration provided for screening visits. Once participants are enrolled in the study,

there will be a remuneration of $20 for each in-office study visit as compensation for the participants' time and inconvenience. For part 1, these visits are baseline and the final visit, for a maximum total of $40. For part 2, these visits are baseline, weeks 4, 8, 12, and 16, and the post-treatment follow-ups every 4-6 months for up to 1 year, for a maximum total of $160.

11 Assessment of Safety

11.1 Definitions

Adverse Event: An AE is any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (eg, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the research.

Adverse Reaction (AR): An AE that is caused by an investigational agent (drug or biologic).

Suspected Adverse Reaction (SAR): An AE for which there is a reasonable possibility that the investigational agent caused the AE. "Reasonable possibility" means that there is evidence to suggest a causal relationship between the drug and the AE. An SAR implies a lesser degree of certainty about causality than AR, which implies a high degree of certainty.

Serious Adverse Event (SAE): An SAE is an AE that results in 1 or more of the following outcomes:

- Death
- A life-threatening event (places the subject at immediate risk of death from the event as it occurred)
- An inpatient hospitalization or prolongation of an existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- A medically important event. Medical and scientific judgment should be exercised in deciding events that may not be immediately life threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed above.

Unexpected Adverse Event: An AE is unexpected if it is not listed in the Investigator's Brochure or Package Insert (for marketed products) or is not listed at the specificity or severity that has been observed. It is the responsibility of the IND sponsor to make this determination.

Serious and Unexpected Suspected Adverse Reaction (SUSAR): A SUSAR is an SAR that is both serious and unexpected.

Unanticipated Problem (UP): Any incident, experience, or outcome that meets all 3 of the following criteria:

1. unexpected in terms of nature, severity, or frequency in relation to
   a. the research risks that are described in the IRB-approved research protocol and informed consent document, Investigator's Brochure, or other study documents; and
   b. the characteristics of the subject population being studied
2. related or possibly related to participation in the research
3. suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Serious Unanticipated Problem: A UP that meets the definition of an SAE or compromises the safety, welfare, or rights of subjects or others.

Unanticipated Problem that is not an Adverse Event (UPnonAE): A UP that does not fit the definition of an AE, but which may, in the opinion of the investigator, involve risk to the subject, affect others in the research study, or significantly impact the integrity of research data. Such events would be considered non-serious UPs. For example, we will report occurrences of breaches of confidentiality, accidental destruction of study records, or unaccounted-for study drug.

11.2 Documenting, Recording, and Reporting Adverse Events

All AEs related to the study agent and research procedures that occur from the time the treatment has initiated through the end of follow-up evaluation (for part 1) or through the end of the 1-year safety follow-up period (for part 2) will be documented, recorded, and reported.

At each contact with the subject, information regarding AEs will be elicited by appropriate questioning and examinations and will be:

- immediately documented in the subject's medical record/source document,
- recorded in the Clinical Research Information Management System of the NIAID (CRIMSON), and
- reported as outlined below (eg, IND sponsor, IRB, and FDA).

If a diagnosis is clinically evident (or subsequently determined), the diagnosis rather than the individual signs and symptoms or lab abnormalities will be recorded as the AE.

A laboratory abnormality will not be reported as an AE if ALL of the following criteria are met:

- It is no more than "grade 1" or "mild" per the protocol-specified toxicity table (or investigator assessment if not listed on the table); AND
- It does NOT require an intervention (eg, discontinuation of treatment, dose reduction/delay, additional assessments, or treatment); AND
- It is assessed by the PI as NOT related to the study agent(s); AND
- It is assessed by the PI as NOT clinically significant (eg, the abnormal value does NOT suggest a disease or organ toxicity).

All abnormal laboratory findings will be reviewed on a routine basis by the PI to identify potential safety signals. Abnormal labs that do meet all of the above criteria will still be recorded in the clinic or phone note documentation. An abnormal lab not included on the toxicity table should be assessed in a similar fashion to the criteria above.

11.3 Investigator Assessment of Adverse Events

The investigator will assess all AEs with respect to seriousness (criteria listed above), severity (intensity or grade), and causality (relationship to study agent and relationship to research) according to the following guidelines.

11.3.1 Severity

The investigator will grade the severity of each AE according to DAIDS Version 2.0, November 2014, which can be found at: http://rsc.tech-res.com/document/safetyandpharmacovigilance/daids_ae_grading_table_v2_nov2014.pdf

Some grade 1 lab parameters on the DAIDS Toxicity Table (fibrinogen, magnesium [low], potassium [low], uric acid [males only, elevated]) fall within the NIH lab reference range for normal values. These normal values will not be reported as grade 1 AEs. The grade 1 values for these tests will be reported as follows:

- Fibrinogen: 100-176 mg/dL
- Magnesium (low): 0.60-0.64 mmol/L
- Potassium (low): 3.0-3.3 mmol/L
- Uric acid (males): 8.7-10.0 mg/dL

11.3.2 Causality

Causality (likelihood that the event is caused by the study agent) will be assessed considering the factors listed under the following categories:

Definitely Related

- reasonable temporal relationship
- follows a known response pattern
- clear evidence to suggest a causal relationship
- there is no alternative etiology

Probably Related

- reasonable temporal relationship
- follows a suspected response pattern (based on similar agents)
- no evidence of a more likely alternative etiology

Possibly Related

- reasonable temporal relationship
- little evidence for a more likely alternative etiology

Unlikely Related

- does not have a reasonable temporal relationship
  OR
- good evidence for a more likely alternative etiology

Not Related

- does not have a temporal relationship
  OR
- definitely due to an alternative etiology

Note: Other factors should also be considered for each causality category when appropriate. Causality assessment is based on available information at the time of the assessment of the AE. The investigator may revise the causality assessment as additional information becomes available.

11.4 Investigator Reporting Responsibilities to the Sponsor

11.4.1 Adverse Events

AE data will be submitted to the IND sponsor when requested for periodic safety assessments, review of IND annual reports, review of IND safety reports, and preparation of final study

reports. Given the baseline pathology in this patient population, DAIDS grade I dermatologic findings will not be reported if the finding pre-dates the study onset.

11.4.2 Serious Adverse Events

All SAEs (regardless of relationship and whether or not they are also UPs) must be reported on the Safety Expedited Report Form (SERF) and sent to CSO by fax or e-mail attachment. Deaths and immediately life threatening SAEs must be reported to the CSO within 1 business day after the site becomes aware of the event. All other SAEs must be reported within 3 business days of site awareness.

CSO CONTACT INFORMATION:

Clinical Safety Office
5705 Industry Lane
Frederick, MD 21704
Phone: 301-846-5301
Fax: 301-846-6224
E-mail: rchspsafety@mail.nih.gov

SAEs that have not resolved by the end of the follow-up period are followed until final outcome is known. If it is not possible to obtain a final outcome for an SAE (eg, the participant is lost to follow-up), then the reason a final outcome could not be obtained will be recorded by the investigator on CRIMSON and the SERF.

SAEs that occur after the 1-year safety follow-up period that are reported to and are assessed by the investigator to be possibly, probably, or definitely related to study drug must be reported to the CSO.

11.4.3 Unanticipated Problems

UPs that are also AEs must be reported to the CSO by fax or e-mail attachment using the NIH Problem Report Form no later than 7 calendar days of site awareness of the event. UPs that are not AEs are not reported to the CSO.

11.4.4 Pregnancy

All pregnancies will be reported on the Pregnancy Notification/Outcome Form to the CSO within 1 business day from site awareness.

Pregnancy outcome data (eg, delivery outcome, spontaneous or elective termination of the pregnancy) will be reported to the CSO within 3 business days of the site's awareness.

Although pregnancy itself is not an AE, events that meet SAE criteria during pregnancy, delivery, or in the neonate (eg, congenital anomaly/birth defect) are reportable on the SERF.

In the event of pregnancy, the following steps will be taken:

- Discontinue the study agent and procedures but continue to follow-up for safety.
- Report to the independent safety monitor (ISM) and IRB.
- Advise research participant to notify the obstetrician of study participation and study agent exposure.

11.5 Investigator Reporting Procedures to the NIAID IRB

11.5.1 Definitions

Protocol Deviation: Any change, divergence, or departure from the IRB-approved study procedures in a research protocol. Protocol deviations are designated as serious or non-serious and further characterized as:

1. Those that occur because a member of the research team deviates from the protocol.
2. Those that are identified before they occur, but cannot be prevented.
3. Those that are discovered after they occur.

Serious Protocol Deviation: A deviation that meets the definition of an SAE or compromises the safety, welfare, or rights of subjects or others.

Non-compliance: The failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human subjects. Non-compliance is further characterized as:

1. Serious: non-compliance that:
   a. increases risks, or causes harm, to participants;
   b. decreases potential benefits to participants;
   c. compromises the integrity of the NIH HRPP; or
   d. invalidates the study data.
2. Continuing: non-compliance that is recurring.
3. Minor: non-compliance that is neither serious nor continuing.

11.5.2 Expedited Reporting to the NIAID IRB

Serious and non-serious UPs, deaths, serious deviations, and serious or continuing non-compliance will be reported within 7 calendar days of investigator awareness. SAEs that are

possibly, probably, or definitely related to the research will be reported to the NIAID IRB within 7 calendar days of investigator's awareness, regardless of expectedness.

11.5.3 Waiver of Reporting Anticipated Protocol Deviations, Expected UPnonAEs, and Deaths to the NIAID IRB

Anticipated deviations in the conduct of the protocol will not be reported to the IRB unless they occur at a rate greater than anticipated by the study team. Deaths unrelated to any research procedures will be reported at the time of continuing review.

11.5.4 Annual Reporting to the NIAID IRB

The following items will be reported to the NIAID IRB in summary at the time of continuing review:

- Serious and non-serious UPs.
- SAEs that are possibly, probably, or definitely related to the research.
- SAEs that are not related to the research.
- Serious and non-serious protocol deviations.
- Serious, continuing, and minor non-compliance.
- Any trends or events which in the opinion of the investigator should be reported.

11.6 Sponsor's Reporting Responsibilities

SUSARs, as defined in Title 21 of the United States Code of Federal Regulations (CFR) Part 312.32 and determined by the IND sponsor, will be reported to FDA and all participating investigators as IND Safety Reports.

The IND sponsor will also submit an IND Annual Report of the progress of the investigation to the FDA as defined in 21 CFR 312.33.

11.7 Pausing Rules for an Individual Participant

Pausing is the suspension of administration of study agent to a single participant until a decision is made whether or not to resume administration of the study agent.

The pausing criteria for a single participant in this study include any of the following:

- A participant experiences an SAE that is unexpected and possibly, probably, or definitely related to a study agent;
- A participant experiences 2 grade 3 or greater AEs that are unexpected and possibly, probably, or definitely related to a study agent;

- Any safety issue that the site investigator determines should pause administration of a study agent to a single participant.

The CSO, in collaboration with the PI, may also pause for an entire group if a safety concern is identified.

11.7.1 Reporting a Pause

If a pausing criterion is met, a description of the AE(s) or safety issue must be reported by the PI within 1 business day to the CSO and the IRB by fax or email.

11.7.2 Resumption of a Paused Study

The CSO, in collaboration with the PI, will determine whether or not it is safe to resume administration of the study agent to the participant. The PI will notify the IRB of the decision on resumption of the study agent.

11.7.3 Discontinuation of Study Agent

A participant who does not resume study agent will continue to be followed for safety.

11.8 Halting Rules for the Protocol

Halting the study requires immediate discontinuation of study agent administered for all participants and suspension of enrollment until a decision is made whether or not to continue enrollment and study agent administration. These rules apply to both part 1 and part 2.

The halting rules are:

1. One participant experiencing any SAEs that are possibly, probably, or definitely related to the study agent;
   OR
2. Three or more of the same or similar AEs in the cohort of participants that are grade 3 or above and are unexpected and possibly, probably, or definitely related to the study agent;
   OR
3. any safety issue that the PI and/or the CSO determines should halt the study.

For the cohort aged 3-6 years, additional rules will apply. Enrollment of children aged 3-6 will halt if:

1. One participant experiencing any SAEs that are possibly, probably, or definitely related to the study agent;
   OR

2. One or more AE in the cohort of participants that are grade 3 or above and are unexpected and possibly, probably, or definitely related to the study agent;
   OR
3. Any safety issue that the PI and/or the CSO determines younger enrollment should halt.

The study may continue with participants older than 7 years, and thus two additional same or similar AEs will cause full halting of the study. Children aged 3-6 that are withdrawn from the study due to any AE in their age cohort will be replaced with children aged 7 and older.

The PI and/or CSO will determine if the study should be halted. In addition, the FDA or IRB may halt the study at any time following review of any safety concerns. The ISM may also recommend a study halt.

11.8.1 Reporting a Study Halt

If a halting rule is met, then a description of the AE(s) or safety issue must be reported by the PI within 1 business day to the CSO and the IRB by fax or email.

11.8.2 Resumption of a Halted Study

The IND sponsor, in collaboration with the PI and any specified safety review team will determine if it is safe to resume the study. The PI will notify the IRB of the decision on resumption of the study.

11.8.3 Discontinuation of Study Agent

Participants who do not resume study agent will continue to be followed for safety.

11.9 Withdrawal Criteria for an Individual Participant

An individual participant will be withdrawn for any of the following:

- An individual participant's decision or the decision of the participant's parent/legal guardian. (The investigator should attempt to determine the reason for the decision.)
- Non-compliance with study procedures to the extent that it is potentially harmful to the participant or to the integrity of the study data.
- A participant becomes pregnant.
- A participant develops a health problem (eg, diagnosis of cancer) that contraindicates their participation in this research study.
- Insertion of any indwelling catheter in the participant or their in-home family members.
- The investigator determines that continued participation in the study would not be in the best interest of the participant.

Children with AD who withdraw from the study before completion of treatment will be followed for 1 calendar year after withdrawal.

11.9.1 Replacement of Withdrawn Participant or Participants Who Discontinue Study Agent

Participants who withdraw from the study will not be replaced. If a participant is withdrawn, then all the data collected from that participant will still be included in analysis.

11.10 Safety Oversight

11.10.1 Safety Review and Communications Plan

A Safety Review and Communications Plan (SRCP) has been developed for the protocol. The SRCP is an internal communications document between the PI and the CSO, which delineates the safety oversight responsibilities of the PI, the CSO, and other stakeholders. The SRCP also includes the overall plan for conducting periodic safety surveillance assessments.

11.10.2 Sponsor Medical Monitor

A medical monitor, representing the IND sponsor (OCRPRO), has been appointed for oversight of safety in this clinical study. The Sponsor Medical Monitor will be responsible for performing safety assessments as outlined in an SRCP.

11.10.3 Independent Safety Monitor

The independent expert does not have direct involvement in the conduct of the study and has no significant conflicts of interests as defined by NIAID policy. An ISM, Hirsh Komarow, MD (staff clinician, Lab of Allergic Diseases) will review the study prior to initiation and at a minimum of once per month while any participant is undergoing treatment, then every 3-4 months thereafter, or as specified by the ISM. The ISM will conduct independent safety monitoring and recommend appropriate action regarding AEs and other safety issues. The ISM is an expert in the field of allergy/immunology including AD.

Prior to each ISM review, the PI will provide photos, SCORAD values, QOL values, lab reports, and AE reports. After each ISM review, a recommendation as to whether the study is to continue as is, be modified, or be terminated will be provided to the PI. All SAEs, all UPs, and all IND Safety Reports will be reported by the PI to the ISM at the same time they are submitted to the IRB or IND sponsor. The ISM will be notified immediately if any pausing or halting rule is met, and the ISM will provide a recommendation for continuation, modification, or termination of the study. The PI will submit the written ISM summary report with recommendations to the IRBs.

12 Site Monitoring Plan

According to the International Council on Harmonisation (ICH) E6(R1), Good Clinical Practice (GCP) guidelines, section 5.18, and 21 CFR 312.50, clinical protocols are required to be adequately monitored by the study sponsor. This study monitoring will be conducted according to the "NIAID Intramural Clinical Monitoring Guidelines." Monitors under contract to OCRPRO/NIAID will visit the clinical research site to monitor aspects of the study in accordance with the appropriate regulations and the approved protocol. The objectives of a monitoring visit will be: 1) to verify the existence of signed informed consent documents and documentation of the consent process for each monitored participant; 2) to verify the prompt and accurate recording of all monitored data points, and prompt reporting of all SAEs; 3) to compare CRIMSON data abstracts with individual participants' records and source documents (participants' charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original participant information); and 4) to help ensure investigators are in compliance with the protocol. The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements (Office for Human Research Protections [OHRP]), FDA, and applicable guidelines (ICH GCP) are being followed. During the monitoring visits, the investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit.

The investigator (and/or designee) will make study documents (eg, consent forms, CRIMSON data abstracts) and pertinent hospital or clinical records readily available for inspection by the IRB, the FDA, the site monitors, and the NIAID staff for confirmation of the study data.

A specific protocol monitoring plan will be discussed with the PI and study staff before enrollment. The plan will outline the frequency of monitoring visits based on such factors as study enrollment, data collection status, and regulatory obligations.

13 Statistical Considerations

Thirty-nine minor participants with AD will be enrolled into part 2 of the study, treated for 4 months, and followed for 1 year. If at the end of the end of the treatment phase, 5 successes are observed (out of 39 particpants) then the treatment would be considered to be active enough for further study.

A success will be defined as at least a 50% reduction in arm-specific SCORAD or total SCORAD during the 4-month period. SCORAD is a well-validated clinical tool and the standard of care for AD assessment. Previous studies on current treatments in AD have similarly targeted the 50% improvement over a similar time span.[41-43] The natural variation in SCORAD can be gleaned from placebo control data and is approximately 5%-30% over studies lasting 1-12 months.[37,44-46] Therefore, a 50% reduction would strongly suggest treatment caution (see

statistical considerations in Section 13 for more detail). **Safety will be characterized based on the frequency of solicited and unsolicited adverse events, SAEs and deaths.**

Standard SCORAD values are calculated by first documenting the areas involved. Head and neck (9%), arms (9% each), legs (18% each), anterior trunk (18%), back (18%), and genitals (1%) for a possible total area score of 100% (the A value in Figure 6). Intensity (B value) is calculated by taking a representative area and scoring redness, swelling, oozing, scratch marks, lichenification (skin thickening), and dryness on a scale enumerated as none (0), mild (1), moderate (2), or severe (3) for a maximal possible value of 18. Lastly, subjective symptoms (C value) are scored on a 0-10 scale for itching and sleep disturbance from the disease (maximal score of 20). Overall score is calculated by the equation A/5 + 7B/2 + C.

Antecubital SCORAD would exclude the A value and focus only on the antecubital area. The antecubital area would be scored for the standard intensity markers for a possible total of 18. This will be added to the subjective itching score (for the antecubital area only) in C as 0-10. Therefore the total possible score for the antecubital SCORAD will be 28.

SCORAD and antecubital SCORAD will be performed independently by 1 primary or associate investigator and 1 research assistant. Scores will be compared for consistency. Further validation will be performed by scoring the intensity of disease depicted in the clinical photographs, which will be blinded for analysis (ie, by individuals who do not know the patients' clinical history or management program). Furthermore, the 50% reduction threshold will apply to the full duration of treatment (ie, change from baseline to 4 month visit score). A 50% reduction in SCORAD at earlier time points that is not sustained over the treatment course will not be considered a success (ie, a 50% reduction from baseline at the second month visit that rebounds to only a 25% reduction from baseline by end of treatment will be considered a treatment failure).

SCORAD is a well-validated clinical tool and the standard of care for AD assessment. Previous studies on current treatments in AD have similarly targeted the 50% improvement over a similar time span.[41-43] The natural variation in SCORAD can be gleaned from placebo control data and is approximately 5%-30% over studies lasting 1-12 months.[37,44-46] Table 3 shows these results. In the placebo arm of 2 studies, the maximum change in SCORAD measured at 4 and 4.5 months was a reduction of 27% and an increase of 1.3%. For other placebo arms, the maximum change was given at timepoints different than this study but the results are still informative. In one study, the maximum reduction was 18% (measured at 6 months) and 40% (measured at 1 month). An observational study had a maximal reduction of 4% measured at 8 months. These data indicate that a 50% reduction at 4 months would be sufficiently large to be considered a success.

Table 3 List of SCORAD Studies

Citation	Treatment	Maximum Change in SCORAD	Time Course
Leloup 2015[41]	Wet wraps and steroids (N = 17)	−54%	4 months
Neuber 2000[42]	Mycophenolate (N = 10)	−64%	3 months
Msika 2008[43]	Topical Steroid (N = 86)	−58%	1 month
Panjo 2007[44]	Placebo (N = 28)	−18%	6 months
	Sub-lingual immunotherapy (N = 28)	−45%	
Janmohamed 2014[45]	Placebo (N = 20)	−40%	1 month
	Wet wraps (N = 19)	−67%	
Weston 2005[37]	Placebo (N = 17)	−27%	4 months
	Oral Probiotics (N = 17)	−42%	
Rosenfeldt 2003[46]	Placebo (N = 20)	+1.3%	4.5 months
	Oral Probiotics (N = 23)	−6%	
Kramer 2005[47]	None (Seasonal evaluation) (N = 1673)	−4%	8 months

The natural history of AD suggests that 50%-90% of patents will self-resolve by approximately 12-15 years of age.[31-33] The pace at which a cohort would be expected to outgrow their disease is approximately 5% per year based on the previous reports.[31-33] Thus we would expect no more than 2 of our cohort to outgrow their disease over the course of a year. However, we are focusing on only the 4-month treatment period, and thus only 1.67% of a cohort would be expected to self-resolve (> 50% improvement) during the 4-month timeframe.

13.1 Study Hypotheses

Treatment with CGN will increase the proportion of patients who have reduced SCORAD levels and antecubital SCORAD levels after 4 months of treatment. Treatment will not be associated with AEs.

13.2 Sample Size Justification

Escalation portion in adults

With 10 patients in the escalation portion, there is a probability 0.1 of observing 1 or more SAEs or Grade 2 or higher scores if the true rate is 1% and a probability of 0.8 of observing 1 or more if the true rate is 15%.

Initial 5 pediatric participants aged 7-17

For the initial 5 pediatric participants (7-17), there is a probability 0.1 of observing 1 or more SAEs or Grade 2 or higher scores if the true rate is 2% and a probability of 0.8 of observing 1 or more if the true rate is 27%.

Overall (39) pediatric participants

Active: With 39 participants, if there are 5 successes observed at the end of the study then there is a 0.9 probability (90% power) of concluding the new treatment is active if the true activity rate is 0.2 and there is a 0.1 probability (significance level of 0.1) of concluding the treatment is active if the true activity rate is 0.05. These calculations are based on the binomial distribution.

Safety: For halting rule 1 of the overall pediatric cohort halting rules with 39 participants, there is a probability of 0.05 of observing one SAE if the true rate is 0.13% and a probability of at least 0.95 of observing at least 1 SAE if the true rate is 7.5%.

For halting rule 2 of the overall pediatric cohort halting rules, with 39 participants there is a probability of 0.043 of observing 3 or more AEs if the true rate is 2% and a probability of 0.95 if the true rate is 15%.

13.3 Description of the Analyses

- Safety Analysis:
  1. Dose-escalation portion (Part 1). If no study agent–related SAEs are observed and no Grade 2 or higher scores on the memory aid/symptoms tracker are observed then the therapy will be considered tolerable.
  2. Activity portion (Part 2). If 1 or more study agent–related SAEs or 2 or more study agent−related AEs are observed then changes in the treatment would be considered due to safety issues.
- Activity Analysis:
  - If 5 or more successes out of 39 patients are observed then the treatment will be considered to be active enough for further study. This criterion has a 0.1 level of

significance if the true activity rate is 0.05. This calculation is based on the exact binomial distribution.

13.4 Final Analysis Plan

- Study Endpoints:
  - Primary:
    - Definition: A success will be defined as a 50% reduction in total-body SCORAD or antecubital-specific SCORAD during the 12-week treatment period
    - Validity and Reliability: SCORAD is a well-validated clinical tool and the standard of care for AD assessment.
    - AEs will be collected
  - Secondary: A success will be defined as a 30% improvement in quality of life as measured by IDQOL or CDLQI, and FDLQI.
  - Exploratory:
    - A 20% reduction in TEWL during the 12-week treatment period
    - A 20% reduction in total serum IgE during the 12-week treatment period
    - Any significant differences in the incidence of atopic disease such as allergy rhinitis or allergic asthma during the course of treatment and follow-up
    - New diagnosis of skin infection and/or *S aureus* skin infection requiring antibiotics during the course of treatment and follow-up

Handling of Missing and Spurious Data: Since this is a phase I/II study, participants who do not receive any study drug will be replaced. All participants that receive any level of treatment will be included, and not replaced; their analysis will be performed evaluating total CFU/weeks of therapy received. However, participants that withdraw or are lost will be assessed as failures or successes based on total response. Thus, a participant that withdraws after only 8 weeks of treatment with < 50% improvement will be documented as a treatment failure. Participants who cannot be evaluated due to toxicities or who drop out of the study will be counted as study failures. A secondary sensitivity analysis will exclude participants who did not receive at least 4 weeks of study drug. The exact binomial test will be performed at the 0.1 level of significance.

14 Ethics/Protection of Human Participants

14.1 Informed Consent Process

Informed consent is a process where information is presented to enable persons to voluntarily decide whether or not to participate as a research subject. It is an ongoing conversation between the human research participant and the researchers that begins before consent is given and continues until the end of the participant's involvement in the research. Discussions about the

research will provide essential information about the study and include: purpose, duration, experimental procedures, alternatives, risks, and benefits. Participants will be given the opportunity to ask questions and have them answered.

The participants will sign the informed consent document before undergoing any research procedures. The participants may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the participants for their records. The researcher will document the signing of the consent form in the participant's medical record. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

14.1.1 Non–English-Speaking Participants

If a non–English-speaking participant is unexpectedly eligible for enrollment, then the participant will be provided with the CC Short Written Consent Form for Non–English-Speaking Research Participants in the participant's native language and a verbal explanation of the purpose, procedures, and risks of the study as described in Medical Administrative Series Policy M77-2, NIH HRPP SOP 12, and 45 CFR 46.117(b)(2). The IRB-approved English consent form will serve as basis for the verbal explanation of the study. The investigator will obtain an interpreter unless the investigator is fluent in the prospective participant's language. Preferably, the interpreter will be someone who is independent of the participant (ie, not a family member). Interpreters provided by the CC will be used whenever possible. The interpreter will interpret all oral communications (English to target language and conversely) between the investigator and a limited English-proficient participant, facilitate discussions, and clarify information as necessary.

The IRB-approved English consent form will be signed by the investigator obtaining consent and a witness to the oral presentation. The CC Short Written Consent Form will be signed by the participant and a witness who observed the presentation of information. The interpreter may sign the consent document as the witness and, in this case, will note "Interpreter" under the signature line. A copy of both signed forms will be provided to the participant to take home.

The investigator obtaining consent will document the consent process in the participant's medical record (CRIMSON), including the name of the interpreter. Further, all instances of use of the CC Short Written Consent Form will be reported to the IRB at the time of annual review. If the CC Short Written Consent Form is used 3 times or more for the same language, this will be reported to the IRB immediately.

14.1.2 Assent Process

If the participant is younger than 18 years of age and older than 7 years, then they will be included along with their parent(s)/legal guardian(s) in discussions about the study. Their written

assent will be obtained if the child has the maturity and psychological ability to give assent, as judged by the study member obtaining consent. The parent(s)/legal guardian(s) will provide permission for the minor participant to participate by signing the consent form. Participants that turn 7 years-old during the course of treatment will sign assent at their first visit after their seventh birthday. Participants that turn 7 years-old after completion of treatment but before the end of study participation will not sign assent.

14.2 Participant Confidentiality

All records will be kept confidential to the extent provided by federal, state, and local law. The study monitors and other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records. Records will be kept locked and all computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the participant, except as necessary for monitoring by IRB, the FDA, NIAID, OHRP, or the sponsor's designee.

15 Data Handling and Record Keeping

15.1 Data Capture and Management

Study data will be maintained in CRIMSON and collected directly from participants during study visits and telephone calls, or will be abstracted from participants' medical records. Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary to confirm the data abstracted for this study. Data entry into CRIMSON will be performed by authorized individuals. The investigator is responsible for assuring that the data collected are complete, accurate, and recorded in a timely manner.

15.2 Record Retention

The investigator is responsible for retaining all essential documents listed in the ICH GCP guidelines. The PI will maintain study records according to the timelines specified in 21 CFR 312.62 or at a minimum of 5 to 7 years and in compliance with institutional, IRB, state, and federal medical records retention requirements, whichever is longest. All stored records will be kept confidential to the extent required by federal, state, and local law.

Should the investigator wish to assign the study records to another party and/or move them to another location, the investigator will provide written notification of such intent to OCRPRO/NIAID with the name of the person who will accept responsibility for the transferred records and/or their new location. Destruction or relocation of research records will not proceed without written permission from OCRPRO/NIAID.

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Appendix A Schedule of Procedures for Adults and Children with Atopic Dermatitis

PART 1

Study Schedule (Weeks)						
	Screening[a]	Baseline[a]	2[b]	4[b]	6[a] (± 10 days)	10[b] (± 10 days)
Physical exam with vital signs	X	X			X	
Medical history	X	X				
Informed consent	X					
Screening labs blood draw[c]	X					
Research blood draw[d]		X				
SCORAD	X	X			X	
Clinical photography		X			X	
Dose escalation			X[e]	X[f]		
Provision of dosing materials[h]		X				
Assessment of AEs			X	X	X	X

AE = adverse event; SCORAD = Severity Scoring of Atopic Dermatitis.

a Study visits to the NIH Clinical Center (CC).

b Procedures will be conducted remotely (via phone/text or email).

c Chemistry panels (acute, hepatic, mineral), complete blood count (CBC) with differential, HIV test (if not documented negative within last 3 months), and pregnancy test (if of childbearing potential).

d For storage of peripheral blood mononuclear cells.

e Escalation to 10^4 CFU.

f Escalation to 10^5 CFU.

h Materials are sterile sprayers pre-filled with lyophilized *R mucosa* (10^3, 10^4, and 10^5 CFU), sterile eye droppers filled with sterile water (1.5 mL), illustrated dosing instruction booklet, and memory aid/symptom card.

PART 2

Study Schedule (Weeks)											
	Screening	Baseline[a]	1-3[b]	4 (± 10 days)	5-7[b]	8 (± 10 days)	9-11[b]	12 (± 10 days)	13-15[b]	16 (± 10 days)	Follow-up[c]
Physical exam with vital signs	X	X		X		X		X		X	X
Medical history	X	X		X		X		X		X	X
Informed consent	X										
Screening labs blood draw[d]	X										
Pregnancy test[e]	X										
TEWL	X	X		X		X		X		X	X
Total body and antecubital-limited SCORAD, EASI	X	X		X		X		X		X	X
Research/safety blood draw[f]		X		X		X[g]		X[g]		X	
Skin swab		X		X		X		X		X	X
Photography		X		X		X		X		X	X
Dose escalation[h]				X		X		X			
Provision of dosing materials[i]		X		X		X				X[j]	
Assessment of AEs			X	X	X	X	X	X	X	X	
Review of memory aid				X		X		X		X	
Environmental exposure questionnaire		X		X		X		X		X	
Quality of life assessments[j]		X		X		X		X		X	X
D-squame sampling		X								X	

AE = adverse event; EASI = Eczema Area and Severity Index; SCORAD = Severity Scoring of Atopic Dermatitis; TEWL = transepidermal water loss.

a If scheduling permits, then screening and baseline may be done on the same day. Any procedures set for both visits will not be duplicated.

b The study team will contact the participant every 12 ± 4 days to assess AEs. Contact will be by phone, text, or email.

c Follow-up will continue for 1 year after treatment ends. Participants will visit the NIH CC once between 4 to 10 months post-treatment.

d Chemistry panels (acute, hepatic, mineral), complete blood count (CBC) with differential, and HIV test (only if sexually active or has history of blood transfusions and does not have documented negative test within last 6 months).

e Urine or serum. Only if participant has had their first period and is sexually active.

f Chemistry panels (acute, hepatic, mineral), CBC with differential, erythrocyte sedimentation rate, C-reactive protein, serum vitamin D, and total IgE as clinically indicated. These may be collected at screening to avoid repeat blood draws on pediatric populations.

g Research/safety blood draw will not be conducted if the dosage was escalated at weeks 4 or 8.

h For part 2A, if the participant tolerates treatment at 10^3 CFU BIW (at week 4), 10^4 CFU BIW (at week 8), or 10^5 CFU BIW (at week 12) but their atopic dermatitis has not substantially improved, then the dosage will be increased to 10^4 CFU BIW for 4 weeks, 10^5 CFU BIW for 4 weeks, or 10^5 CFU once every other day for 4 weeks, respectively. For part 2B, participants will start at 10^5 CFU BIW for 12 weeks, and then either continue at 10^5 CFU BIW for 4 weeks or, if ther atopic dermatitis has not substantially improved, then increase frequency to once every other day for 4 weeks.

i Materials are sterile sprayers filled with lyophilized *R mucosa* (10^3, 10^4, or 10^5 CFU), sterile eye droppers of sterile water (1.5 mL), illustrated dosing instruction booklet, and memory aid/symptom card.

j Validated assessments: Children's Dermatology Life Quality Index and Family Dermatology Life Quality Index.

Appendix B Memory Card and Symptom Tracker

Day	Date	Treatments	Fever?	Pain or Tenderness?	Skin color change (increase or decrease in pigmentation)?	Rash?	Itching?
			0 = None 1 = 100.4°F-101.4°F (38.0°C-38.6°C) 2 = 101.5°F-102.7°F (38.7°C-39.3°C) 3 = 102.8°F-104.0°F (39.4°C-40.0°C) 4 = > 104°F (> 40.0°C)	0 = None 1 = Minimal pain, not interfering with activity 2 = Pain interfering with activity 3 = Severe pain interfering with ability to function 4 = Pain bad enough to seek medical attention	0 = None 1 = Slight and only where treatment applied 2 = Moderate-Severe or whole body	0 = None 1 = Just at treatment site 2 = Area larger then dollar bill 3 = Any ulcers or area half the size of the arm 4= Blackening of skin or whole body involvement	0 = None (or no change from normal) 1 = Minimal itching, not interfering with activity 2 = Itching interfering with activity 3 = Severe itching interfering with ability to function
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Participant name:_____
Return to clinic for follow-up: _____
Please remember to bring this card with you
Number to call with questions or concerns: _____

Appendix C Environmental Exposure Questionnaire

B.A.C.T.E.R.I.A.D. Intake Questionnaire
Ian A Myles, MD
Modified from Dr. Heidi Kong

HISTORY OF OTHER SKIN FINDINGS

1) Has your child ever had bumps filled with pus on the skin (other than acne)?
   a. ☐No ☐Yes ☐Unsure
   b. Frequency: ☐Never ☐<yearly ☐~Yearly ☐>yearly
   c. Most recent example_____
2) Has your child ever had a positive skin culture for *Staph. aureus or MRSA*?
   a. ☐No ☐Yes ☐Unsure
   b. Frequency: ☐Never ☐<yearly ☐Yearly ☐>yearly
   c. Most recent example_____
3) Has your child ever had a skin infection positive for herpes?
   a. ☐No ☐Yes ☐Unsure
   b. Frequency: ☐Never ☐<yearly ☐Yearly ☐>yearly
   c. Most recent example_____
4) Has your child ever had acne or pimples?
   a. ☐No ☐Yes ☐Unsure
   b. Frequency: ☐Never ☐<yearly ☐Yearly ☐>yearly
   c. Most recent example_____

INFECTION HISTORY

Did you ever have a history of infections? ☐No ☐Yes ☐Unsure

Type of infection	Frequency	Number	Treatment

PETS: ☐No ☐Yes _____
SMOKERS IN HOME: ☐No ☐Yes _____
HANDEDNESS: ☐Left ☐Right ☐Ambidextrous

DEMOGRAPHICS:
Hispanic: ☐No ☐Yes
Race (check all that apply):
☐American Indian ☐Asian ☐Black ☐Pacific Islander ☐White ☐Unknown
Place of birth _____
Number of family members in the home:_____

Describe who lives in home (age, gender, etc) _____
_____

BIRTH HISTORY:
Delivery: ☐Vaginal ☐C-Section
Complications? _____

SKIN CARE/TREATMENTS:
Hand sanitizers? ☐No ☐Yes Days since last use (approx.)_____
Name, frequency_____

Topical steroids? ☐No ☐Yes Days since last use (approx.)_____
Name, frequency_____

Topical antibiotics? ☐No ☐Yes Days since last use (approx.)_____
Name, frequency_____

Topical antifungals? ☐No ☐Yes Days since last use (approx.)_____
Name, frequency_____

Other topical treatments? ☐No ☐Yes Days since last use (approx.)_____
Name, frequency_____

Study drugs? ☐No ☐Yes Days since last use (approx.)_____
Name, frequency_____

Oral steroids? ☐No ☐Yes Days since last use (approx.)_____
Name, frequency_____

Oral antibiotics? ☐No ☐Yes Days since last use (approx.)_____
Name, frequency_____

IV antibiotics? ☐No ☐Yes Days since last use (approx.)_____
Name, frequency_____

Oral antifungals? ☐No ☐Yes Days since last use (approx.)_____
Name, frequency_____

Antihistamines? ☐No ☐Yes Days since last use (approx.)_____
Name, frequency_____

Cyclosporin? ☐No ☐Yes Days since last use (approx.)_____
Methotrexate? ☐No ☐Yes Days since last use (approx.)_____
Acyclovir? ☐No ☐Yes Days since last use (approx.)_____
Valtrex? ☐No ☐Yes Days since last use (approx.)_____
Azathioprine? ☐No ☐Yes Days since last use (approx.)_____
Alternative medicines? ☐No ☐Yes Days since last use (approx.)_____
Name, frequency_____

Bleach Baths? ☐No ☐Yes Days since last use (approx.)_____

Usual Bathing: ☐Bath ☐Shower Frequency_____
Soaps Used:
Body? _____
Hand? _____
Shampoo/Conditioner?_____

Swimming pools: ☐No ☐Yes Days since last use (approx.)_____
Hot tubs: ☐No ☐Yes Days since last use (approx.)_____

CONFIDENCE:
On a scale of 0-10, rate your agreement with the following statements (0= completely disagree, 10 = completely agree):

1) I feel that I am in control of my child's skin symptoms: _____
2) I feel that this new approach will improve my child's skin health: _____

DIETARY:
Yogurt? ☐No ☐Yes Frequency (approx.)_____
Sauerkraut? ☐No ☐Yes Frequency (approx.)_____
Kombucha tea? ☐No ☐Yes Frequency (approx.)_____
Describe your typical diet:
_____
_____
_____
_____
_

HOBBIES/ACTIVITIES:
What kind of sports or activities do you do?
_____
_____
_____
_____

B.A.C.T.E.R.I.A.D. Follow-Up Questionnaire
Ian A Myles, MD

Follow-up Visit Number:
☐Month 2 ☐Month 3 ☐Month 4 ☐Post-Treatment (month)_____

Since your last visit have you had:
Any new skin infections? ☐No ☐Yes Details_____
_____

Any new infections of any kind? ☐No ☐Yes Details_____
_____

Any change in home exposures:
New pets? ☐No ☐Yes Details_____
New smokers? ☐No ☐Yes Details_____
Change in school/home? ☐No ☐Yes Details_____
_____
_____

Changes in treatments? ☐No ☐Yes Details_____
_____
_____
_____

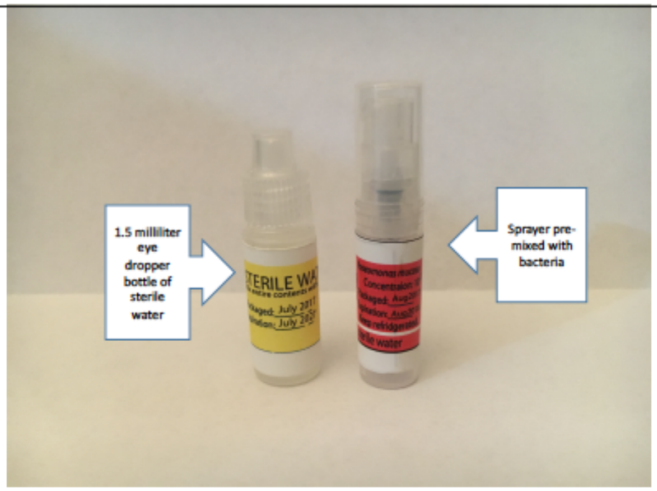
Changes in bathing habits? ☐No ☐Yes Details_____
_____

Changes in activities? ☐No ☐Yes Details_____
_____

Changes in diet? ☐No ☐Yes Details_____
_____

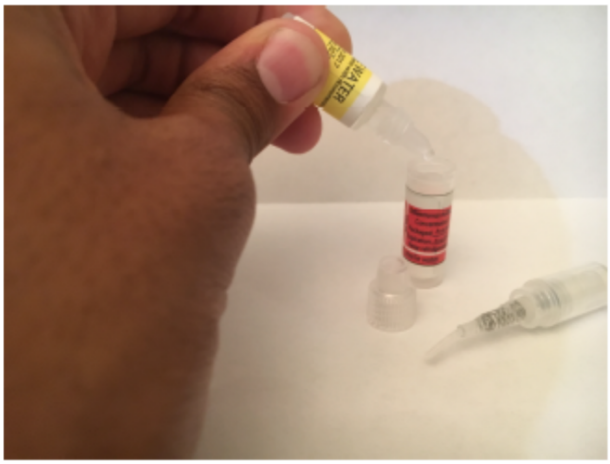
Any other new exposures to discuss? ☐No ☐Yes Details_____
_____
_____
_____

Appendix D Instruction Booklet for Product Administration



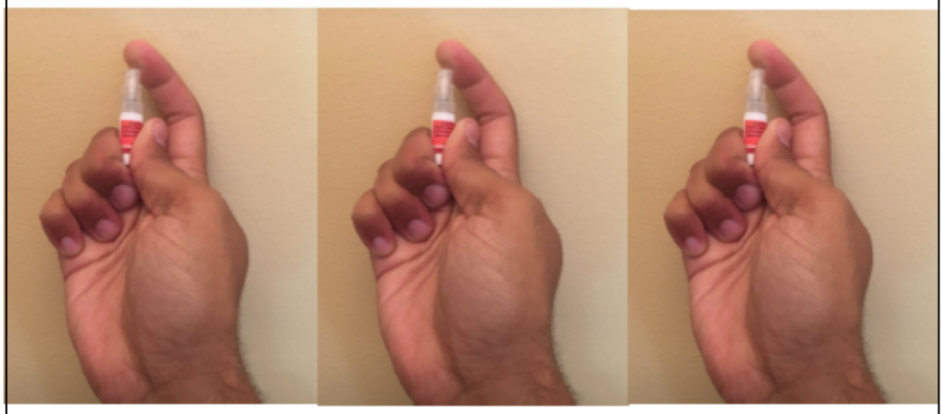
- Your kit should have Two (2) items per treatment

- Open the sprayer (bend just below the cap line)
- Open the eye dropper bottle (unscrew lid)



- Slowly empty the contents of the eye dropper bottle into the sprayer

- Recap the sprayer
- Shake the sprayer for at least 30 seconds, check to make sure all sugar crystals have dissolved



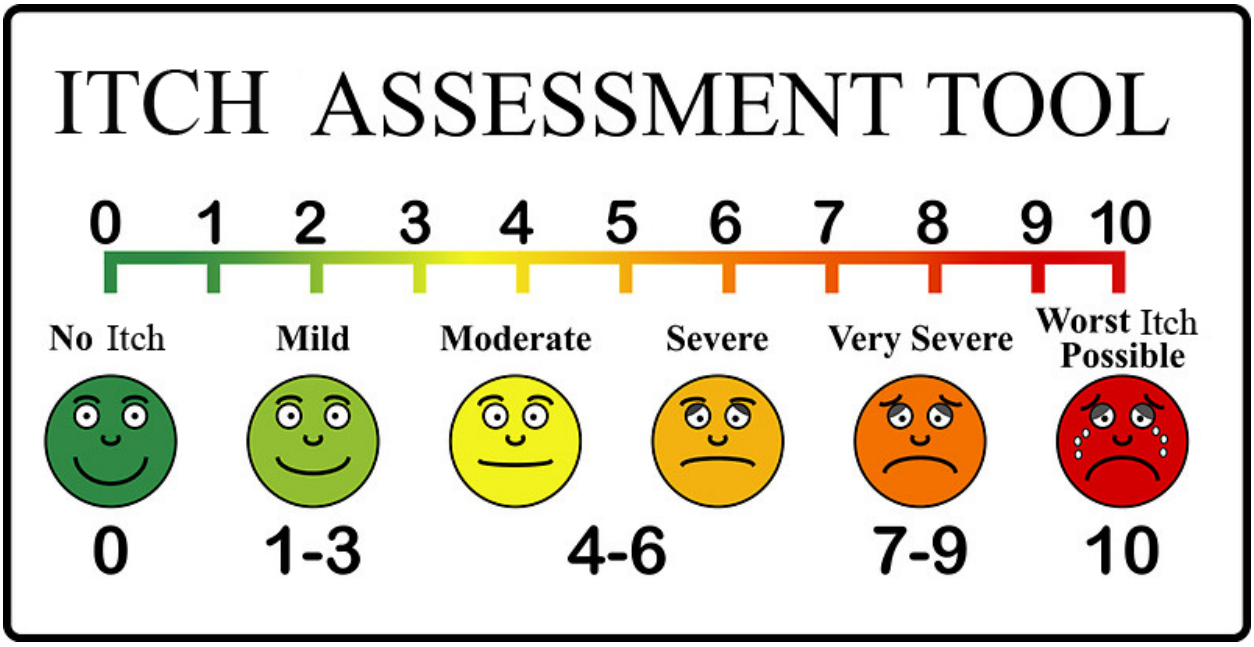
- Prime the sprayer by pumping
- Make sure at least three (3) sprays are made before use

- Hold our arm out straight and aim the sprayer at the inside of your elbow
- Hold the sprayer about 3-6 inches away from your skin and push the plunger down quickly to spray on the study drug treatment. Spray four full spray pumps onto your arm.

- Let the drug treatment sit on your skin and air dry. This will take about 1 minute.
- Repeat the treatment on the elbow crease of the other arm
- Discard all supplies in the regular trash

Appendix E Itch Assessment Tool

Itch assessment tool for younger patients that are unable to reliably comment on the pruritic score (0-10) without visual assistance.



Appendix F Eczema Assessment Severity Index

How to Use EASI

The EASI scoring system uses a **defined process** to grade the **severity of the signs** of eczema and the **extent affected**:

1. Select a body region

Four body regions are considered separately:

- Head and neck
- Trunk (including the genital area)
- Upper extremities
- Lower Extremities (including the buttocks)

2. Assess the extent of eczema in that body region

Each body region has potentially 100% involvement. Using the table below, give each respective body region a **score of between 0 and 6** based on the percentage involvement. Precise measurements are not required.

% involvement	0	1-9%	10 - 29%	30 - 49%	50 - 69%	70 - 89%	90 - 100%
Region score	0	1	2	3	4	5	6

To aid in your body region grading you can use the **diagrams** in Appendix 1.

3. Assess the severity of each of the four signs in that body region:

1. Erythema
2. Edema/papulation
3. Excoriation
4. Lichenification

Further explanations of these terms can be found in FAQ's (Appendix 4)

Grade the severity of each sign on a scale of 0 to 3:

0	None
1	Mild
2	Moderate
3	Severe

- ✓ Take an average of the severity across the involved region.
- ✓ Half points (1.5 and 2.5) may be used. 0.5 is not permitted – if a sign is present it should be at least mild (1)
- ✓ Palpation may be useful in assessing edema/papulation as well as lichenification

To aid your severity grading, a **photographic atlas** of suggested categories is available in **Appendix 2**

.

Remember: ***Include only inflamed areas*** *in your assessment; do not include xerosis (dryness), ichthyosis, keratosis pilaris, urticaria, infection (unless there is underlying eczema), or post inflammatory pigmentation changes.*

EASI guidance December 14

How to record your scores

The assessed parameters are inserted into a table (example shown below for age≥8 years). The final EASI score ranges from 0-72.

Body region	Erythema	Edema/ papulation	Excoriation	Lichenification	Area score	Multiplier	Score
Head/neck	(+	+	+	)	x	x 0.1	
Trunk	(+	+	+	)	x	x 0.3	
Upper extremities	(+	+	+	)	x	x 0.2	
Lower extremities	(+	+	+	)	x	x 0.4	
The final EASI score is the sum of the 4 region scores							——— (0-72)

Two forms of the EASI scoring system are available depending on the age of the patients. The multipliers for the region score are different in the under 8's version to reflect the relative proportion of body regions in young children:

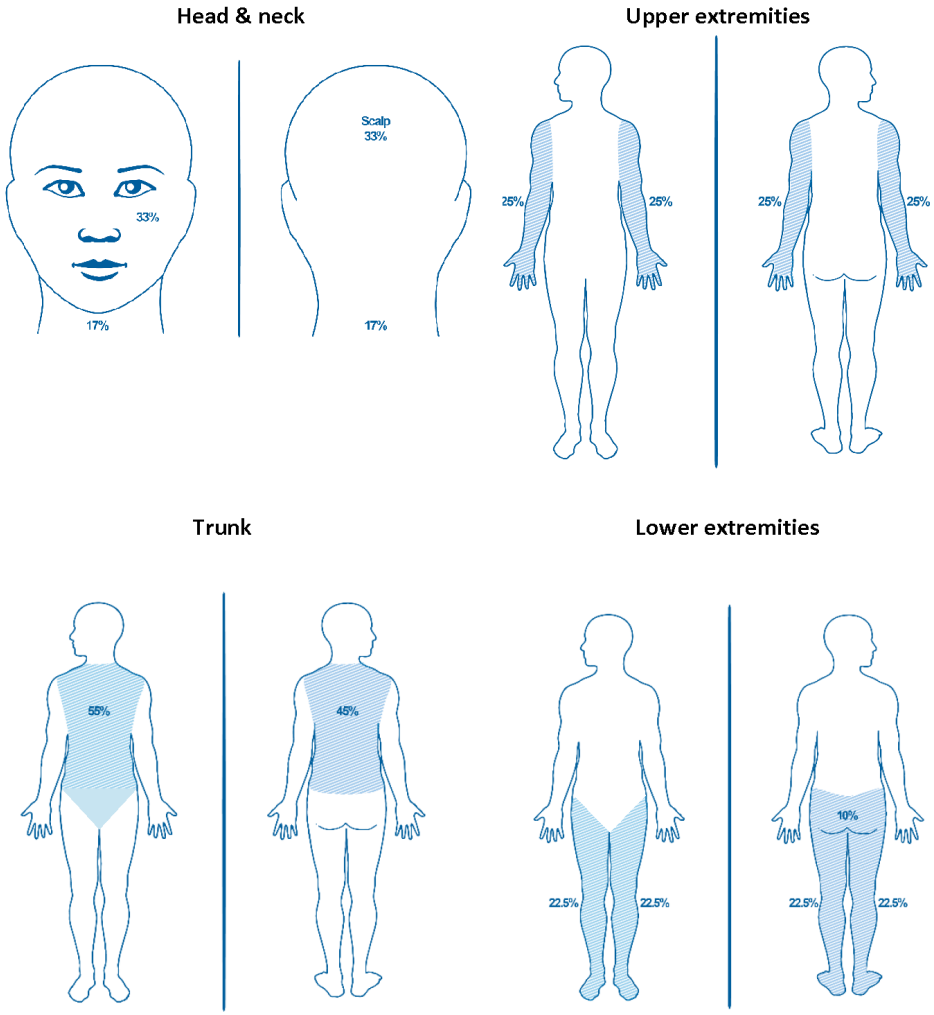
- Patients 8 years or above
- Patients under 8 years of age.

The forms can be found in appendix 3.1 and 3.2 and also as word documents on the HOME website (www.homeforeczema.org)

EASI guidance December 14

Appendix 1: Eczema Area and Severity Index (EASI) - Extent of eczema per body region

Score each region from 0 to 100%



EASI guidance December 14

Appendix 2: Eczema Area and Severity Index (EASI) –lesion severity atlas

EASI guidance December 14

Appendix 3.1: Eczema Area and Severity Index (EASI) case report form – age <8 years

Area of Involvement: Each body region has potentially 100% involvement. Score **0 to 6** based on the following table:

% involvement	0	1-9%	10 - 29%	30 - 49%	50 - 69%	70 - 89%	90 - 100%
Region score	0	1	2	3	4	5	6

Severity of Signs: Grade the severity of each sign on a scale of **0 to 3**:

0	None
1	Mild
2	Moderate
3	Severe

- ✓ Take an average of the severity across the involved area.
- ✓ Half points may be used e.g. 2.5.

Scoring table:

Body region	Erythema (0-3)	Edema/ Papulation (0-3)	Excoriation (0-3)	Lichenification (0-3)	Region score (0-6)	Multiplier	Score per body region
Head/neck	(+	+	+	)	X	X 0.2	
Trunk	(+	+	+	)	X	X 0.3	
Upper extremities	(+	+	+	)	X	X 0.2	
Lower extremities	(+	+	+	)	X	X 0.3	
The final EASI score is the sum of the 4 region scores:							_____ (0-72)

EASI guidance December 14

Appendix 3.2: Eczema Area and Severity Index (EASI) case report form - age≥8 years

Area of Involvement: Each body area has potentially 100% involvement. Score **0 to 6** based on the following table:

% involvement	0	1-9%	10 - 29%	30 - 49%	50 - 69%	70 - 89%	90 - 100%
Region score	0	1	2	3	4	5	6

Severity of Signs: Grade the severity of each sign on a scale of **0 to 3**:

0	None
1	Mild
2	Moderate
3	Severe

- ✓ Take an average of the severity across the involved area.
- ✓ Half points may be used e.g. 2.5.

Scoring table:

Body region	Erythema (0-3)	Edema/ Papulation (0-3)	Excoriation (0-3)	Lichenification(0-3)	Region score (0-6)	Multiplier	Score per body region
Head/neck	(+	+	+	)	X	X 0.1	
Trunk	(+	+	+	)	X	X 0.3	
Upper extremities	(+	+	+	)	X	X 0.2	
Lower extremities	(+	+	+	)	X	X 0.4	
The final EASI score is the sum of the 4 region scores:							_____ (0-72)

EASI guidance December 14

Appendix 4 - Frequently Asked Questions

What is the difference between edema/papulation and lichenification?
Consider edema/papulation as corresponding to the acute signs of atopic dermatitis that reflect histological spongiosis. Lichenification are more firm thickened plaques with accentuation of the skin markings that develop as a result of prolonged scratching or rubbing in chronic disease. In darker skin types, follicular lichenification may present as firm flat-topped discrete papules. Grade these chronic lesions as lichenification.

How do I grade prurigo nodules?
Prurigo nodules are larger, deeper lesions as a result of chronic scratching and are graded as areas of lichenification.

How do I grade erythema in darker skin?
To avoid underestimating inflammation in patients with darker skin tones, take into account the underlying skin pigment when grading erythema. Often this means increasing your erythema grade by one level.

Can half-steps be used to assess lesion severity?
The original EASI validation study allowed for half steps. These may be helpful when trying to average the severity of a parameter over a region. For example, if there are some areas with an erythema grading of 2 and some areas more consistent with a severity of 3, 2.5 may be a good choice.

What if most areas in a region are a severity grade of 1, but there are some areas that are a grade 3?
Attempt to average the severity across the involved areas in that region. If these areas are close to equal in size, a score of 2 would be most appropriate. If the majority of involved areas are a grade 1, a score of 1 or 1.5 is more appropriate. Be careful not to score the highest severity in a region but the average one.

How do I grade xerosis (dryness), ichthyosis and hyperlinear palms?
Unless there is active acute or chronic eczema overlying these findings, they are not included in the EASI assessment.

How precise should my assessment of eczema extent be?
The *region scores*, which reflect the extent of eczema, were designed and validated as rough estimates of the percentage of involved skin. Each region is given a *score* ranging from 0 to 6, based on a "ballpark" estimation of extent (see region score table in page 1). If you find it difficult to provide a rough estimate of disease extent, you can use the schematics in Appendix 1 to guide you. More time-consuming methods for evaluating disease extent such as the rule of nines or the 'palm' method are generally unnecessary, as the EASI was designed to be…easy.

EASI guidance December 14

My patient has responded well to treatment and significantly improved since the last visit. Should I adjust the grading based on the patient's relative improvement?
No. The EASI is a static score, meaning that it is done independently at each time point to reflect current severity. You should grade the EASI per visit regardless of the previous status. Studies have shown that the EASI score has good responsiveness, meaning that overall it is sensitive to change and the improvement will be reflected in the total score.

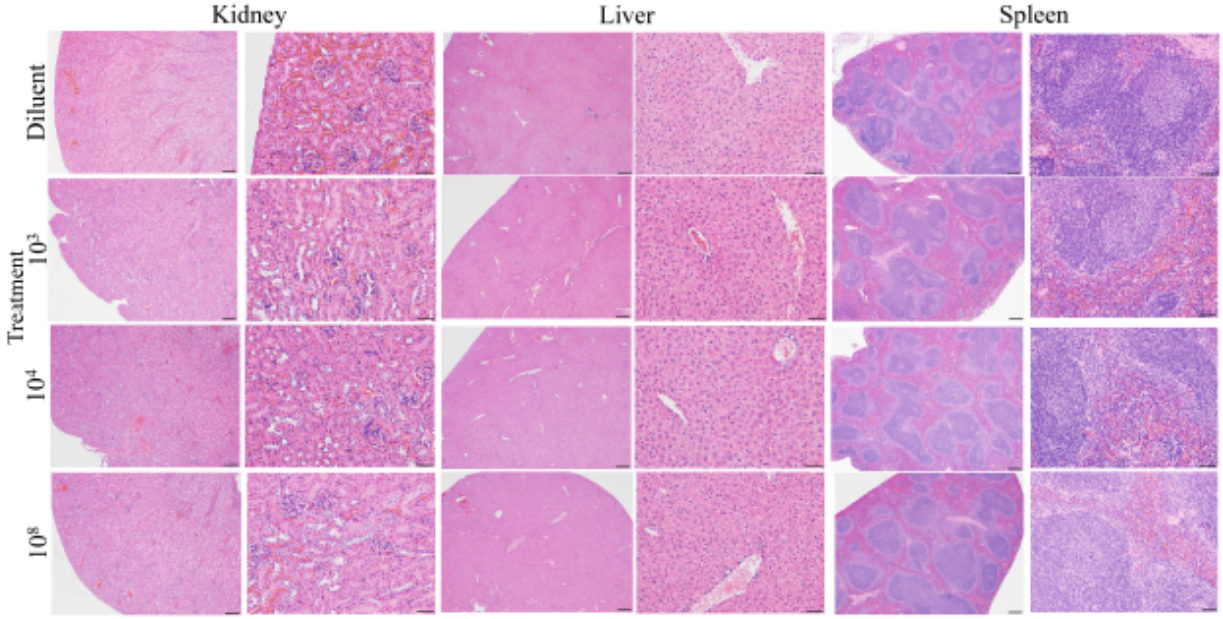
Can the EASI be used in children?
Yes. The EASI is performed in the same method in all age groups, but the calculation of the final EASI score differs by age. When calculating the EASI, each of the 4 region scores is multiplied by a constant which reflects the relative contribution of that region to the total body surface area. For patients 8 years and older the multipliers are 0.1 for the head/neck, 0.2 for the upper extremities, 0.3 for the trunk and 0.4 for the lower extremities. Below 8 years of age the head/neck multiplier is increased to 0.2 while the lower extremities multiplier decreases to 0.3, consistent with the proportions of these regions in childhood. Refer to Appendix 3 for EASI forms by age.

What happens if a child turns 8 during the course of the study? Which EASI formula should I use?
There are no clear-cut definitions for this situation. In general, if the study is a short term study such as an RCT lasting a few months – using the same formula throughout the trial is preferred, even if the child turns 8 during these months. Keeping the EASI formula consistent in this scenario can serve to preserve the EASI sensitivity to change (e.g. its change in response to treatment) without compromising the validity of the score.
In long term studies such as cohort studies lasting a year or longer, it is important to update the EASI formula based on the physical changes children go through. Switching to the age 8+ formula once a child is older is preferred in that scenario.

What do the terms erythema, edema/papulation, excoriation and lichenification mean?
These are key signs of atopic dermatitis. Recognizing and grading them properly requires training on the visual and physical exam consistent with these signs. Generally speaking, erythema is skin redness; edema/papulation refers to an elevation or swelling of the skin (that should be differed from lichenification below); excoriations are scratch marks that have broken the skin surface; and lichenification is a leathery thickening of the skin with exaggerated skin markings.

EASI guidance December 14

Appendix G Intravenous Injection of *R mucosa* is Non-toxic in Mice



Intravenous injection of *R. mucosa* is non-toxic in mice. Wild type mice were injected with diluent or *R. mucosa* IV at 10^3, 10^4, or 10^8 CFU in 100mcL of volume. Weights and activities were monitored daily for 10 days. On day 10 the kidney, liver, and spleen were harvested and compared histologically (N = 5 mice per group). Representative images from one mouse per group at both 4x and 20x magnification are shown.

I.A. Myles, MD, et al, unpublished data, February 2018.

MEDICAL RECORD	CONSENT TO PARTICIPATE IN A CLINICAL RESEARCH STUDY • Adult Patient or • Parent, for Minor Patient

INSTITUTE: National Institute of Allergy and Infectious Diseases

STUDY NUMBER: 17-I-0033 PRINCIPAL INVESTIGATOR: Ian Myles, MD

STUDY TITLE: Beginning Assessment of Cutaneous Treatment Efficacy of Roseomonas in Atopic Dermatitis Phase I/II

Continuing Review Approved by the IRB on 10/29/18
Amendment Approved by the IRB on 05/06/19 (H) Date Posted to Web: 05/15/19
Standard A

INTRODUCTION

We invite you to take part in a research study at the National Institutes of Health (NIH).

First, we want you to know that:

Taking part in NIH research is entirely voluntary.

You may choose not to take part, or you may withdraw from the study at any time. In either case, you will not lose any benefits to which you are otherwise entitled. However, to receive care at the NIH, you must be taking part in a study or be under evaluation for study participation.

You may receive no benefit from taking part. The research may give us knowledge that may help people in the future.

Second, some people have personal, religious or ethical beliefs that may limit the kinds of medical or research treatments they would want to receive (such as blood transfusions). If you have such beliefs, please discuss them with your NIH doctors or research team before you agree to the study.

Now we will describe this research study. Before you decide to take part, please take as much time as you need to ask any questions and discuss this study with anyone at NIH, or with family, friends or your personal physician or other health professional. Throughout this form, "you" refers to you or your minor child.

WHAT IS THE PURPOSE OF THIS STUDY?

We are studying a skin disease called atopic dermatitis (AD), also known as eczema. We are asking you to join this study because you have AD. AD can make skin dry and itchy, cause rashes, and lead to infections. AD is common in children and sometimes gets better on its own. However, chronic AD can result in asthma, hay fever, food allergies, eye infections, and sleep problems. There is no cure for AD. Current treatments only help ease symptoms.

We do not know what causes AD, but studies have shown that it might have to do with bacteria that live on the skin. Certain kinds of bacteria that live on the skin are important for your health. If you do not have those bacteria or if different bacteria get onto your skin, then you can get sick.

In this research study, we want to see if introducing bacteria from healthy skin onto the skin of someone with AD is a safe and effective treatment for the disease. We will use a type of bacterium normally found on healthy skin called *Roseomonas mucosa*, or *R mucosa*. The *R mucosa* we will use in this study is made in an NIH laboratory. It has not yet been tested on people. The United States Food and Drug Administration (FDA) has given us permission to test this *R mucosa* for the first time in people.

This study is divided into 2 parts. In the first part, we will test different doses of *R mucosa* to make sure it does not cause serious side effects in young adults and adults with AD. In the second part, we will test these doses in a larger group of children with AD to see if the *R mucosa* has any effect on AD. We are inviting you to participate in the first part. We plan to enroll a total of 49 people between both parts of the study. Your participation will last about 10 weeks.

WHAT IS THE STUDY SCHEDULE?

Screening:

We first need to make sure that you are eligible to join this study. This is called screening. This visit should take 1-3 hours. We will go over your medical history, give you a physical exam, examine your AD, and draw blood for laboratory tests. These tests will include measuring blood cells, looking at substances in blood to see if your organs are working properly, and testing for HIV. HIV is the virus that causes AIDS. If you are infected with HIV then you will not be able to participate in this study. We will tell you what the results mean, how to find care, how to avoid infecting others, how we report HIV infection, and the importance of informing your partners at possible risk because of your HIV infection. You will not need to have an HIV test if you had one within the last 3 months and it was negative.

If you are able to become pregnant, then you will also have a pregnancy test. You cannot be pregnant or breastfeeding while you are on this study. If you do join then you will be required to use appropriate birth control for the duration of the study. Examples of birth control include an intrauterine device (IUD), hormonal methods (pills, patch, ring, implant, or injection), and barrier methods (condom, cap, or diaphragm with spermicide). If you suspect you became pregnant during the study, then stop taking *R mucosa* and immediately notify the study team.

First Study Visit and Treatment Phase:

If you are eligible to join this study, then you will return to the NIH CC within the next 3 months for a study visit. This visit will take about an hour. We will draw some blood for research tests. We will then give you a 6-week supply of *R mucosa* materials to take home so you can apply it to yourself. We will show you how to apply the *R mucosa* to your arms and we will give you an instruction booklet with pictures so you have a reference at home. We will also give you a memory aid that you will use to keep track of your doses and record how you are feeling.

There are 3 dose levels of *R mucosa*. You will take the low dose for the first 2 weeks. Then you will take the middle dose for 2 more weeks. Finally, you will take the high dose for the last 2 weeks. The vials are labeled with the appropriate dose level. You will spray the *R mucosa* onto the same spot on your arm twice per week.

During this time, you can call or email the study team any time if you have a question or concern. You can also schedule a visit with the study team if you want to meet with someone face to face.

Final Study Visit:

After the 6 weeks, you will return to the NIH CC for a final in-person visit. This visit will take about an hour. We will give you a physical, examine your AD, assess any side effects you might have had, and go over your memory aid. We will contact you one last time approximately 30 days after the 6-week visit (via email, text, or phone) to discuss how you are feeling. After this contact, your involvement in the study will be complete.

WHAT RESEARCH PROCEDURES WILL BE DONE IN THIS STUDY?

Blood draw: Blood will be drawn from a vein in your arm to test for HIV infection, measure blood cells, and look at substances in blood (blood chemistries) to see if your organs are working properly. Some of your blood will be stored for use in future research studies of AD, including genetic testing. The total amount of blood collected during this study will not exceed the guidelines for blood drawn for research purposes at the NIH Clinical Center.

Clinical photography: We will take pictures of your skin. Your face will not be included in any of the photographs. You will be asked to sign a standard NIH photography consent form. These photographs may be published in medical journals, but we will not give your name. We will try to keep your identity unknown as much as possible, while still giving the information needed to support the research being published. You can decide if you want your pictures to be published or not.

WHAT ARE THE RISKS OF THIS STUDY?

***R mucosa*:** Typically, *R mucosa* only poses a health risk to people who have an indwelling catheter or a compromised immune system. You cannot be in this study if you live with someone who has a weakened immune system or an indwelling catheter. Therefore, the risk of infection to you in this study is low. *R mucosa* infection can be treated with antibiotics. There may also be other risks to *R mucosa* that are currently unknown.

Blood draw: Blood draws may cause pain and bruising and, rarely, infection. Sometimes drawing blood causes people to feel lightheaded or even faint. The amount of blood drawn at all visits is within the limits allowed by the NIH Clinical Center for research purposes. While in the study, let the study team know if you are participating in other studies or have blood drawn for any other reason. Some of your blood will be stored for possible future testing.

Clinical photography: Taking pictures of the face and body may be embarrassing to some people. These photographs may be published in medical journals, without identifying the participant. We will attempt to preserve the anonymity of the participant as much as possible, while providing the information needed to support the research being published. We invite you to talk with us about any concerns you have related to photography.

WHAT ARE THE BENEFITS OF BEING IN THIS STUDY?

You may not receive any benefit by participating in this study. There is the possibility that *R mucosa* treatment will help improve your AD, but it may not. The information we obtain in this study will help us learn more about the use of *R mucosa* as treatment for AD.

WHAT ARE THE ALTERNATIVES TO PARTICIPATING IN THIS STUDY?

You may choose not to participate in this study. This decision will not affect any ongoing care or evaluations you may be receiving at the NIH.

HOW CAN PARTICIPATION END EARLY?

Your involvement in this study is voluntary. You can choose to withdraw from the study at any time. This decision will not in any way affect your participation in other protocols at NIH. If you do decide to withdraw from the study, then all further planned study procedures will be immediately cancelled and further follow-up visits will be cancelled. Samples and data collected prior to this request will be stored and used as described above unless you specifically request to remove all of your samples from the study.

You may be removed from the study without your consent if your study doctor feels it is in your best medical interest, if the scientific goals of the study change, or if the study is cancelled or stopped early. You will also be removed if you decide that you do not want your samples stored anymore. If you are removed from the study then that will not affect your participation in other protocols at the NIH.

WILL IT COST TO PARTICIPATE?

There will be no charge to you or your health insurance company for any tests, procedures, or medications conducted or given out at the NIH. We do not pay for your medical care outside the NIH. During the study, medical care and medications provided outside the NIH, including care recommendations from the study and anything not directly related to the study, must continue to be provided by your personal doctor.

IS THERE COMPENSATION FOR THIS STUDY?

You will be reimbursed for your time and inconvenience according to Clinical Center compensation guidelines. You will be compensated $20 for completing the baseline visit, and $20 for completing the final visit. The total maximum compensation is $40.

WHAT WILL HAPPEN TO STORED SAMPLES AND DATA?

If you agree to participate in this study, then you also agree to let us store your samples and data for future research. These stored samples and data may help us learn more about AD. Genetic testing will not be performed. We will label your stored samples and data with a code that only the study team can link to you. We will keep any information that can be traced back to you as private as possible. If you change your mind and decide you do not want us to store your samples or data, then please contact us. We will do our best to comply with your request, but cannot guarantee that we will always be able to destroy all your samples and data.

We might send your samples or data to other investigators for their research, along with the coded label. We might also share information such as your sex, age, health history, or ethnicity. We will not sell your samples or data and you will not be paid for any products that result from the research. Some future studies may need health information (such as smoking history or present health status) that we don't already have. If so, then our study team will contact you. Future research that uses your samples or data will probably not help you, but it may help us learn more about how to treat AD. In general, the research tests we perform are not like routine medical tests, and may not relate directly to your medical care. The greatest risk associated with storing samples and data is that someone may take information from your medical records without your permission. The chances of this happening are very low. If this information becomes available, then you may face discrimination when you apply for insurance or a job. You may also have similar problems if you share the information yourself or let us release your medical records.

HOW WILL DATA BE SHARED?

To advance science, it is helpful for researchers to share information they get from studying human research participants and their data. They do this by putting data into one or more scientific databases, where they are stored along with information from other studies. Researchers can then study the information combined from many studies to learn even more about health and disease.

If you agree to take part in this study, then some of your data will be placed into one or more scientific databases after they have been stripped of identifiers such as name, address, and birth date. Your data may then be used for future research on any topic and shared broadly for research purposes. A researcher who wants to study the information must apply for access to the database. Researchers who are approved to access the database may be able to see and use your

information, along with information from many other people. You will not receive direct benefits from future research that uses your data.

WHAT IF THERE ARE NEW FINDINGS?

Any new findings discovered during this study that are considered relevant to your health or to your decision to continue in the study will be fully discussed with you.

IS THERE A CONFLICT OF INTEREST?

The National Institutes of Health reviews the NIH staff researchers associated with this study at least yearly for conflicts of interest. You may ask your research team for additional information or for a copy of the NIH Guide to Avoiding Financial and Non-Financial Conflicts or Perceived Conflicts of Interest in Clinical Research. This protocol may have investigators who are not NIH employees. Non-NIH investigators are expected to adhere to the principles of this Guide but are not required to report their personal financial holdings to the NIH.

The NIH and the research team for this study are using a laboratory-made strain of *R mucosa*. NIH researchers may plan to use the results, and may get patents, or sell the drug in the future, or make profits other ways. This means it is possible that the results of this study could lead to payments to NIH scientists and to the NIH. By law, government scientists are required to receive such payments for their inventions. You will not receive any money from the development of *R mucosa*.

CLINICALTRIALS.GOV

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by US Law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

MEDICAL RECORD	CONSENT TO PARTICIPATE IN A CLINICAL RESEARCH STUDY • Adult Patient or • Parent, for Minor Patient

STUDY NUMBER: 17-I-0033 CONTINUATION: Page 6 of 6 pages

OTHER PERTINENT INFORMATION

1. Confidentiality. When results of an NIH research study are reported in medical journals or at scientific meetings, the people who take part are not named and identified. In most cases, the NIH will not release any information about your research involvement without your written permission. However, if you sign a release of information form, for example, for an insurance company, the NIH will give the insurance company information from your medical record. This information might affect (either favorably or unfavorably) the willingness of the insurance company to sell you insurance.

The Federal Privacy Act protects the confidentiality of your NIH medical records. However, you should know that the Act allows release of some information from your medical record without your permission, for example, if it is required by the Food and Drug Administration (FDA), members of Congress, law enforcement officials, or authorized hospital accreditation organizations.

2. Policy Regarding Research-Related Injuries. The Clinical Center will provide short-term medical care for any injury resulting from your participation in research here. In general, no long-term medical care or financial compensation for research-related injuries will be provided by the National Institutes of Health, the Clinical Center, or the Federal Government. However, you have the right to pursue legal remedy if you believe that your injury justifies such action.

3. Payments. The amount of payment to research volunteers is guided by the National Institutes of Health policies. In general, patients are not paid for taking part in research studies at the National Institutes of Health. Reimbursement of travel and subsistence will be offered consistent with NIH guidelines.

4. Problems or Questions. If you have any problems or questions about this study, or about your rights as a research participant, or about any research-related injury, contact the Principal Investigator Ian Myles, Building 10, Room 11S256, Telephone (301) 451-8420.

You may also call the Clinical Center Patient Representative at (301) 496-2626.

5. Consent Document. Please keep a copy of this document in case you want to read it again.

COMPLETE APPROPRIATE ITEM(S) BELOW:

A. Adult Patient's Consent

I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby consent to take part in this study.

Signature of Adult Patient/Legal Representative _____ Date _____

Print Name _____

B. Parent's Permission for Minor Patient.

I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby give permission for my child to take part in this study.
(Attach NIH 2514-2, Minor's Assent, if applicable.)

Signature of Parent(s)/Guardian _____ Date _____

Print Name _____

C. Child's Verbal Assent (If Applicable)

The information in the above consent was described to my child and my child agrees to participate in the study.

Signature of Parent(s)/Guardian _____ Date _____ Print Name _____

THIS CONSENT DOCUMENT HAS BEEN APPROVED FOR USE FROM OCTOBER 29, 2018 THROUGH OCTOBER 28, 2019.

Signature of Investigator _____ Date _____ Signature of Witness _____ Date _____

Print Name _____ Print Name _____

MEDICAL RECORD	**CONSENT TO PARTICIPATE IN A CLINICAL RESEARCH STUDY** • Adult Patient or • Parent, for Minor Patient

INSTITUTE: National Institute of Allergy and Infectious Diseases

STUDY NUMBER: 17-I-0033 PRINCIPAL INVESTIGATOR: Ian Myles, MD

STUDY TITLE: Beginning Assessment of Cutaneous Treatment Efficacy of Roseomonas in Atopic Dermatitis Phase I/II

Continuing Review Approved by the IRB on 10/29/18
Amendment Approved by the IRB on 05/06/19 (H) Date Posted to Web: 05/15/19
Standard B

INTRODUCTION

We invite you to take part in a research study at the National Institutes of Health (NIH).

First, we want you to know that:

Taking part in NIH research is entirely voluntary.

You may choose not to take part, or you may withdraw from the study at any time. In either case, you will not lose any benefits to which you are otherwise entitled. However, to receive care at the NIH, you must be taking part in a study or be under evaluation for study participation.

You may receive no benefit from taking part. The research may give us knowledge that may help people in the future.

Second, some people have personal, religious or ethical beliefs that may limit the kinds of medical or research treatments they would want to receive (such as blood transfusions). If you have such beliefs, please discuss them with your NIH doctors or research team before you agree to the study.

Now we will describe this research study. Before you decide to take part, please take as much time as you need to ask any questions and discuss this study with anyone at NIH, or with family, friends or your personal physician or other health professional.

WHAT IS THE PURPOSE OF THIS STUDY?

We are studying a skin disease called atopic dermatitis (AD), also known as eczema. We are asking your child to join this study because they have AD. AD can make skin dry and itchy, cause rashes, and lead to infections. AD is common in children and sometimes gets better on its own. However, chronic AD can result in asthma, food allergies, hay fever, eye infections, and sleep problems. There is no cure for AD. Current treatments only help ease symptoms.

We do not know what causes AD, but studies have shown that it might have to do with bacteria that live on the skin. Certain kinds of bacteria that live on the skin are important for health. If someone does not have those bacteria or if different bacteria get on their skin, then they can get sick.

In this research study, we want to see if introducing bacteria from healthy skin onto the skin of someone with AD is a safe and effective treatment for the disease. We will use a type of bacterium normally found on healthy skin called *Roseomonas mucosa*, or *R mucosa*. The *R mucosa* we will use in this study is made in an NIH laboratory, and we have already tested it on a few people as part of this study. The United States Food and Drug Administration (FDA) has given us permission to test this *R mucosa* in people with AD.

This study is divided into 2 parts. In the first part, we tested different doses of *R mucosa* to make sure it did not cause serious side effects in young adults with AD. In the second part, we will test 1 dose in a larger group of children with AD to see if the *R mucosa* has any effect on AD. We are inviting your child to participate in the second part. We plan to enroll a total of 49 people between both parts of the study. The total duration of your child's participation is about a year and a half. Thus far, a majority of patients have seen their symptoms improve by more than 50% while no patient ha experienced any adverse reactions related to treatment.

WHAT IS THE STUDY SCHEDULE?

Screening

We first need to make sure that your child is eligible to join this study. This is called screening. We will go over their medical history, give them a physical exam, examine their skin, and draw blood for laboratory tests. These tests may include testing for HIV in their blood. HIV is the virus that causes AIDS. If they are infected with HIV then they will not be able to participate in this study. We will tell you what the results mean, how to find care, how to avoid infecting others, how we report HIV infection, and the importance of informing people at possible risk because of their HIV infection. We will only do an HIV test if your child has ever had a blood transfusion or is sexually active, and if they have not had an HIV test within the last 6 months that was negative.

If your child has had their first period and is able to become pregnant, then they will also have a pregnancy test. Your child cannot be pregnant or breastfeeding while they are on this study. If they do join and are sexually active then they will be required to use appropriate birth control for the duration of the study. Examples of birth control include an intrauterine device (IUD), hormonal methods (pills, patch, ring, implant, or injection), and barrier methods (condom, cap, or diaphragm with spermicide). If you or your child suspects they have become pregnant during the study, then they should stop taking *R mucosa* and immediately notify the study team.

The screening visit will take approximately 1-3 hours.

Baseline visit

If your child is eligible to join this study, then you and your child will return to the NIH CC for a baseline visit. If eligibility can be confirmed on the day of the screening visit, then the baseline can be done that same day. Otherwise, baseline will be within 3 months of screening. This visit will take about 3 hours. At this visit, we will do the following:

1. Review of medical history
2. Physical exam
3. Skin tests to examine what bacteria are present and the effects of AD on the skin
4. Photography of the affected skin
5. Questionnaires about your child's symptoms and how their AD affects their everyday life
6. Blood draw for laboratory tests

After we finish these procedures, we will give you a supply of *R mucosa* materials to take home so you can apply it to your child's arms. We will show you how to apply the *R mucosa* and we will give you an instruction booklet with pictures

so you have a reference. We will also give you a memory aid that you will use to keep track of your child's doses and of how your child is feeling. You will need to fill out this card every day.

Treatment phase

For the next 4 weeks, you will spray the *R mucosa* onto the affected area on your child's arm twice per week. The study team will contact you approximately every 10 days by phone, email, or text message to discuss how your child is feeling and how they are responding to the *R mucosa*. You may send us pictures of the affected area on your child's skin if there is something you want us to see.

You and your child will return to the NIH CC around 4, 8, and 12 weeks after the baseline visit. You should bring your child's filled-out memory aid to all visits so we can review it, as well as any leftover *R mucosa* materials. At each visit, your child will undergo the procedures 1-6 listed under Baseline above. We will also examine your child for side effects to the treatment. These visits will each take about 2 hours.

At each visit, we will look to see if the *R mucosa* treatment has had a noticeable effect on your child's AD. If the treatment seems to be working, then we will give you another supply of *R mucosa* at the same dose, and you will keep giving it twice per week. After about 12 weeks, if the *R mucosa* had little or no effect and your child did not have serious side effects, then we will instruct you to spray the *R mucosa* once every other day instead of twice per week.

In between these visits to the NIH CC, the study team will continue to contact you approximately once every 10 days to discuss how your child is feeling and if the *R mucosa* is having any effect. You may send us pictures of the affected area on your child's skin if there is something you want us to see.

After a total of 16 weeks of *R mucosa* treatment, you and your child will return to the NIH CC for another study visit. Your child will undergo procedures 1-6 listed under Baseline above. Your child will stop using the *R mucosa* after week 16.

Follow-up

We will keep in touch with you for 1 year after treatment ends. You may contact the study team at any time during this year if you have any questions or concerns you want to discuss. You and your child will return to the NIH CC for one in-person follow-up visit sometime between 4 and 7 months after the *R mucosa* treatment ends. At this visit, the study doctor will examine your child's AD, provide a questionnaire to fill out, and draw blood for laboratory tests. This visit will take about 1 hour.

At the end of this 1-year follow-up period, your child's participation in this study will end.

WHAT RESEARCH PROCEDURES WILL BE DONE IN THIS STUDY?

Blood draw: Blood will be drawn from a vein in your child's arm to test for HIV infection, measure blood cells, and look at substances in blood (blood chemistries) to see if your child's organs are working properly. Some of your child's blood will be stored for use in future research studies of AD, including genetic testing. The total amount of blood collected during this study will not exceed the guidelines for blood drawn for research purposes at the NIH Clinical Center.

Skin tests: We will conduct some tests on your child's skin to examine their AD symptoms. One test uses a device called a VapoMeter to measure how well your child's skin keeps water inside. "Leaky" skin is a symptom of AD, so this test will help to evaluate your child's condition. We do this test by holding the VapoMeter against your child's skin for approximately 30 seconds.

There are two ways to collect skin cells and bacteria that live on the skin. One is to rub your child's affected skin with a sterile cotton swab. The other is to press a piece of tape to the skin and gently peel it off. We will only use this tape at the baseline visit and the visit at week 16.

Questionnaires: We will ask you to fill out some questionnaires about your child's condition and how it affects their daily life. These should take only a few minutes.

Clinical photography: We will take pictures of your child's skin. Your child's face will not be included in any of the photographs. You will be asked to sign a standard NIH photography consent form. These photographs may be published in medical journals, but we will not give your child's name. We will try to keep your child's identity unknown as much as possible, while still giving the information needed to support the research being published.

WHAT ARE THE RISKS OF THIS STUDY?

***R mucosa*:** Typically, *R mucosa* only poses a health risk to people who have an indwelling catheter or a compromised immune system. Your child cannot be in this study if they live with someone who has a weakened immune system or an indwelling catheter. Therefore, the risk of infection to your child in this study is low. *R mucosa* infection can be treated with antibiotics. Signs of infection may include, but are not limited to, pain and redness at the application site, warmth at the application site, fever, or chills. If you have any of these symptoms, then please contact the research team and your primary care provider as soon as possible. Some patients have reported itching and increased redness at the site of application lasting minutes to hours after treatment. There may also be other risks to *R mucosa* that are currently unknown.

Blood draw: Blood draws may cause pain and bruising and, rarely, infection. Sometimes drawing blood causes people to feel lightheaded or even faint.

Genetic testing: We may use some of the blood we collect for genetic testing. Genetic testing can have emotional and psychological risks, such as:

- Learning your child has a genetic mutation suggesting future health risks or an incurable condition could be upsetting.
- Learning your child has a genetic mutation that they could pass on could be upsetting.
- Other members of your family could be upset to learn that they may be at risk for a genetic condition, which can be revealed through your genetic test results.
- You and your child's other parent may have different views about what to do with the results for your child.

While unlikely, previously unknown family information such as parentage and adoption may be found through genetic testing. We will not discuss this information with you unless it has direct medical implications for your family.

Any genetic information collected or discovered about you or your family will be confidential. Records containing this information will be kept on password-protected computer systems and in locked and secured quarters within the Clinical Center. Genetic results returned to you will be part of your medical record at the NIH. We will not release any information about you or your family to relatives, any insurance company, employer, or your primary care physician without your written permission. Some genetic information, such as the name of your diagnosis, will be in your medical record. There may be a risk that genetic information obtained as a result of participation in research could be misused for discriminatory purposes. However, state and federal laws provide some protections against genetic discrimination. Researchers who will have access to genetic information about you will take measures to maintain the confidentiality of your genetic information.

Although we are committed to confidentiality, a court could still subpoena your medical records.

All of these issues should be considered before participating. Genetic counselors are available to discuss these issues and any other concerns you may have in greater detail.

Skin tests: There are no risks with the VapoMeter or swabbing the skin for cells and bacteria. Removing the strip of medical tape does not hurt but might irritate the skin a little.

Clinical photography: Taking pictures of the face and body may be embarrassing to some people. These photographs may be published in medical journals, without identifying the participant. We will attempt to preserve the anonymity of the participant as much as possible, while providing the information needed to support the research being published. We invite you to talk with us about any concerns you have related to photography.

There are no risks associated with the skin tests or questionnaires.

WHAT ARE THE BENEFITS OF BEING IN THIS STUDY?

Your child may not receive any benefit by participating in this study. There is the possibility that *R mucosa* treatment will help improve your child's AD, but it may not. The information we obtain in this study will help us learn more about the use of *R mucosa* as treatment for AD.

WHAT ARE THE ALTERNATIVES TO PARTICIPATING IN THIS STUDY?

Your child may choose not to participate in this study. This decision will not affect any ongoing care or evaluations your child may be receiving at the NIH.

HOW CAN PARTICIPATION END EARLY?

You may decide to withdraw your child from the study at any time. Your child may also decide to withdraw. This decision will not in any way affect your child's participation in other protocols at NIH. Tell a member of the study team if you or your child decide to do that. If your child does withdraw from the study, then all further planned study procedures will be immediately cancelled and further follow-up visits will be cancelled. Samples and data collected prior to this request will be stored and used as described above unless you or your child specifically requests to remove all of their samples from the study.

Your child may be removed from the study without your or their consent if the study doctor feels it is in their best medical interest, if the scientific goals of the study change, or if the study is cancelled or stopped early. Your child will also be removed if you your child decides to not allow their samples to be stored anymore. If your child is removed from the study then that will not affect your participation in other protocols at the NIH.

WILL IT COST TO PARTICIPATE?

There will be no charge to you or your health insurance company for any tests, procedures, or medications conducted or given out at the NIH. We do not pay for your medical care outside the NIH. During the study, medical care and medications provided outside the NIH, including care recommendations from the study and anything not directly related to the study, must continue to be provided by your child's personal doctor.

IS THERE COMPENSATION FOR THIS STUDY?

You will be reimbursed for travel according to the NIAID/NIH travel policy. You will also be compensated $20 for completing each study visit after the screening visit. The maximum total compensation for this study is $160.

WHAT WILL HAPPEN TO STORED SAMPLES AND DATA?

If you and your child agree to participate in this study, then you also agree to let us store your samples and data for future research. These stored samples and data may help us learn more about AD. Genetic testing may be performed. We will label your stored samples and data with a code that only the study team can link to you. We will keep any information that can be traced back to you as private as possible. If you change your mind and decide you do not want us to store your samples or data, then please contact us. We will do our best to comply with your request, but cannot guarantee that we will always be able to destroy all your samples and data.

We might send your samples or data to other investigators for their research, along with the coded label. We might also share information such as your sex, age, health history, or ethnicity. We will not sell your samples or data and you will not be paid for any products that result from the research. Some future studies may need health information (such as smoking history or present health status) that we don't already have. If so, then our study team will contact you. Future research that uses your samples or data will probably not help you, but it may help us learn more about how to treat AD. In general, the research tests we perform are not like routine medical tests, and may not relate directly to your medical care. The greatest risk associated with storing samples and data is that someone may take information from your medical records without your permission. The chances of this happening are very low. If this information becomes available, then you may face discrimination when you apply for insurance or a job. You may also have similar problems if you share the information yourself or let us release your medical records.

HOW WILL DATA BE SHARED?

To advance science, it is helpful for researchers to share information they get from studying human research participants and their data. They do this by putting data into one or more scientific databases, where they are stored along with information from other studies. Researchers can then study the information combined from many studies to learn even more about health and disease.

If you and your child agree to take part in this study, then some of your child's data will be placed into one or more scientific databases after the data have been stripped of identifiers such as name, address, and birth date. Your child's data may then be used for future research on any topic and shared broadly for research purposes. A researcher who wants to study the information must apply for access to the database. Researchers who are approved to access the database may be able to see and use your child's information, along with information from many other people. Your child will not receive direct benefits from future research that uses your data.

WHAT IF THERE ARE NEW FINDINGS?

Any new findings discovered during this study that are considered relevant to your child's health or to your or your child's decision to continue in the study will be fully discussed with you and your child.

IS THERE A CONFLICT OF INTEREST?

The National Institutes of Health reviews the NIH staff researchers associated with this study at least yearly for conflicts of interest. You may ask your research team for additional information or for a copy of the NIH Guide to Avoiding Financial and Non-Financial Conflicts or Perceived Conflicts of Interest in Clinical Research. This protocol may have investigators who are not NIH employees. Non-NIH investigators are expected to adhere to the principles of this Guide but are not required to report their personal financial holdings to the NIH.

The NIH and the research team for this study are using a laboratory-made strain of *R mucosa*. NIH researchers may plan to use the results, and may get patents, or sell the drug in the future, or make profits other ways. This means it is possible that the results of this study could lead to payments to NIH scientists and to the NIH. By law, government

scientists are required to receive such payments for their inventions. You will not receive any money from the development of *R mucosa*.

CLINICALTRIALS.GOV

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by US Law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

MEDICAL RECORD	CONSENT TO PARTICIPATE IN A CLINICAL RESEARCH STUDY • Adult Patient or • Parent, for Minor Patient

STUDY NUMBER: 17-I-0033 CONTINUATION: Page 8 of 8 pages

OTHER PERTINENT INFORMATION

1. Confidentiality. When results of an NIH research study are reported in medical journals or at scientific meetings, the people who take part are not named and identified. In most cases, the NIH will not release any information about your research involvement without your written permission. However, if you sign a release of information form, for example, for an insurance company, the NIH will give the insurance company information from your medical record. This information might affect (either favorably or unfavorably) the willingness of the insurance company to sell you insurance.

The Federal Privacy Act protects the confidentiality of your NIH medical records. However, you should know that the Act allows release of some information from your medical record without your permission, for example, if it is required by the Food and Drug Administration (FDA), members of Congress, law enforcement officials, or authorized hospital accreditation organizations.

2. Policy Regarding Research-Related Injuries. The Clinical Center will provide short-term medical care for any injury resulting from your participation in research here. In general, no long-term medical care or financial compensation for research-related injuries will be provided by the National Institutes of Health, the Clinical Center, or the Federal Government. However, you have the right to pursue legal remedy if you believe that your injury justifies such action.

3. Payments. The amount of payment to research volunteers is guided by the National Institutes of Health policies. In general, patients are not paid for taking part in research studies at the National Institutes of Health. Reimbursement of travel and subsistence will be offered consistent with NIH guidelines.

4. Problems or Questions. If you have any problems or questions about this study, or about your rights as a research participant, or about any research-related injury, contact the Principal Investigator Ian Myles, Building 10, Room 11S256, Telephone (301) 451-8420.

You may also call the Clinical Center Patient Representative at (301) 496-2626.

5. Consent Document. Please keep a copy of this document in case you want to read it again.

COMPLETE APPROPRIATE ITEM(S) BELOW:

A. Adult Patient's Consent

I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby consent to take part in this study.

Signature of Adult Patient/Legal Representative _____ Date _____

Print Name _____

B. Parent's Permission for Minor Patient.

I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby give permission for my child to take part in this study.
(Attach NIH 2514-2, Minor's Assent, if applicable.)

Signature of Parent(s)/Guardian _____ Date _____

Print Name _____

C. Child's Verbal Assent (If Applicable)

The information in the above consent was described to my child and my child agrees to participate in the study.

Signature of Parent(s)/Guardian _____ Date _____ Print Name _____

THIS CONSENT DOCUMENT HAS BEEN APPROVED FOR USE FROM OCTOBER 29, 2018 THROUGH OCTOBER 28, 2019..

Signature of Investigator _____ Date _____ Signature of Witness _____ Date _____

Print Name _____ Print Name _____

MEDICAL RECORD	**MINOR PATIENT'S ASSENT TO PARTICIPATE IN A CLINICAL RESEARCH STUDY** • Attach to NIH-2514-2, Consent to Participate in a Clinical Research Study

INSTITUTE: National Institute of Allergy and Infectious Diseases

STUDY NUMBER: 17-I-0033 PRINCIPAL INVESTIGATOR: Ian Myles, MD

STUDY TITLE: Beginning Assessment of Cutaneous Treatment Efficacy of Roseomonas in Atopic Dermatitis Phase I/II

Continuing Review Approved by the IRB on 10/29/18
Amendment Approved by the IRB on 05/06/19 (H) Date Posted to Web: 05/15/19
Standard Assent B

Why are we doing this study?

We are asking you to join this study because you have a skin disease called atopic dermatitis. We call this AD for short. AD can make your skin dry and itchy. It can make rashes on your skin. AD also can make it easier for you to get sick from bad germs. There are medicines to make AD easier to deal with, but they do not completely get rid of AD. We do not know what makes people get AD.

All people have germs that live on their skin. Some of these germs are important for your health. They help to fight bad germs that can make you sick. We think that AD might happen because you do not have enough of these good germs.

In this study, we want to see if good germs that we have made in a laboratory can help your skin heal and get rid of your AD. These good germs are called *R mucosa*. Now we will explain the study so you can decide if you want to join. It is your choice to join.

What will happen in this study?

Screening

If you want to be in this study, then first we have to make sure this is an appropriate study for you to join. This is called screening. This visit will take a couple of hours. We will talk with you and your parents or guardians about your health and how you feel. We will give you a checkup. We will use a special machine called a VapoMeter to look more closely at your skin. We will also use a needle to take some blood from your arm for lab tests.

One of these blood tests is to see if you have the virus that causes AIDS. This is called an HIV test. We will share the results of the HIV test with you and your parents or guardians. You cannot be in this study if you have HIV. We will only do an HIV test if you have ever had a blood transfusion or if you are sexually active. If you had an HIV test within the last 6 months and it was negative then we will not do another one.

If you have started your period then we will give you a pregnancy test. If you are pregnant, then you cannot be in this study because the good germs could be dangerous to the fetus. We will share the results of your pregnancy test with you. We will also help you share your test results with your parents or legal guardians.

If you have started your period and are sexually active then you will also need to use birth control while on this study. The study doctors can explain what kinds of birth control are available and how to use them properly. It is important that you contact the study doctor or nurse right away if you think you may be pregnant, if you have missed a period, or if your period is late.

First visit

After we are sure you can join this study, you will have your first study visit. We will do this right after screening if we can. If not then you will come back to the NIH some time within 3 months. This visit will take 1 to 3 hours. We will give you a checkup and ask some questions about your AD. We will use the VapoMeter to look at your skin. We will collect some skin cells and germs on your skin by rubbing a cotton swab (like a Q-tip) on your arm. We will also take pictures of your skin and use a needle to take some blood from your arm for lab tests.

We will then show you and your parents or guardians how to put the good germs onto your arm. The good germs are in a liquid. Your parent or guardian will use a small spray bottle to put it onto your arm. It will dry very quickly and might feel a little sticky. You should not to touch it for a few minutes so you do not accidentally wipe it off.

You parent or guardian will spray the good germs onto your arm twice a week for 4 weeks. We will call, email, or text your parent or guardian during this time so they can ask us questions. You can talk to us, too, if you have a question.

Next visits

You will come back to the NIH four more times, about once per month. At these visits, we will give you a checkup, talk to you about your AD, use the VapoMeter to look at your skin, collect skin cells and skin germs with a cotton swab, take pictures, and take some blood. At all but the last visit, we will give you more of the good germs to take home. These visits will each take about 2 hours.

After these 4 months end, you will stop spraying the good germs onto your skin.

Follow-up visit

We will keep in contact with you and your parents or guardians for another year so we can see how the good germs have affected you. You will come to the NIH one more time during this year. We will give you a checkup and collect some blood for lab tests. This visit will take about 1 hour. You will be done with this study 1 year after finishing the good germs.

Will it hurt to be in this study?

R mucosa is a type of bacteria that normally lives on healthy skin. It does not usually make people sick. The people who can get sick from it are usually already very sick with something else. They are not able to fight off good or bad germs. So we do not think you will get sick from these good germs. Some patients have complained of itching or increased redness at the places where they have applied the bacteria – this has lasted from a few minutes to one day but has not been bad enough for the patients to want to quit treatment.

There may be other risks with these good germs we do not know yet. If we do find anything new then we will let you know so you can decide if you want to keep taking it.

We will collect some blood from your arm for lab tests. You will feel a pinch when we use the needle to get the blood and this could leave a black and blue spot on the skin. We can put a special cream on your skin before this so it does not hurt as much. You can ask your doctor or nurse about it.

We may want to use some of your blood or skin samples to test your genes. Genes are instructions that tell the body how to work and grow. Some genes can make you sick. We may do tests to look at your genes. If we learn something that could help you, then we will give this information to you and your parents.

We will take pictures of your skin. These pictures will allow us to keep a good record of your illness and see how it changes over time. We will try very hard to make sure that you cannot be identified in the pictures. We may also want to publish these pictures in medical journals. You can decide if you want your pictures to be published or not.

The VapoMeter is a small machine that looks like a pen. We press it against your arm for a few seconds so it can look at your skin. This does not hurt. Rubbing the cotton swab on your skin to collect cells also will not hurt.

Will this study help me?

We do not know if spraying the good germs onto your skin will help with your AD. It might or it might not. The information we collect from you on this study will help us learn more about using good germs like *R mucosa* to help people with AD.

Do I have to be in this study?

You do not have to be in this study if you do not want to. It is your decision. You should talk to your parents or guardians and your doctor about it. You can always ask us questions if you have any and we will answer them. If you decide not to be in the study, then you may continue to take the medicines you are on now if that is what your doctor thinks is best. If you say you want to join the study now, but you decide that you want to stop later, that is okay too, just tell your doctor or nurse. We will respect whatever you and your parents or guardians think is best for you.

I have had this study explained to me in a way that I understand, and I have had the chance to ask questions. I agree to take part in this study.

Signature of Minor Patient:_____Date:_____

Print Name:_____

Signature of Investigator:_____Date:_____

Print Name:_____

MEDICAL RECORD	**MINOR PATIENT'S ASSENT TO PARTICIPATE IN A CLINICAL RESEARCH STUDY** • Attach to NIH-2514-2, Consent to Participate in a Clinical Research Study

INSTITUTE: National Institute of Allergy and Infectious Diseases

STUDY NUMBER: 17-I-0033 PRINCIPAL INVESTIGATOR: Ian Myles, MD

STUDY TITLE: Beginning Assessment of Cutaneous Treatment Efficacy of Roseomonas in Atopic Dermatitis Phase I/II

Continuing Review Approved by the IRB on 10/29/18
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Standard Assent 7-10 years old

Why are we doing this study?

We are asking you to join this study because you have a skin disease called atopic dermatitis. We call this AD for short. AD can make your skin dry and itchy, and cause rashes. It also make it easier for you to get sick from bad germs. There are medicines you can take to help, but they do not completely make you better. We do not know what makes people get AD.

All people have germs that live on their skin. Some of these germs are good for your health. They help to fight bad germs that can make you sick. We think you have AD because you do not have enough of these good germs.

In this study, we want to see if good germs that we have made in a laboratory can help your skin heal and get rid of your AD. Now we will explain the study so you can decide if you want to join. It is your choice to join.

What will happen in this study?

The good germs are in a spray that your parents or guardians will put on your arm. We will show you and your parents or guardians how to do it. The spray will dry very quickly and might feel a little sticky. You should not touch it for a few minutes so you do not accidentally wipe it off.

Your parents or guardians will spray the good germs onto your arm twice a week for 4 weeks. We will call, email, or text your parents or guardians during this time so they can ask us questions. You can talk to us, too, if you have a question.

You will have to come to the NIH every month for 4 months for a checkup. At these visits, we will talk to you about how you are feeling and do some tests. These visits will each take about 2 hours.

After these 4 months end, you will stop spraying the good germs onto your skin.

We will keep in contact with you and your parents or guardians for another year so we can see how the good germs have affected you. You will come to the NIH one more time during this year for one last checkup. This visit will take about 1 hour. You will be done with this study 1 year after finishing the good germs.

Will it hurt to be in this study?

The good germs we use do not usually make people sick. The people who can get sick from it are usually already very sick with something else. They are not able to fight off good or bad germs. So we do not think you will get sick from

these good germs. Some patients have complained of itching or increased redness at the places where they have applied the bacteria – this has lasted from a few minutes to one day but has not been bad enough for the patients to want to quit treatment.

The good germs might do things we do not know about yet. If we do find anything new then we will let you and your parents or guardians know so you can decide if you want to keep taking it.

When you come to the NIH for checkups, we will take some blood from your arm for lab tests. You will feel a pinch when we use the needle to get the blood and this could leave a black and blue spot on the skin. We can put a special cream on your skin before this so it does not hurt as much. You can ask your doctor or nurse about it.

We will collect some skin cells and germs from your skin. There are two ways we do this. One is to rub a cotton swab (like a Q-tip) on your arm, which does not hurt. The other way is to press a special piece of tape to your arm. It might hurt a little when we take the tape off. We will also touch your arm with a small machine that looks like a pen to measure how much water is in your skin. This does not hurt.

We may want to do some tests on your blood and skin samples to look at your genes. Genes are instructions that tell the body how to work and grow. Some genes can make you sick. If we learn something about your genes that could help you, then we will give this information to you and your parents or guardians.

We will take pictures of your skin so we can see how your AD changes while you are in this study. We will try very hard to make sure that no one else can tell it is you in the pictures.

Will this study help me?

We do not know if spraying the good germs onto your skin will help with your AD. It might or it might not. The information we learn from this study will help us learn more about using good germs to help people with AD.

Do I have to be in this study?

You do not have to be in this study if you do not want to. It is your decision. You should talk to your parents or guardians and your doctor about it. You can always ask us questions if you have any and we will answer them. If you decide not to be in the study, then you may continue to take the medicines you are on now if that is what your doctor thinks is best. If you say you want to join the study now, but you decide that you want to stop later, that is okay too, just tell your doctor or nurse. We will respect whatever you and your parents or guardians think is best for you.

MEDICAL RECORD	**CONTINUATION SHEET for either:** **NIH 2514-1, Consent to Participate in A Clinical Research Study** **NIH 2514-2, Minor Patient's Assent to Participate In A Clinical Research Study**

STUDY NUMBER: 17-I-0033

I have had this study explained to me in a way that I understand, and I have had the chance to ask questions. I agree to take part in this study.

Signature of Minor Patient:_____Date:_____

Print Name:_____

Signature of Investigator:_____Date:_____

Print Name:_____

MEDICAL RECORD	**MINOR PATIENT'S ASSENT TO PARTICIPATE IN A CLINICAL RESEARCH STUDY** • Attach to NIH-2514-2, Consent to Participate in a Clinical Research Study

INSTITUTE: National Institute of Allergy and Infectious Diseases

STUDY NUMBER: 17-I-0033 PRINCIPAL INVESTIGATOR: Ian Myles, MD

STUDY TITLE: Beginning Assessment of Cutaneous Treatment Efficacy of Roseomonas in Atopic Dermatitis Phase I/II

Continuing Review Approved by the IRB on 10/29/18
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Standard Assent 11-17 years old

Why are we doing this study?

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All people have germs that live on their skin. Some of these germs are important for your health. They help to fight bad germs that can make you sick. We think that AD might happen because you do not have enough of these good germs.

In this study, we want to see if good germs that we have made in a laboratory can help your skin heal and get rid of your AD. These good germs are called *R mucosa*. Now we will explain the study so you can decide if you want to join. It is your choice to join.

What will happen in this study?

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If you want to be in this study, then first we have to make sure this is an appropriate study for you to join. This is called screening. This visit will take a couple of hours. We will talk with you and your parents or guardians about your health and how you feel. We will give you a checkup. We will use a special machine called a VapoMeter to look more closely at your skin. We will also use a needle to take some blood from your arm for lab tests.

One of these blood tests is to see if you have the virus that causes AIDS. This is called an HIV test. We will share the results of the HIV test with you and your parents or guardians. You cannot be in this study if you have HIV. We will only do an HIV test if you have ever had a blood transfusion or if you are sexually active. If you had an HIV test within the last 6 months and it was negative then we will not do another one.

If you have started your period then we will give you a pregnancy test. If you are pregnant, then you cannot be in this study because the good germs could be dangerous to the fetus. We will share the results of your pregnancy test with you. We will also help you share your test results with your parents or legal guardians.

If you have started your period and are sexually active then you will also need to use birth control while on this study. The study doctors can explain what kinds of birth control are available and how to use them properly. It is important that you contact the study doctor or nurse right away if you think you may be pregnant, if you have missed a period, or if your period is late.

First visit

After we are sure you can join this study, you will have your first study visit. We will do this right after screening if we can. If not then you will come back to the NIH some time within 3 months. This visit will take 1 to 3 hours. We will give you a checkup and ask some questions about your AD. We will use the VapoMeter to look at your skin. We will collect some skin cells and germs on your skin by rubbing a cotton swab (like a Q-tip) on your arm. Another way we will collect skin cells is to press a special strip of tape to your arm. We will also take pictures of your skin and use a needle to take some blood from your arm for lab tests.

We will then show you and your parents or guardians how to put the good germs onto your arm. The good germs are in a liquid. Your parent or guardian will use a small spray bottle to put it onto your arm. It will dry very quickly and might feel a little sticky. You should not to touch it for a few minutes so you do not accidentally wipe it off.

Your parent or guardian will spray the good germs onto your arm twice a week for 4 weeks. We will call, email, or text your parent or guardian during this time so they can ask us questions. You can talk to us, too, if you have a question.

Next visits

You will come back to the NIH four more times, about once per month. At these visits, we will give you a checkup, talk to you about your AD, use the VapoMeter to look at your skin, collect skin cells and skin germs with a cotton swab, take pictures, and take some blood. At all but the last visit, we will give you more of the good germs to take home. And at the last visit, we will use the special tape to collect cells from your skin, too. These visits will each take about 2 hours.

After these 4 months end, you will stop spraying the good germs onto your skin.

Follow-up visit

We will keep in contact with you and your parents or guardians for another year so we can see how the good germs have affected you. You will come to the NIH one more time during this year. We will give you a checkup and collect some blood for lab tests. This visit will take about 1 hour. You will be done with this study 1 year after finishing the good germs.

Will it hurt to be in this study?

R mucosa is a type of bacteria that normally lives on healthy skin. It does not usually make people sick. The people who can get sick from it are usually already very sick with something else. They are not able to fight off good or bad germs. So we do not think you will get sick from these good germs. Some patients have complained of itching or increased redness at the places where they have applied the bacteria – this has lasted from a few minutes to one day but has not been bad enough for the patients to want to quit treatment.

There may be other risks with these good germs we do not know yet. If we do find anything new then we will let you know so you can decide if you want to keep taking it.

We will collect some blood from your arm for lab tests. You will feel a pinch when we use the needle to get the blood and this could leave a black and blue spot on the skin. We can put a special cream on your skin before this so it does not hurt as much. You can ask your doctor or nurse about it.

MEDICAL RECORD	**CONTINUATION SHEET for either:** **NIH 2514-1, Consent to Participate in A Clinical Research Study** **NIH 2514-2, Minor Patient's Assent to Participate In A Clinical Research Study**

STUDY NUMBER: 17-I-0033 CONTINUATION: Page 3 of 3 pages

We may want to use some of your blood or skin samples to test your genes. Genes are instructions that tell the body how to work and grow. Some genes can make you sick. We may do tests to look at your genes. If we learn something that could help you, then we will give this information to you and your parents or guardians.

We will take pictures of your skin. These pictures will allow us to keep a good record of your illness and see how it changes over time. We will try very hard to make sure that you cannot be identified in the pictures. We may also want to publish these pictures in medical journals. You can decide if you want your pictures to be published or not.

The VapoMeter is a small machine that looks like a pen. We press it against your arm for a few seconds so it can look at your skin. This does not hurt.

Rubbing the cotton swab on your skin to collect cells also will not hurt. The special tape to collect skin cells might be a little uncomfortable, but is less painful than peeling off a bandage.

Will this study help me?

We do not know if spraying the good germs onto your skin will help with your AD. It might or it might not. The information we collect from you on this study will help us learn more about using good germs like *R mucosa* to help people with AD.

Do I have to be in this study?

You do not have to be in this study if you do not want to. It is your decision. You should talk to your parents or guardians and your doctor about it. You can always ask us questions if you have any and we will answer them. If you decide not to be in the study, then you may continue to take the medicines you are on now if that is what your doctor thinks is best. If you say you want to join the study now, but you decide that you want to stop later, that is okay too, just tell your doctor or nurse. We will respect whatever you and your parents or guardians think is best for you.

I have had this study explained to me in a way that I understand, and I have had the chance to ask questions. I agree to take part in this study.

Signature of Minor Patient:_____Date:_____

Print Name:_____

Signature of Investigator:_____Date:_____

Print Name:_____