PROTOCOL ADRN-12

LONGITUDINAL ENDOTYPING OF ATOPIC DERMATITIS THROUGH TRANSCRIPTOMIC SKIN ANALYSIS

LEADS STUDY

Version Number: 6.0 **Version Date**: 06 Mar 2025

Investigational New Drug (IND): Exempt

National Clinical Trial (NCT): NCT05436535

Study Sponsor: The National Institute of Allergy and Infectious Diseases (NIAID)

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Consortium/Network: Atopic Dermatitis Research Network (ADRN)

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Protocol Number: ADRN-12	**Version Number/Date:** 6.0/06 Mar 2025
Protocol Title: Longitudinal Endotyping of Atopic Dermatitis through Transcriptomic Skin Analysis (LEADS)	
Study Sponsor: The National Institute of Allergy and Infectious Diseases (NIAID)	

INSTRUCTIONS:

*The original signature page must be kept for your records. Return an electronic PDF copy of the signed signature page (*as described below) to the DAIT Regulatory Management Center via the applicable DAIT RMC email address for the protocol/network:* [redacted]

I confirm that I have read the above protocol in the latest version. I understand it, and I will work according to the principles of Good Clinical Practice (GCP) as described in the United States Code of Federal Regulations (CFR) – 45 CFR part 46 and 21 CFR parts 50, 56, 312, and 812 and in the International Conference for Harmonisation (ICH) document entitled *Integrated Addendum to ICH E6(R1): Guideline for Good Clinical Practice E6(R2)*. Further, I will conduct the study in keeping with local legal and regulatory requirements.

I agree to ensure that all staff members involved in the conduct of this study are informed about their obligations in meeting the above commitments.

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

*[*The site Principal Investigator should sign and date at the indicated location below. A written signature/date is acceptable (e.g., scanned and sent via email as a PDF version). An electronic signature is also acceptable (e.g., sent via email as a PDF version).]*

_____

Site Principal Investigator (Print)

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Site Principal Investigator (Signature) **Date**

Protocol Synopsis

<table>
<tr><td>Title</td><td>Longitudinal Endotyping of Atopic Dermatitis through Transcriptomic Skin Analysis</td></tr>
<tr><td>Short Title</td><td>LEADS Study</td></tr>
<tr><td>Clinical Phase</td><td>Post-Marketing Mechanistic Study</td></tr>
<tr><td>Number of Sites</td><td>Up to 15 clinical sites in the United States</td></tr>
<tr><td>Sponsor</td><td>National Institute of Allergy and Infectious Diseases</td></tr>
<tr><td>Study Design</td><td>This is a multi-center, prospective, longitudinal study which will characterize the gene expression profiles and transcriptomic endotypes that underlie mild and moderate-to-severe AD and evaluate changes in these expression patterns and endotypes in response to standard-of-care treatment as delineated in the protocol. The study will enroll participants in three groups:
Participants with AD that is not currently being managed with dupilumab (dupilumab-naïve AD; DNAD)
Participants with AD that is currently being treated with dupilumab and has been treated with dupilumab for at least 4 months prior to enrollment (long-term dupilumab use; LTD)
Participants without AD (non-AD; NAD)
DNAD participants will complete up to ten scheduled study visits with assessment of topical steroid response and, if uncontrolled with topical steroids, dupilumab response. Skin samples will be collected at all study visits to determine the gene expression profiles and transcriptomic endotypes that underlie mild vs. moderate-to-severe AD disease. We may also evaluate the lipidomic, metabolomic, proteomic, and microbiome profiles of AD skin endotypes associated with mild and moderate-to-severe AD disease. NAD participants will complete up to seven scheduled study visits and will serve as a control population. LTD participants will complete up to six scheduled study visits, which will allow comparison of their skin gene expression profiles to those of participants who begin dupilumab treatment during this study.</td></tr>
<tr><td>Study Objectives & Endpoints</td><td>The primary and secondary objectives for the trial are listed below in two tables, separated by priority and including the related hypotheses for each analysis. Additional exploratory endpoints are outlined in Section 2.1.

Endpoints will be collected based on enrollment group and treatment course as delineated in the schedules of events (Appendix A), and analysis timepoints for each exploratory objective will be noted in the statistical analysis plan (SAP).

<table>
<tr><th>Primary Objective:</th><th>Related Endpoint:</th><th>Related Hypothesis:</th></tr>
<tr><td>1. To determine if the type 2-high non-lesional skin (skin tape) endotype is associated with current mild versus moderate-to-severe AD disease</td><td>1. Non-lesional skin tape transcriptome at Day 7 (long-term dupilumab participants excluded)</td><td>1. The type 2-high skin endotype in non-lesional skin is associated with severity of AD disease.</td></tr>
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<tr><td></td><td>
<table>
<tr><th>Secondary Objectives:</th><th>Related Endpoints:</th><th>Related Hypotheses:</th></tr>
<tr><td>1. To determine how gene expression in the skin (skin tape) differs between non-AD participants and those with current mild or moderate-to-severe AD disease</td><td>1. Non-lesional and lesional skin tape transcriptome at Day 7 and Day 168-224 (long-term dupilumab participants excluded)</td><td>1. Distinct transcriptomic signatures detectable in tape collected skin underlie mild and moderate-to-severe AD.</td></tr>
<tr><td>2. To determine how gene expression in the skin (skin tape) changes with standard-of-care treatment (two timepoints) among the study outcome groups: (1) topical steroid responders, (2) dupilumab responders, (3) dupilumab non-responders, (4) non-AD, and (5) long-term dupilumab participants</td><td>2. Non-lesional and lesional skin tape transcriptome at Day 7 and Day 168-224</td><td>2. The clinical response to protocol's standard-of-care treatment among different AD participants will be associated with distinct transcriptional shifts in tape collected skin over time.</td></tr>
</table>
</td></tr>
<tr><td>Accrual Objective</td><td>This study aims to enroll approximately 600 participants total, 6 years of age and older. These 600 participants will be enrolled as a part of the three planned groups as indicated below:
• Approximately 390 participants who have AD that is not currently being managed with dupilumab, including:
o 160 adults with moderate-to-severe AD (EASI > 7).
o 80 pediatric participants (6-17 years old) with moderate-to-severe AD.
o 100 adults with mild AD (EASI ≤ 7).
o 50 pediatric participants with mild AD.
• Approximately 60 LTD participants, including 20 pediatric and 40 adult participants.
• Approximately 150 NAD participants, including 50 pediatric and 100 adult participants.

The subtotals above based on enrollment group and age are estimates, but will be targeted closely to accommodate necessary sample sizes for each planned analysis.</td></tr>
<tr><td>Study Duration</td><td>This study will take approximately 36 months to complete, with enrollment expected to take approximately 29 months. An individual participant's involvement in the study will take approximately 6-8 months, depending on enrollment group and including the week-long screening period.</td></tr>
<tr><td>Treatment Description</td><td>Study medications for this protocol are all FDA approved and are being used as indicated. Participants will receive standard-of-care treatment as delineated in Section 3.1.

Vanicream™ Moisturizing Cream will be provided for all study participants.</td></tr>
</table>

	Triamcinolone 0.1% ointment (for non-sensitive body regions), hydrocortisone 2.5% ointment (for sensitive body regions), and dupilumab will be provided to DNAD participants, as applicable. DNAD participants will initially receive treatment with topical steroids. Those who do not initially respond to topical steroids and those who subsequently have AD that becomes uncontrolled (EASI >7) on or before the 12 Week Steroid Assessment (approximately Day 91) will begin treatment with dupilumab. LTD participants will use dupilumab and topical corticosteroid treatments provided by their physicians outside of the study. LTD participants will continue dupilumab treatment as prescribed by their physician outside of the study. These participants may apply topical steroids prescribed by their primary care physician to non-target skin as needed.
Inclusion Criteria	Individuals who meet all of the following criteria are eligible for enrollment as study participants: <u>All Participants:</u> 1. Participant and/or parent guardian must be able to understand and provide informed consent and assent (if applicable) 2. Male or female, 6 years of age or older inclusive at the Screening Visit 3. Participants must agree to apply a stable dose of the study provided topical moisturizer (Vanicream™ Moisturizing Cream) at least twice daily between the Baseline Assessment and Day 7 Visits to a specified skin target area. 4. Individuals with persistent asthma must adhere to asthma controller medication(s) as prescribed by their physician for the duration of the study. 5. Individuals who can become pregnant, as defined in the study manual of procedures, must have a negative pregnancy test at the Baseline Assessment and Day 7 Visits if they do not self-report as pregnant. 6. Individuals who can become pregnant, as defined in the study manual of procedures, must meet either of the following criteria prior to Baseline: a) Willing to remain abstinent from intercourse that may result in a pregnancy. b) Willing to use an FDA-approved method of contraception for the duration of study participation. Acceptable methods include the following: • Permanent sterilization of partner • Long-acting reversible contraceptives (e.g., intrauterine devices or systems, implantable rods, contraceptive injections) when used as directed for at least 7 days prior to Baseline. • Short-acting hormonal contraceptives (e.g., oral contraceptive pills, patch, vaginal ring) when used as directed for at least 30 days prior to Baseline. • Barrier methods (e.g., condoms; diaphragm, sponge, or cervical cap with spermicide) 7. Participant and/or parent guardian must be able to understand and complete study-related questionnaires. 8. Participants must have non-lesional skin on the upper or lower extremities or trunk of sufficient size and in the required locations, as specified in the study manual of procedures (MOP), for specimen collection at the Baseline Assessment and Day 7 Visits. <u>Non-AD Participants:</u> 9. NAD participants must have no history of AD or food allergy as diagnosed by a physician.

	All AD Participants (DNAD and LTD): 10. DNAD and LTD participants must have a history of chronic AD, (according to the Atopic Dermatitis Research Network [ADRN] Standard Diagnostic Criteria [Appendix B]), that has been present for at least 1 year before the Screening Visit. 11. DNAD and LTD participants must agree to refrain from applying topical steroid to a specified target area between the Baseline Assessment and Day 7 Visits. DNAD Participants: 12. DNAD participants must have active lesions on the upper or lower extremities or trunk of sufficient size and in the required locations, as specified in the study manual of procedures (MOP), for specimen collection at the Baseline Assessment and Steroid Initiation (Day 7) Visits. LTD Participants: 13. LTD participants must be currently receiving dupilumab and must have started dupilumab treatment ≥ 4 months prior to the Screening Visit.
Exclusion Criteria	Individuals who meet any of these criteria are not eligible for enrollment: 1. Inability or unwillingness of a participant or parent guardian to comply with the study protocol 2. Have a genetic relative (e.g., parent, sibling, grandchild, half-sibling) or household member (e.g., spouse) already enrolled in the study 3. Weight less than 15 kg 4. Known systemic hypersensitivity to any of the excipients of the study treatments (Vanicream™ Moisturizing Cream, hydrocortisone, triamcinolone, or dupilumab) 5. Have any skin disease other than AD that might compromise the stratum corneum barrier (e.g., bullous diseases, psoriasis, cutaneous T cell lymphoma [also called Mycosis Fungoides or Sezary syndrome], dermatitis herpetiformis, Hailey-Hailey, or Darier's disease) 6. Known or suspected immunosuppression, including history of invasive opportunistic infections (e.g., tuberculosis, histoplasmosis, listeriosis, coccidioidomycosis, pneumocystosis, aspergillosis) despite infection resolution, or otherwise recurrent immune-compromised status, as judged by investigator. 7. Known history of human immunodeficiency virus (HIV) infection 8. Ocular disorder that in the opinion of the investigator could adversely affect the individual's risk for study participation. Examples include, but are not limited to, individuals with a history of or active case of herpes keratitis; Sjogren's Syndrome, Keratoconjunctivitis Sicca, or Dry Eye Syndrome that require daily use of supplemental lubrication; or individuals with ocular conditions that require the regular use of ocular corticosteroids or cyclosporine. 9. Parasitic infection, except for vaginal trichomoniasis, within 12 months of the Screening Visit, or high risk for contracting parasitic infections (e.g., traveling to endemic areas) 10. History of malignancy within 5 years before the Screening Visit (completely treated in situ carcinoma of the cervix, and completely treated and resolved non-metastatic squamous or basal cell carcinoma of the skin or melanoma in situ are not exclusionary) 11. History of non-malignant lymphoproliferative disorders 12. History of alcohol or drug abuse within 2 years before the Screening Visit 13. History of keloid formation in adult participants 14. History of hypersensitivity to local anesthetics (e.g., lidocaine or Novocain), bleeding disorders, or treatment with anticoagulants or other conditions in adult participants that would make the biopsy procedure inadvisable

15. History of serious life-threatening reaction to tape or adhesives
16. Individuals with asthma who have required use of a systemic corticosteroid within 3 months prior to the Screening Visit or who require a dose greater than 880 mcg/day of fluticasone propionate or equivalent inhaled corticosteroid to maintain asthma control
17. Planned major surgical procedure that could affect study participation or outcome assessment, per PI discretion
18. Chronic or acute infection requiring treatment with systemic antibiotics, antivirals, antiparasitics, antiprotozoals, or antifungals within 4 weeks before the Baseline Assessment, or superficial skin infection within 1 week before the Baseline Assessment
19. Pregnant or breast-feeding women, or women planning to become pregnant or breastfeed during the study
20. Use of any systemic (oral, IV, IM) immunosuppressive/immunomodulating therapies (e.g., steroids, cyclosporine, Janus kinase (JAK) inhibitors, mycophenolate, azathioprine, or methotrexate) within 4 weeks of the Baseline Assessment, or any condition that, in the opinion of the investigator, will likely require such treatment(s) during study participation
21. Treatment with biologics (other than dupilumab) as follows:
    a. Any cell-depleting agents, including but not limited to rituximab, within 6 months before the Baseline Assessment, or until lymphocyte and CD19+ lymphocyte count returns to normal, whichever is longer
    b. Omalizumab, infliximab, adalimumab, golimumab, certolizumab pegol, abatacept, etanercept, anakinra within 16 weeks before the Baseline Assessment for any indication
    c. Other biologics within 5 half-lives (if known) or 16 weeks before the Baseline Assessment, whichever is longer
22. Treatment with a live (attenuated) vaccine within 7 weeks before the Baseline Assessment or planning to receive a live vaccine during the study
23. Ongoing participation in another research study involving any of the following:
    a. Current or planned use of an investigational drug or device
    b. Current or planned use of prohibited medications or procedures
    c. Substantial time commitment and/or study requirements that, in the opinion of the investigator, may interfere with the participant's ability to comply with LEADS study requirements
24. Use of an investigational drug within 8 weeks or within 5 half-lives (if known), whichever is longer, before the Baseline Assessment
25. Use of topical calcineurin inhibitors (tacrolimus or pimecrolimus), topical phosphodiesterase inhibitors (crisaborale), topical JAK inhibitors (ruxolitinib), or topical aryl hydrocarbon receptor (AhR) agonists (tapinarof) within 1 week before the Baseline Assessment. Use of these medications is not exclusionary when only applied to the face, neck, palms, or soles.
26. Use of phototherapy (such as narrowband ultraviolet B [NBUVB], ultraviolet B [UVB], ultraviolet A1 [UVA1], psoralen + UVA [PUVA]) or a tanning booth/parlor within 4 weeks of the Baseline Assessment
27. Treatment with bleach bath within 1 week before the Baseline Assessment
28. Use of a chlorinated hot tub or pool within 1 week before the Baseline Assessment
29. Initiation of treatment with prescription moisturizers or moisturizers containing ceramide, hyaluronic acid, urea, or filaggrin (FLG) during the study.
    a. Participants may continue using stable doses of such moisturizers on body areas other than the target area if initiated before the Baseline Assessment.

	b. Initiation of prescription moisturizer is not exclusionary when only applied to the face, neck, palms, or soles. 30. Planned or anticipated use of any prohibited medications or procedures during study participation. 31. Past or current medical problems or findings from physical examination that are not listed above, which, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the participant's ability to comply with study requirements or that may impact the quality or interpretation of the data obtained from the study.
Study Stopping Rules	Study enrollment will be suspended pending Division of Allergy, Immunology, and Transplantation (DAIT) National Institute of Allergy and Infectious Diseases (NIAID) and NIAID Allergy and Asthma Data Safety Monitoring Board (DSMB) expedited review of all pertinent data if any of the following occur: 1. One death, or life-threatening adverse event (AE), that is at least possibly related to the study therapy regimen or procedures 2. A grade 3 or higher AE that is at least possibly related to the study therapy regimen or procedures in two or more participants.

Table of Contents

Glossary of Abbreviations

AD	Atopic Dermatitis
ADR	Adverse Drug Reaction
ADRN	Atopic Dermatitis Research Network
AE	Adverse Event
AhR	Aryl hydrocarbon Receptor
AR	Adverse Reaction
CBC	Complete Blood Count
CFR	Code of Federal Regulations
CFU	Colony Forming Unit
CoNS	Coagulase Negative Staphylococcal Species
CPC	Clinical Product Center
CRA	Clinical Research Associate
CRSwNP	Chronic rhinosinusitis with nasal polyps
CTCAE	Common Terminology Criteria for Adverse Events
DAIT	Division of Allergy, Immunology, and Transplantation
DNA	Deoxyribonucleic Acid
DNAD	Dupilumab-Naïve Atopic Dermatitis; identifier for the group of participants to be enrolled in the trial who have AD that is not currently being managed with dupilumab
DoR	Delegation of Responsibility Log
DSMB	Data Safety Monitoring Board
EASI	Eczema Area and Severity Index
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EO	Exploratory Objective
EoE	Eosinophilic Esophagitis
FDA	Food and Drug Administration
FLG	Filaggrin
GCP	Good Clinical Practice
GLM	Generalized Linear Model
GWAS	Genome-wide Association Study
HIV	Human Immunodeficiency Virus
IGA	Investigator Global Assessment
IgE	Immunoglobulin E
IL	Interleukin
IRB	Institutional Review Board
ISR	Injection Site Reaction
JAK	Janus kinase

LTD	Long-term Dupilumab; identifier for the group of participants to be enrolled in the trial who have AD that is currently being treated with dupilumab and has been treated with dupilumab for at least 4 months prior to enrollment
mAb	Monoclonal Antibody
MOP	Manual of Procedures
NAD	Non-AD; identifier for the group of participants to be enrolled in the trial without AD
NBUVB	Narrowband Ultraviolet B
NCI	National Cancer Institute
NESS	Nottingham Eczema Severity Score
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NRS	Numerical Rating Scale; as in Pruritus NRS
OHRP	Office for Human Research Protections
PCR	Polymerase Chain Reaction
PI	[Site] Principal Investigator
PP-NRS	Peak Pruritus Numerical Rating Scale
PUVA	Psoralen and Ultraviolet A
Q2W	Every other week
Q4W	Every 4 weeks
RNA	Ribonucleic Acid
S. aureus	*Staphylococcus aureus*
SCCC	Statistical and Clinical Coordinating Center
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Suspected Adverse Reaction
SCORAD	SCOring Atopic Dermatitis
SO	Secondary Objective
SOP	Standard Operating Procedure
STS	Skin tape strip
TAA	Target Area Assessment
TCS	Topical Corticosteroids
Th	T Helper
TSB	Tryptic Soy Broth
US	United States
UVA1	Ultraviolet A1
UVB	Ultraviolet B
WGCNA	Weighted Gene Co-expression Network Analysis
WTS	Whole Transcriptome Sequence

1 Background and Rationale

1.1 Background and Scientific Rationale

Atopic dermatitis (AD) is a highly prevalent skin disorder that imposes onerous and lifelong psychosocial, financial, educational, and occupational impacts on individuals and families, as well as high economic costs on society. AD is also a complex disease phenotype, with patients exhibiting dramatic differences in severity, degree of itching, *Staphylococcus aureus* (*S. aureus)* colonization, duration of illness, age of onset, and associated co-morbidities (including food allergy and asthma). This phenotypic heterogeneity, moreover, is associated with heterogeneity in response to treatment, with many patients responding well to conventional topical therapies and others requiring novel or yet-to-be-developed systemic biologics, and finding the best treatment for a patient can itself impose a costly and exhausting burden.

This clinical heterogeneity underlying AD suggests that the AD population is composed of multiple subgroups, each with distinct pathobiological mechanisms driving their disease (i.e., endotypes). In support of this, we have previously used transcriptional profiling of the skin to identify a subgroup (50%) of AD subjects with exuberant type 2 cytokine-driven inflammation (Dyjack et al, 2018). In another recent study that transcriptionally endotyped AD subjects, we identified not only type 2-high subjects, but also subjects with expression patterns characteristic of excessive Interleukin (IL)-36γ activity and/or viral driven inflammation (unpublished). Interestingly, we found that most AD subjects with a history of eczema herpeticum (EH) infection exhibited all three endotype patterns (unpublished). Moreover, the literature suggests the existence of additional AD disease endotypes driven, for example, by T helper (Th) 1 and Th17 cells (Brunner et al, 2019a; Czarnowicki et al, 2019; Noda et al, 2015) or by skin colonization with pathogenic bacteria (Simpson et al, 2018), such as *S. aureus*. These endotypic groups are likely, in part, shaped by genetic variation that interacts with environmental factors. For example, strong associations reported between microbial dysbiosis and filaggrin (FLG) null genetic mutations could underlie a distinct AD molecular endotype (Leung et al, 2019; Irvine et al, 2011; Paller et al, 2019). In addition, although FLG mutations are highly associated with disease risk, only 20% of AD subjects exhibit these variants (Irvine et al, 2011), and a recent large AD genome-wide association study (GWAS) (>100,000 subjects) found >30 genetic loci associated with disease risk (Paternoster et al, 2015). Thus, multiple pathobiological mechanisms, or endotypes, may be caused by different constellations of genetic and environmental factors. Yet the precise clinical characteristics and molecular mechanisms of these endotypes, the degree to which they occur and interact within different patients, and measures by which patients can be endotypically profiled all remain unknown.

Clinical heterogeneity in response to therapy is also consistent with AD being a complex, multi-factorial disease, caused by the interaction of a large number of genetic and environmental risk factors. Selection of new mechanistically directed medications, like the type 2-inhibitor, dupilumab, based on carried endotype, would greatly benefit patients. For example, the type 2-high AD endotype, which exhibits more severe disease (Dyjack et al, 2018), likely represents a patient subgroup who would be responsive to such emerging type 2 inhibitor therapies (Guttman-Yassky et al, 2019). In support of this, dupilumab therapy has been shown to only clear or nearly clear approximately 40% of subjects (Beck et al, 2014), nearly matching the AD type 2-high frequency observed in our study. Other endotypes, yet to be discovered, may be poorly served by any existing therapies.

The overall premise of this study is that clinical management of AD patients will require person-level delineation of the multiple endotypes driving disease in this group. Elucidation of these endotypes will occur by way of a well-controlled examination of skin function at the whole transcriptome sequence (WTS) level, allowing for the sampling of possible epidermal, immune, and mesenchymal components of AD skin. Advanced technologies like single cell Ribonucleic Acid sequencing (scRNA-seq) will enable a comprehensive determination of the cell types and functions that are altered to drive particular disease endotypes. We posit that many of these endotypes will modify the lipid and protein components of the epidermis, which then go on to alter skin phenotype and function. Lastly, a combination of genetic risk and non-atopic clinical features such as skin microbial dysbiosis may underlie AD endotypes and/or disease severity. Our ultimate goal through these examinations will be to develop evidence-based, precision medicine approaches to address disease in the most burdened AD patients. Ultimately, knowledge from this study will facilitate development of biomarkers and discovery of pathways to target for the treatment of AD subjects who are not responsive to currently available topical and systemic therapies.

1.2 Rationale for Selection of Intervention

Treatment of AD includes use of moisturizers as a component of basic management and step up to topical anti-inflammatory medications with increased severity (Boguniewicz et al, 2018). Topical corticosteroids (TCS) are considered the mainstay of therapy with hydrocortisone 2.5%, a low potency TCS, used on sensitive areas such as the face and intertriginous areas or for mild AD, and triamcinolone 0.1%, a mid-potency TCS, used for moderate-to-severe AD, but typically avoiding sensitive areas. Both of these TCS have been widely used in clinical practice for many years. Dupilumab, a fully human monoclonal antibody (mAb) binding IL-4 receptor alpha, thus blocking type 2 cytokines IL-4 and IL-13, has been approved by the FDA for patients 6 years and older with moderate-to-severe AD whose disease is not adequately controlled with topical prescription therapies or when those therapies are not advisable. Adult dosing is an initial dose of 600 mg, followed by 300 mg given every other week (Q2W) by subcutaneous injection, with pediatric dosing weight based (≥ 60 kg , 600 mg followed by 300 mg Q2W; 30 to < 60 kg, 400 mg followed by 200 mg Q2W; 15 to < 30 kg, 600 mg followed by 300 mg every 4 weeks [Q4W]). Dupilumab has been shown to improve immune dysregulation and skin barrier abnormalities in patients with AD (Guttman-Yassky et al, 2019).

1.3 Clinical Studies

The pathophysiology of AD is complex and associated with highly variable treatment responses. As such, a stepwise approach is required for management of AD (Boguniewicz et al, 2018; Boguniewicz et al, 2011). Initial steps involve use of skin emollients (moisturizers) to maximize moisturization and topical medications to control local skin inflammation. The systemic nature of AD is increasingly recognized with inflammatory changes that can be measured in a blood proteomic signature at an early age (Brunner et al, 2019b). Recent studies point to systemic T cell activation with expansion of circulating Th2 and Th22 cells (Czarnowicki et al, 2015). Furthermore, non-lesional AD skin is characterized by broad terminal differentiation defects in addition to immune abnormalities (Suárez-Fariñas et al, 2011). While a number of clinical phenotypes and endotypes have been described, type 2 immunity appears to be central to all of them (Czarnowicki et al, 2019).

Dupilumab is a fully human mAb directed at interleukin-4 receptor alpha (IL-4Rα), thus interfering with signaling by both IL-4 and IL-13, two key type 2 cytokines (Hamilton et al, 2015). Treatment of AD patients with dupilumab was shown to suppress molecular markers of cutaneous and systemic type 2 inflammation, as well as reverse epidermal abnormalities that coincided with clinical improvement (Guttman-Yassky et al, 2019). In two phase 3 trials, adult patients with moderate-to-severe AD inadequately controlled on topical treatment were treated with dupilumab, 600 mg loading dose followed by 300 mg weekly or Q2W, or placebo by subcutaneous injection (Simpson et al, 2016). The primary outcome of an Investigator Global Assessment (IGA) of 0 or 1 (clear or almost clear) and a reduction of 2 points or more in that score from baseline at week 16 was achieved by 36-38% of patients on dupilumab monotherapy at week 16 versus 8-10% on placebo ($p<0.001$). In addition, improvement of at least 75% in Eczema Area and Severity Index (EASI-75) from baseline to week 16 was reported in ~50% of patients on dupilumab. A number of other clinically relevant outcome measures including pruritus scores and patient reported outcome measures were also significantly improved in the patients treated with the biologic. Injection-site reactions and conjunctivitis were more frequent in the dupilumab treated patients than in the placebo groups. The conjunctivitis was for the most part self-limited, and only 1 patient in the phase 3 monotherapy trials discontinued study treatment. A recent review of randomized placebo-controlled trials of dupilumab in AD, asthma, chronic rhinosinusitis with nasal polyps (CRSwNP) and eosinophilic esophagitis (EoE) found that the incidence of conjunctivitis was more frequent with dupilumab treatment in most AD trials but very low and similar to that seen in placebo treated patients in the asthma, CRSwNP and EoE trials (Akinlade et al, 2019). Greater baseline AD disease severity and history of prior conjunctivitis were associated with increased conjunctivitis incidence. Of note, conjunctivitis was mostly mild-to-moderate in severity, and most cases recovered or resolved while continuing on dupilumab. Data from a blinded, placebo-controlled trial demonstrated that patients treated with dupilumab had

decreased *S. aureus* colonization and increased microbial diversity that correlated with clinical improvement of AD and biomarkers of type 2 immunity (Callewaert et al, 2020).

In a randomized, double-blind, parallel-group, phase 3 monotherapy trial in 251 adolescent patients ages 12-17 years with moderate-to-severe AD, patients were stratified to 16-weeks of treatment with 1 of 4 regimens: dupilumab 400 mg loading dose, then 200 mg Q2W (baseline weight <60 kg); dupilumab 600 mg loading dose, then 300 mg (baseline weight ≥60 kg) Q2W; dupilumab 600 mg loading dose, then 300 mg Q4W; or placebo with all patients receiving injections Q2W to maintain study blinding (Simpson et al, 2020). A significantly higher proportion of patients treated with dupilumab achieved EASI-75 and IGA 0 or 1 at week 16 vs placebo treated patients. Efficacy of the Q2W regimen was generally superior to the Q4W regimen. The incidence of conjunctivitis in the dupilumab treated patients (~10%) was similar to that seen in the adult trials. Most recently, dupilumab together with TCS was studied in children 6-11 years with severe AD; 367 patients were randomized 1:1:1 to dupilumab 300 mg Q4W, weight based dupilumab (100 mg Q2W, baseline weight <30 kg; 200 mg Q2W, baseline weight ≥30 kg), or placebo with concomitant medium-potency TCS in a double-blind 16 week phase 3 trial (Paller et al, 2020). Both the Q4W and Q2W dupilumab + TCS regimens resulted in clinically meaningful and statistically significant improvement in signs, symptoms, and quality of life (QOL) versus placebo + TCS in all pre-specified endpoints. For Q4W, Q2W, and placebo, 32.8%, 29.5%, and 11.4% of patients, respectively, achieved IGA scores of 0 or 1; 69.7%, 67.2%, and 26.8% achieved ≥75% improvement in EASI scores; and 50.8%, 58.3%, and 12.3% achieved ≥4-point reduction in worst itch score. Conjunctivitis and injection-site reactions were more common with dupilumab + TCS than with placebo + TCS. Dupilumab is approved in the United States (US) for patients age ≥ 6 months with moderate-to-severe AD uncontrolled by topical prescription medicines or when those medications are not advised. The reason patients with AD do not consistently respond to dupilumab is unknown.

2 Study Objectives, Endpoints, and Hypotheses

2.1 Study Objectives, Endpoints, and Hypotheses

Below are three tables (Tables 2.1.1 – 2.1.3) that include the 26 objectives and 35 related endpoints for the study, separated by priority and including the related hypotheses for each analysis. Endpoints will be collected based on enrollment group and treatment course as delineated in the schedules of events (Appendix A), and analysis timepoints for each exploratory objective will be noted in the statistical analysis plan (SAP).

Table 2.1.1. Primary Objective and Related Endpoint, Hypothesis

Primary Objective:	Related Endpoint:	Related Hypothesis:
1. To determine if the type 2-high non-lesional skin (skin tape) endotype is associated with current mild versus moderate-to-severe AD disease	1. Non-lesional skin tape transcriptome at Day 7 (long-term dupilumab participants excluded)	1. The type 2-high skin endotype in non-lesional skin is associated with severity of AD disease.

Table 2.1.2. Secondary Objectives and Related Endpoints, Hypotheses

Secondary Objectives:	Related Endpoints:	Related Hypotheses:
1. To determine how gene expression in the skin (skin tape) differs between non-AD participants and those with	1. Non-lesional and lesional skin tape transcriptome at Day 7 and Day 168-224 (long-term dupilumab participants excluded)	1. Distinct transcriptomic signatures detectable in tape collected skin underlie mild and moderate-to-severe AD.

Secondary Objectives:	Related Endpoints:	Related Hypotheses:
current mild or moderate-to-severe AD disease		
2. To determine how gene expression in the skin (skin tape) changes with standard-of-care treatment (two timepoints) among the study outcome groups: (1) topical steroid responders, (2) dupilumab responders, (3) dupilumab non-responders, (4) non-AD, and (5) long-term dupilumab participants	2. Non-lesional and lesional skin tape transcriptome at Day 7 and Day 168-224	2. The clinical response to protocol's standard-of-care treatment among different AD participants will be associated with distinct transcriptional shifts in tape collected skin over time.

Table 2.1.3. Exploratory Objectives and Related Endpoints, Hypotheses

Exploratory Objectives:	Related Endpoints:	Related Hypotheses:
1. To determine how gene expression in the skin (skin biopsy) differs between non-AD participants and those with current mild or moderate-to-severe AD disease	1. Non-lesional and lesional skin biopsy transcriptome (adults only, long-term dupilumab participants excluded)	1. Distinct transcriptomic signatures detectable in skin biopsies underlie mild and moderate-to-severe AD.
2. To determine how gene expression in the skin (skin biopsy) changes with standard-of-care treatment (two timepoints) among the study outcome groups: (1) topical steroid responders, (2) dupilumab responders, (3) dupilumab non-responders, (4) non-AD, and (5) long-term dupilumab participants.	2. Non-lesional and lesional skin biopsy transcriptome (adults only)	2. The clinical response to protocol's standard-of-care treatment among different AD participants will be associated with distinct transcriptional shifts in skin biopsies over time.
3. To compare gene expression in the skin (skin tape) between participants who have recently started dupilumab (naïve at study start) and those who have been treated with dupilumab long term (at least 4 months of dupilumab treatment at study start)	3. Non-lesional and lesional skin tape transcriptome (only participants on dupilumab)	3. Participants previously naïve to dupilumab will exhibit distinct skin gene expression profiles under dupilumab from those who have been taking dupilumab long term.
4. To identify novel AD skin endotypes at all skin sampling time points	4. Non-lesional and lesional skin tape transcriptome 5. Non-lesional and lesional skin biopsy transcriptome (adults only)	4. Distinct AD disease endotypes exist that can be characterized using skin transcriptome profiles.

Exploratory Objectives:	Related Endpoints:	Related Hypotheses:
5. To determine whether novel AD skin endotypes are associated with current mild or moderate-to-severe AD disease	6. Non-lesional and lesional skin tape endotypes determined in exploratory objective 4 (long-term dupilumab participants excluded) 7. Non-lesional and lesional skin biopsy endotypes determined in exploratory objective 4 (adults only, long-term dupilumab participants excluded)	5. Novel AD skin endotypes are associated with AD status and severity.
6. To determine how AD skin endotypes and gene expression differ among the study outcome groups: (1) topical steroid responders, (2) dupilumab responders, (3) dupilumab non-responders, (4) non-AD, and (5) long-term dupilumab participants	8. Non-lesional and lesional skin tape endotypes determined in exploratory objective 4 9. Non-lesional and lesional skin biopsy endotypes determined in exploratory objective 4 (adults only) 10. Non-lesional and lesional skin tape transcriptome 11. Non-lesional and lesional skin biopsy transcriptome (adults only)	6. The clinical response to protocol's standard-of-care treatment among different AD participants will be associated with distinct AD skin endotypes and transcriptional profiles.
7. To determine skin gene expression changes that are associated with topical moisturizer treatment	12. Non-lesional and lesional skin tape transcriptome (long-term dupilumab participants excluded)	7. Clinical treatment with moisturizer is associated with changes in transcriptional profiles in the skin.
8. To determine skin gene expression changes that are associated with topical steroid response and non-response	13. Non-lesional and lesional skin tape transcriptome (non-AD and long-term dupilumab participants excluded)	8. Clinical response to topical steroids is associated with transcriptional profiles in the skin.
9. To determine skin gene expression changes that are associated with dupilumab response and non-response	14. Non-lesional and lesional skin tape transcriptome (dupilumab-naïve participants only) 15. Non-lesional and lesional skin biopsy transcriptome (adults only, dupilumab-naïve participants only)	9. Clinical response to dupilumab is associated with transcriptional profiles in the skin.
10. To determine the changes in cell type-specific gene and protein expression and cellular composition that are associated with dupilumab response and non-response	16. Single cell non-lesional and lesional skin biopsy transcriptome and proteome (dupilumab-naïve adults only)	10. Shifts in the cellular composition of skin and cell type-specific gene and protein expression changes underlie clinical response to dupilumab.
11. To determine whether AD skin endotypes are associated with topical steroid response	17. Non-lesional and lesional skin tape endotypes determined in exploratory objective 4	11. The clinical response to topical steroid treatment will be

Exploratory Objectives:	Related Endpoints:	Related Hypotheses:
	(dupilumab-naïve participants only) 18. Non-lesional and lesional skin biopsy endotypes determined in exploratory objective 4 (dupilumab-naïve adults only)	determined by skin endotype status.
12. To determine whether AD skin endotypes are associated with dupilumab response	19. Non-lesional and lesional skin tape endotypes determined in exploratory objective 4 (dupilumab-naïve participants only) 20. Non-lesional and lesional skin biopsy endotypes determined in exploratory objective 4 (dupilumab-naïve adults only)	12. The clinical response to dupilumab treatment will be determined by skin endotype status.
13. To determine whether cell type-specific gene and protein expression and cellular composition differ among AD skin endotypes	21. Single cell non-lesional and lesional skin biopsy transcriptome and proteome (adults only)	13. Shifts in the cellular composition of skin and cell type-specific gene and protein expression changes underlie skin endotypes.
14. To determine longitudinal changes in skin (skin tape) gene expression across standard-of-care treatment (all timepoints) in the study outcome groups: (1) topical steroid responders, (2) dupilumab responders, (3) dupilumab non-responders, (4) non-AD, and (5) long-term dupilumab participants	22. Non-lesional and lesional skin tape transcriptome	14. The clinical response to protocol's standard-of-care treatment among different AD participants will be associated with distinct transcriptional trajectories generated across these standard-of-care treatments.
15. To determine whether genetic variation that regulates skin gene expression is associated with AD disease	23. Non-lesional and lesional skin tape and skin biopsy transcriptome 24. Genome-wide genotypes from blood samples	15. Genetic variation drives particular AD skin endotypes.
16. To determine the proteomic profiles of AD lesional and non-lesional skin endotypes	25. Non-lesional and lesional skin tape proteome	16. AD skin endotypes exhibit distinct epidermal protein profiles.
17. To determine the lipidomic and metabolomic properties of AD lesional and non-lesional skin endotypes	26. Non-lesional and lesional skin tape lipidome and metabolome	17. AD skin endotypes exhibit distinct lipid and metabolome profiles.
18. To assess the relationship between *Staphylococcus aureus* (*S. aureus*) abundance and AD lesional and non-lesional skin endotypes	27. *S. aureus* abundance as measured by microbial Deoxyribonucleic Acid (DNA) (femA qPCR (rCFU/cm^2)) and colony forming units	18. AD skin endotypes exhibit different *S. aureus* levels in the skin.

Exploratory Objectives:	Related Endpoints:	Related Hypotheses:
	(CFU/cm^2) on non-lesional and lesional skin	
19. To determine the association between intensity of pruritus and AD lesional and non-lesional skin endotypes	28. Peak Pruritus numerical rating scale [PP-NRS]	19. AD skin endotypes exhibit different levels of pruritus.
20. To determine the association between blood biomarker levels and AD lesional and non-lesional skin endotypes	29. Blood biomarker levels, including but not limited to total Immunoglobulin E (IgE), allergen-specific IgE, CCL17, CCL22, and eosinophil count	20. AD skin endotypes exhibit different levels of serum/plasma biomarkers.
21. To determine the association between clinical phenotypes and AD lesional and non-lesional skin endotypes	30. Clinical traits, including but not limited to history of S. aureus infection, eczema herpeticum, food allergy, or asthma	21. AD skin endotypes exhibit different clinical phenotypes.
22. To determine differences in microbiomes among AD lesional and non-lesional skin endotypes, pending availability of funding	31. Lesional and non-lesional skin swab microbiomes	22. AD skin endotypes exhibit different microbiomes.
23. To determine spatial transcriptomic and proteomic associations with AD disease severity, endotypes, outcome groups, and drug treatment in lesional and non-lesional biopsy skin	32. Non-lesional and lesional skin biopsy single cell, cell type, as well as histological feature transcriptome and proteome data (adults only)	23. AD skin biopsies will exhibit different transcriptome, proteome, and cell type spatial patterns by severity, outcome group, drug response, and endotype.

3 Study Design

3.1 Description of Study Design

This is a multi-center, prospective longitudinal study which will characterize the gene expression profiles and transcriptomic endotypes that underlie mild and moderate-to-severe AD and will evaluate changes in these expression patterns and endotypes in response to standard-of-care treatment as delineated in the protocol. The study will enroll participants in three groups:

- Participants with AD that is not currently being managed with dupilumab (dupilumab-naïve AD; DNAD)
- Participants with AD that is currently being treated with dupilumab and has been treated with dupilumab for at least 4 months prior to enrollment (long-term dupilumab use; LTD)
- Participants without AD (non-AD; NAD)

DNAD participants will complete up to ten scheduled study visits with assessment of topical steroid response and, if uncontrolled with topical steroids, dupilumab response. Skin samples will be collected at all study visits to determine the gene expression profiles and transcriptomic endotypes that underlie mild vs. moderate-to-severe AD disease. We may also evaluate the lipidomic, metabolomic, proteomic, and microbiome profiles of AD skin endotypes associated with mild and moderate-to-severe AD disease. NAD participants will complete up to seven scheduled study visits and will serve as a control population. LTD participants will complete up to six scheduled study visits, which will allow

comparison of their skin gene expression profiles to those of participants who begin dupilumab treatment during this study.

Potential participants will be recruited in person or over the phone and assessed for eligibility. Following recruitment, all potentially eligible participants will first attend a Screening Visit during which the participant and/or guardian will provide informed consent and assent (if applicable) for study participation. Consented and assented participants will be assessed for eligibility through the collection of medical history and medication use. If eligible, participants will then be assigned to one of three groups (NAD, DNAD, LTD) and asked to come in for a Baseline Assessment. Recruitment, Screening, and Baseline Assessment may be conducted on the same day if the participant meets all eligibility criteria.

At the Baseline Assessment (Day 0), medical history, medication use, vital signs, and a pregnancy test (if applicable, as defined in the study manual of procedures) will be collected to confirm eligibility. Participants who meet eligibility after completing baseline study assessments will be considered enrolled. Several standard AD severity scoring measures will also be taken for participants who have AD (DNAD, LTD). Although the participant's AD will be monitored and treated body-wide over the course of the study, in consultation between the participant and investigator, a target skin region will be selected that will act as the controlled focal study area to be sampled, helping to facilitate compliance and consistency of topical treatment application which will be essential to the integrity of study outcomes. For this targeted region, an area will be selected to ensure availability of sufficient skin surface to sample as symptoms fluctuate over time and with treatment. In addition to body-wide measures of AD severity, targeted severity measures (targeted area assessment [TAA] and targeted EASI) will also be collected, enabling us to track clinical responses in AD severity to treatment within this more controlled target skin region.

Enrolled participants will be asked to begin applying Vanicream™ Moisturizing Cream (provided by the study) at least twice daily to the specified target area for approximately one week (until 1 day prior to their 1 Week Assessment/Steroid Initiation) and refrain from applying other moisturizers and topical steroids to the target skin area during this time. AD participants may apply topical steroids prescribed by their physician and moisturizer to non-target skin as needed during this time.

All enrolled participants will be permitted to apply Vanicream™ Moisturizing Cream to all other body sites as needed throughout the duration of the study.

3.1.1 DNAD Participants

On Day 7, DNAD participants will begin applying triamcinolone 0.1% ointment (provided by the study) twice daily to the specified target area. Additionally, DNAD participants will apply triamcinolone 0.1% ointment (non-sensitive regions)/ hydrocortisone 2.5% ointment (sensitive regions) twice daily to active lesions on non-target skin.

On Day 35, DNAD participants will return for a 4 Week Steroid Assessment, where response to triamcinolone will be evaluated at the target site by TAA and targeted EASI scoring, and overall management of AD body-wide by topical steroid/moisturizer treatment will be evaluated by EASI score. DNAD participants who have an inadequate body-wide response to topical steroid/moisturizer treatment (EASI > 7) will be designated as "topical steroid non-responders" and will begin treatment with dupilumab. DNAD participants with EASI ≤ 7 will be designated as "topical steroid responders" and will continue to apply triamcinolone/hydrocortisone to active lesions.

<u>Topical Steroid Responders</u>

After the 4 Week Steroid Assessment, topical steroid responders will discontinue applying triamcinolone 0.1% ointment to non-lesional skin on the target area. Topical steroid responders will begin to apply triamcinolone 0.1% ointment (non-sensitive regions)/ hydrocortisone 2.5% ointment (sensitive regions) once or twice daily, per clinician discretion, to active lesions body-wide. They will return for up to three additional scheduled visits prior to their End of Study Visit: an Optional Steroid Assessment, 12 Week Steroid Assessment, and a 19 Week Steroid Assessment.

If symptoms worsen at any time between planned study visits, participants can be scheduled for an Unscheduled Visit. If their EASI score is > 7 at any of the scheduled or unscheduled visits beginning at the 4 Week Steroid Assessment through the 12 Week Steroid Assessment, they will be designated as a "topical steroid non-responder" and will begin treatment with dupilumab. After the 12 Week Steroid Assessment, if the participant's EASI score is > 7 at any point, they will be withdrawn from the study.

At Day 140, topical steroid responders will come in for a 19 Week Steroid Assessment when they will discontinue applying triamcinolone/hydrocortisone to active lesions on the target skin area and begin to apply Vanicream™ Moisturizing Cream at least twice daily to target skin. Triamcinolone/hydrocortisone may continue to be used on active lesions on non-target skin. Participants will return for an End of Study Assessment at Day 168.

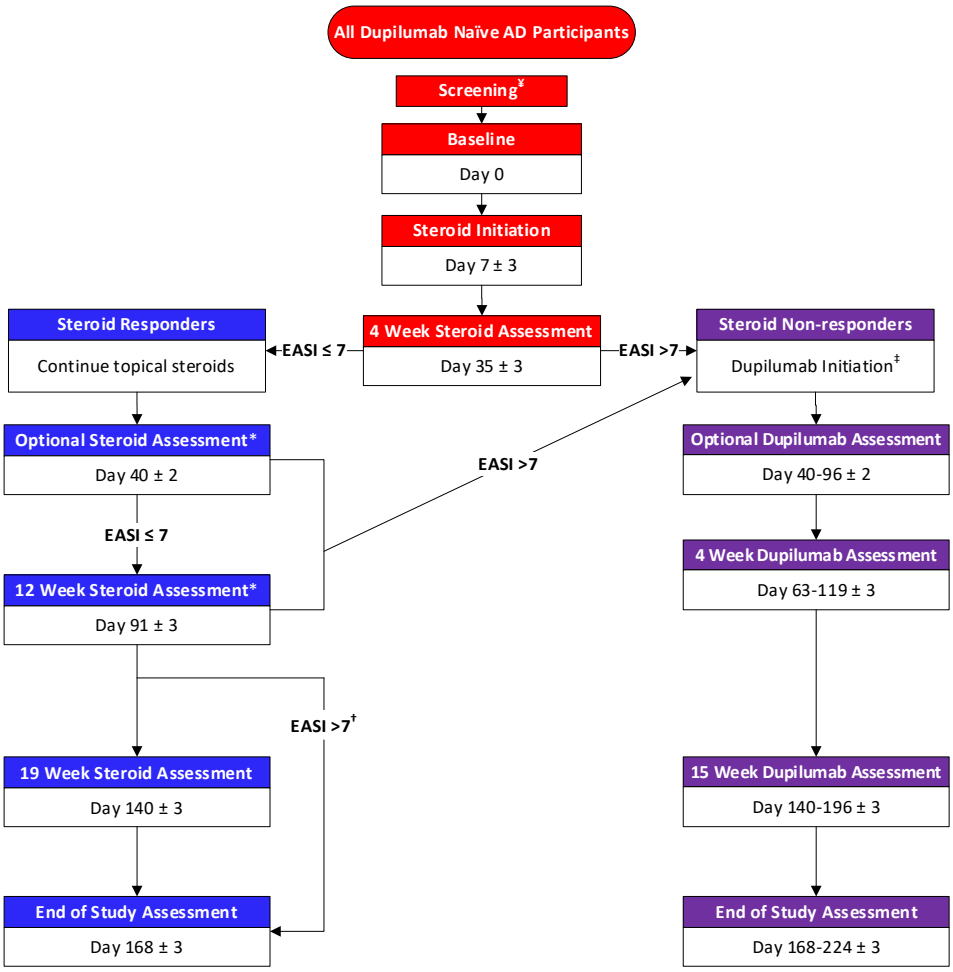
Topical Steroid Non-responders

Topical steroid non-responders will discontinue application of triamcinolone to the target area and continue to refrain from applying Vanicream™ Moisturizing Cream to the target area, unless required at any point as a rescue treatment at the discretion of the investigator. They may apply triamcinolone/hydrocortisone to active lesions on non-target areas as needed. Topical steroid non-responders beginning treatment with dupilumab will initially receive a loading dose of two subcutaneous injections at the Dupilumab Initiation Visit within 3 days of the qualifying EASI score (EASI > 7 beginning at the 4 Week Steroid Assessment through the 12 Week Steroid Assessment). They will be provided with additional doses to take home to self-administer by subcutaneous injection according to their dose schedule outlined in Section 6.1.3. Topical steroid non-responders will return for up to three additional scheduled visits prior to their End of Study Visit: an Optional Dupilumab Assessment, 4 Week Dupilumab Assessment, and a 15 Week Dupilumab Assessment.

If symptoms worsen at any time between planned study visits, participants can be scheduled for an Unscheduled Visit. If disease becomes uncontrolled in a participant on dupilumab despite treatment with rescue medications (Section 7.4), per investigator judgment, the participant will complete an Early Termination Visit and will be classified as having experienced a treatment failure.

By the 15 Week Dupilumab Assessment, participants will have concluded treatment with dupilumab. During this visit, the topical steroid non-responders will be instructed to begin to apply Vanicream™ Moisturizing Cream at least twice daily to target skin. Participants will return for an End of Study Assessment approximately 133 days after Dupilumab Initiation.

Figure 3.1.1 Dupilumab-naïve AD Participant Flow Chart



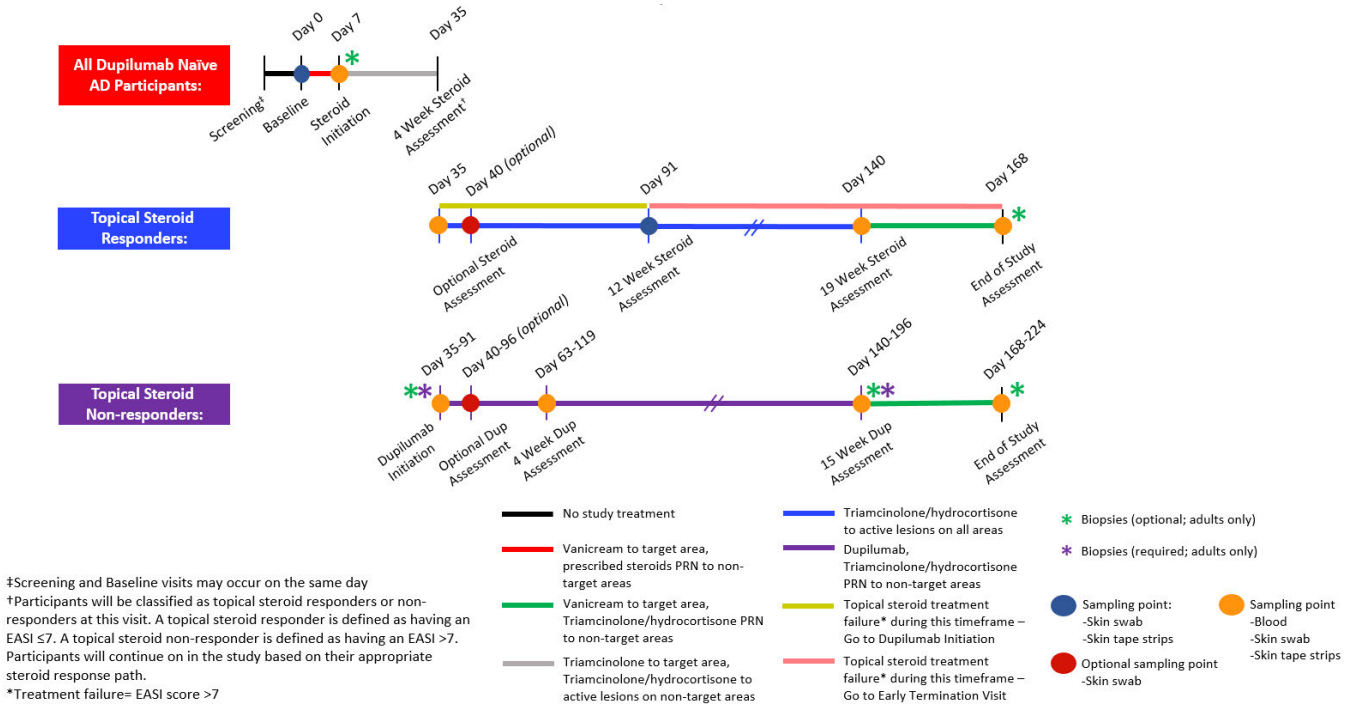
¥Screening and Baseline Visits may occur on the same day

*If the participant is found to be non-responsive to steroid treatment (EASI > 7) at any time between the 4 Week Steroid Assessment and the 12 Week Steroid Assessment Visit, inclusive, the participant will begin Dupilumab treatment.

†If the participant is found to be non-responsive to steroid treatment (EASI > 7) after the 12 Week Steroid Assessment Visit but prior to the End of Study Assessment Visit, the participant will complete an Early Termination Visit and be withdrawn from the study.

‡If at any point the participant discontinues Dupilumab prior to completing the study, the participant will complete an Early Termination Visit and be withdrawn from the study.

Figure 3.1.1.2 Overview of study design and skin sampling of the target skin area for dupilumab-naïve AD participants

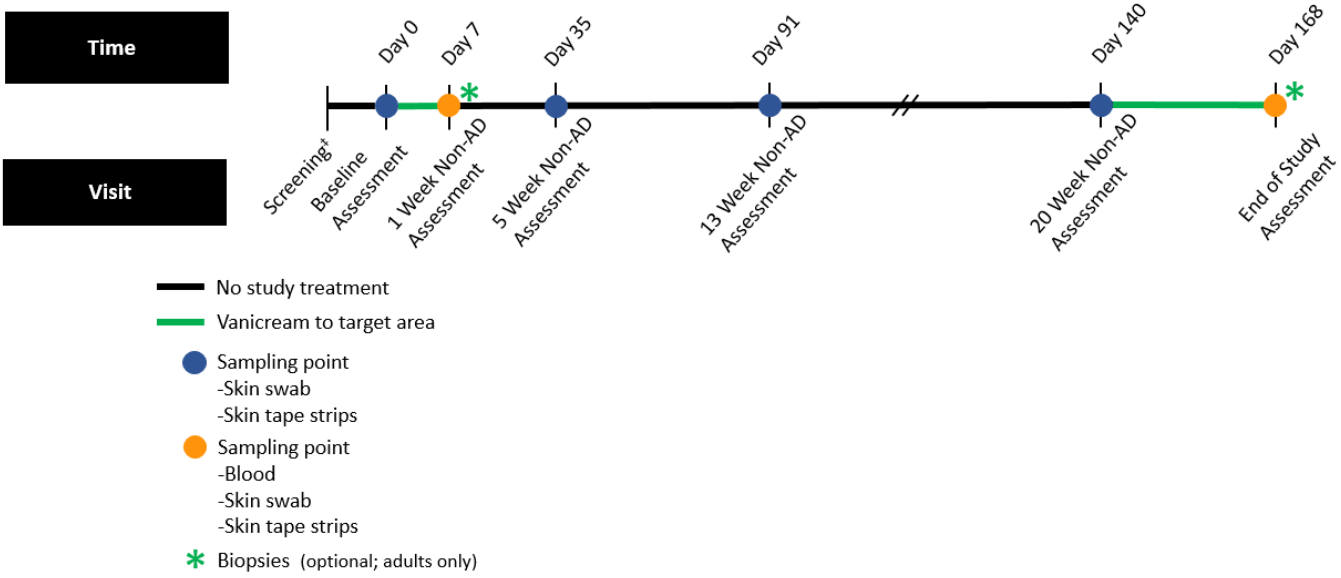


‡Screening and Baseline visits may occur on the same day
†Participants will be classified as topical steroid responders or non-responders at this visit. A topical steroid responder is defined as having an EASI ≤7. A topical steroid non-responder is defined as having an EASI >7. Participants will continue on in the study based on their appropriate steroid response path.
*Treatment failure= EASI score >7

3.1.2 NAD Participants

After the 1 Week Non-AD Assessment, NAD participants will return to clinic for four scheduled interim visits including the 5 Week, 13 Week, and 20 Week Assessments ahead of their End of Study Assessment. NAD participants will apply Vanicream™ Moisturizing Cream at least twice daily to the specified target skin area starting at their 20 Week Assessment (Day 140) through the End of Study Assessment (Day 168).

Figure 3.1.2 Overview of study design and skin sampling of the target skin area for non-AD participants

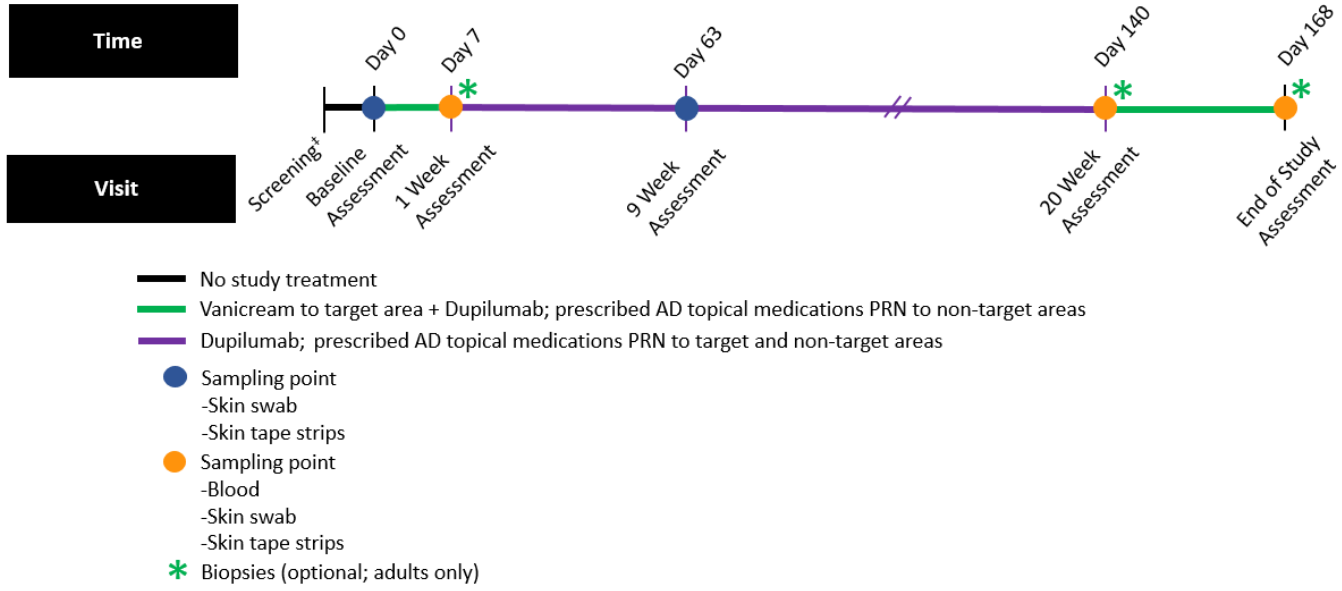


‡ Screening and Baseline may occur on the same day

3.1.3 LTD Participants

AD participants already on dupilumab (for ≥ 4 months) at the start of the study will continue dupilumab treatment as prescribed by their physician outside of the study. After the 1 Week Assessment, LTD participants may continue to apply topical steroids/moisturizer body-wide as needed per their physician's orders. They will return for a 9 Week (Day 63) and 20 Week Assessment (Day 140). At their penultimate scheduled visit, LTD participants will discontinue applying any topical steroids to the specified target area and begin to apply Vanicream™ Moisturizing Cream at least twice daily on the target skin area until their End of Study Assessment (Day 168). If the participant discontinues their dupilumab treatment at any point in the study, the participant will be withdrawn from the study.

Figure 3.1.3 Overview of study design and skin sampling of the target skin area for long-term dupilumab participants



‡ Screening and Baseline may occur on the same day

3.2 Stratification, Randomization, and Blinding/Masking

Laboratory personnel conducting the assays will be blinded to diagnostic group (NAD, mild DNAD, moderate-to-severe DNAD, or LTD), study therapy regimen, and treatment response group (topical steroid responsive/non-responsive and/or dupilumab responsive/non-responsive), until after samples are processed. Analytical personnel who are unblinded for quality control purposes or to complete interim analyses of the data will not be involved in processing samples.

4 Selection of Participants and Clinical Sites/Laboratories

4.1 Rationale for Study Population

This study will enroll male and female, adult and pediatric participants, 6 years of age and older. Participants with mild (EASI ≤7) and moderate-to-severe (EASI >7) AD will be enrolled as our objective is to study all severities of AD and their response to all steps in AD management per protocol standard-of-care. NAD participants will be needed as a control population to compare to AD. As AD is often transient at younger ages and we are trying to study more stable endotypes/phenotypes, enrollment will be limited to those 6 years of age and older. Both sexes and all racial groups will

be included to study the full spectrum of clinical AD. We will monitor the enrollment distribution by age, sex, and race/ethnicity in the three enrollment groups (NAD, DNAD, LTD). Any detected skews accruing during enrollment will be addressed by instructing sites to target unrepresented groups.

4.2 Inclusion Criteria

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

All Participants:

1. Participant and/or parent guardian must be able to understand and provide informed consent and assent (if applicable)
2. Male or female, 6 years of age or older inclusive at the Screening Visit
3. Participants must agree to apply a stable dose of the study provided topical moisturizer (Vanicream™ Moisturizing Cream) at least twice daily between the Baseline Assessment and Day 7 Visits to a specified skin target area.
4. Individuals with persistent asthma must adhere to asthma controller medication(s) as prescribed by their physician for the duration of the study.
5. Individuals who can become pregnant, as defined in the study manual of procedures, must have a negative pregnancy test at the Baseline Assessment and Day 7 Visits if they do not self-report as pregnant.
6. Individuals who can become pregnant, as defined in the study manual of procedures, must meet either of the following criteria prior to Baseline:
    a) Willing to remain abstinent from intercourse that may result in a pregnancy.
    b) Willing to use an FDA-approved method of contraception for the duration of study participation. Acceptable methods include the following:
        - Permanent sterilization of partner
        - Long-acting reversible contraceptives (e.g., intrauterine devices or systems, implantable rods, contraceptive injections) when used as directed for at least 7 days prior to Baseline.
        - Short-acting hormonal contraceptives (e.g., oral contraceptive pills, patch, vaginal ring) when used as directed for at least 30 days prior to Baseline.
        - Barrier methods (e.g., condoms; diaphragm, sponge, or cervical cap with spermicide)
7. Participant and/or parent guardian must be able to understand and complete study-related questionnaires.
8. Participants must have non-lesional skin on the upper or lower extremities or trunk of sufficient size and in the required locations, as specified in the study manual of procedures (MOP), for specimen collection at the Baseline Assessment and Day 7 Visits.

NAD Participants:

9. NAD participants must have no history of AD or food allergy as diagnosed by a physician.

All AD Participants (DNAD and LTD):

10. DNAD and LTD participants must have a history of chronic AD, (according to the Atopic Dermatitis Research Network [ADRN] Standard Diagnostic Criteria [Appendix B]), that has been present for at least 1 year before the Screening Visit.
11. DNAD and LTD participants must agree to refrain from applying topical steroid to a specified target area between the Baseline Assessment and Day 7 Visits.

DNAD Participants:

12. DNAD participants must have active lesions on the upper or lower extremities or trunk of sufficient size and in the required locations, as specified in the study manual of procedures (MOP), for specimen collection at the Baseline Assessment and Steroid Initiation (Day 7) Visits.

LTD Participants:

13. LTD participants must be currently receiving dupilumab and must have started dupilumab treatment ≥ 4 months prior to the Screening Visit.

4.3 Exclusion Criteria

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

1. Inability or unwillingness of a participant or parent guardian to comply with the study protocol
2. Have a genetic relative (e.g., parent, sibling, grandchild, half-sibling) or household member (e.g., spouse) already enrolled in the study
3. Weight less than 15 kg
4. Known systemic hypersensitivity to any of the excipients of the study treatments (Vanicream™ Moisturizing Cream, hydrocortisone, triamcinolone, or dupilumab)
5. Have any skin disease other than AD that might compromise the stratum corneum barrier (e.g., bullous diseases, psoriasis, cutaneous T cell lymphoma [also called Mycosis Fungoides or Sezary syndrome], dermatitis herpetiformis, Hailey-Hailey, or Darier's disease)
6. Known or suspected immunosuppression, including history of invasive opportunistic infections (e.g., tuberculosis, histoplasmosis, listeriosis, coccidioidomycosis, pneumocystosis, aspergillosis) despite infection resolution, or otherwise recurrent immune-compromised status, as judged by investigator.
7. Known history of human immunodeficiency virus (HIV) infection
8. Ocular disorder that in the opinion of the investigator could adversely affect the individual's risk for study participation. Examples include, but are not limited to, individuals with a history of or active case of herpes keratitis; Sjogren's Syndrome, Keratoconjunctivitis Sicca, or Dry Eye Syndrome that require daily use of supplemental lubrication; or individuals with ocular conditions that require the regular use of ocular corticosteroids or cyclosporine.
9. Parasitic infection, except for vaginal trichomoniasis, within 12 months of the Screening Visit, or high risk for contracting parasitic infections (e.g., traveling to endemic areas)
10. History of malignancy within 5 years before the Screening Visit (completely treated in situ carcinoma of the cervix, and completely treated and resolved non-metastatic squamous or basal cell carcinoma of the skin or melanoma in situ are not exclusionary)
11. History of non-malignant lymphoproliferative disorders
12. History of alcohol or drug abuse within 2 years before the Screening Visit
13. History of keloid formation in adult participants
14. History of hypersensitivity to local anesthetics (e.g., lidocaine or Novocain), bleeding disorders, or treatment with anticoagulants or other conditions in adult participants that would make the biopsy procedure inadvisable
15. Individuals with asthma who have required use of a systemic corticosteroid within 3 months prior to the Screening Visit or who require a dose greater than 880 mcg/day of fluticasone propionate or equivalent inhaled corticosteroid to maintain asthma control
16. Treatment with biologics (other than dupilumab) as follows:
    a. Any cell-depleting agents, including but not limited to rituximab, within 6 months before the Baseline Assessment, or until lymphocyte and CD19+ lymphocyte count returns to normal, whichever is longer
    b. Omalizumab, infliximab, adalimumab, golimumab, certolizumab pegol, abatacept, etanercept, anakinra within 16 weeks before the Baseline Assessment for any indication
    c. Other biologics within 5 half-lives (if known) or 16 weeks before the Baseline Assessment, whichever is longer

17. Ongoing participation in another research study involving any of the following:
    a. Current or planned use of an investigational drug or device
    b. Current or planned use of prohibited medications or procedures
    c. Substantial time commitment and/or study requirements that, in the opinion of the investigator, may interfere with the participant's ability to comply with LEADS study requirements
18. Use of an investigational drug within 8 weeks or within 5 half-lives (if known), whichever is longer, before the Baseline Assessment
19. Use of topical calcineurin inhibitors (tacrolimus or pimecrolimus), topical phosphodiesterase inhibitors (crisaborale), topical JAK inhibitors (ruxolitinib), or topical Ahr agonists (tapinarof) within 1 week before the Baseline Assessment. Use of these medications is not exclusionary when only applied to the face, neck, palms, or soles.
20. Treatment with bleach bath within 1 week before the Baseline Assessment
21. Use of a chlorinated hot tub or pool within 1 week before the Baseline Assessment
22. Initiation of treatment with prescription moisturizers or moisturizers containing ceramide, hyaluronic acid, urea, or filaggrin (FLG) during the study.
    a. Participants may continue using stable doses of such moisturizers on body areas other than the target area if initiated before the Baseline Assessment.
    b. Initiation of prescription moisturizer is not exclusionary when only applied to the face, neck, palms, or soles.
23. Planned or anticipated use of any prohibited medications or procedures during study participation.
24. Past or current medical problems or findings from physical examination that are not listed above, which, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the participant's ability to comply with study requirements or that may impact the quality or interpretation of the data obtained from the study.

4.4 Selection of Clinical Sites/Labs

This will be a multicenter study in the US. These sites have access to large adult and pediatric AD populations, experience with clinical trials and performing required study procedures including skin biopsies, skin tape stripping (STS), AD severity scoring, blood/tissue processing, and biomaterials shipping.

The laboratories processing and analyzing the samples for this study are discussed in Section 9. The proposed techniques for successful implementation and completion of the proposed studies have been established in preliminary studies at the respective laboratories.

5 Known and Potential Risks and Benefits to Participants

5.1 Risks of Triamcinolone Acetonide Ointment 0.1% and Hydrocortisone 2.5% Ointment

The following local adverse reactions (AR) are reported infrequently with TCS, but may occur more frequently with the use of occlusive dressings. These reactions are listed in an approximate decreasing order of occurrence: burning, itching, irritation, dryness, folliculitis, hypertrichosis, acneiform eruptions, hypopigmentation, perioral dermatitis, allergic contact dermatitis, maceration of the skin, secondary infection, skin atrophy, striae, and miliaria. No occlusive dressings will be used in this protocol so risk for these local Ars is low.

Although rare, systemic absorption of TCS has produced reversible hypothalamic-pituitary-adrenal (HPA) axis suppression, manifestations of Cushing's syndrome, hyperglycemia, and glucosuria in some patients. Conditions which augment systemic absorption include the application of the more potent steroids, use over large surface areas, prolonged use, and the addition of occlusive dressings. Participants will be instructed to apply small amounts of mid- to low-potency topical steroids to lesions only to minimize systemic absorption. Children may absorb proportionally larger amounts of TCS and thus be more susceptible to systemic toxicity. If irritation develops, TCS may be discontinued, and appropriate physician defined therapy instituted.

5.2 Risks of Dupilumab as cited in the Package Insert

As per the September 2024 dupilumab package insert, the most common (occurring in ≥ 1% of participants) adverse drug reactions (ADRs) in those using dupilumab for AD were injection site reactions (ISRs), conjunctivitis, blepharitis, oral herpes, keratitis, eye pruritus, other herpes simplex virus infections, dry eyes, and eosinophilia.

Conjunctivitis and keratitis were found to occur more frequently in dupilumab-treated participants. Conjunctivitis was the most common occurring ocular ADR, having been reported in up to 16% of participants receiving dupilumab in one trial over 52 weeks (CHRONOS, NCT02260986). Keratitis was reported in up to 4% of those participants in the same trial.

Hypersensitivity reactions, such as generalized urticaria, rash, erythema nodosum, and serum sickness or serum sickness-like reactions were specified as ADRs, but noted to occur in less than 1% of the participants who received dupilumab in clinical trials. The two cases of serum sickness/sickness-like reactions were associated with high titers of anti-drug antibodies (ADA). A trial evaluating dupilumab for use in patients with asthma had one participant who experienced anaphylaxis.

An increase in the eosinophil count from baseline was noted in the dupilumab monotherapy trials within the dupilumab-treated participants. However, this increase returned to near baseline levels by the end of treatment. Cases of eosinophilic pneumonia and vasculitis consistent with eosinophilic granulomatosis with polyangiitis have been reported in adult asthma and chronic rhinosinusitis with nasal polyposis, but a causal association with dupilumab has not been established.

Dupilumab can improve asthma and chronic rhinosinusitis with nasal polyposis symptoms, but medication changes should only be made in consultation with a physician. Participants will be made aware of this guidance. For those participants with asthma, a letter will be provided to alert the physician managing their asthma to contact the study physician if changes to the participant's asthma medication regimen will be made as a safety precaution.

Arthralgia has been reported with the use of dupilumab leading to gait disturbances or decreased mobility associated with joint symptoms. In post-marketing reports, onset of arthralgia was variable, ranging from days to months after the first dose of dupilumab. Some patients' symptoms resolved while continuing treatment with dupilumab, and other patients recovered or were recovering following discontinuation of dupilumab. Participants will be advised to report any new onset or worsening joint pain.

Additionally, it is not known whether the immune response to a helminth infection is altered in the presence of dupilumab, but this possibility is a theoretical risk. Participants will be advised of situations that would put them at high risk for contracting a parasitic infection.

5.3 Risks of Study Procedures

5.3.1 Risks Associated with Stopping the Use of Protocol Prohibited Medications/Procedures

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

5.3.2 Risks Associated with Physical Exam

There are no known risks associated with the physical exam.

5.3.3 Risks Associated with Health Questionnaires

There is a possibility that participants may find questions too personal. Participants may refuse to answer any questions that make them feel uncomfortable. There is also a possibility that a participant's answers may be read by others; however, participants' records are carefully protected so this is very unlikely. See Section 17.3 for more information on confidentiality.

5.3.4 Risks Associated with Blood Collection

Risks associated with drawing blood include possible pain when the needle is inserted, as well as bleeding, bruising and/or infection at the puncture site. Some people may experience lightheadedness, nausea, or fainting. A topical anesthetic may be placed on the skin before the blood draw to reduce the pain of the stick. Side effects from this cream (mainly skin rash) may occur. National Institutes of Health (NIH) guidelines for blood collection (amount and frequency based on age) will be followed (NIH, 2009).

5.3.5 Risks Associated with Skin Swab Collection

There are no significant risks associated with skin swab collection.

5.3.6 Risks Associated with STS Collection

Risks associated with STS, theoretically, include the rare possibility of an allergic reaction to the tape or a skin infection. Since the tape does not contain latex and is removed immediately after application, the risk of an allergic reaction is extremely low. However, in previous and ongoing studies involving tape stripping, it has been noted that a very mild erythema may develop immediately after a series of tape strips on one localized area of skin, presumably due to the mild mechanical disturbance. The erythema is expected to resolve within 12 hours without sequelae. The risk of skin infection or scarring is extremely low since only superficial skin layers are removed. Some people may experience lightheadedness, nausea, or fainting. Possible bleeding and/or bruising may also occur at the area. Participants with a history of serious life-threatening reaction to tape or adhesives will be excluded from participating, per study exclusion criteria.

5.3.7 Risks Associated with Skin Biopsy Collection

Exclusively adult participants (18+ years old) may provide skin biopsies, whether designated optional or required, in ADRN-12/LEADS.

Risks of skin biopsy include pain and the possibility of an AR consisting of local swelling, bleeding, infection, and scar formation. The pain associated with injection of a local anesthetic, such as lidocaine, is mild and transitory. Allergic reactions to lidocaine are extremely rare and occur in less than 1 in 10,000 individuals who receive lidocaine. Reactions can be mild to life-threatening. Allergic reactions could result in hives, shortness of breath, an asthma attack, or anaphylactic shock. Anaphylactic shock is the most severe form of an allergic reaction. It could lead to complete failure of the heart and circulation and could result in more health problems, or death. However, anaphylactic shock is extremely rare and occurs in less than 30 in 100,000 individuals who receive

lidocaine. Symptoms occur within minutes to 2 hours, but in rare instances may occur up to 4 hours later. Occasionally, participants may experience swelling at the injection site. Significant bleeding from the biopsy site(s) is rare and infrequent. Infection of a biopsy site is unusual, but may occur. A small scar may result at the biopsy site. Participants who receive skin biopsies will be given wound care instructions. Participants who have a lidocaine or Novocain allergy or those with a history of keloid formation will be excluded from participating, per study exclusion criteria.

5.4 Potential Benefits

There may or may not be any direct benefits for the participants who elect to enroll in this study. AD participants who receive treatment medications through this study may benefit from the medications to treat their AD. One potential benefit for participants is that their AD may improve, and their itch may be reduced while on TCS or dupilumab; however, there is no guarantee that the medications will help the participant's condition. The participant's skin condition may even get worse by withholding his/her previous/regular AD treatment.

The potential benefit to society is significant if it improves our understanding of what affects AD disease severity and response to treatment. Therefore, the expectation is that the results will benefit others in the future. Information obtained from these studies will improve our understanding of the immune and epidermal defects observed in AD participants. Our ultimate goal through these studies will be to develop evidence-based, precision medicine approaches to address disease in the most burdened AD patients. Knowledge from this protocol may allow development of biomarkers for steroid-resistant AD patients and dupilumab non-responders.

6 Study Treatments

6.1 Study Treatments

Study medications for this protocol are all FDA approved and are being used as indicated. Vanicream™ Moisturizing Cream will be provided for all study participants. Triamcinolone 0.1% ointment, hydrocortisone 2.5% ointment, and dupilumab prefilled syringes (PFS) will be provided to the dupilumab-naïve AD participants, as applicable; long-term dupilumab AD participants will use AD treatments provided by their physicians outside of the study.

6.1.1 Vanicream™ Moisturizing Cream

Commercially available Vanicream™ Moisturizing Cream will be provided to NIAID by Pharmaceutical Specialties, Inc., labeled for the study and distributed to the sites by the DAIT/NIAID Clinical Product Center (CPC), EMINENT Services Corporation.

Vanicream™ Moisturizing Cream should be stored between 68 - 77 degrees Fahrenheit (°F) (20 - 25 degrees Celsius [°C]).

Participants will be asked to apply Vanicream™ Moisturizing Cream at least twice daily to a specified target area per timeframes specified in Section 3.1. Participants will be permitted to apply Vanicream™ Moisturizing Cream (or other moisturizer) to all other body sites as needed throughout the duration of the study.

6.1.2 Triamcinolone 0.1% Ointment and Hydrocortisone 2.5% Ointment

Commercially available triamcinolone 0.1% ointment and hydrocortisone 2.5% ointment (generic brands) will be purchased through the DAIT/NIAID Investigational Product Procurement Center (IPPC) and distributed to the sites by the DAIT/NIAID CPC, EMINENT Services Corporation.

Triamcinolone 0.1% ointment and hydrocortisone 2.5% ointment should be stored between 68° - 77°F (20° - 25°C).

DNAD participants will apply triamcinolone 0.1% ointment to the specified target area twice daily per the timelines as specified in Section 3.1. They will apply triamcinolone 0.1% ointment (non-sensitive regions) and hydrocortisone 2.5% ointment (sensitive regions) to active lesions once or twice daily – specifics dependent on time enrolled and clinician discretion – as described in Section 3.1

6.1.3 Dupilumab

DUPIXENT® (dupilumab) clinical grade drug product is available in 2 pre-filled syringe (PFS) doses. Regeneron Pharmaceuticals will be providing the following two dosage forms of the single use dupilumab PFS for Protocol ADRN-12:

- 200 mg clinical grade drug product: Dupilumab 175 mg/mL (200 mg/1.14 mL) solution for subcutaneous administration is supplied in a single-dose pre-filled glass syringe with plunger rod.
- 300 mg clinical grade drug product: Dupilumab 150 mg/mL (300 mg / 2 mL) solution for subcutaneous administration is supplied in a single-dose pre-filled glass syringe with plunger rod.

Dupilumab should be stored in a refrigerator between 36° - 46°F (2°- 8°C) in its original carton with protection from light. Syringes should not be exposed to heat or direct sunlight. They should not be frozen or shaken and should be kept out of the reach of children.

Adult dupilumab-naïve topical steroid non-responder (EASI > 7) participants beginning treatment with dupilumab will initially receive a loading dose of 600 mg (two 300 mg subcutaneous injections). The two injections will be administered at different sites in the abdomen, thighs, or upper arms. Subsequently, they will receive 300 mg every two weeks by subcutaneous injection in the abdomen, thigh, or upper arm, rotating the injection site as described in the Package Insert.

Pediatric dupilumab-naïve topical steroid non-responder (EASI > 7) participants beginning treatment with dupilumab will receive a loading dose, according to their weight (Table 6.1.3), by subcutaneous injection. The two injections will be administered at different sites in the abdomen, thighs, or upper arms. Participants who weigh 30 kg or more will receive subsequent dupilumab doses based on weight (Table 6.1.3) every two weeks by subcutaneous injection in the abdomen, thigh, or upper arm. Pediatric participants weighing 15 to <30 kg will receive subsequent dupilumab 300 mg doses every four weeks.

The selected injection site for a given dose should not be the same as the injection site selected for the previous dose. Injections should occur at minimum 7 days apart. If a participant misses a dose, it should be administered within 7 days from the missed dose. If the missed dose is not administered within 7 days, the participant will be instructed to wait until their next scheduled dose based on their original dosing schedule.

Table 6.1.3: Dose of Dupilumab for Subcutaneous Administration in Pediatric Patients (6 to 17 Years of Age)

Body Weight	Initial Dose	Subsequent Doses
15 to less than 30 kg	600 mg (two 300 mg injections)	300 mg Q4W
30 to less than 60 kg	400 mg (two 200 mg injections)	200 mg Q2W
60 kg or more	600 mg (two 300 mg injections)	300 mg Q2W

LTD participants will use dupilumab as prescribed by their primary care physician.

6.2 Drug Accountability

Under Title 21 of the Code of Federal Regulations (21CFR §312.62) the investigator will maintain adequate records of the disposition of the study medications, including the date and quantity of the drug received, to whom the drug was dispensed (participant-by-participant accounting), and a detailed accounting of any drug accidentally or deliberately destroyed. The investigator must delegate the drug accountability responsibility to a licensed/registered pharmacist at the registered investigational pharmacy at the clinical research site. Please refer to the DAIT Pharmacy Guidelines (https://www.niaid.nih.gov/sites/default/files/pharmacy.pdf).

All personnel involved in drug management and preparation must receive proper training based on DAIT and site requirements prior to study initiation. The Pharmacist of Record should be listed on the Delegation of Responsibility Log (DoR) and is responsible to ensure that any pharmacy personnel involved in any aspect of the drug management, preparation, and dispensing process have completed and documented all DAIT required trainings (GCP/HSP, protocol/pharmacy manual, DAIT Pharmacy Guidelines). Only individuals listed on the DoR log and/or IoR may manage drug products for this study.

All drug disposition and dispensing (receipt, storage, use, return, and disposition) will be maintained by the study site using accountability logs that are approved by DAIT/NIAID and 21 CFR Part 11 compliant. Additionally, a participant-specific accountability log must be completed and will contain the identification of each participant and the date and quantity of drug(s) dispensed.

The study clinical research associate (CRA) will conduct accountability of the drugs by monitoring the main accountability records and participant-specific accountability log. The study CRA will also review other pharmacy logs, as applicable.

All records regarding the disposition of study medications will be available for monitoring and inspection.

6.3 Assessment of Participant Adherence with Study Treatment

Adherence with topical steroid treatment will be based on participant self-report.

Dupilumab will be administered in clinic at the Dupilumab Initiation Visit and thus is an observed adherence. It may also be observed when given in clinic, as it will be for participants who are able to be seen within window, at the 4 Week Dupilumab Assessment. Adherence for doses administered at home will be based on participant self-report. Reminder calls will be provided for self-administered doses as outlined in the study manual of procedures (MOP). Adherence will be determined by the percentage of injections received.

6.4 Toxicity Prevention and Management

Dose modification for an individual participant is not allowed. Study drugs may be prematurely discontinued as delineated in Section 6.5. If absolutely necessary (e.g., for treatment of intolerable AD symptoms or super-infection), a prohibited medication or procedure (as defined in Section 7.3) may be allowed at the discretion of the investigator (See Section 7.4).

At present, treatment with dupilumab does not require any laboratory monitoring. In clinical trials of patients with AD treated with dupilumab, approximately 10% developed conjunctivitis. For most patients, this is mild and manifests as dry eyes or ocular irritation and does not require discontinuation or change in dosing frequency. Patients typically have resolution of this AE with over-the-counter lubricating eye drops. Infrequently, patients may have more severe

conjunctivitis or other ocular symptoms that may need to be evaluated by an ophthalmologist. Most of these patients can continue the same dosing of dupilumab while being treated, most commonly with ophthalmic corticosteroids (Akinlade B, et al, 2019).

6.5 Premature Discontinuation of Study Medication(s)

Study therapy may be prematurely discontinued for any participant for any of the following reasons:

- Diagnosis of a malignancy during study, excluding carcinoma in situ of the cervix, or squamous or basal cell carcinoma of the skin
- Evidence of pregnancy
- Participant no longer meets study eligibility at 1 Week Assessment/Steroid Initiation, after initiation of study treatment (Vanicream™ Moisturizing Cream) at the Baseline Study Visit
- Severe worsening of AD, which in the opinion of the investigator requires stopping of the study medication(s)
- Participant is determined to be a treatment failure based on study criteria. If an AD flare cannot be controlled with a topical rescue medication and treatment with a systemic medication is required, the participant will be considered a treatment failure. Treatment with oral antibiotics for an infection will not require discontinuation.
- Evidence of non-compliance to study protocol that in the opinion of the investigator requires discontinuation of study medication(s)
- Any serious AE that is possibly or definitely related to a study medication
- Reasons specific to dupilumab treatment:
    - Anaphylactic reaction to dupilumab injection
    - Any infection that:
        - Requires parenteral treatment with an antibiotic, antifungal, antiviral, antiparasitic, or antiprotozoal agent
        - Requires oral treatment with such agents for longer than 2 weeks
        - Is opportunistic, such as tuberculosis and other infections whose nature or course may suggest an immune-compromised status
    - Evidence of severe and prolonged ISRs to dupilumab

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

If a participant is prematurely discontinued from study treatment, the participant will be asked to come in for an Early Termination Visit and will be withdrawn from the study.

7 Other Medications

7.1 Concomitant Medications

Any treatment (including nutritional supplements) or procedure administered from the time of consent to the end of study visits is considered a concomitant medication/procedure. This includes permitted medications ongoing at the time of consent.

7.1.1 Protocol-mandated

All protocol-mandated study treatments are described in Section 6.

7.1.2 Other permitted concomitant medications

Other than the prohibited medications and procedures listed in Section 7.3, treatment with concomitant medications and procedures is permitted during the study. This includes treatment with contraceptives, nasal and inhaled corticosteroids, and oral antihistamines.

Participants are prohibited from initiating treatment with prescription moisturizers or moisturizers containing ceramide, hyaluronic acid, urea, or FLG, but may continue using stable doses of such moisturizers on areas other than the targeted skin region if initiated before the Baseline Assessment. Note that initiation of such moisturizers is not prohibited when exclusively used on the face, neck, palms, or soles.

7.2 Prophylactic Medications

Individuals who can become pregnant, as defined in the study manual of procedures, must use an effective method of contraception (e.g., oral contraceptive, intrauterine device (IUD), barrier method with spermicide, surgical sterilization or surgically sterilized partner, Depo-Provera, Norplant, NuvaRing, or hormonal implant) for the duration of study participation.

Pregnancy tests will be performed on individuals who can become pregnant, as defined in the study manual of procedures, who do not self-report as pregnant at the Baseline Assessment (Day 0) and Day 7 Visits. Topical steroid non-responders who can become pregnant and do not self-report as pregnant will have a pregnancy test performed prior to initiating dupilumab treatment. Individuals who can become pregnant, as defined in the study manual of procedures, will also be asked during their other clinic visits as to whether they have tested positive to a pregnancy test since their last study visit. At investigator discretion and subject to each individual clinical institution's policies, individuals may be asked to take additional pregnancy tests where not required per protocol at in-person clinic visits.

All reported participant pregnancies, but not partner pregnancies, will be followed as described in Section 12.6.

7.3 Prohibited Medications/Procedures

Treatment with the following concomitant medications and procedures is prohibited through the End of Study Assessment (Day 168-224).

- Topical medications, including those listed below, when used on the arms, legs, or trunk. These medications are not considered prohibited when their use is limited exclusively to the face, neck, palms, or soles. Note that these medications may be used for the treatment of AD or super-infection within the trial at investigator discretion, though additional documentation is required in these cases; see Section 7.4.
    - Topical calcineurin inhibitors (tacrolimus or pimecrolimus)
    - Topical phosphodiesterase inhibitors (crisaborole)
    - Topical JAK inhibitors (ruxolitinib)
    - Topical AhR agonists (tapinarof)
    - TCS, other than the protocol-mandated study medications outlined in Section 6 (not applicable to the long-term dupilumab participants who can continue to use TCS as prescribed by their physicians outside of the study)
    - Topical antibiotics
    - Initiation of prescription moisturizers or moisturizers containing ceramide, hyaluronic acid, urea, or FLG. Participants may continue using stable doses of such moisturizers on body areas other than the target skin region if initiated before the Baseline Assessment

- Systemic treatment with an immunosuppressive/immunomodulating agent (including, but not limited to, systemic corticosteroids, cyclosporine, JAK inhibitors, mycophenolate-mofetil, azathioprine, methotrexate, IFN-γ, or other biologics)
- Treatment with biologics (other than dupilumab) including, but not limited to, the following:
    - Omalizumab
    - Any cell-depleting agents (e.g., rituximab)
    - Infliximab, adalimumab, golimumab, certolizumab pegol, abatacept, etanercept, or anakinra
- Medications used for the treatment of asthma:
    - Systemic corticosteroids
    - Inhaled corticosteroids at a dose greater than 880 mcg/day of fluticasone propionate or equivalent
- Systemic antibiotics, antivirals, antiparasitics, antiprotozoals, or antifungals
- Allergen immunotherapy (AIT), unless the participant has been on maintenance therapy for at least six months prior to the Screening Visit. Participants on maintenance therapy for at least six months can continue AIT as long as no changes are made to dosing through Day 168-224.
- Treatment with an investigational drug
- Major elective surgical procedures that could affect study participation or outcome assessment, per PI discretion
- Procedures listed below. Note that these procedures may be used for the treatment of AD within the trial at investigator discretion, though additional documentation is required in these cases; see Section 7.4.
    - Phototherapy (such as NBUVB, UVB, UVA1, or PUVA)
    - Bleach baths
    - Use of a tanning booth/parlor

In addition, participants will be asked to abstain from live (attenuated) vaccinations through the End of Study Assessment (Day 168-224). If a participant requires a vaccination prior to 12 weeks after discontinuing treatment with dupilumab, titers should be checked post-vaccination. Live (attenuated) vaccinations include but are not limited to the following:

- BCG
- Chickenpox (Varicella)
- FluMist-Influenza
- Intranasal influenza
- Measles (Rubeola)
- Measles-mumps-rubella (MMR) combination
- Measles-mumps-rubella-varicella (MMRV) combination
- Mumps
- Oral polio (Sabin)
- Oral typhoid
- Rotavirus
- Rubella
- Smallpox (Vaccinia)
- Yellow fever

7.3.1 Mitigation Following Use of Prohibited Medications/Procedures

The prohibited medications and procedures defined in Section 7.3 should be avoided whenever possible during the study. Use of prohibited medications and procedures represents a deviation from the planned protocol and may impact sample collections and/or the ability to evaluate treatment efficacy.

However, there may be times when use of prohibited medications cannot be avoided while ensuring that clinical best practices are followed for the safety of the participants. Moreover, use of prohibited medications/procedures alone is not a participant stopping rule, as delineated in Section 11.2. Considering these factors, the following actions may be taken following use of prohibited medications/procedures by participants to mitigate the potential impact on the study while maintaining the safety, rights, and wellbeing of participants.

Early Use of Prohibited Medications/Procedures in Dupilumab Naïve Participants

If a dupilumab-naïve participant uses a prohibited medication with a washout period greater than one week prior to the 4-Week Steroid Assessment, that participant may repeat Screening and the Baseline Assessment with prior approval by the protocol co-chairs. Participants who gain prior approval from the protocol co-chairs must wait until their washout periods conclude, but may then repeat the enrollment procedures beginning with Screening. Washout periods for prohibited medications/procedures are described within the study MOP.

Eligibility criteria as described in Section 4.1 and Section 4.2 will be re-evaluated during this reset of the participant's timeline, with the sole exception of exclusion criteria #15; participants will not need to wait an additional three months prior to repeating Screening procedures if they are conducting the repeat due to systemic corticosteroid use. The three-month avoidance of systemic corticosteroid use required upon initial enrollment is longer than necessary strictly to washout of systemic corticosteroids (typically 4 weeks). This is because exclusion criterion #15 is designed to discourage enrollment of asthmatic individuals whose condition is uncontrolled. Not all enrolled participants who use a systemic corticosteroid in the study, however, will do so because of uncontrolled asthma. As such, there is not a consistent need to re-establish each participant's ability to refrain from systemic corticosteroids for three months when repeating Screening procedures. Investigator discretion may be used to discontinue participants whose systemic corticosteroid use is, in fact, an indication of reduced asthma control. Additional detail may be found in the study MOP.

Implementation of this mitigation plan, whenever approved, does not negate the requirement to file protocol deviations in accordance with Section 16, as the original prohibited medication/procedure use is out of alignment with the planned protocol and documentation will be required for the visit schedule reset.

Other Use of Prohibited Medications/Procedures

If a participant uses a prohibited medication/procedure with a washout of four weeks or less during the study, and the medication use and washout occur completely between scheduled visits, the participant may continue being seen in clinic on schedule. However, this authorization for continued enrollment does not negate the requirement to file protocol deviations in accordance with Section 16, as the original prohibited medication use is out of alignment with the planned protocol.

If a participant uses a prohibited medication/procedure with a washout of four weeks or less during the protocol, and the medication/procedure use or washout will overlap with scheduled sample collections, visits may be delayed and/or rescheduled at investigator discretion. Samples collected while a participant is using or washing out of a prohibited medication/procedure are often significantly impacted such that their utility is reduced

substantially, so alterations to the visit schedule may be appropriate. In such cases, protocol deviations must be filed in accordance with Section 16, as the original prohibited medication/procedure use and subsequent delays to sample collection are both out of alignment with the planned protocol.

7.4 Rescue Medications

If absolutely necessary (e.g., for treatment of intolerable AD symptoms or super-infection), a topical prohibited medication or procedure (as defined in Section 7.3) may be allowed at the discretion of the investigator. Recommended guidelines for the treatment of flares are outlined in the study MOP. Participants who receive rescue treatment with a topical prohibited medication or procedure through the End of Study Assessment (Day 168-224) will be asked to continue with study treatment and procedures per the Schedule of Events (Appendix A) unless they meet a Participant Stopping Rule (Section 11.2). Participants whose condition requires treatment with a topical prohibited medication or procedure are subject to guidance in Section 7.3.1, meaning that scheduled visits may be delayed at investigator discretion and/or that participants may repeat Screening and Baseline Assessment with prior approval from the protocol co-chairs and Medical Officer.

If an AD flare cannot be controlled with a topical rescue medication and treatment with a systemic medication is required, the participant will be considered a treatment failure, and the participant will complete an Early Termination Visit. Treatment with oral antibiotics for an infection will not require discontinuation.

In the event of an infection or allergic reaction, best clinical practices will be followed for participant treatment. Infections will be treated with anti-bacterial medications based on the antibiotic sensitivity of the infection. Allergic reactions to the topical anesthetic, lidocaine, tape strips, or study drug will be treated with antihistamine and/or epinephrine depending on the severity of the reaction.

8 Study Procedures

A summary of complete study procedures is included in the Schedules of Events (Appendix A). Details regarding sample collection are included in the study MOP. The skin type (e.g., non-lesional or lesional), as well as the type and number of skin samples (e.g., swabs, STS, biopsies) to be collected, will depend on the enrollment group (NAD, DNAD, or LTD) and response to treatment.

To ensure participant safety, participants will be asked to comply with the clinical centers' current institutional policies and procedures related to COVID-19, when scheduling and attending their appointments.

8.1 Recruitment

Potential participants will be recruited using standardized questionnaires that collect contact information and medical history related to inclusion and exclusion criteria. Participants may be recruited by phone or in person. The research study will be explained in lay terms to each potential research participant. Once recruitment has been initiated, the participant will be assigned a unique participant identification (ID) number. Those who have no obvious characteristics making them ineligible for the study and who are interested in participating will be invited to clinic to complete the Screening Visit.

8.2 Screening Visit

The purpose of the Screening Visit is to confirm eligibility to continue in the study. The study will be explained in lay terms to each potential participant and/or parent guardian. During the visit, written informed consent will be obtained from the participant and/or parent guardian, and assent as applicable from the participant prior to performing any study

procedures. If it is possible to provide a physical copy of the consent and verify who is consenting, remote consenting may be conducted. In the event of a remote consent, the participant will need to return the signed consent to the clinic, prior to the conduct of any study procedures. The signed consent may be returned via an approved electronic platform, in person, and/or by mail. The process for remote consenting will be further defined in the study MOP.

The following assessments will be conducted to determine participant eligibility:

- Collection of demographics and contact information
- Medical history
- Confirm pregnancy status (if applicable)
    - At investigator discretion and subject to each individual clinical institution's policies, individuals may be asked to complete a urine pregnancy test.
- Assessment of current medications

Participants who do not meet inclusion and exclusion criteria due to assessment of their current medications/procedures will be asked whether they would be willing to come off their medications/procedures for a washout period. Participants who do not wish to washout of the prohibited medications/procedures will be identified as screen failures and will not continue in the study.

At the conclusion of the Screening Visit, participants who are eligible will be assigned to one of three enrollment groups: NAD, DNAD, or LTD. When participants are unable to return for a Baseline Assessment within 7 days, they may repeat the Screening Visit. Participants may also repeat the Screening Visit with prior approval to mitigate some instances of prohibited medication/procedure use, as described in Section 7.3.1.

Participants will be assessed for adverse events (AEs) at the conclusion of the Screening Visit. Participants will be provided with instructions on when to contact the research clinic should they experience any AEs. Contact information for the study physician and the 24-hour on-call physician will also be provided. The physician receiving the call will assess if the participant needs to be seen in clinic or if any further treatment is necessary.

8.3 Baseline Assessment

The purpose of the Baseline Assessment is to confirm eligibility and conduct baseline clinical assessments, for study enrollment. Baseline Assessment will serve as Day 0 for scheduling the remaining trial activities. The following procedures and assessments will be conducted at the Baseline Assessment:

- Interim medical history
- Full physical examination
- Assessment of AD severity (EASI, IGA, SCORAD, NESS, PP-NRS, TAA, and targeted EASI), AD participants only
- Allergic disease questionnaires
- Confirm pregnancy status (if applicable), including performing a urine pregnancy test
- Assessment of concomitant medications
- Photographs will be taken of the selected and marked targeted skin areas (non-lesional and lesional, as applicable)
- Photographs will be taken of the measured lesional and non-lesional sites for collection of skin swabs and tape strips, as applicable
- Vital signs, including temperature, blood pressure, heart rate, and respiratory rate, and growth parameters (height and weight)
- Skin swab collection; up to 6 swabs

- STS collection; up to 40 STS
- Distribute Vanicream™ Moisturizing Cream
- Assessment of AEs

The Baseline Assessment can be completed on the same day as the Screening Visit; assessments that are completed at the Screening Visit will not be repeated.

Participants who do not meet inclusion and exclusion criteria due to assessment of their current medications/procedures will be asked whether they would be willing to come off their medications/procedures for a washout period. Participants who do not wish to washout of the prohibited medications/procedures will be identified as screen failures and will not continue in the study. Participants who are willing to washout of medications/procedures may repeat the Screening Visit once washout periods have been met.

At the conclusion of the Baseline Assessment, all eligible and enrolled participants will be instructed to apply Vanicream™ Moisturizing Cream twice daily to their target area to maintain adequate hydration of the epidermis and improve barrier function until their Day 7 Visit.

Participants will be reminded to refrain from the use of prohibited medications/procedures as described in Section 7.3. Participants will be given instructions regarding restrictions on bathing, showering, use of sunscreen, and use of moisturizers. For example, all participants will be instructed to avoid application of moisturizer (including Vanicream™ Moisturizing Cream) to their target area for 1 day prior to study visits; LTD participants will be further instructed to avoid application of moisturizer to any area on the upper/lower extremities or trunk for 1 day prior to study visits.

Use of chlorinated pools and hot tubs will also be prohibited 2 days prior to study visits. Individuals who can become pregnant, as defined in the study manual of procedures, will be instructed to use an acceptable method of contraception as described in Section 4.2.

Participants will be provided with instructions on when to contact the research clinic should they experience any adverse events (AE). Contact information for the study physician and the 24-hour on-call physician will also be provided. The physician receiving the call will assess if the participant needs to be seen in clinic or if any further treatment is necessary.

Participant visit schedules after the Baseline Assessment will depend on the clinical group (e.g., steroid responders and non-responders) to which they are assigned.

8.4 1 Week Assessment (Steroid Initiation)

Participants of all clinical groups will have a visit at Day 7. For DNAD participants, the purpose of the 1 Week Assessment at Day 7 is to initiate treatment with topical steroids; this visit may be called the “Steroid Initiation.” Participants in other clinical groups have visits at this time so that Day 7 can serve as a standardized sampling point after consistent moisturization. The following procedures, assessments, and laboratory measures will be conducted at the visit:

- Interim medical history
- Physical examination (abbreviated at investigator discretion)
- Assessments of AD severity (EASI, IGA, SCORAD, PP-NRS, TAA, and targeted EASI), AD participants only
- Allergic disease questionnaires
- Confirm pregnancy status (if applicable), including performing a urine pregnancy test
- Assessment of concomitant medications

- Vital signs, including temperature, blood pressure, heart rate, and respiratory rate
- Blood collection; approximately 20 mL for serum/plasma biomarkers, approximately 2 mL for genetics (if unable to collect at this visit, genetics may be collected at any point during study participation), and approximately 2 mL for a Complete Blood Count (CBC) with differential
- Photographs will be taken of the measured lesional and non-lesional sites for collection of skin swabs, tape strips, and biopsies, as applicable
- Skin swab collection; up to 9 swabs
- STS collection; up to 60 STS
- Up to three optional 2.5 mm skin biopsies for molecular assays, adults only
- Triamcinolone and hydrocortisone distribution for dupilumab-naïve AD participants
- Assessment of AEs

Participants will be reminded to refrain from the use of prohibited medications/procedures as described in Section 7.3. Participants will be given instructions regarding restrictions on bathing, showering, use of sunscreen, and use of moisturizers. For example, all participants will be instructed to avoid application of moisturizer (including Vanicream™ Moisturizing Cream) to their target area for 1 day prior to study visits; LTD participants will be further instructed to avoid application of moisturizer to any area on the upper/lower extremities or trunk for 1 day prior to study visits.

Use of chlorinated pools and hot tubs will also be prohibited 2 days prior to study visits. Individuals who can become pregnant, as defined in the study manual of procedures, will be instructed to use an acceptable method of contraception as described in Section 4.2.

Participants will be provided with instructions on when to contact the research clinic should they experience any AEs. Contact information for the study physician and the 24-hour on-call physician will also be provided. The physician receiving the call will assess if the participant needs to be seen in clinic or if any further treatment is necessary.

8.5 4 Week Steroid Assessment

Participants in the DNAD clinical group will have a visit at Day 35 called the 4 Week Steroid Assessment. The purpose of the 4 Week Steroid Assessment is to assess response to topical corticosteroid treatment and conduct clinical assessments. The following procedures and assessments will be conducted at the 4 Week Steroid Assessment:

- Interim medical history
- Physical examination (abbreviated at investigator discretion)
- Assessments of AD severity (EASI, IGA, SCORAD, PP-NRS, TAA, and targeted EASI)
- Allergic disease questionnaires
- Confirm pregnancy status (if applicable)
    - At investigator discretion and subject to each individual clinical institution’s policies, individuals may be asked to complete a urine pregnancy test.
- Assessment of concomitant medications
- Vital signs, including temperature, blood pressure, heart rate, and respiratory rate
- Blood collection; approximately 20 mL for serum/plasma biomarkers and approximately 2 mL for a CBC with differential
- Photographs will be taken of the measured lesional and non-lesional sites for collection of skin swabs and tape strips, as applicable
- Skin swab collection; up to 6 swabs

- STS collection; up to 40 STS
- Distribute triamcinolone and hydrocortisone, as applicable
- Assessment of AEs

Participants will be reminded to refrain from the use of prohibited medications/procedures as described in Section 7.3. Participants will be given instructions regarding restrictions on bathing, showering, use of sunscreen, and use of moisturizers – including instructions to avoid application of moisturizer (including Vanicream™ Moisturizing Cream) to their target area for 1 day prior to study visits.

Use of chlorinated pools and hot tubs will be prohibited 2 days prior to study visits. Individuals who can become pregnant, as defined in the study manual of procedures, will be instructed to use an acceptable method of contraception as described in Section 4.2.

Participants will be provided with instructions on when to contact the research clinic should they experience any AEs. Contact information for the study physician and the 24-hour on-call physician will also be provided. The physician receiving the call will assess if the participant needs to be seen in clinic or if any further treatment is necessary.

DNAD participants who respond to topical steroid treatment (EASI ≤7) will continue topical steroid treatment. One optional Steroid Assessment (Day 40), a 12 Week Steroid Assessment (Day 91), and a 19 Week Steroid Assessment (Day 140) will be conducted after the 4 Week Steroid Assessment. The optional Steroid Assessment is described in Section 8.7, and the two required Steroid Assessments are described in Section 8.8.

DNAD participants who do not respond or cease to respond to topical steroid treatment (EASI >7 any time from the 4 Week Steroid Assessment through the 12 Week Steroid Assessment) will cross over to the topical steroid non-responder schedule of events and begin dupilumab treatment (Section 8.6).

8.6 Dupilumab Initiation Visit

DNAD participants classified as Topical Steroid Non-Responders (EASI score >7 any time from the 4 Week Steroid Assessment through the 12 Week Steroid Assessment) will begin treatment with dupilumab.

The Dupilumab Initiation Visit will ideally be completed on the same day as the study visit at which the qualifying EASI score was obtained (e.g., Day 35 if the score was elevated at the 4 Week Steroid Assessment) and must be completed within 3 days of a qualifying EASI score (see Section 8.13 for Visit Windows). When dupilumab initiation is completed within window (Section 8.13), activities that overlap will not be repeated. The following procedures and assessments will be conducted at the Dupilumab Initiation Visit:

- Interim medical history
- Physical examination (abbreviated at investigator discretion)
- Assessments of AD severity (EASI, IGA, SCORAD, PP-NRS, TAA, and targeted EASI)
- Allergic disease questionnaires
- Confirm pregnancy status (if applicable), including performing a urine pregnancy test
- Assessment of concomitant medications
- Vital signs, including temperature, blood pressure, heart rate, and respiratory rate; and growth parameters, including height and weight
- Blood collection; approximately 20 mL for serum/plasma biomarkers and approximately 2 mL for a CBC with differential

- Photographs will be taken of the measured lesional and non-lesional sites for collection of skin swabs, tape strips, and biopsies, as applicable
- Skin swab collection; up to 6 swabs
- STS collection; up to 40 STS
- Two required 2.5 mm skin biopsies for molecular assays, adults only
- Up to two optional 2.5 mm skin biopsies for molecular assays, adults only
- Distribute triamcinolone and hydrocortisone, as applicable
- Administer and distribute dupilumab
- Assessment of AEs

Participants will be provided with their dupilumab dosing schedule and instructions for at-home self-injection. Reminder phone calls will be made to the participant to remind them of their at-home injections. Adult participants will be provided with an additional 300 mg dose to take home to self-administer two weeks after their Dupilumab Initiation Visit. Pediatric participants who weigh 30 kg or more will be provided an additional dose based on their weight (200 mg dose for those weighing 30 to less than 60 kg, or 300 mg dose for those weighing 60 kg or more) to take home to self-administer after their Dupilumab Initiation Visit. If a participant is not comfortable self-administering injections and does not have a caretaker willing to administer injections, the participant may return to the study clinic to have their injection administered by study staff.

Participants will be reminded to refrain from the use of prohibited medications/procedures as described in Section 7.3. Participants will be given instructions regarding restrictions on bathing, showering, use of sunscreen, and use of moisturizers – including instructions to avoid application of moisturizer (including Vanicream™ Moisturizing Cream) to any area on the upper/lower extremities or trunk for 1 day prior to study visits.

Use of chlorinated pools and hot tubs will also be prohibited 2 days prior to study visits. Individuals who can become pregnant, as defined in the study manual of procedures, will be instructed to use an acceptable method of contraception as described in Section 4.2.

Participants will be provided with instructions on when to contact the research clinic should they experience any AEs. Contact information for the study physician and the 24-hour on-call physician will also be provided. The physician receiving the call will assess if the participant needs to be seen in clinic or if any further treatment is necessary.

One optional Dupilumab Assessment (Day 40-96), a 4 Week Dupilumab Assessment (Day 63-119), and a 15 Week Dupilumab Assessment (Day 140-196) will be conducted after the Dupilumab Initiation Visit. The optional Dupilumab Assessment is described in Section 8.7, and the two required Dupilumab Assessments are described in Section 8.8.

8.7 Optional Steroid Assessment / Optional Dupilumab Assessment

After the 4 Week Steroid Assessment and/or after the Dupilumab Initiation Visit (as applicable), DNAD participants will have the option to return to the clinic for an Optional Assessment during which the following procedures, assessments and laboratory measures will be conducted:

- Interim medical history
- Physical examination (abbreviated at investigator discretion)
- Assessments of AD severity (EASI, IGA, SCORAD, PP-NRS, TAA, and targeted EASI)
- Confirm pregnancy status (if applicable)

  - At investigator discretion and subject to each individual clinical institution's policies, individuals may be asked to complete a urine pregnancy test.
- Assessment of concomitant medications
- Vital signs, including temperature, blood pressure, heart rate, and respiratory rate
- Photographs will be taken of the measured lesional and non-lesional sites for collection of skin swabs, as applicable
- Skin swab collection; up to 6 swabs
- Assessment of AEs

Participants will be reminded to refrain from the use of prohibited medications/procedures as described in Section 7.3. Participants will be given instructions regarding restrictions on bathing, showering, use of sunscreen, and use of moisturizers. For example, all participants will be instructed to avoid application of moisturizer (including Vanicream™ Moisturizing Cream) to their target area for 1 day prior to study visits; LTD participants and topical steroid non-responders will be further instructed to avoid application of moisturizer to any area on the upper/lower extremities or trunk for 1 day prior to study visits.

Use of chlorinated pools and hot tubs will also be prohibited 2 days prior to study visits. Individuals who can become pregnant, as defined in the study manual of procedures, will be instructed to use an acceptable method of contraception as described in Section 4.2.

Participants will be provided with instructions on when to contact the research clinic should they experience any AEs. Contact information for the study physician and the 24-hour on-call physician will also be provided. The physician receiving the call will assess if the participant needs to be seen in clinic or if any further treatment is necessary.

DNAD participants who continue to be responsive to topical steroid treatment (EASI ≤7) will continue topical steroid treatment after the Optional Steroid Assessment. If EASI >7 at the Optional Steroid Assessment for DNAD participants previously classified as topical steroid responders, they will cross over to the topical steroid non-responder schedule of events and begin dupilumab treatment (Section 8.6).

8.8 Additional Assessments

Throughout the study, each group will have additional assessment visits. NAD participants will have 5 Week, 13 Week, and 20 Week Assessments. Dupilumab-naïve topical steroid responders will have a 12 Week Steroid Assessment and a 19 Week Steroid Assessment. Dupilumab-naïve topical steroid non-responders will have a 4 Week Dupilumab Assessment and a 15 Week Dupilumab Assessment. LTD participants will have a 9 Week Assessment and a 20 Week Assessment.

The following procedures and assessments will be conducted at these additional interim assessment visits:

- Interim medical history
- Physical examination (abbreviated at investigator discretion)
- Assessments of AD severity (EASI, IGA, SCORAD, PP-NRS, TAA, and targeted EASI) for DNAD and LTD participants only
- Allergic disease questionnaires
- Confirm pregnancy status (if applicable)

  - At investigator discretion and subject to each individual clinical institution's policies, individuals may be asked to complete a urine pregnancy test.
- Assessment of concomitant medications
- Vital signs, including temperature, blood pressure, heart rate, and respiratory rate
- Blood collection for serum/plasma biomarkers (approximately 20 mL) at the 4 Week Dupilumab Assessment and 15 Week Dupilumab Assessment for topical steroid non-responders, 19 Week Steroid Assessment for topical steroid responders, and 20 Week Assessment for LTD participants.
- Blood collection for CBC with differential (approximately 2 mL) at the 15 Week Dupilumab Assessment for topical steroid non-responders, 19 Week Steroid Assessment for topical steroid responders, and 20 Week Assessment for LTD participants.
- Photographs will be taken of the measured lesional and non-lesional sites for collection of skin swabs, tape strips, and biopsies, as applicable
- Skin swab collection; up to 9 swabs
- STS collection; up to 60 STS
- Skin biopsy collection for adult participants, dependent on group and respective visit, is described in the Schedule of Events (Appendix A)
- Distribute triamcinolone and hydrocortisone, as applicable, for topical steroid responders and non-responders
- Distribute Vanicream™ Moisturizing Cream, as applicable, at the 15 Week Dupilumab Assessment for topical steroid non-responders, 19 Week Steroid Assessment for topical steroid responders, and 20 Week Assessment for NAD and LTD participants.
- Administer and distribute dupilumab, as applicable, for dupilumab-naïve topical steroid non-responders only
- Assessment of AEs

At the 4 Week Dupilumab Assessment (Day 63-119), adult dupilumab-naïve topical steroid non-responders will be provided with five additional 300 mg doses to take home to self-administer every two weeks (Days 77-133, 91-147, 105-161, 119-175, and 133-189). Pediatric dupilumab-naïve topical steroid non-responders weighing 15 to <30 kg will be provided with an additional two 300 mg doses to take home to self-administer every four weeks (Days 91-147 and 119-175) while participants weighing 30 kg or more will be provided with five additional doses, depending on weight (200 mg dose for those weighing 30 to less than 60 kg, or 300 mg dose for those weighing 60 kg or more), to administer every two weeks (Days 77-133, 91-147, 105-161, 119-175, and 133-189). Participants will be provided with their dosing schedule and reminder phone calls will be made to the participant to remind them of their at-home injections. If a participant is not comfortable self-administering injections and does not have a caretaker willing to administer injections, the participant may return to the study clinic to have their injection administered by study staff.

Participants will be reminded to refrain from the use of prohibited medications/procedures as described in Section 7.3. Participants will be given instructions regarding restrictions on bathing, showering, use of sunscreen, and use of moisturizers. For example, all participants will be instructed to avoid application of moisturizer (including Vanicream™ Moisturizing Cream) to their target area for 1 day prior to study visits; long-term dupilumab users and topical steroid non-responders will be further instructed to avoid application of moisturizer to any area on the upper/lower extremities or trunk for 1 day prior to study visits.

Use of chlorinated pools and hot tubs will also be prohibited 2 days prior to study visits. Individuals who can become pregnant, as defined in the study manual of procedures, will be instructed to use an acceptable method of contraception as described in Section 4.2.

Participants will be provided with instructions on when to contact the research clinic should they experience any AEs. Contact information for the study physician and the 24-hour on-call physician will also be provided. The physician receiving the call will assess if the participant needs to be seen in clinic or if any further treatment is necessary.

DNAD participants who continue to respond to topical steroid treatment (EASI ≤7) will continue topical steroid treatment after their 12 Week Steroid Assessment. If EASI >7 at or before the 12 Week Steroid Assessment, AD participants will cross over to the topical steroid non-responder schedule of events and begin dupilumab treatment (Section 8.6). If this occurs after the 12 Week Steroid Assessment, the participant will be considered a treatment failure and will complete the Early Termination Visit (Section 8.10) and be withdrawn from the study.

8.9 End of Study Assessment

The following procedures and assessments will be conducted at the End of Study Assessment:

- Interim medical history
- Physical examination (abbreviated at investigator discretion)
- Assessments of AD severity (EASI, IGA, SCORAD, NESS, PP-NRS, TAA, and targeted EASI) for DNAD and LTD participants only
- Allergic disease questionnaires
- Confirm pregnancy status (if applicable)
    - At investigator discretion and subject to each individual clinical institution's policies, individuals may be asked to complete a urine pregnancy test.
- Assessment of concomitant medications
- Vital signs, including temperature, blood pressure, heart rate, and respiratory rate
- Blood collection; approximately 20 mL for serum/plasma biomarkers and approximately 2 mL for a CBC with differential
- Photographs will be taken of the measured lesional and non-lesional sites for collection of skin swabs, tape strips, and biopsies, as applicable
- Skin swab collection; up to 9 swabs
- STS collection; up to 60 STS
- Up to three optional 2.5 mm skin biopsies for molecular assays, adults only
- Assessment of AEs

At the conclusion of this visit, participants will be allowed to resume use of prohibited medications/procedures as described in Section 7.3. Participants will be allowed to receive live (attenuated) vaccinations, as needed. They will be allowed to resume bathing/showering, use of chlorinated pools and hot tubs, sunscreen, and moisturizers without restrictions.

8.10 Early Termination Visit

An Early Termination Visit will occur if disease becomes uncontrolled in participants on topical steroids any time after the 12 Week Steroid Assessment; if disease becomes uncontrolled, even with rescue, in participants on study-provided dupilumab; if a participant stops treatment with dupilumab; if an enrolled participant no longer meets eligibility at their 1 Week Assessment; or if a participant discontinues study participation without withdrawing consent. The following procedures and assessments may be conducted at the Early Termination Visit:

- Interim medical history
- Physical examination (abbreviated at investigator discretion)

- Assessments of AD severity (EASI, IGA, SCORAD, NESS, PP-NRS, TAA, and targeted EASI) for DNAD and LTD participants only
- Allergic disease questionnaires
- Confirm pregnancy status (if applicable)
    - At investigator discretion and subject to each individual clinical institution's policies, individuals may be asked to complete a urine pregnancy test.
- Assessment of concomitant medications
- Vital signs, including temperature, blood pressure, heart rate, and respiratory rate
- Blood collection; approximately 20 mL for serum/plasma biomarkers and approximately 2 mL for a CBC with differential
- Photographs will be taken of the measured lesional and non-lesional sites for collection of skin swabs, tape strips, and biopsies, as applicable
- Skin swab collection; up to 9 swabs
- STS collection; up to 60 STS
- Up to three optional 2.5 mm skin biopsies for molecular assays, adults only
- Assessment of AEs

Based on the reason for participant withdrawal or early termination, study samples may not be collected during this visit. The criteria for sample collection will be defined in the study MOP.

At the conclusion of this visit, participants will be allowed to resume use of prohibited medications/procedures as described in Section 7.3. Participants will be allowed to receive live (attenuated) vaccinations, as needed. They will be allowed to resume bathing/showering, use of chlorinated pools and hot tubs, sunscreen, and moisturizers without restrictions.

8.11 Early Withdrawal/Termination Follow-Up Phone Call

A final telephone visit will occur for participants who prematurely withdraw or are withdrawn, any time after Day 7. The timing of this visit will vary per participant, based on received study treatments and/or completed study procedures. This visit will occur 30 days after the last dose of treatment *and* after the longest AE observation period for any completed study procedures. The visit will be brief, and participants will be asked to report any new AEs, answer questions regarding the status of their AD, as applicable, and report on any new concomitant medication use. Individuals who can become pregnant, as defined in the study manual of procedures, will be asked whether they have tested positive to a pregnancy test since their last visit.

AEs discovered and/or reviewed during this phone call will be followed as described in Section 12.4.3. For many participants who prematurely withdraw or are withdrawn, this final phone call will coincide with the conclusion of all non-serious AE surveillance. However, any participant with an ongoing SAE will be followed until it resolves with or without sequelae, or the SAE stabilizes, or until the participant withdraws consent, whichever occurs first. Monitoring of a pregnant participant shall continue until the conclusion of the pregnancy.

8.12 Unscheduled Visits

If disease activity increases (e.g., AD flare) or other concerns arise (e.g., symptoms at the biopsy or STS sites) between regularly scheduled visits, participants should be instructed to contact study personnel and may be asked to return to the study site for an "Unscheduled Visit." Participants may also be asked to return to the clinic for Unscheduled Visits, as

needed, to provide additional skin swabs, STS, and/or skin biopsies for studies, or if samples are lost or destroyed, or if insufficient yields were obtained at a previous study visit.

The following procedures, assessments, and laboratory measures may be conducted at an Unscheduled Visit:

- Interim medical history
- Physical examination (abbreviated at investigator discretion)
- Assessments of AD severity (EASI, IGA, SCORAD, NESS, PP-NRS, TAA, and targeted EASI) for DNAD and LTD participants only
- Allergic disease questionnaires
- Confirm pregnancy status (if applicable)
    - At investigator discretion and subject to each individual clinical institution's policies, individuals may be asked to complete a urine pregnancy test.
- Assessment of concomitant medications
- Vital signs, including temperature, blood pressure, heart rate, and respiratory rate; and growth parameters, including height and weight
- Administer and distribute triamcinolone, hydrocortisone, dupilumab, and Vanicream™ Moisturizing Cream, as applicable
- Assessment of AEs
- Blood collection; approximately 20 mL for serum/plasma biomarkers; approximately 2 mL for genetics if not collected at a prior visit; and approximately 2 mL for a CBC with differential, per investigator discretion
- Photographs will be taken of the measured lesional and non-lesional sites for collection of skin swabs, tape strips, and biopsies, as applicable
- Skin swab collection; up to 9 swabs
- STS collection; up to 60 STS
- Up to three optional 2.5 mm skin biopsies for molecular assays, adults only
- Up to three required 2.5 mm skin biopsies for molecular assays, adult dupilumab-naïve topical steroid non-responders only

Refer to the study MOP which details procedures that should be conducted based on the type of visit.

Participants will be reminded to refrain from the use of prohibited medications/procedures as described in Section 7.3. Participants will be given instructions regarding restrictions on bathing, showering, use of sunscreen, and use of moisturizers. For example, all participants will be instructed to avoid application of moisturizer (including Vanicream™ Moisturizing Cream) to their target area for 1 day prior to study visits; participants taking dupilumab will be further instructed to avoid application of moisturizer to any area on the upper/lower extremities or trunk for 1 day prior to study visits.

Use of chlorinated pools and hot tubs will also be prohibited 2 days prior to study visits. Individuals who can become pregnant, as defined in the study manual of procedures, will be instructed to use an acceptable method of contraception as described in Section 4.2.

Participants will be provided with instructions on when to contact the research clinic should they experience any AEs. Contact information for the study physician and the 24 hour on call physician will also be provided. The physician receiving the call will assess if the participant needs to be seen in clinic or if any further treatment is necessary.

8.13 Visit Windows

Study visits should take place within the time limits specified below.

Relevant Clinical Group	Visit/Event	Target	Window
All	Recruitment	N/A	N/A
All	Screening	N/A	N/A
All	Baseline Assessment	Day 0	Within 7 days of Screening
All	1 Week Assessment	Day 7	± 3 days
Dupilumab Naïve AD	4 Week Steroid Assessment	Day 35	-3, +6 days
Topical Steroid Responders	Optional Steroid Assessment	Day 40	± 2 days
Topical Steroid Responders	12 Week Steroid Assessment	Day 91	± 3 days
Topical Steroid Responders	19 Week Steroid Assessment	Day 140	± 3 days
Topical Steroid Responders	End of Study Assessment	Day 168	± 3 days
Topical Steroid Non-Responders	Dupilumab Initiation	Day 35*	Within 3 days of qualifying EASI score
Topical Steroid Non-Responders	Optional Dupilumab Assessment	5 days after Dupilumab Initiation	3-7 days after Dupilumab Initiation
Topical Steroid Non-Responders	4 Week Dupilumab Assessment	28 days after Dupilumab Initiation	25-31 days after Dupilumab Initiation
Topical Steroid Non-Responders	15 Week Dupilumab Assessment	105 days after Dupilumab Initiation	102-108 days after Dupilumab Initiation
Topical Steroid Non-Responders	End of Study Assessment	133 days after Dupilumab Initiation	130-136 days after Dupilumab Initiation
Non-AD	5 Week Assessment	Day 35	± 3 days
Non-AD	13 Week Assessment	Day 91	± 3 days
Non-AD	20 Week Assessment	Day 140	± 3 days
Non-AD	End of Study Assessment	Day 168	± 3 days
Long-term Dupilumab	9 Week Assessment	Day 63	± 3 days
Long-term Dupilumab	20 Week Assessment	Day 140	± 3 days
Long-term Dupilumab	End of Study Assessment	Day 168	± 3 days

**Dupilumab Initiation is dependent on obtaining a qualifying EASI score (>7). It may happen as early as same-day on the 4-Week Steroid Assessment, whether it is held on Day 32 or Day 41. It may be further delayed if participants obtain a qualifying EASI score after the 4 Week Steroid Assessment (e.g., Optional Steroid Assessment, 12-Week Steroid Assessment, Unscheduled Visit prior to 12 Week Steroid Assessment). Participants may cross over to complete visits for Topical Steroid Non-Responders any time through the 12 Week Steroid Assessment, which is targeted at Day 91.*

9 Mechanistic Assays

This section describes the proposed methodologies for this study. The techniques are state-of-the-art at the time of the writing of this protocol. Even so, the techniques completed will be updated and/or changed should there be additional technical breakthroughs in this area of research and are subject to budgetary limitations. The same methodologies/techniques will be utilized for all samples of a given type to ensure standardization and reduce variability. Some assays may only be conducted on a subset of participants (e.g., single cell and spatial transcriptome and proteome assays). Details of the laboratory processes are described in Standard Operating Procedures (SOPs) maintained by each laboratory.

9.1 Whole Transcriptome RNA-sequencing

Whole-transcriptome RNA-sequencing will be performed at National Jewish Health (NJH). Skin biopsies from lesional and non-lesional sites will be enzymatically digested using an adaptation of a recently developed cold active protease treatment method (Adam et al, 2017). A fraction of cells will be cryopreserved and will be later used for the single cell transcriptome RNA-seq (as described in Section 9.2), and the remainder of the cells will be pooled for whole transcriptome RNA-seq, or other assays. The pooled cells will be preserved in RLT lysis buffer (Qiagen) and stored at -80°C, followed by RNA extraction using RNeasy Micro Kits (Qiagen) in accordance with the manufacturer's protocol.

STS from lesional and non-lesional sites will be cryopreserved. STS samples will be lysed in RLT lysis buffer (Qiagen) and stored at -80°C, followed by RNA extraction using RNeasy Micro Kits.

Whole-transcriptome libraries will be constructed from both skin biopsy and tape extracted RNA using Nex-Generation sequencing libraries kits. Barcoded libraries will then be pooled and sequenced. High quality sequencing reads will be mapped using the STAR software package (Dobin et al, 2013) and quantified using Salmon (Patro et al, 2017), or equivalent software to produce the gene count matrices used for all analyses. Data will be analyzed as described in Section 13.4.2.1 and in the Statistical Analysis Plan (SAP).

9.2 Single Cell Proteogenomics

Single-cell proteogenomics (scProteogenomics), in which RNA and protein are sequenced for the same set of cells, will be performed at NJH. Cryopreserved cells from dissociated skin biopsies (see Section 9.1) will be used to perform sample cell capture. We will stain pooled samples with the Biolegend TotalSeq-A Universal Cocktail and perform single cell capture using the 10X Genomics 3' scRNA-seq workflow, after which the cDNA library and antibody-derived tag libraries will be sequenced on an Illumina NovaSeq 6000. We will use 10X Genomics Cell Ranger software (v3.0.2) to perform all initial data processing to create gene and protein count matrices. Quality control filtering will then be performed to remove suspected doublets, low count cells, and genes/proteins expressed in less than 0.1% of cells. Quality controlled data will be further processed and analyzed as described in Section 13.4.4.10 and in the SAP.

9.3 Genome-wide Genotyping

Genomic DNA will be extracted from whole blood collected from all participants at the 1 Week Assessment (Day 7) using the Wizard Genomic DNA Purification kit (Promega, Fitchburg, WI). Whole genome sequencing or genotyping may then be performed, followed by phasing using EAGLE 2.4, and imputation using MiniMac4 (HRC r1.1 panel), implemented on the Michigan Imputation Server (https://imputationserver.sph.umich.edu). Analysis will then be carried out as described in Section 13.4.4.14 and in the SAP.

9.4 Proteomics

Proteomic analysis of repeated tape strips from lesional and non-lesional skin will be conducted by mass spectrometry analysis following procedures established by NJH research laboratories (Goleva et al, 2020). Additional targeted assessment of the inflammatory cytokines produced in STS samples will be performed by multiplex Meso Scale Discovery assays as carried out by NJH groups previously (Lyubchenko et al, 2021).

9.5 Lipidomics

Lipid profiling will be conducted at the NJH site. Lipids will be extracted from the tape strips and quantified using mass spectrometry based on established protocols (Berdyshev et al, 2021; Berdyshev et al, 2018; Leung et al, 2019).

9.6 Metabolomics

Metabolomics profiling will be conducted at NJH. The water-soluble fraction from lesional and non-lesional skin tape lipidomic analysis will be utilized for the metabolomic profiling. Metabolomics analyses will be performed using a

Vanquish UHPLC coupled to a Q Exactive mass spectrometer (Thermo Fisher Scientific) as described previously (Nemkov et al, 2019). For targeted quantitation, extraction solutions will be supplemented with stable isotope-labeled standards of metabolites.

9.7 Measurement of S. aureus Abundance

Measurement of *S. aureus* abundance will be conducted at the Schlievert laboratory at the University of Iowa by qPCR (rCFU/cm^2) and CFU/cm^2. DNA extracted from skin swabs will be assayed by qPCR (rCFU/cm^2) to quantitate the abundance of *S. aureus* at each collection site. Quantitation will be done using the *S. aureus* gene target (femA) in a multiplex TaqMan® gene expression assay. A minimum of three technical replicates will be used for each participant sample. A standard curve of the femA Polymerase Chain Reaction (PCR) product will be used to quantify the *S. aureus* copy number or abundance. Live CFUs of coagulase positive *S. aureus* will be determined by rapid thawing of frozen swab samples and then immediate inoculation on a bacterial culture plate containing mannitol salt agar with egg yolk for selective growth of *Staphylococcus spp*.

9.8 Analysis of Serum/Plasma Biomarkers

Biomarkers will be analyzed at a central laboratory, which will be determined for each biomarker. Th2 biomarkers to be assessed may include, but are not limited to, the following: thymus and activation-regulated chemokine (TARC/CCL17), pulmonary and activation-regulated chemokine (PARC/CCL18), total serum immunoglobulin E (IgE), and antigen-specific IgE.

9.9 Microbiome

Targeted 16S and shotgun metagenomic sequencing of nucleic acids extracted from skin swab samples will be performed to identify species of bacteria carried on participants' skin, the level of carriage, and the expression, metabolism, and function of the carried microbes.

9.9.1 Microbiome Assessment by 16S rRNA

Lesional and non-lesional skin swabs will be collected per the Schedules of Events (Appendix A). DNA extracted from skin swabs will be assayed at a central laboratory by sequencing the V1-V3 16S rRNA hypervariable region to assess composition and diversity of the AD and non-AD microbiomes. The V1-V3 region will be amplified with PHusion High-Fidelity DNA Polymerase (New England Biolabs) using dual-indexed bar-coded primers (Fadrosh et al, 2014). Amplified products will be purified and normalized on SequalPrep™ plates (Life Technologies) and pooled for 300 bp paired-end sequencing on an Illumina MiSeq. Quality and quantity of the libraries will be evaluated using an Agilent BioAnalyzer. This approach routinely yields high quality sequence data, with ~40K reads per sample and assembly of 250-300 bp overlapping amplicons from the paired-end reads for each sample (Merkley et al, 2015).

9.9.2 Metagenomic Analysis of Microbiome

Metagenome analysis of skin samples collected will identify genomic-level functional features of the microbiome. Metagenome libraries will be constructed at a central laboratory, using Illumina Nextera XT (Illumina), and sequenced on an Illumina NovaSeq6000 using standard protocols for 150 bp paired-end sequencing. Samples will be multiplexed to provide a minimum of 80 M mappable reads/sample. Based on ongoing metagenome analysis of AD samples, we anticipate that ~20% of the 80 M reads will represent non-human or bacterial DNA. This will result in ~16 M high-quality bacterial reads for metagenome analysis.

9.9.3 Functional Assessment of Coagulase Negative Staphylococcal Species (CoNS) Isolates for Antimicrobial Activity

CoNS will be isolated from each swab sample by a central laboratory. CoNS strains will be stored at -80°C for potential future analysis of functional assessment for antimicrobial activity by a central laboratory.

Up to 84 individual colonies of CoNS will be randomly selected from each sample and transferred to Tryptic Soy Broth (TSB) (400 µL) in a 96-well cluster tube for analysis. For analysis, each assay plate will contain internal controls of a non-antimicrobial strain of *Staphylococcus epidermidis* (ATCC1457) as negative control, a known antimicrobial strain of *Staphylococcus hominis* producing Sh-lantibiotics as positive control, and blank wells without bacteria. CoNS clones will be expanded in a 96-well cluster tube at 37°C overnight with shaking at 250 rpm. Bacterial growth will be evaluated by measuring OD600 and only CoNS grown to (OD600 > 0.6) will be used for the following analysis. This will be performed by removing bacteria by centrifugation followed by sterile filtration by a 96-well filter plate with 0.22 µm polyvinylidene difluoride membrane (Corning Inc). The antimicrobial activity in each sterile filtered media (100 µL) will then be evaluated by mixing with fresh TSB (10 µL) containing 1×10^4 CFUs of *S. aureus* (ATCC35556). Antimicrobial CoNS strains will be defined as those that suppress *S. aureus* growth after 22 hours to less than 50% (I_{50}) of average growth seen in negative controls. The frequency of antimicrobial CoNS will be determined to total CoNS numbers subjected to the assay. CoNS isolates may then be processed for species identification by metagenomic sequencing.

9.10 Spatial transcriptomics and proteomics

Spatial proteogenomics, in which RNA and protein expression levels are determined in the context of certain cell types and histological features within skin biopsies, will be performed at NJH. Formalin-fixed skin biopsies will be used for these analyses. Data will be generated using the Nanostring GeoMx, 10X Genomics Visium, or equivalent technology, using standard protocols.

10 Biospecimen Storage

During the consent process, participants will be asked to give permission for long-term storage and future use of samples for research in the fields of AD and immunology. The following biospecimens will be stored:

- Serum
- Plasma
- DNA/RNA
- STS samples and any derivatives
- Skin biopsies and any derivatives
- Skin swabs and any derivatives

Instructions for sample preparation, handling, storage, and shipping are included in the study MOP. Principal Investigators (PIs) will be responsible for being aware of and observing all the regulations for classification, packaging, and labeling, permits or authorizations, and personnel training for shipment of biological and hazardous materials required for the conduct of this study.

11 Criteria for Participant and Study Completion and Premature Study Termination

11.1 Participant Completion

Participation will be considered complete for each participant at the conclusion of the End of Study Assessment (Day 168-224). Participants that complete an Early Termination Visit will not be considered as having completed the study.

11.2 Participant Stopping Rules and Withdrawal Criteria

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

1. The participant elects to withdraw consent from all future study activities, including follow-up.
2. The participant is "lost to follow-up" (i.e., no further follow-up is possible because attempts to reestablish contact with the participant have failed).
3. The participant dies.
4. The Investigator no longer believes participation is in the best interest of the participant.
5. Medical condition for which continued participation, in the opinion of the Investigator, would pose a risk to the participant or would be likely to confound interpretation of the results.
6. The participant has an anaphylactic reaction to study drug.
7. The participant shows evidence of non-compliance to study protocol that in the opinion of the investigator requires discontinuation.
8. DNAD participants terminate study treatment prior to their last scheduled dose (i.e., ahead of the 15 Week Dupilumab Assessment for topical steroid non-responders, ahead of the 19-Week Steroid Assessment for topical steroid responders)
9. LTD participants terminate dupilumab treatment prior to their End of Study Assessment.

11.3 Participant Replacement

Participants who continue to meet eligibility post completion of the Baseline and 1 Week Assessment/Steroid Initiation (Day 7) study visits and who subsequently withdraw, are withdrawn, or early terminate will not be replaced. Additional participants may be enrolled as necessary to obtain the required sample size as defined in Section 13.7.1.

11.4 Follow-up after Early Study Withdrawal

If a participant is withdrawn from the study for any reason or early terminates, the participant will be asked to complete a final visit by phone to assess any AEs and concomitant medications since their last visit. For participants who received any study medication (Vanicream™ Moisturizing Cream, triamcinolone, hydrocortisone, or dupilumab provided by the study) prior to withdrawal, this visit will occur 30 days after their last study treatment dose. If a participant withdraws prior to receiving study treatment, this visit will occur after the participant completes the timeframe specified for AE collection based on study procedures completed (Refer to Section 12.2.1).

AEs discovered and/or reviewed during this phone call will be followed as described in Section 12.4.3. For many participants who prematurely withdraw or are withdrawn, this final phone call will coincide with the conclusion of all non-serious AE surveillance. However, any participant with an ongoing SAE will be followed until it resolves with or without sequelae, or the SAE stabilizes, or until the participant withdraws consent, whichever occurs first. Monitoring of a pregnant participant shall continue until the conclusion of the pregnancy.

11.5 Study Stopping Rules

Study enrollment will be suspended pending Division of Allergy, Immunology, and Transplantation (DAIT)/NIAID and NIAID Allergy and Asthma Data Safety Monitoring Board (DSMB) expedited review of all pertinent data if any of the following occur:

- 1 death, or life-threatening AE, that is at least possibly related to the study therapy regimen or procedures
- A grade 3 or higher AE that is at least possibly related to the study therapy regimen or procedures in two or more participants

The study may not be resumed until all pertinent information is reviewed by DAIT/NIAID, NIAID Allergy and Asthma DSMB, and the central Institutional Review Board (IRB), and all parties concur with the resumption of the study. Local IRBs will be informed of the study suspension and the NIAID/DSMB/Central IRB decision on resumption of the study.

The study may be suspended or terminated by DAIT/NIAID upon review of any observations, events, or new information that merits such action.

12 Safety Monitoring and Reporting

12.1 Overview

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

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

12.2 Definitions

12.2.1 Adverse Event (AE)

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

The investigator must report AEs regardless of relationship to study therapy regimen or study mandated procedures.

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

- **Study therapy regimen:**
  - Vanicream™ Moisturizing Cream, triamcinolone, hydrocortisone, and dupilumab: An AE occurring after treatment initiation

    Vanicream™ Moisturizing Cream will be the only study therapy for the non-AD and long-term dupilumab participants. Medications not provided as part of the study, even if the same as study medications, will not be considered as study treatment.

- **Study mandated procedures:**
  - Blood Draw: The following events related to the blood draw procedure will be considered AEs if they occur within 48 hours of the blood draw:
    - Fainting / Vasovagal Events
    - Bruising at the puncture site larger than 2 cm in diameter

- Bleeding from the puncture site lasting more than 30 minutes
- Swelling at the puncture site larger than 2 cm
- Allergic reaction to topical anesthetic that requires use of rescue medications, detailed in Section 7.4

- STS: The following events related to the STS collection procedure will be considered AEs if they occur within 48 hours of the tape stripping:
  - Fainting / Vasovagal Events
  - Bruising at the tape stripping site larger than 2 cm in diameter
  - Bleeding from the tape stripping site lasting more than 30 minutes
  - Redness or swelling at the tape stripping site larger than 3 cm
  - Fever (> 100.4°F) x two readings separated by more than 10 hours
  - Allergic reaction to STS that requires use of rescue medications, detailed in Section 7.4

  Purulent drainage from a tape stripping site within 2 weeks of the procedure will also be considered an AE.

- Skin Biopsy: The following events related to the skin biopsies will be considered AEs if they occur within 48 hours of the skin biopsies:
  - Fainting / Vasovagal Events
  - Bleeding at skin biopsy site lasting more than 6 hours
  - Redness or swelling at biopsy site larger than 2 cm in diameter
  - Fever (> 100.4°F) x two readings separated by more than 10 hours
  - Allergic reaction to lidocaine that requires use of rescue medications, detailed in Section 7.4

  Purulent drainage from a skin biopsy site within 2 weeks of the procedure will also be considered an AE.

Suspected Adverse Reaction (SAR)
Any AE for which there is a reasonable possibility that the study drug caused the AE. For the purposes of safety reporting, 'reasonable possibility' means 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 means any AE caused by a drug (21 CFR 312.32(a)).

Unexpected Adverse Event
An AE or SAR is considered "unexpected" if it is not listed in the package insert or is not listed at the specificity, severity, or rate of occurrence that has been observed.

12.2.2 Serious Adverse Event (SAE)

An AE or SAR is considered "serious" if, in the view of either the investigator or the Sponsor, it results in any of the following outcomes: Death, a life-threatening AE, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the

patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse (21 CFR 312.32(a)).

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

12.3 Grading and Attribution of Adverse Events

12.3.1 Grading Criteria

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

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

Grade 1 = Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2 = Moderate; minimal, local, or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily life (e.g., preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.).

Grade 3 = Severe or medically significant but not immediately life-threatening = Hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily life (e.g., bathing, dressing, feeding self, taking medications, etc.).

Grade 4 = Life-threatening consequences; urgent intervention indicated.

Grade 5 = Death.

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

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

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

12.3.2 Attribution Definitions

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

therapy regimen or procedures will be determined using the descriptors and definitions provided in Table 12.3.2.

Table 12.3.2 Attribution of Adverse Events

Code	Descriptor	Relationship (to study treatment medications and/or other study procedure)
UNRELATED CATEGORY		
1	Not Related	The AE is clearly not related: there is insufficient evidence to suggest a causal relationship.
RELATED CATEGORIES		
2	Possibly Related	The AE has a reasonable possibility to be related; there is evidence to suggest a causal relationship.
3	Related	The AE is clearly related.

12.4 Collection and Recording of Adverse Events

12.4.1 Collection Period

AEs will be collected from the time of consent, until a participant completes study participation, or until 30 days after their last study treatment dose if he/she prematurely withdraws (without withdrawing consent) or is withdrawn from the study. Prior to study treatment, AEs will be collected according to the study mandated procedures he/she last completed, as applicable. AE collection periods for study mandated procedures are described in Section 12.2.1.

12.4.2 Collecting Adverse Events

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

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

12.4.3 Recording Adverse Events

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

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

Once recorded, an SAE will be followed until it resolves with or without sequelae, or the SAE stabilizes, or until the participant withdraws consent, whichever occurs first.

Monitoring of a pregnant participant shall continue until the conclusion of the pregnancy.

12.5 Reporting of Serious Adverse Events and Adverse Events

12.5.1 Reporting of Serious Adverse Events to DAIT/NIAID

This section describes the responsibilities of the site investigator to report SAEs to the DAIT/NIAID and the Statistical and Clinical Coordinating Center (SACCC) via the SAE eCRF. Timely reporting of AEs is required by 21 CFR and ICH E6 guidelines.

Site investigators will report all SAEs (see Section 12.2.2), regardless of relationship or expectedness within 24 hours of discovering the event.

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

- AE term
- Relationship to study therapy regimen and procedures
- Reason why the event is serious
- Supplementary CRF pages that are current at the time of SAE reporting: medical history, concomitant medications, demographics, study treatment administration

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

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

[redacted]

12.5.2 Reporting to Health Authority

Not applicable

12.5.3 Reporting of Adverse Events to IRBs

All investigators shall report AEs in a timely fashion to their respective IRBs and central IRB in accordance with applicable regulations and guidelines.

12.6 Pregnancy Reporting

The investigator shall be informed of any pregnancy in a study participant immediately upon becoming aware of the event. Study treatment will be discontinued for the pregnant participant. The investigator shall counsel the participant and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the pregnant participant shall continue until the conclusion of the pregnancy. Note that partner pregnancies will not be monitored.

The investigator shall report to the SACCC all pregnancies within 24 hours of becoming aware of the event using the Pregnancy eCRF. All pregnancies identified during the study shall be followed to conclusion, and the outcome of each must be reported. The Pregnancy eCRF shall be updated and submitted to the SACCC when details about the outcome are available.

Information requested about the delivery shall include:

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

Any pregnancy complication that results in a congenital abnormality, birth defect, miscarriage, or medically indicated abortion will be reported as an SAE and shall be submitted to the SACCC using the SAE reporting procedures described above.

12.7 Reporting of Other Safety Information

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

12.8 Review of Safety Information

12.8.1 Medical Monitor Review

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

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

12.8.2 DSMB Review

The SACCC will provide the NIAID Allergy and Asthma DSMB with a listing of all AEs and SAEs on an ongoing basis (at least annually).

12.8.3 Planned DSMB Reviews

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

12.8.4 *Ad Hoc* DSMB Reviews

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

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

Temporary Suspension of Enrollment for ad hoc DSMB Safety Review

A temporary halt in enrollment will be implemented if an *ad hoc* DSMB safety review is required. New participants will not be consented for study participation during the enrollment halt. Participants already on therapy will continue treatment.

13 Statistical Considerations and Analytical Plan

13.1 Overview

This is a multi-center, longitudinal study that will characterize the gene expression profiles and transcriptomic endotypes that underlie mild and moderate-to-severe AD and will determine changes in these expression patterns and endotypes in response to standard-of-care treatment as delineated in the protocol. The primary objective of this study is to determine whether the type 2-high non-lesional skin endotype is associated with current mild versus moderate-to-severe AD disease.

Throughout analysis, our approach will be to analyze pediatric and adult populations separately to better model and discover any enrollment-group-specific endotypes and phenotypes. We will then compare how effects of interest differ between the two age cohorts. In regard to skin sampling, in addition to STS samples collected from all children and adults at each sample point, we will be collecting required and optional biopsy samples from adults, depending on time point and course of treatment. We may in certain instances compare biopsy and STS samples, as information from these samples can be complementary. For AD participants, skin samples will be collected from both lesional and non-lesional skin, as applicable. Although main analyses will focus on non-lesional skin, such that signatures of AD disease and severity can specifically be tied to systemic effects on skin tissue rather than to the unique character of lesional skin, we will also investigate how AD and treatment effects differ between the two skin types. Finally, as transcriptional responses to AD or AD treatments can also be influenced by individual traits (e.g., age, sex, race/ethnicity, concomitant therapies, etc.), we may include these as covariates in the models below where appropriate, to ensure that such variables don't confound our inferences. Where significant, effects of these covariates on responses will be investigated by running sensitivity analyses on datasets stratified based on these covariates.

13.2 Objectives, Endpoints, and Hypotheses

Study objectives and related endpoints are outlined in three tables displayed in Section 2.1. The study is designed to address 26 objectives and 35 related endpoints for the study, separated by priority and including the related hypotheses for each analysis.

13.3 Measures to Minimize Bias

Laboratory personnel conducting the assays will be blinded to diagnostic group (NAD, mild DNAD, moderate-to-severe DNAD, or LTD), study therapy regimen, and treatment response group (topical steroid responsive/non-responsive and/or dupilumab responsive/non-responsive), until after samples are processed. Analytical personnel who are unblinded for quality control purposes or to complete interim analyses of the data will not be involved in processing samples.

13.4 Analysis Plan

13.4.1 Analysis Populations

The study population consists of children and adults with mild and moderate-to-severe AD, as well as children and adults with no AD. The total sample size will be approximately 600 children and adults (200 children and 400 adults). Formal intent-to-treat and per protocol populations do not apply to this study. Because we want a balanced population with regard to age, sex, and race/ethnicity between the three enrollment groups, we will monitor these variables during the enrollment period. Any detected skews accruing during enrollment will be addressed by instructing sites to target unrepresented groups. When assigning participants as "child" or "adult" for the analyses, the age at the Baseline Assessment will be used (6-17 years at baseline = child; ≥ 18 at baseline = adult).

13.4.2 Analysis of Primary Objective (To determine if the type 2-high non-lesional skin [skin tape] endotype is associated with current mild versus moderate-to-severe AD disease)

This analysis will utilize WTS data from the Day 7 Visit. All analyses for this objective will be performed separately for children and adults. We will first define the type 2-high skin endotype in the transcriptome dataset by identifying a network of co-expressed genes enriched in known markers of type 2-inflammation using weighted gene co-expression analysis, or similar analysis (Weighted Gene Co-expression Network Analysis (WGCNA); Langfelder and Horvath, 2008). These networks should capture distinct biological processes taking place in these participants, including type 2-high inflammation. The type 2-high network will be identified as the network with strongest enrichment of published type 2 inflammation gene sets (Dyjack et al, 2018; Leung et al, 2019), based on hypergeometric tests of over-representation.

We will next test for association between type 2 inflammation and AD status and severity by treating type 2 inflammation as a dichotomous or continuous variable, in turn:

1. *Type 2 inflammation as a dichotomous variable:*
   We will divide individuals into type 2-high and type 2-low expression categories by clustering all participants based on gene-level expression of all genes in the type 2 inflammation gene network. Thus, these dichotomous group assignments will emerge from the transcriptome data based on individuals' relative expression of the network of genes most enriched in known type 2 markers. We will then test whether type 2-high status (high vs. low) is associated with disease group identity (AD vs. non-AD, mild AD vs. non-AD, moderate-to-severe AD vs. non-AD, and mild AD vs. moderate-to-severe AD) using logistic regression, with type 2 high status as the predictor and disease group as the outcome. We will also include relevant covariates, such as age, sex, race/ethnicity, and concomitant therapies as discussed above in the Overview (Section 13.1).

2. *Type 2 inflammation as a continuous variable:*
   We will calculate the geometric mean of expression across the type 2 inflammation gene network and then test whether this mean expression is associated with disease group identity using logistic regression, with mean expression (and covariates) as the predictor and disease group as the outcome.

13.4.3 Analyses of Secondary Objectives (SO)

Determining how gene expression in the skin (skin tape) differs between non-AD participants and those with current mild or moderate-to-severe AD disease [SO #1]

This analysis will utilize WTS data from non-lesional and lesional skin tape samples collected at the Day 7 and End of Study Assessment (Day 168-224) Visits. To investigate whether skin gene expression differs due to AD status and severity of disease at each time point, we will carry out gene differential expression (DE) analysis using a generalized linear model (GLM) comparing participants grouped by a disease class variable (non-AD, mild AD, and moderate-to-severe AD), accounting for covariates. To assess effects of AD status, we will contrast AD vs. non-AD, mild AD vs. non-AD, and moderate-to-severe AD vs. non-AD. We will contrast mild AD vs. moderate-to-severe AD to explicitly test for differences based on AD severity.

The above tests will be performed separately by visit, for lesional and non-lesional skin, and for children and adults, after which expression differences by AD severity group will be compared both between skin types and age cohorts.

Pathway and enrichment analyses will be performed on differentially expressed gene (DEG) sets to determine the biological mechanisms that underlie transcriptional differences.

Determining how gene expression in the skin (skin tape) changes with standard-of-care treatment (two timepoints) among the study outcome groups: (1) topical steroid responders, (2) dupilumab responders, (3) dupilumab non-responders, (4) non-AD, and (5) long-term dupilumab responders [SO #2]
To investigate changes in skin gene expression due to standard-of-care treatment as delineated in the protocol and how these changes may differ with study outcome groups (i.e., groups based on treatment regimen and response), we will model gene expression at both Day 7 and End of Study (Day 168-224) Visits using a linear mixed model based on normalized data that includes variables for visit, study outcome group (topical steroid responders, dupilumab-naïve responders, dupilumab-naïve non-responders, non-AD, and long-term dupilumab participants), and visit x study outcome group interaction, along with relevant covariates and a random intercept for each participant. Using this model, we will examine responses to standard-of-care treatment as delineated in the protocol by contrasting Day 7 and End of Study Assessments within outcome groups. We will also examine differences in response to standard-of-care treatment between any two outcome groups by testing the interaction term for visit effect in one outcome group vs. another.

The above tests will be performed separately for lesional and non-lesional skin and for children and adults, after which expression changes over time will be compared both between skin types and age cohorts (among all the outcome groups combined or within a given outcome group). Note that for lesional skin, we will compare target skin sampled at Day 168-224 that corresponds to the original lesional skin sampled in the target area at Day 7, even if the lesion has resolved over the course of treatment.

Pathway and enrichment analyses will be performed on DEG sets.

13.4.4 Analyses of Exploratory Objectives (EO)

Determining how gene expression in the skin (skin biopsy) differs between non-AD participants and those with current mild or moderate-to-severe AD disease [EO#1]
For this objective, we will repeat the analyses described for SO1 (Section 13.4.3.1) assessing the effects of AD status/severity on gene expression as well as differences in disease signatures between lesional and non-lesional skin using skin biopsies in place of STS samples.

We will also use linear mixed models (for non-lesional and lesional skin separately) based on normalized data to examine differences in disease signatures between STS and biopsy skin samples in participants from whom we have both types of samples by including terms for sample type (STS or biopsy), disease severity (mild or moderate-to-severe), and sample type x disease severity interaction, along with a random intercept for each participant. The interaction term will allow us to explicitly test for different changes between mild and moderate-to-severe AD in STS vs. biopsy samples.

Because all analyses are focused on skin biopsies, only adults will be included. Note that each of the above tests will be assessed on a gene level where different time points will be analyzed separately.

Determining how gene expression in the skin (skin biopsy) changes with standard-of-care treatment (two timepoints) among the study outcome groups: (1) topical steroid responders, (2) dupilumab responders, (3) dupilumab non-responders, (4) non-AD, and (5) long-term dupilumab participants [EO #2]
For this objective, we will repeat the analyses described for SO2 (Section 13.4.3.2) assessing changes in gene expression by group and differences between lesional and non-lesional skin samples using skin biopsy WTS data in place of STS WTS data.

For each outcome group separately, we will also examine differences in response to standard-of-care treatment between STS and biopsy samples in participants from whom we have both types of samples by fitting a mixed model based on normalized data that includes a random intercept for each participant and fixed effect terms for sample type (STS or biopsy), visit, and skin sample x visit interaction.

Comparing gene expression in the skin (skin tape) between participants who have recently started dupilumab (naïve at study start) and those who have been treated with dupilumab long term (at least 4 months of dupilumab treatment at study start) [EO #3]
We will obtain WTS data to investigate transcriptional differences between naïve and long-term users of dupilumab. We will use a GLM to compare gene expression between naïve and long-term users at each visit separately.

The above tests will be performed separately for lesional and non-lesional skin and for children and adults, after which expression differences between naïve and long-term dupilumab users will be compared both between skin types and age cohorts.

Identifying novel AD skin endotypes at all skin sampling time points [EO #4]
Endotype identification analysis will be carried out using WTS data collected from non-lesional/lesional STS and non-lesional/lesional biopsy (adults only) samples at each of the sample time points. Separate analyses will be carried out for children and adults, for STS and biopsy samples, and for non-lesional and lesional skin.

There is no straightforward way to identify endotypes of complex disease. Therefore, we will employ a series of complementary approaches to identify participants with previously defined and novel disease endotypes, which we detail in the SAP. These approaches will either yield gene sets (based on previous studies, DE analysis, or WGCNA) or summary expression states (based on topic modeling; (Dey et al, 2017) that alone or in combination may capture distinct pathobiological mechanisms of disease.

For each gene set-based endotype identification analysis, we will derive a summary representation of the endotype by treating expression of each gene set as a continuous or a dichotomous variable, in turn. In the case of a continuous variable, we will calculate the geometric mean of expression across each functional gene set. In the case of a dichotomous variable, for each set of endotype genes, we will divide individuals into endotype-high and endotype-low expression categories by hierarchically clustering all participants into $K = 2$ groups based on gene-level expression.

Finally, for expression state-based endotype identification, all participants will be profiled based on their mixture of proportional expression across a range of expression states identified. These membership

proportions will form the basis of hierarchical clustering of participants into *K* clusters that correspond to biological subtypes, where the number of groups, *K*, will be determined based on the data. For each endotype cluster identified using this approach, participants will be dichotomously classified as either endotype-high (a member of the endotype cluster) or endotype-low (not a member of the endotype cluster).

Determining whether novel AD skin endotypes are associated with current mild or moderate-to-severe AD disease [EO #5]

For continuous measures of gene set-based candidate disease endotypes obtained as noted in Section 13.4.4.4, we will determine whether each gene set is associated with AD status or severity by testing whether mean expression across an endotype gene set is associated with disease group identity (mild AD vs. non-AD, moderate-to-severe AD vs. non-AD, and mild AD vs. moderate-to-severe AD) using logistic regression, with expression as the predictor and disease group as the outcome. In the case of a dichotomous variable (high versus low expression of an endotype), we will test whether endotype status is correlated with disease group identity using logistic regression, with endotype status as the predictor and disease group as the outcome.

All analyses will be performed at each time point separately, using current AD status/severity defined at the corresponding visit. Endotypes for lesional/non-lesional skin, children/adults, and STS/biopsies will be analyzed separately.

Determining how AD skin endotypes and gene expression differ among the study outcome groups: (1) topical steroid responders, (2) dupilumab responders, (3) dupilumab non-responders, (4) non-AD, and (5) long-term dupilumab participants [EO #6]

We will determine how AD skin endotypes differ among study outcome groups using the exact same approach as detailed in Section 13.4.4.5, except we will substitute in study outcome group for disease group.

To determine which genes are differentially expressed based on study outcome group (at each timepoint analyzed separately), we will use the same approach as in Section 13.4.3.1, except we will substitute in study outcome group for disease group. We will test for differences in one outcome group compared to the remaining outcome groups combined as well as for differences among pairwise outcome groups. These comparisons will be carried out separately for lesional and non-lesional skin, STS and biopsy samples, and for children and adults, after which expression changes by study outcome group will be compared both between skin types, sample types, and age cohorts.

Finally, for both endotypes and gene expression, we may compare groups of interest that arise beyond the five main outcome groups listed above. For example, for dupilumab-naïve participants, we may compare lesional skin endotypes or gene expression prior to dupilumab treatment between participants whose skin lesions subsequently resolve during treatment and those whose skin lesions remain. For any such comparisons, we will investigate different skin types, sample types, and age cohorts separately, as appropriate.

Determining skin gene expression changes that are associated with topical moisturizer treatment [EO #7]

For this objective, we will use WTS data from STS samples collected from lesional and non-lesional skin. We will measure clinical response to moisturizer in both the target area (lesional skin only) and body-

wide. Target area skin response will be measured by calculating the shift in two different AD severity scores between visits: the targeted area assessment (TAA) and targeted EASI. For body-wide assessment, we will both calculate the shift in EASI between visits as well as classify participants as either "responder" or "non-responder" if EASI ≤ 7 or > 7, respectively. We will then determine how topical moisturizer use is associated with skin expression changes and how these changes may differ based on clinical response to treatment by modeling gene expression both before and after treatment, using a linear mixed model based on normalized data that includes a participant-specific random intercept and fixed effects for visit, clinical response, disease group, and visit x clinical response interaction. Using this model, we will examine transcriptional responses to moisturizer by contrasting samples collected before and after treatment within AD disease groups. We will also examine differences in response to moisturizer based on clinical response by testing the interaction term. We will also construct models that predict different moisturizer clinical responses as a function of gene expression at Day 0. This will enable us to investigate any associations between clinical response to treatment and baseline expression. For each dataset, we will run different models using the four different clinical response measures (two continuous target lesional skin measures and both continuous and dichotomous body-wide measures). Note that clinical response measures from lesional skin will be used both in models with lesional skin gene expression and non-lesional skin gene expression.

Lesional and non-lesional skin, and children and adults will be modeled separately, after which expression changes in response to treatment with moisturizer will be compared between skin types and age cohorts.

Determining skin gene expression changes that are associated with topical steroid response and non-response [EO #8]

For this objective, we will use WTS data from STS samples collected from lesional and non-lesional skin to describe clinical response to topical steroids, using the same four targeted and body-wide skin measures of clinical response as described in Section 13.4.4.7. We will determine how topical steroid use changes skin gene expression, and how these changes may differ based on clinical response to treatment by modeling gene expression both before and after treatment using a linear mixed model based on normalized data that includes a random intercept for each participant and fixed effects for visit, clinical response, disease group, and visit x clinical response interaction. Using this model, we will examine transcriptional responses to topical steroids by contrasting samples collected by timepoint within AD disease groups. We will also examine differences in response to topical steroids based on clinical response by testing the interaction term. We will also construct models that predict different topical steroid clinical responses as a function of gene expression before treatment. Different clinical response measures will be modeled separately.

Lesional and non-lesional skin, and children and adults will be analyzed separately, after which expression changes in response to treatment with topical steroids will be compared between skin types and age cohorts.

Determining skin gene expression changes that are associated with dupilumab response and non-response [EO #9]

For this objective, we will use WTS data from STS and skin biopsy (adults only) samples collected from lesional and non-lesional skin to describe the clinical response to dupilumab, using both continuous and dichotomous body-wide measures described in Section 13.4.4.7. We will then determine how dupilumab

use changes skin gene expression over time and how these changes may differ based on clinical response to treatment by modeling gene expression before, during (for STS), and after treatment using a linear mixed model based on normalized data that includes a random intercept for each participant and fixed effects for visit, clinical response, disease group, and visit x clinical response group interaction. This model will enable us to examine transcriptional responses to dupilumab treatment between contrasting visits as well as differing transcriptional responses based on clinical response. We will also construct models that predict dupilumab clinical responses as a function of gene expression before treatment. Different clinical response measures will be modeled separately.

Lesional and non-lesional skin, children and adults, and STS and biopsy samples will be analyzed separately, after which expression changes in response to treatment with dupilumab will be compared between skin types, age cohorts, and tissue types.

Determining the changes in cell type-specific gene and protein expression and cellular composition that are associated with dupilumab response and non-response [EO #10]
Adult skin biopsy samples collected from lesional and non-lesional skin will be cryopreserved. Once we have characterized all individuals as being clinical responders (EASI ≤ 7) or non-responders (EASI > 7) to dupilumab, we will select archived lesional/non-lesional sample pairs from participants from each group for single cell proteogenomics (scProteogenomics).

Gene and protein count matrices obtained from Cell Ranger (Section 9.2) will be separately batch-corrected and normalized, after which gene and protein modalities collected from the same cells will be integrated (Hao et al, 2021). Dimensionality reduction, cell clustering, and cell type identification based on gene and protein levels will be carried out using a pipeline optimized in the Seibold Lab. All samples (both visits, skin types, and responders/non-responders) will be clustered into cell types together.

To assess changes in cellular composition between visits, between responder and non-responder skin, or between lesional and non-lesional skin, we will explore whether cell types are selectively present or absent within a group. We will calculate cell type composition in each participant sample and then compare individual cell type proportions between visits (for each skin type and response status separately), response statuses (for each visit and skin type separately), and skin types (for each visit and response status separately). Cell type composition will also be compared between groups using multivariate linear mixed models for compositional data. We will also construct models that predict dupilumab clinical response as a function of cell type proportion prior to treatment.

To assess differences in gene/protein expression due to treatment, response status, or skin type, we will identify differentially-expressed genes/proteins between visits (for each skin type separately, controlling for response group), between response groups (for each skin type and visit separately), or between skin types (for each visit separately, controlling for response group) for each cell type separately using a mixed model.

Determining whether AD skin endotypes are associated with topical steroid response [EO #11]
Based on AD endotypes identified using both lesional and non-lesional STS and biopsy samples (Section 13.4.4.4) (dupilumab-naïve participants only), we will test for an association between skin endotypes and visit, clinical response to topical steroid use, and an interaction between the two. To describe clinical response to topical steroids, we will use in turn the same four targeted and body-wide skin measures of

clinical response described in Section 13.4.4.7. Specifically, we will use a logistic regression model appropriate for repeated measures to predict endotype status (high or low) as a function of visit, clinical response, disease group, and visit x clinical response interaction. The interaction term enables testing for whether the relationship between visit and endotype differs based on clinical response. We will also construct models that predict steroid clinical response as a function of endotype status before treatment. This will enable us to investigate any associations between clinical response to treatment and baseline endotype. Separate analyses will be performed for children and adults, for each endotype (stratified by lesional and non-lesional skin), and for STS and biopsy samples.

Determining whether AD skin endotypes are associated with dupilumab response [EO #12]
Based on AD endotypes identified using both lesional and non-lesional STS and biopsy samples (Section 13.4.4.4) (dupilumab-naïve topical steroid non-responders only), we will test for an association between skin endotypes and visit, clinical response to dupilumab (using in turn the two body-wide measures described in Section 13.4.4.7), and an interaction between the two. Specifically, we will use a logistic regression model appropriate for repeated measures to predict endotype status (high or low) as a function of visit, clinical response, disease group, and visit x clinical response interaction. This model will enable us to examine relationships between endotype status and visit as well as determine how these relationships change with clinical response. We will also construct models that predict dupilumab clinical response as a function of endotype status before treatment. Separate analyses will be performed for children and adults, for each endotype (stratified by lesional and non-lesional skin), and for STS and biopsy samples.

Determining whether cell type-specific gene and protein expression and cellular composition differ among AD skin endotypes [EO #13]
Using adult skin biopsy samples, we will select archived lesional and non-lesional sample pairs from participants that best represent each of the discovered non-lesional biopsy-based AD endotypes, as well as non-AD samples that contain none of the AD endotypes, for scProteogenomics. Note that if some endotypes exist only in combination with others, we will select the most endotypically pure participants for analysis. We will also consider performing scProteogenomics on participants carrying combinations of AD endotypes that are most strongly associated with AD and/or AD severity.

These selected samples will be processed, integrated, and clustered into cell populations using the same approach described in Section 13.4.4.10, where samples from both visits and skin types will be combined for cell type inference. To then assess changes in cellular composition that underlie AD skin endotypes, we will first explore whether cell types are selectively present or absent in certain AD endotypes. For example, ILC2s or tissue resident T cells may selectively be present in participants who are type 2-high. We will calculate cell type composition in each participant sample and then compare individual cell type proportions between each AD endotype and non-AD controls using beta regression, where different visits and skin types will be analyzed separately. Cell type composition will also be compared between different AD endotypes and non-AD controls using multivariate linear mixed models for compositional data. We will use a similar approach to assess cellular compositional differences between lesional and non-lesional skin and between Day 7 and End of Study Assessments.

To assess differences in gene/protein expression underlying AD endotype, we will identify differentially-expressed genes/proteins between endotype groups (for each cell type, visit, and skin type separately)

using a mixed model. We will assess cell type-specific differences in expression between time points and skin types using a similar approach.

Determining longitudinal changes in skin (skin tape) gene expression across standard-of-care treatment (all timepoints) in the study outcome groups: (1) topical steroid responders, (2) dupilumab responders, (3) dupilumab non-responders, (4) non-AD, and (5) long-term dupilumab participants [EO #14]
In addition to establishing transcriptomic changes between targeted visits (e.g., Steroid Initiation vs. Steroid Assessments, before and after treatment), we will also examine the trajectory of gene expression changes among different study outcome groups across 25+ weeks of standard-of-care treatment as delineated in the protocol (up to seven scheduled sample visits) in a single analysis using WTS data from all STS samples, potentially revealing the regulatory pathways that govern disease and response/non-response to treatment across samples. To do this, we will use a linear mixed model based on normalized data to predict gene expression as a function of visit, outcome group, and their interaction (with a participant-specific random intercept). This model will enable us to identify differences in expression trajectories among outcome groups. Children and adults, and lesional and non-lesional skin will be analyzed separately.

Determining whether genetic variation that regulates skin gene expression is associated with AD disease [EO #15]
To identify genetic variants that may determine skin gene and endotype expression in different outcome groups, we will use imputed genome-wide genotype data (obtained as detailed in Section 9.3) and the WTS expression data to perform a cis-eQTL analysis. The eQTL analysis will be performed separately for children and adults, lesional and non-lesional skin, different visits, and different outcome groups. We will perform a meta-analysis of the test statistics for different sample groups. The final output will include a list of general skin eQTLs and eQTLs that are specific to different outcome groups.

To put the eQTLs discovered in the context of the AD endotypes, we will examine whether endotype genes contain significant eQTLs and determine whether eQTLs are harbored in genes that are enriched in cell types that define particular AD endotypes (based on the scRNA-seq data). Additionally, we will perform eQTL analyses separately for the most common endotype groups (i.e., for endotype-high individuals within endotypes with n > 50 high individuals). These analyses will identify genetic variants that affect gene expression specifically in AD endotype groups, which should be enriched for endotype genetic risk variants.

We will next leverage our skin cis-eQTL database to perform a transcriptome-wide association study (TWAS) on existing, publicly available GWAS data for AD cases and healthy controls. Briefly, an expression prediction model will be built with our skin cis-eQTL data, which will be used to impute expression on AD and control samples in the UK Biobank (AD=9,321, healthy controls=351,820). We will test for association predicted expression of all genes and AD using GWAS summary statistics from UKBiobank. Results will be interfaced with endotype data generated from WTS data above to provide endotype context to the findings.

Determining the proteomic profiles of AD lesional and non-lesional skin endotypes [EO #16]
For all participants, we will generate proteomic profiles based on cryopreserved lesional and non-lesional STS samples collected during sampling visits (up to seven), as described in Section 9.4. Specifically, we will measure the abundance of different proteins, including those involved in barrier function (e.g., keratins,

FLG, FLG2, hornerin, late cornified envelop proteins, transglutaminases), proteases and protease inhibitors involved in skin desquamation, and inflammatory cytokines. We will test for differential abundance of these proteins by endotype and outcome group at each time point using a GLM and longitudinal changes in these profiles by endotype and outcome group similar to Section 13.4.4.14.

Analyses will be performed separately for lesional and non-lesional skin, and for children and adults.

Determining the lipidomic and metabolomic profiles of AD lesional and non-lesional skin endotypes [EO #17]

Similar to the proteomic data, we will also generate lipid and metabolomic profiles based on cryopreserved lesional and non-lesional STS samples collected during sampling visits (up to seven). Once these assays are complete, we will examine: 1) FLG breakdown products as a surrogate measure of FLG, a key barrier protein in the skin, 2) profiles of the levels of various classes of ceramides, lipids that are critical for skin barrier regulation, 3) the effects of cytokine environment as based on fatty acid chain length assessment in various types of sphingolipids (ceramides, sphingomyelins) and glycerolipids (lisophosphatidylcholine), and 4) amino acids, glycolysis, tricarboxylic acid cycle and other polar metabolites. Lipid and metabolome abundance data will be analyzed as described for proteomic data in Section 13.4.4.16 (differential abundance based on each endotype and outcome group using a GLM, separate analyses for each time point; longitudinal changes in these profiles by endotype and outcome group similar to Section 13.4.4.14; separate analyses for lesional and non-lesional skin, and for children and adults).

Assessing the relationship between S. aureus abundance and AD lesional and non-lesional skin endotypes [EO #18]

We will test for differential abundance of *S. aureus* between each endotype group as described for proteomic data in Section 13.4.4.16 (differential abundance based on each endotype and outcome group using a GLM, separate analyses for each time point; longitudinal changes in these profiles by endotype and outcome group similar to Section 13.4.4.14; separate analyses for lesional and non-lesional skin, and for children and adults).

Determining the association between intensity of pruritus and AD lesional and non-lesional skin endotypes [EO #19]

Intensity of pruritus will be measured for all study participants at each sample visit using the peak pruritus numerical rating scale (PP-NRS). We will assess the relationship between intensity of pruritus and AD endotypes at each timepoint, as described in Section 13.4.4.16 (differential pruritus intensity based on each endotype and outcome group using a GLM, separate analyses for each time point; longitudinal changes in these intensities by endotype and outcome group similar to Section 13.4.4.14; separate analyses for lesional and non-lesional skin, and for children and adults).

Determining the association between blood biomarker levels and AD lesional and non-lesional skin endotypes [EO #20]

Levels of biomarkers in the blood will be measured for all study participants at timepoints delineated in the schedule of events (Appendix A). We will assess the relationship between biomarker levels at each time point and each endotype as described in Section 13.4.4.16 (differential biomarker levels based on each endotype and outcome group using a GLM, separate analyses for each time point; longitudinal

changes in these levels by endotype and outcome group similar to Section 13.4.4.14; separate analyses for lesional and non-lesional skin, and for children and adults).

Determining the association between clinical phenotypes and AD lesional and non-lesional skin endotypes [EO #21]
Measures for various clinical (e.g., history of *S. aureus* infection, eczema herpeticum, food allergy, asthma) and demographic traits (e.g., obesity, ethnicity, sex, and age) will be gathered for all study participants during the Screening Visits and Baseline Assessments. For each endotype, we will assess the relationships between these traits and endotype status.

Determining differences in microbiomes among AD lesional and non-lesional skin endotypes [EO #22]
Skin swabs collected for all participants from lesional and non-lesional skin will be used to generate microbiome data from each visit in order to examine microbial associations with endotypes and how these associations change across standard-of-care. We will test for differential microbial abundance based on each endotype as described in Section 13.4.4.16 (differential abundance based on each endotype and outcome group using a GLM, separate analyses for each time point; longitudinal changes in these profiles by endotype and outcome group similar to Section 13.4.4.14; separate analyses for lesional and non-lesional skin, and for children and adults).

Determining spatial transcriptomic and proteomic associations with AD disease severity, endotypes, outcome groups, and drug treatment in lesional and non-lesional biopsy skin [EO #23]
Adult skin biopsy samples collected from lesional and non-lesional skin will be preserved. We will select archived lesional/non-lesional sample pairs from participants that represent the different disease outcome groups and endotypes characterized and carry out spatial transcriptomics and proteomics on these samples using spatial transcriptomic/proteomic technology.

Using these data, we will investigate how cell type-specific gene and protein expression in the context of histological features within biopsy tissue differ based on disease severity, endotype, outcome groups, and drug treatment using the GeoMX software package or equivalent combined with appropriate linear and mixed models, as described above for previous objectives.

13.4.5 Descriptive Analyses

Descriptive analyses will be reported separately by baseline severity group and study outcome group. Continuous baseline measures will be reported as 1) mean (or geometric mean) with standard deviation, or 2) median with 1st and 3rd quartile, as appropriate. Categorical baseline and demographic characteristics and participant disposition will be reported as frequencies and proportions.

13.5 [redacted]

[redacted]

[redacted]

13.6 Statistical Hypotheses

13.6.1 Hypotheses for the Primary Objective

The null and alternative hypotheses for the primary objectives will be assessed for children and adults separately and are as follows:

Test for differences in type 2-high status by baseline severity:

- H_0: The percentage of participants with type 2-high status does not differ between severity groups.
- H_A: The percentage of participants with type 2-high status differs between severity groups.

Test for differences in type 2 overall gene expression by baseline severity:

- H_0: Geometric mean of expression for the type 2 inflammation network genes does not differ between severity groups.
- H_A: Geometric mean of expression for the type 2 inflammation network genes differs between severity groups.

13.6.2 Hypotheses for Secondary Objective 1

The null and alternative hypotheses for the SOs will be assessed on a gene level, for each visit separately, and are as follows:

Test for differences in gene expression by current severity group (pairwise differences, stratified by skin type and age cohort):

- H_0: Gene expression does not differ between severity groups.
- H_A: Gene expression differs between severity groups.

Test for whether differences between severity groups (pairwise) are differential by skin type (lesional/non-lesional):

- H_0: Differences in gene expression between severity groups do not differ by skin type (lesional/non-lesional).
- H_A: Differences in gene expression between severity groups differ by skin type (lesional/non-lesional).

Test for whether differences between severity groups (pairwise) are differential by age cohort (children/adults):

- H_0: Differences in gene expression between severity groups do not differ by age cohort (children/adults).
- H_A: Differences in gene expression between severity groups differ by age cohort (children/adults).

13.6.3 Hypotheses for Secondary Objective 2

The null and alternative hypotheses for the SOs will be assessed on a gene level and are as follows:

Test for changes in gene expression over time (stratified by outcome group, skin type, and age cohort):

- H_0: Gene expression does not differ between Day 7 and end of study.
- H_A: Gene expression differs between Day 7 and end of study.

Test for whether changes in gene expression over time are differential by study outcome group:

- H_0: Changes in gene expression between Day 7 and end of study do not differ between study outcome groups.
- H_A: Changes in gene expression between Day 7 and end of study differ between study outcome groups.

Test for whether changes in gene expression over time are differential by skin type (lesional/non-lesional):

- H_0: Changes in gene expression between Day 7 and end of study do not differ by skin type (lesional/non-lesional).
- H_A: Changes in gene expression between Day 7 and end of study differ by skin type (lesional/non-lesional).

Test for whether changes in gene expression over time are differential by age cohort (children/adults):

- H_0: Changes in gene expression between Day 7 and end of study do not differ by age cohort (children/adults).
- H_A: Changes in gene expression between Day 7 and end of study differ by age cohort (children/adults).

13.7 Sample Size Considerations

13.7.1 Statistical power for study analyses

The study will enroll 600 participants in total, including: 300 adults with AD, 100 non-AD adults, 150 children with AD, and 50 non-AD children. Of the 450 AD participants, approximately 390 will be dupilumab-naïve. Dupilumab-naïve participant enrollment will be targeted to achieve approximately two thirds moderate-to-severe participants (moderate-to-severe AD adults = 160, moderate-to-severe AD children = 80) and one third mild participants (mild AD adults = 100, mild AD children = 50). We will target approximately 60 long-term dupilumab participants who are currently being treated with dupilumab and have received dupilumab for ≥ 4 months (20 children, 40 adults). These long-term dupilumab participants will be excluded from the primary objective and secondary objective #1.

Based on these population targets, we make several additional assumptions regarding sizes for the study outcome groups. Among the dupilumab-naïve AD participants, only those who fail topical steroid treatment will initiate dupilumab treatment. Currently, we project a 50% failure rate for topical steroids among the dupilumab-naïve moderate-to-severe participants and a 5% failure for the dupilumab-naïve mild AD participants (Brunner et al, 2016). Therefore, we anticipate topical steroid non-response in 85 adult AD participants and 43 children with AD, who will go on to receive dupilumab. Finally, of these dupilumab-naïve participants who receive dupilumab

during the study, we expect that approximately 40% will achieve EASI ≤ 7, per the following reference (Beck et al, 2014).

This sample size powers the primary comparison of type 2-high inflammation status by baseline severity group at 80%. Specific details about power and sample size for the primary and secondary objectives are below.

Power analysis for Primary Objective

The primary objective for the study is to determine if the type 2-high non-lesional skin (skin tape) endotype is associated with current mild versus moderate-to-severe AD disease.

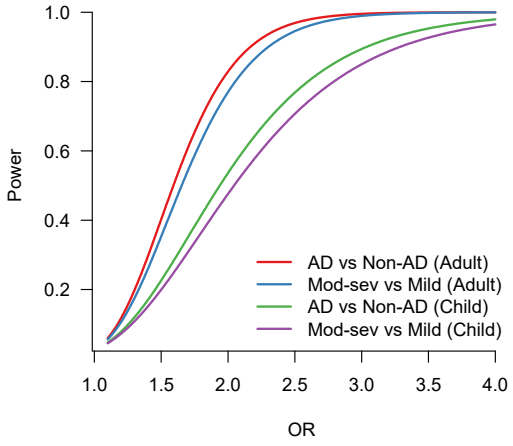
Assuming a prevalence of 50% for type 2-high status, as well as the group-level sample sizes in Table 13.7.1.1, we have 80% power to detect statistical significance for the odds ratios listed in the table below (we also provide calculations assuming 5% dropout after the first week). Power was calculated based on simple logistic regression with a binary predictor assuming an alpha level of 0.05 (Hsieh et al, 1998).

Table 13.7.1.1 Power justification of the Primary Objective in adults and children

				Minimum detectable odds ratio at 80% power	
Population	Comparison	Comparison group sample size	Reference group sample size	Assuming target sample size	Assuming 5% dropout
Adults	AD vs. Non-AD	260	100	1.95	1.99
	Moderate-to-severe AD vs. Mild AD	160	100	2.06	2.10
Children	AD vs. Non-AD	130	50	2.60	2.68
	Moderate-to-severe AD vs. Mild AD	80	50	2.80	2.89

Additionally, Figure 13.7.1.1 displays a range of power scenarios for these comparisons of interest.

Figure 13.7.1.1 Power curves for association tests in the Primary Objective



Note that the primary objective includes Day 7 data only, and therefore will not be impacted by participant dropout during the study.

Power analysis for Secondary Objective #1

SO1 is as follows: to determine how gene expression in the skin (skin tape) differs between non-AD participants and those with current mild or moderate-to-severe AD disease.

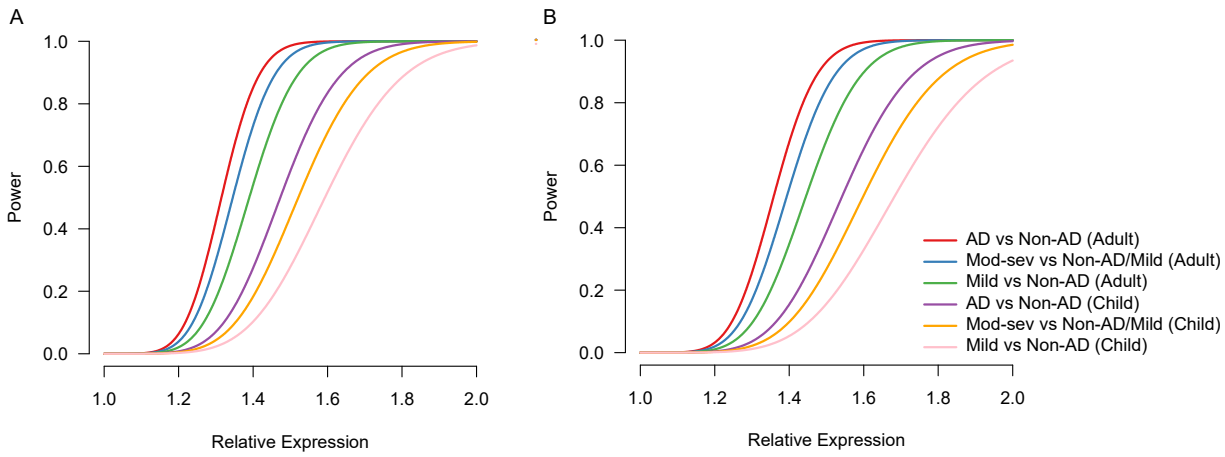
Assuming a gene expression coefficient of variation (CV) of 0.4 to approximate the observed median CV value across genes in humans (Hart et al, 2013), as well as the group-level sample sizes in Table 13.7.1.2, we have 80% power to detect statistical significance for the fold changes listed in Table 13.7.1.2. Power was calculated using the RNA-seq power computation method of Hart et al, 2013. The significance threshold (α) was set at 0.05/10,000, assuming ~10,000 expressed genes with sufficient variability to test. Finally, we assumed the least powerful situation for identifying differential expression, which is for genes that are lowly expressed (10 reads per gene). We calculated power assuming both our target sample sizes as well as for the case of 20% dropout by end of study.

Table 13.7.1.2 Power justification of Secondary Objective #1 in adults and children

				Minimum detectable fold change at 80% power	
Population	Comparison	Comparison group sample size	Reference group sample size	Assuming target sample size	Assuming 20% dropout
Adults	AD vs. non-AD	260	100	1.38	1.44
	Mild AD vs. non-AD	100	100	1.48	1.55
	Moderate-to-severe AD vs. non-AD	160	100	1.42	1.48
	Moderate-to-severe AD vs. Mild AD	160	100	1.42	1.48
Children	AD vs. non-AD	130	50	1.58	1.67
	Mild AD vs. non-AD	50	50	1.74	1.85
	Moderate-to-severe AD vs. non-AD	80	50	1.64	1.74
	Moderate-to-severe AD vs. Mild AD	80	50	1.64	1.74

Additionally, Figure 13.7.1.2 displays a range of power scenarios for these comparisons of interest.

Figure 13.7.1.2 Power curves for differential expression analyses in SO1. A) Assuming target sample sizes. B) Assuming sample sizes after 20% dropout by end of study.



Note that SO1 will assess differences in gene expression at both Day 7 and End of Study (Day 168-224).

Power analysis for Secondary Objective #2

SO2 is as follows: to determine how gene expression in the skin (skin tape) changes over time among the study outcome groups: (1) topical steroid responders, (2) dupilumab responders, (3) dupilumab non-responders, (4) non-AD, and (5) long-term dupilumab participants.

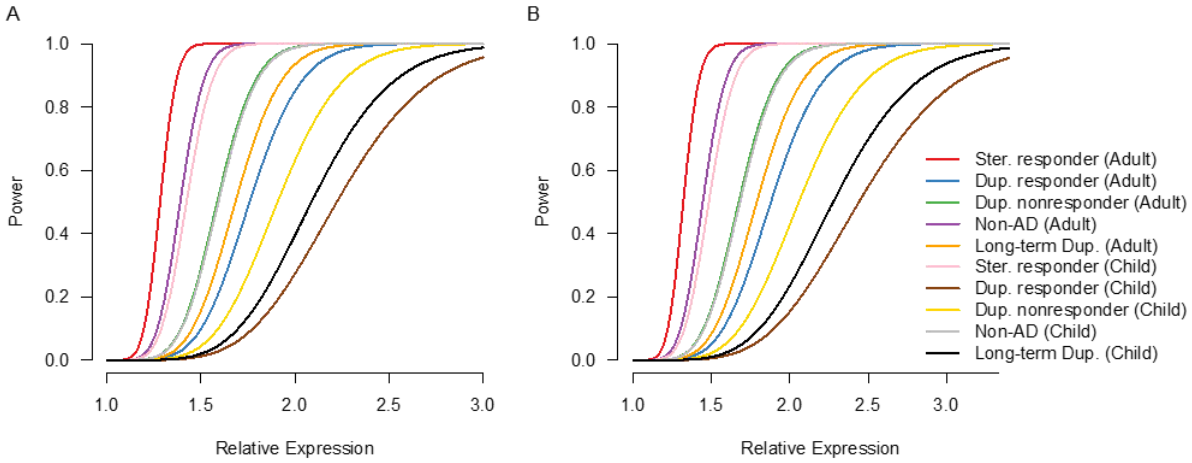
Anticipated group sample sizes are based on the targeted population sample sizes and assumed treatment response rates described in Section 13.7.1. The method for power calculation and underlying assumptions (gene expression CV, alpha, etc.) were identical to those used for SO1. Based on these assumptions, we have 80% power to detect statistically significant fold changes in gene expression between the study start and end points for the groups and fold changes listed in Table 13.7.1.3. Again, we calculated power for both our target samples sizes as well as for the case of 20% dropout by end of study.

Table 13.7.1.3 Power justification for Secondary Objective #2 in adults and children

			Minimum detectable fold change at 80% power	
Population	Study outcome group	Sample size	Assuming target sample size	Assuming 20% dropout
Adults	Topical steroid responders	175	1.34	1.39
	Dupilumab responders	34	1.95	2.11
	Dupilumab non-responders	51	1.73	1.84
	Non-AD	100	1.48	1.55
	Long-term dupilumab	40	1.85	1.99
Children	Topical steroid responders	87	1.52	1.60
	Dupilumab responders	17	2.57	2.88
	Dupilumab non-responders	26	2.15	2.35
	Non-AD	50	1.74	1.85
	Long-term dupilumab	20	2.39	2.65

Additionally, Figure 13.7.1.3 displays a range of power scenarios for these expression changes of interest.

Figure 13.7.1.3 Power curves for differential expression analyses in SO2. A) Assuming target sample sizes. B) Assuming sample sizes after 20% dropout by end of study.



Note that SO2 will assess differences in gene expression between Day 7 and End of Study (Day 168-224).

14 Identification and Access to Source Data

14.1 Source Data

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

14.2 Access to Source Data

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

15 Quality Assurance and Quality Control

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

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

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

16 Protocol Deviations

16.1 Protocol Deviation Definitions

16.1.1 Protocol Deviation

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

16.1.2 Major Protocol Deviation (Protocol Violation)

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

16.1.3 Non-Major Protocol Deviation

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

16.2 Reporting and Managing Protocol Deviations

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

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

17 Ethical Considerations and Compliance with Good Clinical Practice

17.1 Statement of Compliance

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

17.2 Informed Consent Process

The consent process will provide information about the study to a prospective participant and will allow adequate time for review and discussion prior to his/her decision. The PI or designee listed on the Investigator of Record (IoR) form will review the consent and/or assent and answer questions. Consent designees must be listed on the site delegation of responsibilities log and have demonstrated knowledge of the protocol and study procedures. The prospective participant will be told that being in the trial is voluntary and that he or she may withdraw from the study at any time, for any reason. All participants (and/or their legal guardians) will read, sign, and date a consent or assent (if applicable) form before undergoing any study procedures. Consent and assent (if applicable) materials will be presented in participants' primary language. A copy of the signed consent and assent (if applicable) form will be given to the participant.

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

Documentation of the consent and assent (if applicable) process must be entered on a source document.

17.3 Privacy and Confidentiality

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

18 Publication Policy

The ADRN Publications Policy will apply to presentations and publications of this study.

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Appendix A: Schedule of Events

NAD Control Participants:

Study Visit	Recruitment	Screening	Baseline Assessment	1 Week Non-AD Assessment	5 Week Non-AD Assessment	13 Week NAD Assessment	20 Week NAD Assessment	End of Study Assessment	Early Termination[1]	Early Withdrawal/ Termination Follow-Up[2]	Unscheduled Visit[3]
Target Day[12]		-7	0	7	35	91	140	168			
Study Procedures/Evaluations											
Recruitment	X										
Informed Consent		X									
Demographics		X									
Adverse Events		X	X	X	X	X	X	X	X	X	X
Concomitant Medications		X	X	X	X	X	X	X	X	X	X
Pregnancy Status		X	X	X	X	X	X	X	X	X	X
Pregnancy Test[4]			X	X							
Medical History		X	X[5]	X[5]	X[5]	X[5]	X[5]	X[5]	X[5]		X[5]
Physical Exam			X	X[5]	X[5]	X[5]	X[5]	X[5]	X[5]		X[5]
Vital Signs/Growth Parameters[6]			X	X	X	X	X	X	X		X
Allergic Disease Questionnaires			X	X	X	X	X	X	X		X
Blood Sample Collection											
CBC w/differential				X				X	X		X[7]
Genetics[8]				X							X
Serum/Plasma Biomarkers				X				X	X		X
Skin Sampling Location Photographs			X	X	X	X	X	X	X		X
Skin Swab Collection											
S. aureus Abundance (NL)			X	X	X	X	X	X	X		X
Microbiome 16S rRNA analysis (NL)			X	X	X	X	X	X	X		X
Metagenomics (NL)			X	X	X	X	X	X	X		X
Microbiome Functional Assessment (NL)			X	X	X	X	X	X	X		X
Skin Tape Stripping (STS)											
Transcriptomics (NL)			X	X	X	X	X	X	X		X
Lipidomics (NL)			X	X	X	X	X	X	X		X
Proteomics (NL)			X	X	X	X	X	X	X		X

Study Visit	Recruitment	Screening	Baseline Assessment	1 Week Non-AD Assessment	5 Week Non-AD Assessment	13 Week NAD Assessment	20 Week NAD Assessment	End of Study Assessment	Early Termination[1]	Early Withdrawal/ Termination Follow-Up[2]	Unscheduled Visit[3]
Skin Biopsy[9]											
Optional Biopsy (NL)				X				X	X		X
Treatment Distribution											
Vanicream™ Moisturizing Cream			X[10]				X[11]				

1. Based on the reason for participant withdrawal or early termination, study samples may not be collected during this visit. The criteria for sample collection will be defined in the study MOP.
2. A final phone visit will be completed for participants who withdraw or complete an Early Termination Visit, any time after the 1 Week Assessment. The timing of this visit will be based on the participant's last treatment dose or completed study procedure and respective AE observation period.
3. Participants will be instructed to contact study personnel if they experience any symptoms of concern at the tape stripping or skin biopsy sites and may be asked to return to the clinical site for an Unscheduled Visit. If an Unscheduled Visit is required for the purpose of collecting additional samples, only the required samples should be collected. This could include the collection of blood, skin swabs, STS and/or skin biopsies. Refer to the study MOP which details procedures that should be conducted based on the type of visit.
4. A urine pregnancy test will be completed for all individuals who can become pregnant, as defined in the study manual of procedures, who do not self-report as pregnant. At investigator discretion and subject to each individual clinical institution's policies, individuals may be asked to take additional pregnancy tests where not required per protocol at in-person clinic visits.
5. Initial medical history assessments and physical exams will be comprehensive. An abbreviated medical history and a physical exam, abbreviated at investigator discretion, will be conducted at subsequent scheduled visits and may be conducted at any unscheduled visits.
6. Temperature, blood pressure, heart rate, and respiratory rate will be measured. Growth parameters to include height and weight will only be measured at the Baseline Assessment and per PI discretion at an Unscheduled Visit.
7. A CBC with differential may be collected at an Unscheduled Visit per investigator discretion.
8. Blood for genetics may be collected at any time during study participation.
9. 2.5 mm skin biopsy (NL) will be collected for molecular assays for adult participants only.
10. Participants will be provided Vanicream™ Moisturizing Cream and will be required to apply it at least twice daily on the skin target area from their Baseline Assessment until their 1 Week Assessment (Day 7).
11. Vanicream™ Moisturizing Cream will be provided to participants at the 20 Week Non-AD Assessment, if needed, to begin applying at least twice daily on the skin target area until their End of Study Assessment (Day 168).
12. See Section 8.13 for detailed visit windows.

Dupilumab-Naïve AD Participants:

						Topical Steroid Responders						
Study Visit	**Recruitment**	**Screening**	**Baseline Assessment**	**Steroid Initiation**	**4 Week Steroid Assessment[1]**	**Optional Steroid Assessment**	**12 Week Steroid Assessment [5]**	**19 Week Steroid Assessment**	**End of Study Assessment**	**Early Termination[2]**	**Early Withdrawal/ Termination Follow-Up[3]**	**Unscheduled Visit[4]**
Target Day[17]		-7	0	7	35	40	91	140	168			
Study Procedures/Evaluations												
Recruitment	X											
Informed Consent		X										
Demographics		X										
Adverse Events		X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications		X	X	X	X	X	X	X	X	X	X	X
Pregnancy Status		X	X	X	X	X	X	X	X	X	X	X
Pregnancy Test[6]			X	X								
Medical History		X	X[7]	X[7]	X[7]	X[7]	X[7]	X[7]	X[7]	X[7]		X[7]
Physical Exam			X	X[7]	X[7]	X[7]	X[7]	X[7]	X[7]	X[7]		X[7]
Vital Signs/Growth Parameters[8]			X	X	X	X	X	X	X	X		X
Allergic Disease Questionnaires			X	X	X		X	X	X	X		X
AD Severity Assessments												
Eczema Area and Severity Index (EASI)			X	X	X	X	X	X	X	X		X
Scoring Atopic Dermatitis (SCORAD)			X	X	X	X	X	X	X	X		X
Investigator Global Assessment (IGA)			X	X	X	X	X	X	X	X		X
Peak Pruritus Numerical Rating Scale (PP-NRS)			X	X	X	X	X	X	X	X		X
Skin Target Area Assessment (TAA)			X	X	X	X	X	X	X	X		X
Nottingham Eczema Severity Score (NESS)			X						X	X		X
Blood Sample Collection												
CBC w/differential				X	X			X	X	X		X[9]
Genetics[10]				X								X
Serum/Plasma Biomarkers				X	X			X	X	X		X
Skin Sampling Location Photographs			X	X	X	X	X	X	X	X		X
Skin Swab Collection												
S. aureus Abundance (Les, NL)			X	X	X	X	X	X	X	X		X
Microbiome 16S rRNA analysis (Les, NL)			X	X	X	X	X	X	X	X		X
Metagenomics (Les, NL)			X	X	X	X	X	X	X	X		X
Microbiome Functional Assessment (Les, NL)			X	X	X	X	X	X	X	X		X

						Topical Steroid Responders						
Study Visit	**Recruitment**	**Screening**	**Baseline Assessment**	**Steroid Initiation**	**4 Week Steroid Assessment[1]**	**Optional Steroid Assessment**	**12 Week Steroid Assessment [5]**	**19 Week Steroid Assessment**	**End of Study Assessment**	**Early Termination[2]**	**Early Withdrawal/ Termination Follow-Up[3]**	**Unscheduled Visit[4]**
Skin Tape Stripping												
Transcriptomics (Les, NL)			X	X	X		X	X	X	X		X
Lipidomics (Les, NL)			X	X	X		X	X	X	X		X
Proteomics (Les, NL)			X	X	X		X	X	X	X		X
Skin Biopsy[11]												
Optional Biopsies (Les, NL)				X					X	X		X[12]
Treatment Distribution												
Vanicream™ Moisturizing Cream			X[13]					X[14]				
Triamcinolone / Hydrocortisone				X[15]	X[16]		X[16]	X[16]				

1. Participants will be initially classified as topical steroid responders or non-responders based on their EASI score at the 4 Week Steroid Assessment. A topical steroid responder is defined as having an EASI score of ≤7. A topical steroid non-responder is defined as having an EASI score of >7. Topical steroid non-responders will cross over to the topical steroid non-responder schedule of events beginning with the Dupilumab Initiation Visit.
2. Based on the reason for participant withdrawal or early termination, study samples may not be collected during this visit. The criteria for sample collection will be defined in the study MOP.
3. A final phone visit will be completed for participants who withdraw or complete an Early Termination Visit, any time after their 1 Week Assessment (Steroid Initiation). The timing of this visit will be based on the participant's last treatment dose or completed study procedure and respective AE observation period.
4. If medical concerns arise between regularly scheduled visits, participants will be instructed to contact study personnel and may be asked to return to the clinical site for an Unscheduled Visit. If an Unscheduled Visit is required for the purpose of collecting additional samples, only the required samples should be collected. This could include the collection of blood, skin swabs, skin tape strips (STS) and/or skin biopsies. Refer to the study MOP which details procedures that should be conducted based on the type of visit.
5. Last point for crossing over to the topical steroid non-responder treatment group. Participants who experience topical steroid treatment failure after the 12 Week Steroid Assessment will have an Early Termination Visit.
6. A urine pregnancy test will be completed for all individuals who can become pregnant, as defined in the study manual of procedures, who do not self-report as pregnant. At investigator discretion and subject to each individual clinical institution's policies, individuals may be asked to take additional pregnancy tests where not required per protocol at in-person clinic visits.
7. Initial medical history assessments and physical exams will be comprehensive. An abbreviated medical history and a physical exam, abbreviated at investigator discretion, will be conducted at subsequent scheduled visits and may be conducted at any unscheduled visits.
8. Temperature, blood pressure, heart rate, and respiratory rate will be measured. Growth parameters to include height and weight will only be measured at the Baseline Assessment and per PI discretion at an Unscheduled Visit.
9. A CBC with differential may be collected at an Unscheduled Visit per investigator discretion.
10. Blood for genetics may be collected at any time during study participation.
11. 2.5 mm skin biopsies (Les, NL) will be collected for molecular assays for adult participants only.
12. Biopsies should not be collected at an Unscheduled Visit to address an AD flare.

13. Participants will be provided Vanicream™ Moisturizing Cream and will be required to apply it at least twice daily on the skin target area from their Baseline Assessment until their 1 Week Assessment (Steroid Initiation).
14. Participants will be provided Vanicream™ Moisturizing Cream at the 19 Week Steroid Assessment, if needed, and will be required to apply it at least twice daily on the skin target area until their End of Study Assessment.
15. Participants will be given triamcinolone 0.1% ointment (for non-sensitive body regions) and hydrocortisone 2.5% ointment (for sensitive body regions). Participants will apply triamcinolone/hydrocortisone on the skin target area regardless of lesional status, as well as to actively lesional skin body-wide, twice daily between Steroid Initiation and the 4 Week Steroid Assessment.
16. Participants who respond to topical steroid treatment (EASI ≤7) will continue topical steroid treatment by applying triamcinolone/hydrocortisone to lesional skin body-wide once or twice daily, per clinician discretion, until the 19 Week Steroid Assessment. If EASI >7 at or before the 12 Week Steroid Assessment, participant will cross over to the topical steroid non-responder schedule of events and begin dupilumab treatment. If EASI >7 after the 12 Week Steroid Assessment, the participant will complete an Early Termination Visit. Triamcinolone (for non-sensitive body regions) and hydrocortisone (for sensitive body regions) will continue to be distributed to participants as needed.
17. See Section 8.13 for detailed visit windows.

Dupilumab-Naïve AD Participants, Continued for Topical Steroid Non-Responders[1]:

Study Visit	Dupilumab Initiation	Optional Dupilumab Assessment	4 Week Dupilumab Assessment	15 Week Dupilumab Assessment	End of Study Assessment	Early Termination[3]	Early Withdrawal/ Termination Follow-Up[4]	Unscheduled Visit[5]
Target Day[17]	35[2]	40	63	140	168			
Study Procedures/Evaluations								
Adverse Events	X	X	X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X	X
Pregnancy Status	X	X	X	X	X	X	X	X
Pregnancy Test[7]	X							
Abbreviated Medical History	X	X	X	X	X	X		X
Physical Exam	X[8]	X[8]	X[8]	X[8]	X[8]	X[8]		X[8]
Vital Signs[9]	X	X	X	X	X	X		X
Allergic Disease Questionnaires	X		X	X	X	X		X
AD Severity Assessments								
Eczema Area and Severity Index (EASI)	X	X	X	X	X	X		X
Scoring Atopic Dermatitis (SCORAD)	X	X	X	X	X	X		X
Investigator Global Assessment (IGA)	X	X	X	X	X	X		X
Peak Pruritus Numerical Rating Scale (PP-NRS)	X	X	X	X	X	X		X
Skin Target Area Assessment (TAA)	X	X	X	X	X	X		X
Nottingham Eczema Severity Score (NESS)					X	X		X
Blood Sample Collection								
CBC w/ differential	X			X	X	X		X[10]
Serum/Plasma Biomarkers	X		X	X	X	X		X
Skin Sampling Location Photographs	X	X	X	X	X	X		X
Skin Swab Collection								
S. aureus Abundance (Les, NL)	X	X	X	X	X	X		X
Microbiome 16S rRNA analysis (Les, NL)	X	X	X	X	X	X		X
Metagenomics (Les, NL)	X	X	X	X	X	X		X
Microbiome Functional Assessment (Les, NL)	X	X	X	X	X	X		X
Skin Tape Stripping								
Transcriptomics (Les, NL)	X		X	X	X	X		X
Lipidomics (Les, NL)	X		X	X	X	X		X
Proteomics (Les, NL)	X		X	X	X	X		X

Study Visit	Dupilumab Initiation	Optional Dupilumab Assessment	4 Week Dupilumab Assessment	15 Week Dupilumab Assessment	End of Study Assessment	Early Termination[3]	Early Withdrawal/ Termination Follow-Up[4]	Unscheduled Visit[5]
Skin Biopsy								
Required Biopsies (Les, NL)	X[11]			X[11]				X[11,12]
Optional Biopsies (Les, NL)	X[11]			X[11]	X[11]	X[11]		X[11,12]
Treatment Administration/Distribution								
Vanicream™ Moisturizing Cream				X[13]				
Triamcinolone / Hydrocortisone	X[14]		X[14]	X[14]				
Dupilumab	X[15,16]		X[16]					X

1. Only topical steroid non-responders will continue the study through this schedule of events. A topical steroid non-responder is defined as having an EASI score >7.
2. Dupilumab initiation will ideally be completed on the same day as the 4 Week Steroid Assessment, though it may be delayed three days after the 12 Week Steroid Assessment depending on when a topical steroid non-responsive participant experiences their qualifying EASI score. See Section 8.13 for more detail.
3. Based on the reason for participant withdrawal or early termination, study samples may not be collected during this visit. The criteria for sample collection will be defined in the study MOP.
4. A final phone visit will be completed for participants who withdraw or complete an Early Termination Visit, any time after their 1 Week Assessment (Steroid Initiation). The timing of this visit will be based on the participant's last treatment dose or completed study procedure and respective AE observation period.
5. If medical concerns arise between regularly scheduled visits, participants will be instructed to contact study personnel and may be asked to return to the clinical site for an Unscheduled Visit. If an Unscheduled Visit is required for the purpose of collecting additional samples, only the required samples should be collected. This could include the collection of blood, skin swabs, skin tape strips (STS), and/or skin biopsies. Refer to the study MOP which details procedures that should be conducted based on the type of visit.
6. Visit windows for non-responders are determined by the day the Dupilumab Initiation Visit is completed.
7. A urine pregnancy test will be performed for individuals who can become pregnant, as defined in the study manual of procedures, who do not self-report as pregnant prior to dupilumab initiation. At investigator discretion and subject to each individual clinical institution's policies, individuals may be asked to take additional pregnancy tests where not required per protocol at in-person clinic visits.
8. Physical exams scheduled for topical steroid non-responders may be abbreviated at investigator discretion.
9. Temperature, blood pressure, heart rate, and respiratory rate will be measured. Growth parameters including height and weight will be assessed only at the Dupilumab Initiation Visit and per PI discretion at an Unscheduled Visit.
10. A CBC with differential may be collected at an Unscheduled Visit per investigator discretion.
11. 2.5 mm skin biopsies (Les, NL) will be collected for molecular assays for adult participants only.
12. Biopsies should not be collected at an Unscheduled Visit to address an AD flare.
13. Participants will be provided Vanicream™ Moisturizing Cream at their 15 Week Dupilumab Assessment (Day 140-196), if needed, and will be required to apply it at least twice daily on the skin target area until their End of Study Assessment (Day 168-224).
14. Participants will be given triamcinolone 0.1% ointment (for non-sensitive body regions) and hydrocortisone 2.5% (for sensitive body regions) at their 1 Week Assessment (Steroid Initiation) and will be able to use it throughout the study on their non-target skin body regions as needed until the 15 Week Dupilumab Assessment. In the event of a flare in the target area, rescue treatment can be applied per investigator discretion. Triamcinolone (for non-sensitive body regions) and hydrocortisone (for sensitive body regions) will continue to be distributed to participants as needed.

15. When beginning dupilumab treatment, two dupilumab subcutaneous injections (loading dose) will be administered in the abdomen, thighs, or upper arms. The two injections should be administered in different locations (e.g., 2 different abdominal quadrants).
16. Dupilumab will be administered in clinic and additional doses will be distributed for home injections as appropriate according to the participant's age and body weight as outlined in Section 6.1 of the protocol.
17. Target days for topical steroid non-responders may diverge substantially from those indicated in this table, as they are based on when the participant obtains a qualifying EASI score. See Section 8.13 for detailed visit windows.

Long-term Dupilumab Participants:

Study Visit	Recruitment	Screening	Baseline Assessment	1 Week Assessment	9 Week Assessment	20 Week Assessment	End of Study Assessment	Early Termination[1]	Early Withdrawal/ Termination Follow-Up[2]	Unscheduled Visit[3]
Target Day[13]		-7	0	7	63	140	168			
Study Procedures/Evaluations										
Recruitment	X									
Informed Consent		X								
Demographics		X								
Adverse Events		X	X	X	X	X	X	X	X	X
Concomitant Medications		X	X	X	X	X	X	X	X	X
Pregnancy Status		X	X	X	X	X	X	X	X	X
Pregnancy Test[4]			X	X						
Medical History		X	X[5]	X[5]	X[5]	X[5]	X[5]	X[5]		X[5]
Physical Exam			X	X[5]	X[5]	X[5]	X[5]	X[5]		X[5]
Vital Signs/Growth Parameters[6]			X	X	X	X	X	X		X
Allergic Disease Questionnaires			X	X	X	X	X	X		X
AD Severity Assessments										
Eczema Area and Severity Index (EASI)			X	X	X	X	X	X		X
Scoring Atopic Dermatitis (SCORAD)			X	X	X	X	X	X		X
Investigator Global Assessment (IGA)			X	X	X	X	X	X		X
Peak Pruritus Numerical Rating Scale (PP-NRS)			X	X	X	X	X	X		X
Skin Target Area Assessment (TAA)			X	X	X	X	X	X		X
Nottingham Eczema Severity Score (NESS)			X				X	X		X
Blood Sample Collection										
CBC w/differential				X		X	X	X		X[7]
Genetics[8]				X						X
Serum/Plasma Biomarkers				X		X	X	X		X
Skin Sampling Location Photographs			X	X	X	X	X	X		X
Skin Swab Collection										
S. aureus Abundance (Les, NL)			X	X	X	X	X	X		X
Microbiome 16S rRNA analysis (Les, NL)			X	X	X	X	X	X		X
Metagenomics (Les, NL)			X	X	X	X	X	X		X
Microbiome Functional Assessment (Les, NL)			X	X	X	X	X	X		X

Study Visit	Recruitment	Screening	Baseline Assessment	1 Week Assessment	9 Week Assessment	20 Week Assessment	End of Study Assessment	Early Termination[1]	Early Withdrawal/ Termination Follow-Up[2]	Unscheduled Visit[3]
Skin Tape Stripping										
Transcriptomics (Les, NL)			X	X	X	X	X	X		X
Lipidomics (Les, NL)			X	X	X	X	X	X		X
Proteomics (Les, NL)			X	X	X	X	X	X		X
Skin Biopsy[9]										
Optional Biopsies (Les, NL)				X		X	X	X		X[10]
Treatment Administration/Distribution										
Vanicream™ Moisturizing Cream			X[11]			X[12]				

1. Based on the reason for participant withdrawal or early termination, study samples may not be collected during this visit. The criteria for sample collection will be defined in the study MOP.
2. A final phone visit will be completed for participants who withdraw or complete an Early Termination Visit, any time after the 1 Week Assessment. The timing of this visit will be based on the participant's last treatment dose or completed study procedure and respective AE observation period.
3. Participants will be instructed to contact study personnel if they experience any symptoms of concern at the tape stripping or skin biopsy sites and may be asked to return to the clinical site for an Unscheduled Visit. If an Unscheduled Visit is required for the purpose of collecting additional samples, only the required samples should be collected. This could include the collection of blood, skin swabs, skin tape strips (STS), and/or skin biopsies. Refer to the study MOP which details procedures that should be conducted based on the type of visit.
4. A urine pregnancy test will be completed for all individuals who can become pregnant, as defined in the study manual of procedures, who do not self-report as pregnant. At investigator discretion and subject to each individual clinical institution's policies, individuals may be asked to take additional pregnancy tests where not required per protocol at in-person clinic visits.
5. Initial medical history assessments and physical exams will be comprehensive. An abbreviated medical history and a physical exam, abbreviated at investigator discretion, will be conducted at subsequent scheduled visits and may be conducted at any unscheduled visits.
6. Temperature, blood pressure, heart rate, and respiratory rate will be measured. Growth parameters to include height and weight will only be measured at the Baseline Assessment and per PI discretion at an Unscheduled Visit.
7. A CBC with differential may be collected at an Unscheduled Visit per investigator discretion.
8. Blood for genetics may be collected at any time during study participation.
9. 2.5 mm skin biopsies (Les, NL), as applicable, will be collected for molecular assays for adult participants only.
10. Biopsies should not be collected at an Unscheduled Visit to address an AD flare.
11. Participants will be provided Vanicream™ Moisturizing Cream and will be required to apply it at least twice daily on the skin target area from their Baseline Assessment until their 1 Week Assessment.
12. Participants will be provided Vanicream™ Moisturizing Cream at the 20 Week Assessment, if needed, and will be required to apply it at least twice daily on the skin target area until their End of Study Assessment.
13. See Section 8.13 for detailed visit windows.

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

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

I. Atopic Dermatitis (AD)[1]

A. Active Atopic Dermatitis (AD)[1]

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

B. Inactive Atopic Dermatitis (AD)[1]

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

C. Definition

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

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

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

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

References

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