Immunogenetic Profiling of Dupilumab for the Treatment of Atopic Dermatitis

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Clinical Research Protocol

Immunogenetic profiling of dupilumab for the treatment of atopic dermatitis

Version Date:	November 8, 2020
Investigational Product:	Dupilumab
Sponsor:	Regeneron Pharmaceuticals and Sanofi-Genzyme
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Funding Organization:	Regeneron Pharmaceuticals and Sanofi-Genzyme

Approval:

PI or Sponsor Signature (Name and Title) *Date*

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PROTOCOL AGREEMENT

I have read the protocol specified below. In my formal capacity as Investigator, my duties include ensuring the safety of the study subjects enrolled under my supervision and providing Regeneron and Sanofi-Genzyme with complete and timely information, as outlined in the protocol. It is understood that all information pertaining to the study will be held strictly confidential and that this confidentiality requirement applies to all study staff at this site. Furthermore, on behalf of the study staff and myself, I agree to maintain the procedures required to carry out the study in accordance with accepted GCP principles and to abide by the terms of this protocol.

Protocol Title: Immunogenetic profiling of dupilumab for the treatment of atopic dermatitis

Protocol Date: November 8, 2020

Investigator Signature	*Date*

Print Name and Title

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LIST OF ABBREVIATIONS

AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BUN	blood urea nitrogen
CBC	Complete blood count
CFR	Code of Federal Regulations
CyTOF	Cytometry by Time of Flight
DMC	Data Monitoring Committee
DSMB	Data Safety Monitoring Board
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GGT	gamma-glutamyl transferase
HIPAA	Health Insurance Portability and Accountability Act of 1996
ICF	informed consent form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IgG4	Immunoglobulin-G4
IL-4	Interleukin-4
IL-13	Interleukin-13
IRB	Institutional Review Board
IUD	Intrauterine device
EASI	Eczema Area and Severity Index
IGA	Investigator Global Assessment
PI	Principal Investigator
PK	Pharmacokinetic
QOL	Quality of life
RNA-seq	RNA-sequencing
RT-PCR	Reverse transcriptase polymerase chain reaction
SAE	serious adverse experience
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamate pyruvate transaminase
SC	subcutaneously

PROTOCOL SYNOPSIS

TITLE	Immunogenetic profiling of dupilumab for the treatment of atopic dermatitis
SPONSOR	Regeneron Pharmaceuticals and Sanofi-Genzyme
FUNDING	Regeneron Pharmaceuticals and Sanofi-Genzyme
STUDY DESIGN & OVERVIEW	This is a single-arm, open-label study, which will examine the effect of dupilumab on the immunologic and genetic environment within atopic dermatitis lesions.
PRIMARY OBJECTIVE	Perform quantitative analysis of the immunologic and genetic changes in immune cell populations in lesional and non-lesional skin after dupilumab treatment (15 AD patients) at weeks 2, 4, 12 compared to baseline week 0. The immunologic and genetic profiles in AD patients will also be compared to healthy control skin surgical discard specimens (n=15). The primary endpoint is the percent change from pre-treatment baseline of CD4+ T effector cells expressing IL-4 at weeks 2, 4, 12.
SECONDARY OBJECTIVES	Use RNA-seq to identify differentially expressed genes and pathways in each cell population at weeks 2, 4, 12 compared pre-treatment baseline in dupilumab-treated patients. We will explore whether early molecular changes are predictive of the clinical response to dupilumab at 52 weeks (reduction of EASI and improvement in pruritus score), which could lead to the identification of novel biomarkers. Microbiome samples from skin and stool at weeks 0, 2, 4, 12, and 52 will be banked for future analysis. Allergic contact dermatitis patch testing will be performed prior to and during treatment with dupilumab in order to gain a better understanding of how dupilumab affects contact allergy.
NUMBER OF SUBJECTS	15

SUBJECT SELECTION CRITERIA	Inclusion Criteria: For subjects with atopic dermatitis: 1. Ability to provide written informed consent and comply with the protocol. 2. At least 18 years of age. 3. Diagnosis of chronic atopic dermatitis for at least 3 years prior to enrollment. 4. Subject is considered a candidate for phototherapy or systemic therapy 5. Eczema Area and Severity Index (EASI) score ≥ 12. 6. Investigator Global Assessment (IGA) ≥ 3. 7. 10% body surface area (BSA) or greater. 8. Subject is unlikely to conceive due to male, post-menopausal, or using adequate contraceptive (barrier, hormonal, implant, or permanent sterilization methods). 9. Physical exam within clinically acceptable limits. For healthy control subjects: 1. Sample of non-inflamed skin. 2. No history of autoimmune disease. 3. At least 18 years of age. Exclusion Criteria: 1. Subject is unable to provide written informed consent or comply with the protocol. 2. Subject is younger than 18 years of age. 3. Subject has had atopic dermatitis for less than 3 years prior to enrollment. 4. Subject with mild atopic dermatitis (EASI<12 and IGA<3) or is not a candidate for phototherapy or systemic treatments. 5. Subject with current, or a history of, severe atopic dermatitis well controlled on current therapy. 6. Serious known infection. 7. History of immunosuppression (including human immunodeficiency virus (HIV)). 8. History of malignancy within 5 years before the screening visit, except completely treated in situ carcinoma of the cervix, completely treated and resolved non-metastatic squamous or basal cell carcinoma of the skin. 9. Severe concomitant illnesses. 10. Having used immunosuppressive/immunomodulating drugs (eg, systemic corticosteroids, cyclosporine, mycophenolate-mofetil, IFN-γ, Janus kinase inhibitors, azathioprine, methotrexate, etc.) or phototherapy within 4 weeks before the baseline visit. 11. Treatment with topical corticosteroid or topical calcineurin inhibitor within 1 week before the baseline visit.

	12. Treatment with oral antibiotics within 2 weeks of the baseline visit. 13. Treatment with any cell-depleting agents including but not limited to rituximab: within 6 months before the baseline visit, or until lymphocyte count returns to normal, whichever is longer, or use of other biologics: within 5 half-lives (if known) or 16 weeks prior to baseline visit, whichever is longer. 14. Physical or laboratory exam not within clinically acceptable limits. 15. Subjects possess other diagnoses that, in the investigator's opinion, preclude him/her from safely participating in this study or interfere with the evaluation of the subject's atopic dermatitis. 16. At baseline visit, the subject does not have sufficient clear skin for which to perform patch testing as determined by the investigator's opinion, which is also defined as approximately 50-75% of skin on the back clear of atopic dermatitis. 17. History of known or suspected intolerance to any of the ingredients of the investigational study product. 18. Pregnant or nursing (lactating) women, where pregnancy is defined as the state of a female after conception and until the termination of gestation, confirmed by a positive hCG laboratory test (>10 mIU/mL). 19. Women of child-bearing potential, defined as all women physiologically capable of becoming pregnant, unwilling to use effective contraception during the study and for 6 weeks after stopping treatment. For healthy control subjects: 1. No areas of non-inflamed skin. 2. History of autoimmune disease. 3. Subject is younger than 18 years of age.
STUDY TREATMENT	Dupilumab (Dupixent ®) 600 mg SC at week 0, then 300 mg SC every other week.
STUDY DURATION	The total duration of the study is expected to be 104 weeks: 52 weeks for recruitment and 52 weeks for site visits.
PRIMARY ENDPOINT	Percentage change from pre-treatment baseline of CD4+ T effector cells in lesional and non-lesional skin expressing IL4 at weeks 2, 4, 12 in dupilumab-treated patients.
SECNDARY ENDPOINT	Differentially expressed genes and pathways in each cell population at weeks 2, 4, 12 compared to pre-treatment baseline using RNA-seq.
SAFETY EVALUATIONS	Safety and tolerability will be assessed by adverse events, vital signs, physical examinations (including skin examinations and injection-site evaluations), and concomitant medication review. Laboratory assessments

	will be performed at screening.
STATISTICS **Primary Analysis Plan**	CyTOF: One sample paired t test comparing mean change in lesional and non-lesional IL-4 positive, CD4+ T effector cells compared to baseline. RNA-seq: Identification of differentially expressed genes compared to baseline within each immune population studied using the statistical program Cuffdiff (paired analysis).
ETHICAL CONSIDER-ATIONS	This study will be conducted in accordance with applicable laws and regulations and according to the recommendations of International Conference on Harmonization Good Clinical Practice (ICH GCP) guidelines and those of the Declaration of Helsinki (Edinburgh, 2000); only after approval for the study has been obtained from the relevant regulatory authority and relevant independent ethics committee (IEC). The institutional review board (IRB)/IEC must review and approve the protocol and informed consent form (ICF) before any subjects are enrolled. The subject must be consented using the approved ICF before any procedures specified in the protocol are performed.

1 BACKGROUND

Atopic dermatitis is a chronic, relapsing inflammatory skin disease characterized by intense pruritus and skin xerosis. Dupilumab is the first FDA-approved therapy for adult patients with moderate-to-severe atopic dermatitis in the class of recombinant high-affinity, fully human immunoglobulin-G4 (IgG4) monoclonal antibody that selectively targets the receptor alpha subunit of Interleukin-4 (IL-4). By binding to the IL-4 receptor alpha subunit, dupilumab modulates both the IL4 and Interleukin-13 (IL-13) pathways, which type 2/Th2 cytokines implicated in numerous allergic diseases. Through this blockade, dupilumab inhibits the inflammatory response that plays a role in the development of atopic dermatitis.

Non-clinical studies have not shown any impediment to using dupilumab administered subcutaneously in man.

The approval of dupilumab (Dupixent ®) was based on the safety and efficacy outcomes from 3 Phase III studies known as SOLO 1, SOLO 2, and CHRONOS. These trials included over 2,119 patients with moderate-to-severe atopic dermatitis not adequately controlled by topical medications.

For more detail refer to the Prescribing Information.

2 STUDY RATIONALE

The University of California San Francisco (UCSF) Department of Dermatology has expertise in immunologic/genetic analyses of inflammatory skin conditions such as atopic dermatitis (AD) and psoriasis. This unique proposal will examine the effect of dupilumab on the immunologic and genetic environment within atopic dermatitis lesional and non-lesional skin. While dupilumab is a targeted biologic that is expected to have a rapid and sustained impact on the Th2 axis, the full scope of dupilumab's molecular mechanism of action on multiple cell types is not yet known.

This proposal represents a translational collaborative effort between a nationally recognized clinical center specializing in atopic dermatitis and strong research enterprise. The UCSF Psoriasis and Skin Treatment Center, directed by the principal investigator Dr. Liao, is a high-volume atopic dermatitis referral center whose activities include outpatient care, a phototherapy unit, a Goeckerman tar treatment program, and over 20 clinical trials conducted over the past three years. Staff for the center includes 6 attending physicians, 8 nurses, 3 administrators, and 2 full time research fellows. Over 500 atopic dermatitis patients are seen each year. The infrastructure at the Center will facilitate ready recruitment of subjects and will ensure accurate, detailed AD phenotypes are ascertained for this study. Dr. Michael Rosenblum is a board-certified dermatologist and basic scientist with expertise in cutaneous immunology. His lab has identified a new population of regulatory cells in the skin, termed memory Tregs, which play an important role in chronic inflammatory skin diseases such as psoriasis. Dr. Rosenblum has helped pioneer a novel technology which allows for immunologic profiling of freshly isolated human skin leukocytes as detailed in this application. Dr. Wilson Liao is a board-certified dermatologist and geneticist who has contributed to the discovery of over 20 psoriasis genes. Dr. Liao's group has expertise in the analysis of genetic and genomic datasets and

has collaborated extensively with Dr. Rosenblum.

2.1 Impact of Targeted Biologics on Atopic Dermatitis Skin

Regarding understanding the impact of biologics on the molecular profile of AD skin, a fundamental question that has not yet been adequately answered is: What is the response of specific cell populations in lesional skin to biologic treatment?

A number of recent studies have used techniques such as RT-PCR, gene microarrays, and immunohistochemistry to evaluate the immunologic environment of AD lesions and its changes with treatments such as ultraviolet light, cyclosporine, and dupilumab (Ungar, Tintle, Rozenblit, Hamilton). **However, these studies are limited by the use of bulk skin tissue, which does not permit precise quantitative analysis of individual cell types, and by the reliance on limited gene probe libraries used in RT-PCR or microarray analysis.** A more recent study of AD used the more sensitive technique of RNA-sequencing (Suárez-Fariñas), but also used bulk tissue. Ideally, in order to understand the fundamental biology of how a biologic medication improves AD, one would like to be able to isolate the different cell populations and study their individual characteristics.

At UCSF, we have developed innovative technologies to characterize **individual skin cell populations** at the protein and RNA level. Using an optimized flow cytometry protocol, we are able to separately analyze distinct cell populations in human skin **(Figure 1).** Distinct skin populations such as CD4+ T effector cells, CD8+ T cells, T regulatory cells, and innate lymphoid type 2 cells can be separated and the production of proteins such as IL-4, IL-13, IL-17, IFN-γ, and IL-22 can be measured in parallel. Because this technique is based on protein expression, it offers a truly functional view of the activity of cell populations in AD and healthy skin. We have used this technique to publish on the analysis of psoriasis and hidradenitis suppurativa skin in high impact journals such as the ***Journal of the American Academy of Dermatology***, ***JAMA Dermatology***, **and** ***Journal of Clinical Investigation*** (Cordoro et al; Debbaneh et al; Leslie et al; Sanchez Rodriguez et al).

Moreover, we have recently achieved a **major breakthrough** in upgrading this technology to the **Cytometry by Time-Of-Flight (CyTOF)**, which allows for the simultaneous measurement of 40+ molecular markers as compared to 15-16 markers with flow cytometry (**Figures 2A and 2B**). CyTOF is a fusion of flow cytometry and mass spectrometry whereby cells of interest are stained with antibodies conjugated to stable heavy metal isotopes. Cells are then passed in a single-cell suspension into a nebulizer, which places cells into droplets for ionization by argon plasma, which allows the heavy metals to be detected by their mass-to-charge ratio in a time-of-flight mass spectrometer. The measurement of over 40 simultaneous cellular parameters at single-cell resolution significantly augments our ability of cytometry to evaluate complex cellular systems and processes.

To our knowledge, we are the first group to adapt CyTOF to human skin samples and biopsies. Tissue is finely minced, resuspended in a cocktail of enzymes (collagenase and DNase) and incubated overnight at 37˚C to yield a single-cell suspension. A viability

stain, cisplatin, is added to mark dead cells for exclusion from the downstream analysis. Cells are then fixed in 1.5% paraformaldehyde to preserve the cell state and to enable banking of samples. Samples are then stained with a cocktail of antibodies targeting extracellular molecules of interest, permeabilized, and stained again with antibodies, this time targeting intracellular molecules. Finally, samples are incubated with a DNA intercalator containing iridium ions in fixative before analysis on the instrument. Data generation on the mass cytometer requires approximately 1 hr per 10^6 cells. Upon data collection, data are normalized and de-barcoded into their respective conditions using methods we have previously published and open-source software in MATLAB. After data pre-processing, we utilize an analytical pipeline we have previously developed to create maps of the immune cells from each sample called Scaffold maps (**Figure 2B**). Using this approach, groups of similar cells are identified in the data using an unsupervised clustering algorithm. The relationship between these cell subsets is visualized as a force-directed graph, in which related cell types form an edge between them and appear adjacent to one another in the display. These graphs also incorporate classical immune cell populations identified manually in the data as reference points to help orient the researcher. The result is a robust and thorough evaluation of all immune cells present in different samples, which has resulted in powerful mechanistic insights in a number of contexts.

In addition, we are able to perform RNA-sequencing on distinct cell populations. RNA-seq is a novel technique of quantifying gene expression in which RNA species are sequenced rather than hybridized as in microarrays. **RNA-seq provides greater sensitivity than microarrays, particularly for low-expressed transcripts (Li et al)**.

At UCSF, the Liao Lab has developed expertise in the analysis of RNA-seq data. First, our group has shown that RNA-seq can also identify the expression of noncoding transcripts in inflammatory skin disease that are absent on microarray platforms (Gupta et al). Our group is one of the few that has published on non-coding RNA in human skin. These non-coding transcripts are important for the regulation of coding transcripts and may play an important role in the pathogenesis of inflammatory skin disease.

Second, in addition to performing standard differential gene analysis (e.g. between lesional and healthy skin), we apply state-of-the-art gene network analysis, which can identify modules of significantly correlated genes and define key driver genes. At UCSF we conducted whole transcriptome RNA-seq analysis on 18 psoriasis patients (pre-treatment and post-treatment with the TNF-α inhibitor adalimumab) and 16 healthy controls, generating an average of 52.3 million 100-bp paired-end reads per sample. Using Weighted Gene Co-expression Network Analysis (WGCNA) (Horvath et al), we identified 3 network modules that were significantly associated with psoriasis and 6 network modules significantly associated with biologic treatment, with only 16% of the psoriasis-associated and 5% of the treatment-associated co-expressed genes being identified by differential expression analysis. Dissection of the network modules identified short-chain fatty acid metabolism as a pathway highly associated with psoriasis, while regulation of leukocyte mediated cytotoxicity was highly associated with biologic treatment (Ahn et al, 2016).

Third, our group has expertise in the analysis of **RNA-seq data derived from specific cell populations in human skin** (Ahn et al, 2017). **A limitation of all previously published RNA-seq studies is that they have relied on bulk skin biopsies,** which contain many cell types, thus **making it difficult to discern the contributions of individual cell populations.** We performed RNA-seq on **keratinocytes, dendritic cell, CD4+ T effector cells, and CD8+ T effector cells** (**Figure 3**) from human skin and demonstrated that RNA-seq of bulk skin primarily captures gene expression from keratinocytes, but is less sensitive in detecting gene expression from immune cell populations such as T cells. Thus, previous gene expression studies of bulk skin (e.g. following dupilumab treatment) are skewed towards studying keratinocyte biology, while potentially missing out on important biology occurring within T cells. We are able to identify "transcriptional signatures" within each cell type, rather than in bulk skin. Importantly, the cell type-level RNA-seq analysis proposed here has never previously been utilized to evaluate patients with AD undergoing biologic therapy.

CyTOF panel for AD:

CD3, CD19, CD117, CD11b, CD4, CD8a, CD11c, CD14, IL-4, TNFa, CD123, gdTCR, GATA3, IFNg, IL-13, IL-10, IL-4Ra, CD27, IL-33, Tbet, CD152 (CTLA-4), Foxp3, CD33, CD45RO, CD127, IL-25, IL-17A, Ki-67, CD25, RORgt, TSLP, CD38, IL-2, HLA-DR, PD-1, CD56, CD16

RNA-seq cell types for AD

CD4+ T effectors, CD4+ T Regs, CD8+ T effectors

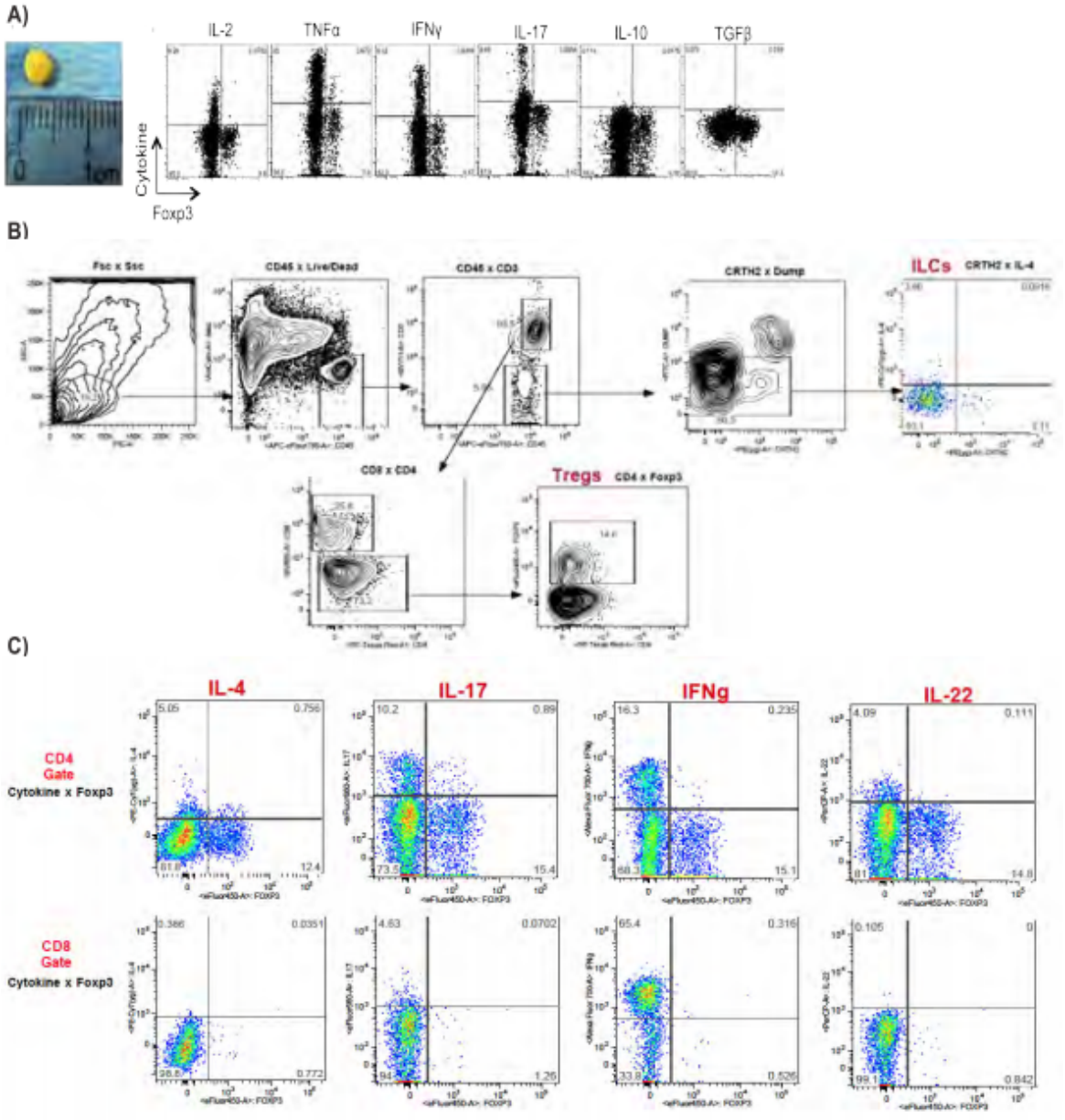

Figure 1. Multi-parameter flow cytometric analysis of human skin and atopic dermatitis markers. (A) Example of functional immunoflow cytometry on 4 mm punch biopsy specimen of normal human skin. Tissue was minced and digested for 12 hrs in a digestion cocktail containing collagenase. Cells were washed, stimulated with PMA/ionomycin, and stained for 13-color flow cytometry to detect cell surface proteins and intracellular cytokine production. In contrast to other methods, our protocol does not rely on cell culture or addition of exogenous growth factors such as IL-2, which can substantially alter the phenotype of cells. Foxp3 is a transcription factor that drives the differentiation of regulatory T cells. (B) Gating strategy for atopic dermatitis flow panel. Live CD45+ leukocytes are further subsetted as CD4+ T effector cells, CD4+ T regulatory cells, and CD8+ T effector cells, or innate lymphoid cells type 2 (ILC2). (C). This technique is extremely sensitive, as a biopsy specimen from normal skin (having no appreciable inflammatory infiltrate by routine histology) shows a defined CD4+ and CD8+ T cell population capable of producing specific effector cytokines such as IL-4, IL-17, IFN-gamma, and IL-22.

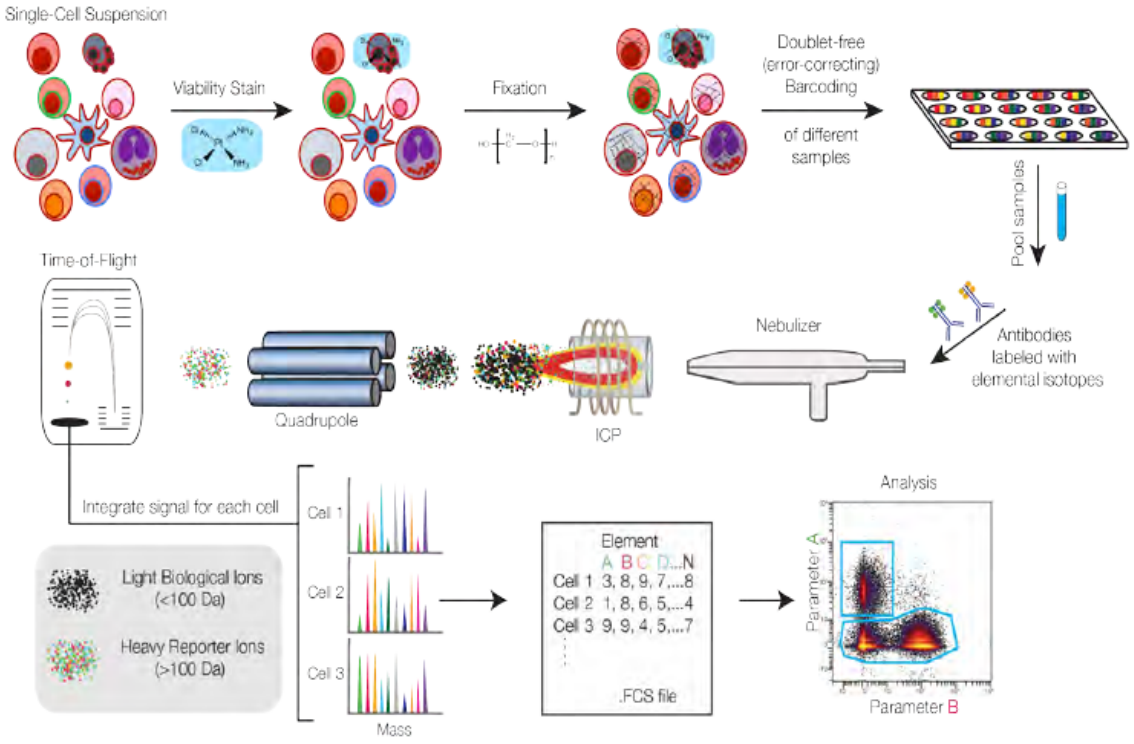

Figure 2A. Workflow of a cytometry experiment. Cells are placed into a single cell suspension, and a viability stain is added to exclude dead cells from the analysis. Cells are then fixed to preserve the endogenous cell state. Barcodes are applied to different samples to enable pooling, which minimizes technical variability between samples. Pooled samples are then stained with a panel of antibodies targeting molecules of interest as well as a DNA intercalator. Samples are then introduced into the mass cytometer by a nebulizer, which creates fine droplets containing cells. Cells travel though an inductively coupled argon plasma heated to 7500K, which breaks covalent bonds to form an ion cloud. Low molecular weight ions are filtered out, enriching for heavy metal reporter ions that were bound to antibodies. Ions are quantified using a time-of-flight mass spectrometer and signal is integrated for each cell event. Data are stored as a conventional "flow cytometry standard" file, which can be separated into samples based on the unique barcodes and then analyzed. From Spitzer et al., *Cell*, 2016.

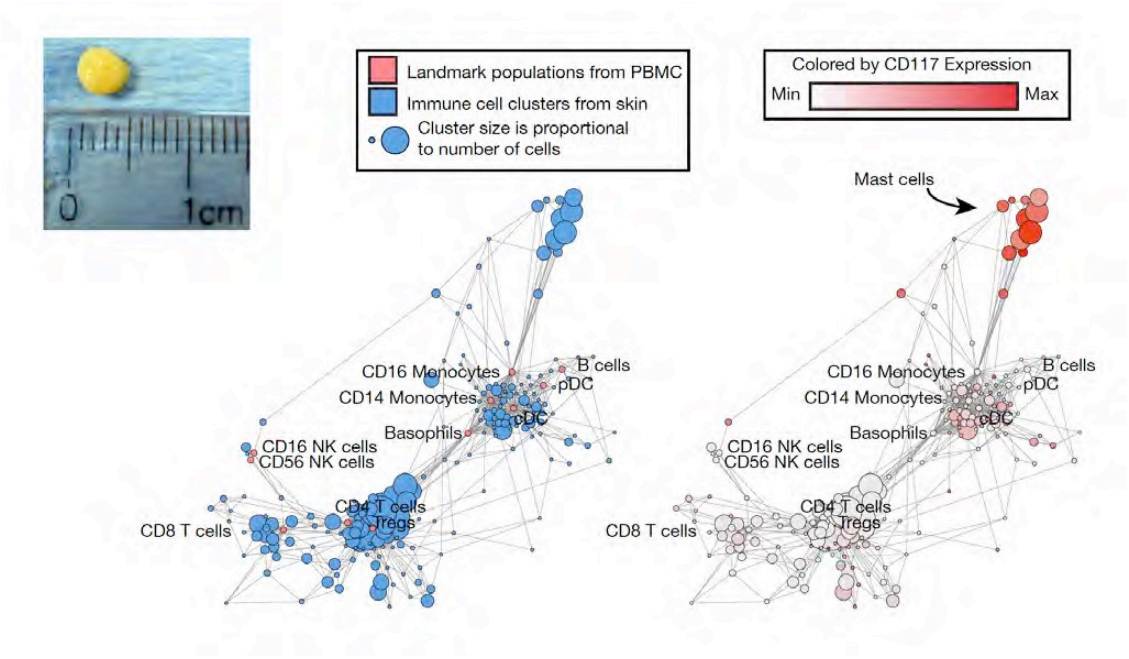

Figure 2B. Immune cells in human skin analyzed by mass cytometry (CyTOF). We have recently pioneered and optimized comprehensive analysis of immune cell subsets from 4mm skin punch biopsy specimens (inset) using CyTOF. Cell subsets in skin are visualized as a Scaffold map (Spitzer et al., Science, 2015). **Left:** Red nodes in the graph represent manually identified landmark immune cell types from human peripheral blood (PBMC). Blue nodes represent unsupervised clusters of immune cells identified in healthy human skin. **Right:** Scaffold map colored by CD117, highlighting a connected group of clusters exhibiting high expression. These cells also express FceR1 (data not shown) and represent skin-resident mast cells that are distinct from any cell type found in peripheral blood.

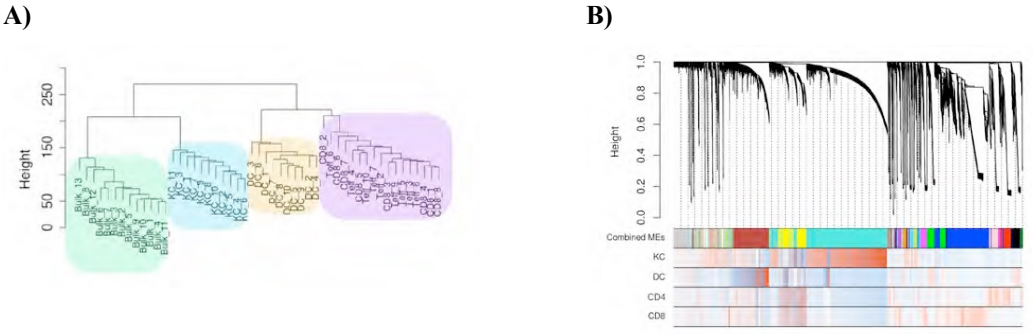

Figure 3. RNA-sequencing of distinct cell populations within human skin (Ahn et al, 2017). (A) As opposed to examining the transcriptome of bulk skin, we have pioneered the capability to perform transcriptome profiling in specific cell populations. (A) A comparison of bulk vs cell population-specific RNA-seq was performed. Unsupervised hierarchical clustering reveals that the bulk skin samples (green) are most similar to keratinocytes (blue), whereas dendritic cells (beige) and T cells (purple) cluster distinctly. This suggests that prior gene expression studies of bulk skin to not adequately capture immune cell profiles. (B) Gene network analysis of cell population-specific RNA-seq data. Each vertical line represents a different gene. We are able to identify gene modules that are "signatures" of each cell population (black boxes).

2.2 Risk / Benefit Assessment

Details of the risk and benefits of dupilumab are outlined in the Prescribing Information. The most common adverse effects (incidence ≥ 1%) of dupilumab include injection site reactions, conjunctivitis, blepharitis, oral herpes, keratitis, eye pruritus, other herpes simplex infection, and dry eye. Other adverse effects include hypersensitivity reactions, including generalized urticarial and serum sickness or serum sickness-like reactions.

The risk to subjects in this trial will be minimized by compliance with the inclusion/exclusion criteria, proper study design, and close clinical monitoring.

In regards to skin biopsy procedures, as in any surgical procedure, there are certain inherent risks including bleeding, post-operative pain, infection, reactions to sutures, anesthetics or topical antibiotics, and scarring. All reasonable efforts will be made to minimize the possibility of these potential complications.

In regard to patch testing, in rare cases there may be blistering reactions, in highly allergic patients. It is possible in these instances for scarring to occur at the site of the offending allergen. Sometimes, particularly in patients with photodamaged skin temporary bruising can occur with removal of the tape from the patches. Patch testing, in rare circumstances, can lead to excited skin syndrome (typically in patients who have significant active dermatitis at the time of testing). To mitigate this, patients deemed to have no clear areas for testing due to significant active dermatitis will not be patch tested. Patients who are pregnant will also be excluded, as patch testing has not been studied in this population. We believe there is only minimal risk to those participating in any and all parts of this study. We also believe that the minimal risk is reasonable for the benefits gained by a better understanding of how dupilumab affects contact allergy. Additionally, patients will receive patch testing and allergen avoidance which is of immense benefit to improving their skin disease and quality of life going forward.

The benefit to subjects is that they will be receiving an approved therapy for moderate-to-severe atopic dermatitis. Although it cannot be guaranteed, dupilumab has shown to be safe and effective in moderate-to-severe atopic dermatitis. An additional benefit to patients is that they are contributing to society by helping the enhancement of science.

3 STUDY OBJECTIVES

3.1 Primary Objective

Perform quantitative analysis of the immunologic and genetic changes in immune cell populations in lesional and non-lesional skin after dupilumab treatment (15 AD patients) at weeks 2, 4, 12 compared to baseline week 0. The immunologic and genetic profiles in AD patients will also be compared to healthy control skin surgical discard specimens (n=15). The primary endpoint is the percent change from pre-treatment baseline of CD4+ T effector cells expressing IL-4 at weeks 2, 4, 12 in dupilumab-treated patients.

3.2 Secondary Objectives

Use RNA-seq to identify differentially expressed genes and pathways in each cell population at weeks 2, 4, 12 compared to pre-treatment baseline in dupilumab-treated patients. We will explore whether early molecular changes are predictive of the clinical response to dupilumab (reduction of EASI/IGA and improvement in pruritus score), which could lead to the identification of novel biomarkers. Microbiome samples from skin and stool at weeks 0, 2, 4, 12, and 52 will be banked for future analysis. Allergic contact dermatitis patch testing will be performed prior to and after 6 months of treatment with dupilumab to gain a better understanding of how dupilumab affects contact allergy.

4 STUDY DESIGN

4.1 Study Overview

This is a single center, open-label study. Fifteen subjects with moderate to severe AD will receive dupilumab for a treatment period of 52 weeks (i.e. last injection on week 48). Surgical discard specimens will also be collected from fifteen healthy subjects. Biopsy samples from AD subjects and surgical discard samples will undergo molecular profiling. Skin swabs and stool samples will be collected and banked for future analysis. **The reason to treat patients for 52 weeks is to have the ability to correlate early molecular events with clinical outcomes (EASI, IGA, itch score) at week 52**. **In addition, collection of microbiome samples will be performed at week 52.** See Flowchart for timeline of study activities.

5 CRITERIA FOR EVALUATION

5.1 Primary Analysis Endpoint

Percentage change from pre-treatment baseline of CD4+ T effector cells expressing IL-4 at weeks 2, 4, 12 in dupilumab-treated patients.

5.2 Secondary Analysis Endpoint

Number of differentially expressed genes and pathways in each cell population at weeks 2, 4, 12 compared to pre-treatment baseline using RNA-seq.

We will also explore whether early molecular changes are predictive of the clinical response to dupilumab at 52 weeks (reduction of EASI and improvement in pruritus score), which could lead to the identification of novel biomarkers.

5.3 Safety Evaluations

Safety and tolerability to dupilumab will be assessed by adverse events, vital signs, physical examinations (including skin examinations and injection-site evaluations), and concomitant medication review. Laboratory assessments will be performed at screening.

6 SUBJECT SELECTION

6.1 Study Population

Subjects with a diagnosis of moderate to severe atopic dermatitis for at least 3 years prior to enrollment who meet the inclusion and exclusion criteria will be eligible for participation in this study.

6.2 Inclusion Criteria

Inclusion criteria for AD subjects:

1. Ability to provide written informed consent and comply with the protocol.
2. At least 18 years of age.
3. Diagnosis of chronic atopic dermatitis for at least 3 years prior to enrollment.
4. Subject is considered a candidate for phototherapy or systemic therapy
5. Eczema Area and Severity Index (EASI) score ≥ 12
6. Investigator Global Assessment (IGA) ≥ 3
7. 10% body surface area (BSA) or greater
8. Subject is unlikely to conceive due to male, post-menopausal, or using adequate contraceptive (barrier, hormonal, implant, or permanent sterilization methods).
9. Physical exam within clinically acceptable limits.

Inclusion criteria for healthy controls obtained by surgeons (surgical specimens):

4. Sample of non-inflamed skin.
5. No history of autoimmune disease.
6. At least 18 years of age.

6.3 Exclusion Criteria

Exclusion criteria for AD subjects:

1. Subject is unable to provide written informed consent or comply with the protocol.
2. Subject is younger than 18 years of age.
3. Subject with mild atopic dermatitis (EASI<12 and IGA<3) or is not a candidate for phototherapy or systemic treatments.
4. Subject with current, or a history of, severe atopic dermatitis well controlled on current therapy.
5. Serious known infection.
6. History of immunosuppression (including human immunodeficiency virus (HIV)).
7. History of malignancy within 5 years before the screening visit, except completely treated in situ carcinoma of the cervix, completely treated and resolved non-metastatic squamous or basal cell carcinoma of the skin.
8. Severe concomitant illnesses.
9. Having used immunosuppressive/immunomodulating drugs (e.g., systemic corticosteroids, cyclosporine, mycophenolate-mofetil, IFN-γ, Janus kinase inhibitors, azathioprine, methotrexate, etc.) or phototherapy within 4 weeks before the baseline visit.
10. Treatment with topical corticosteroid or topical calcineurin inhibitor within 1 week

before the baseline visit.

11. Treatment with any cell-depleting agents including but not limited to rituximab: within 6 months before the baseline visit, or until lymphocyte count returns to normal, whichever is longer, or use of other biologics: within 5 half-lives (if known) or 16 weeks prior to baseline visit, whichever is longer.
12. Treatment with oral antibiotics within 2 weeks of the baseline visit.
13. Physical exam not within clinically acceptable limits.
14. Subjects possess other diagnoses that, in the investigator's opinion, preclude him/her from safely participating in this study or interfere with the evaluation of the subject's atopic dermatitis.
15. Subjects possess other diagnoses that, in the investigator's opinion, preclude him/her from safely participating in this study or interfere with the evaluation of the subject's atopic dermatitis.
16. History of known or suspected intolerance to any of the ingredients of the investigational study product.
17. Pregnant or nursing (lactating) women, where pregnancy is defined as the state of a female after conception and until the termination of gestation, confirmed by a positive hCG laboratory test (>10 mIU/mL).
18. Women of child-bearing potential, defined as all women physiologically capable of becoming pregnant, unwilling to use effective contraception during the study and for 6 weeks after stopping treatment. Effective contraception is defined as either:

    - Barrier method: Condom or occlusive cap (diaphragm or cervical/vault caps) with spermicide (where available). Spermicides alone are not a barrier method of contraception and should not be used alone.

The following methods are considered more effective than the barrier method and are also acceptable:

- Total abstinence: When this is in line with the preferred and usual lifestyle of the subject (Periodic abstinence [e.g. calendar, ovulation, symptothermal, post-ovulation methods] and withdrawal are not acceptable methods of contraception)
- Female sterilization: have had a surgical bilateral oophorectomy (with or without hysterectomy) or tubal ligation at least six weeks before taking study treatment. In case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow up hormone level assessment.
- Male partner sterilization (at least 6 months prior to screening). For female subjects on the study, the vasectomized male partner should be the sole partner for that subject.
- Use of established oral, injected or implanted hormonal methods of contraception, intrauterine device (IUD) or intrauterine system (IUS)

NOTE: Women are considered post-menopausal and not of child bearing potential if they have had: 12 months of natural (spontaneous) amenorrhea with an appropriate clinical profile (e.g. age appropriate, history of vasomotor symptoms) or six months of

spontaneous amenorrhea with serum FSH levels >40 mIU/mL and estradiol <20 pg/mL or surgical bilateral oophorectomy (with or without hysterectomy) or tubal ligation at least six weeks before taking study treatment. In case of oophorectomy alone - only when the reproductive status of the woman has been confirmed.

Exclusion criteria for healthy controls obtained by surgeons (surgical specimens):
1. No areas of non-inflamed skin.
2. History of autoimmune disease.
3. Subject is younger than 18 years of age.

7 CONCURRENT MEDICATIONS

7.1 Allowed Medications and Treatments

All prior concomitant medications must be on a stable dose for at least 4 weeks before the first study treatment administration. All subjects should be maintained on the same medications throughout the entire study period, as medically feasible. Any changes in medications and medication dosing will be recorded on the CRF. After the screening period, the use of concomitant medication for atopic dermatitis in all body regions is restricted to bland emollients and other non-medicated interventions.

7.2 Prohibited Medications and Treatments

The following are prohibited during the study and prior to the study for a set duration as follows:

- Systemic immunosuppressive or immunomodulatory treatments: 4 weeks.
- Phototherapy: 4 weeks.
- Cell-depleting agents including but not limited to rituximab: 6 months or until lymphocyte count returns to normal, whichever is longer.
- Biologics: 16 weeks or within 5 half-lives (if known), whichever is longer.
- Topical treatment that is likely to impact signs and symptoms of atopic dermatitis such as corticosteroids, vitamin D analogues, pimecrolimus, retinoids, salicylic acid, lactic acid, tacrolimus, tar, urea, α-hydroxy or fruit acids: 1 week.
- Live vaccinations: 6 weeks.
- Any investigational treatment or participation in any interventional trial: 4 weeks or 5 half-lives, whichever is longer.
- Any oral antibiotic: 2 weeks.
- Subjects will be asked to avoid bathing areas of microbiome skin swabbing for a 24 hour period prior to swabbing.

8 STUDY TREATMENTS

Dupilumab (Dupixent ®)

8.1 Supply of Study Drug at the Site

Regeneron and Sanofi-Genzyme will ship the study drug to the investigational sites. The

study drug is supplied in cartons containing either two 300 mg/2 mL solution pre-filled syringes with needle shield or 2 pre-filled syringes. Each kit of study drug will be labeled with the required FDA warning statement, the protocol number, and directions for use and storage.

8.2 Storage

Dupilumab must be refrigerated at 36°F to 46°F (2°C to 8°C) in the original carton to protect from light. The medication will be kept in a temperature-monitored refrigerator at the study site. If necessary, pre-filled syringes may be kept at room temperature up to 77°F (25°C) for a maximum of 14 days. The product will be kept in the original carton until the time of use.

8.3 Dosage/Dosage Regimen

Dupilumab will be administered at an initial dose of 600 mg subcutaneously followed by 300mg subcutaneously through the study, which is the FDA-approved and recommended dose for dupilumab. There is no weight-based dosing. The dosing schedule will be as follows: dupilumab 600mg subcutaneously once at week 0, followed by dupilumab 300 mg subcutaneously every other week until week 50.

8.4 Administration Instructions

All doses of study treatment will be administered at the study site after the study assessments for the visit have been completed at visits occurring between week 0 and 4, as well as weeks 8, 12, 16, 24, 36, and 44. After week 4 subjects will administer the medication independently every other week (weeks 6, 10, 14, 18, 20 22, 26, 28, 30, 32, 34, 38, 40, 42, 46, 48, and 50) only after they have shown the competency to self-administer the treatment outside of the study site. The medication will be administered via subcutaneous injection into the thigh or abdomen, except for the 2 inches (5 cm) around the navel. The upper arm can also be used if a caregiver administers the injection. There will be no medication given after week 50.

The subject should be instructed to contact the investigator if he/she is unable for any reason to attend a study visit as scheduled. All dates and times of injections done to the subject during the study must be recorded on the Dosage Administration Record CRF. The investigator will promote compliance by instructing the subject to attend the study visits as scheduled and by stating that compliance is necessary for the subject's safety and the validity of the study.

8.5 Study Drug Accountability

The qualified site personnel will maintain an accurate record of the shipment and dispensing of study treatment in a treatment accountability log. All study treatment kits assigned to the subject during the study will be recorded.

8.6 Measures of Treatment Compliance

Subjects will be given scheduled doses of dupilumab at each visit from visit 1 (week 0)

until visit 9 (week 44). After week 4, subjects will be given a dosing diary to record their scheduled injections administered at home every other week.

9 STUDY PROCEDURES AND GUIDELINES

A Schedule of Events representing the required testing procedures to be performed for the duration of the study is diagrammed in Appendix 1.

Prior to conducting any study-related activities, written informed consent and the Health Insurance Portability and Accountability Act (HIPAA) authorization must be signed and dated by the subject.

All clinical assessments will take place at the study site: 515 Spruce St., San Francisco, CCA 94118

All blood draws will take place at the UCSF Lab at Mount Zion: 2330 Post Street, San Francisco, CA 94115

All patch testing will take place at UCSF Department of Dermatology, 1701 Divisadero 4th floor, San Francisco, CA 94115.

9.1 Clinical Assessments

For subjects with a diagnosis of atopic dermatitis:

9.1.1 Medical History

Relevant medical history, including history of current disease, and information regarding underlying diseases will be recorded at Screening.

1.1.2 Concomitant Medications

All concomitant medication and concurrent therapies will be documented at Screening, at every site visit, and at early termination when applicable. Dose, route, unit frequency of administration, and indication for administration and dates of medication will be captured.

9.1.3 Demographics

Demographic information (date of birth/age, gender, ethnicity, age of onset, vital signs, body mass index, body surface area, EASI, IGA, pruritus numerical rating, laboratory values (CBC with differential, BUN, Cr, AST/ALT/Alk Phos), baseline medications)) will be recorded at Screening.

9.1.4 Physical Examination

A complete physical examination will be performed by either the investigator or a sub-investigator who is a physician at screening and weeks 0, 12, 24, and 52. New abnormal physical exam findings must be documented and will be followed by a physician or other qualified staff at the next scheduled visit.

9.1.5 Vital Signs

Body temperature, blood pressure, pulse and respirations will be performed after resting for 5 minutes at all site visits.

9.1.6 Adverse Events

Information regarding occurrence of adverse events will be captured throughout the study. Duration (start and stop dates and times), severity/grade, outcome, treatment and relation to study drug will be recorded on the case report form (CRF).

9.1.7 EASI and IGA

Eczema Area and Severity Index (EASI) and Investigator Global Assessment (IGA) will be completed at screening and weeks 0, 2, 4, 8, 12, 16, 24, 36, 44, and 52.

9.1.8 Pruritus Numerical Rating

Pruritus numerical rating will be completed at weeks 0, 2, 4, 8, 12, 16, 24, 36, 44, and 52.

9.1.9 Photography

Photographs (full front and back) will be taken at weeks 0, 2, 4, 12, and 52.

9.2 Clinical Laboratory Measurements

9.2.1 Hematology

Blood will be obtained and sent to the clinical hematology lab for a complete blood count (hemoglobin, hematocrit, red blood cell count, white blood cell count, white blood cell differential, and platelet count) determinations for assessment of systemic evidence for infection and/or inflammation at Screening.

9.2.2 Blood Chemistry Profile

Blood will be obtained and sent to the clinical chemistry lab for determination of electrolyte levels, serum BUN, creatinine, AST, ALT, and alkaline phosphatase at screening.

9.2.3 Pregnancy Test

A urine pregnancy test will be obtained at the study site for female subjects of childbearing age at screening and weeks 0, 4, 8, 12, 16, 24, 36, 44, and 52.

9.2.4 Microbiome Sampling

Sterile-technique skin swabbing and collection of stool samples will be performed at weeks 0, 2, 4, 12, and 52. Specimens will be stored at -80 C for future analysis.

9.2.5 Skin biopsy procedure

At weeks 0, 2, 4, and 12, four standard 5mm punch biopsies (the approximate size of a pencil-end eraser) will be performed. Of the biopsies performed at each visit, 3 will be from atopic dermatitis lesional skin and 1 will be from non-lesional (clear) skin. After selecting biopsy sites on trunk or extremities (subject preference), the area will be

cleansed with an antiseptic solution and a numbing medication (lidocaine or lidocaine with epinephrine) will be injected to the biopsy area. Each 5mm piece of skin will be removed via punch biopsy and a sterile gauze pad will be placed over the site to control bleeding. The biopsy site may be closed with a stitch. The parents will be provided with post-biopsy care instructions. All standard of care guidelines and sterile technique will be used. These specimens will be analyzed by CyTOF and RNAseq.

9.2.6 Pre-treatment and post-treatment dupilumab testing

Pre-dupilumab patch testing:
Each of the 15 patients will be patch tested to a core series of allergens (see below). The allergens will be applied to the back of the patient using Finn Chambers (SmartPractice, Phoenix, AZ) on Scanpor tape (Norgesplaster Alpharma AS, Vennesla, Norway). More Scanpor tape will be applied over the patches to secure them to the back. 48 hours later the patient will return for patch removal and photography. Readings will be graded as follows: ? (weak/doubtful), + (mild), ++ (strong), or +++ (very strong) on the basis of degrees of induration, papules, vesicles, and/or spreading beyond the disc space. A final determination of ‘‘allergic/positive’’ or ‘‘not allergic’’ will be determined on the basis of the pattern (crescendo/decrescendo), patch test appearance, and known characteristics of that allergen. For example, a weak/doubtful (macular erythema) reaction to a formaldehyde-releasing agent could be determined to be an allergic/positive reaction in the setting of multiple or stronger reactions to related formaldehyde-releasing allergens. The clinical relevance of the positive patch test reaction will be determined using the patient’s history and clinical skin examination. Current relevance is defined as definite (a use test with the suspected item containing the putative allergen was positive, or a patch test to the object or product was positive), probable (the allergen can be verified as present in known skin contactants, and the clinical presentation was consistent), or possible (the patient is exposed to circumstances in which skin contact with materials known to contain the allergen was likely to occur). Past relevance will also be indicated. At the final visit the patient will be counseled regarding allergen avoidance.

Post-dupilumab patch testing:
After 6 months of dupilumab therapy the patient will undergo a second round of patch testing, to the same core series of allergens and using the same procedures described above. If new allergens are identified, additional avoidance counseling will occur.

Timing and logistics:
Each patch test will require 2 three-part patient visits. Application of allergens will take approximately 20 minutes. Removal and photography (48 hours later) approximately 10 minutes. Final reading (120 hours after placement) and allergen avoidance counseling will occur at the final visit (approximately 30 minutes).

ACD Core 80 Series

1. Benzocaine, 5.0% pet.
2. 2-Mercaptobenzothiazole, 1.0% pet.
3. Colophonium (rosin), 20.0% pet.
4. 4-phenylenediamine base, 1.0% pet.
5. Benzyl Alcohol 10.0%
6. Fragrance mix II, 14.0% pet.
7. Lanolin alcohol (Amerchol L101), 50% pet.
8. Carba mix, 3.0% pet.
9. Neomycin sulfate, 20.0% pet.
10. Thiuram mix, 1.0% pet.
11. Oleamidopropyl Dimethylamine 0.1% aq.
12. Ethylenediamine dihydrochloride, 1.0% pet.
13. Bisphenol A epoxy resin, 1.0% pet.
14. Quaternium-15, 2.0% pet.
15. 4-tert-butylphenol formaldehyde resin, 1.0%
16. Ethylhexyl Salicylate, 5.0% pet.
17. Fusidic acid sodium salt, 2% pet.
18. Potassium dichromate, 0.25% pet.
19. Myroxylon Pereirae resin (Balsam of Peru)
20. Nickel sulfate hexahydrate, 2.5% pet.
21. Diazolidinyl urea (Germall II), 1.0% pet.
22. DMDM hydantoin (Germall 115), 1.0% pet.
23. Imidazolidinyl urea, 2.0% pet.
24. Bacitracin, 20.0% pet.
25. Mixed dialkyl thioureas, 1.0% pet.
26. Methylchloroisothiazolinone/methylisothiazolinone,
27. Paraben mix, 12.0% pet.
28. Cinnamal (Cinnamic aldehyde), 1.0% pet.
29. Fragrance mix I, 8.0% pet.
30. Amidoamine(stearamidopropyl dimethylamine)
31. 2-Bromo-2-nitropropane -1,3-diol (Bronopol)
32. Sesquiterpenelactone mix, 0.1% pet.
33. 2-Hydroxyethyl methacrylate, 2.0% pet.
34. Propylene glycol, 30.0% aq.
35. Benzophenone-3 (Oxybenzone)
36. Chloroxylenol (4-Chloro-3,5-xylenol), 1.0% pet.
37. Isopropyl myristate, 20.0% pet.
38. Methylisothiazolinone, 0.2% aq. (2000 ppm)
39. Desoximetasone, 1.0% pet.
40. Methyldibromo glutaronitrile/Phenoxyethanol, (MDBGN/PE) (Euxyl K 400), 2.0% pet
41. Triethanolamine, 2% pet.
42. Tocopherol (DL-a-Tocopherol),, 100.0%
43. Iodopropynyl butylcarbamate, 0.5% pet.
44. Ethyl acrylate, 0.1% pet.
45. Polysorbate 40, 5% pet.
46. Tosylamide /formaldehyde resin, 10.0% pet.
47. Methyl methacrylate, 2.0% pet.
48. Cobalt (ii) chloride hexahydrate, 1.0% pet.
49. Tixocortol-21-pivalate, 1.0% pet.

50. Budesonide, 0.1% pet.
51. Hydroperoxide of linalool, 1% pet.
52. Disperse blue mix 124/106, 1.0% pet.
53. Propolis, 10.0% pet.
54. Lidocaine-HCI, 15.0% pet.
55. Hydroperoxied of limonene, 0.3 pet.
56. Clobetasol-17-propionate, 1.0% pet.
57. Cocamidopropyl betaine, 1.0% aq.
58. Formaldehyde, 2% aq.
59. Disperse orange 3, 1% pet.
61. Cocamide DEA
62. Compositae mix, 6.0% pet.
63. 2-n-octyl-4-isothiazolin-3-one, 0.1% pet.
64. Melaleuca Alternifolia (tea tree leaf oil)
65. Cananga Odorata Flower oil (ylang –ylang oil)
66. Benzyl salicylate, 10% pet.
67. Disperse yellow 3, 1% pet.
68. Decyl glucoside, 5.0% pet.
69. Mercapto mix, 1.0% pet.
70. Glyceryl thiogycolate, 1% pet.
71. Benzoyl peroxide, 1% pet.
72. Dibucaine hydrochloride, 2.5% pet.
73. 2-tert-buytl-4-methoxylphenol, 2% pet.
74. Isoamyl-p-methoxycinnamate, 10% pet.
75. Ethyleneurea, melamine formaldehyde mix, 5%
76. Gold sodium thiosulfate, 0.5% pet.
77. Hydroxyisohexyl 3-cyclohexene carboxaldehyde, 5% pet.
78. Texile mix, 6.6% pet.
79. Glutural, 0.5% pet.
80. Diphenylguanidine, 1.0% pet.

10 EVALUATIONS BY VISIT

See Appendix 1.

10.1 Visit 0 (Screening)

1. Review inclusion/exclusion criteria.
2. Review the study with the subject and obtain written informed consent and HIPAA authorization and assent, if appropriate.
3. Assign the subject a unique screening number.
4. Record demographics data.
5. Record medical history, including a history of atopic dermatitis, diagnosis date, and prior treatments for atopic dermatitis.
6. Record concomitant medications.
7. Perform and record vital signs.
8. Perform a complete physical examination.
9. Perform EASI and IGA.

10. Perform pruritus numerical rating.
11. Collect blood for clinical laboratory tests (CBC, serum electrolytes, BUN, Creatinine, AST, ALT, alkaline phosphatase).
12. Perform urine pregnancy test (if applicable).

10.2 Visit 1: PRE-TREATMENT PATCH TESTING

1. Application of contact allergens (20 minutes).
2. 48 hours later: removal of allergens and photography (30 minutes).
3. 120 hours later: final reading of allergens (10 minutes) and allergen avoidance counseling (approximately 30 minutes).

10.3 Visit 2 (week 0)

1. Record any Adverse Experiences.
2. Concomitant medications review.
3. Perform and record vital signs.
4. Perform a complete physical examination.
5. Perform EASI and IGA.
6. Perform pruritus numerical rating.
7. Perform urine pregnancy test (if applicable).
8. Perform skin biopsy procedure.
9. Obtain photographs.
10. Dispense study medication.
11. Collect skin swabs and stool sample.

10.4 Visit 3 (week 2)

1. Record any Adverse Experiences.
2. Concomitant medications review.
3. Perform and record vital signs.
4. Perform EASI and IGA.
5. Perform pruritus numerical rating.
6. Perform skin biopsy procedure.
7. Obtain photographs.
8. Dispense study medication.
9. Collect skin swabs and stool sample.

10.5 Visit 4 (week 4)

1. Record any Adverse Experiences
2. Concomitant medications review.
3. Perform and record vital signs.
4. Perform EASI and IGA.
5. Perform pruritus numerical rating.
6. Perform urine pregnancy test (if applicable).

7. Perform skin biopsy procedure.
8. Obtain photographs.
9. Dispense study medication.
10. Collect skin swabs and stool sample.

10.6 Visit 5 (week 8)

1. Record any Adverse Experiences.
2. Concomitant medication review.
3. Perform and record vital signs.
4. Perform EASI and IGA.
5. Perform pruritus numerical rating.
6. Perform urine pregnancy test (if applicable).
7. Dispense study medication.

10.7 Visit 6 (week 12)

1. Record any Adverse Experiences.
2. Concomitant medications review.
3. Perform and record vital signs.
4. Perform a complete physical examination.
5. Perform EASI and IGA.
6. Perform pruritus numerical rating.
7. Perform urine pregnancy test (if applicable).
8. Perform skin biopsy procedure.
9. Obtain photographs.
10. Dispense study medication.
11. Collect skin swabs and stool sample.

10.8 Visit 7 (week 16)

1. Record any Adverse Experiences.
2. Concomitant medications review.
3. Perform and record vital signs.
4. Perform EASI and IGA assessments.
5. Perform pruritus numerical rating.
6. Perform urine pregnancy test (if applicable).
7. Dispense study medication.

10.9 Visit 8 (week 24)

1. Record any Adverse Experiences.
2. Concomitant medications review.
3. Perform and record vital signs.
4. Perform a complete physical examination.
5. Perform EASI and IGA.

6. Perform pruritus numerical rating.
7. Perform urine pregnancy test (if applicable).
8. Dispense study medication

10.10 Visit 9 POST-TREATMENT PATCH TESTING (between weeks 24 and 36)

1. Application of contact allergens (20 minutes).
2. 48 hours later: removal of allergens and photography (30 minutes).
3. 120 hours later: final reading of allergens (10 minutes) and allergen avoidance counseling (approximately 30 minutes).

10.11 Visit 10 (week 36)

1. Record any Adverse Experiences.
2. Concomitant medications review.
3. Perform and record vital signs.
4. Perform EASI and IGA.
5. Perform pruritus numerical rating.
6. Perform urine pregnancy test (if applicable).
7. Dispense study medication.

10.12 Visit 11 (week 44)

1. Record any Adverse Experiences.
2. Concomitant medications review.
3. Perform and record vital signs.
4. Perform EASI and IGA.
5. Perform pruritus numerical rating.
6. Perform urine pregnancy test (if applicable).
7. Dispense study medication.

10.13 Visit 12: END OF TREATMENT (week 52)

1. Record any Adverse Experiences.
2. Concomitant medications review.
3. Perform and record vital signs.
4. Perform a complete physical examination.
5. Perform EASI and IGA.
6. Perform pruritus numerical rating.
7. Perform urine pregnancy test (if applicable).
8. Obtain photographs.
9. Collect skin swabs and stool sample.

10.14 EARLY WITHDRAWAL / TERMINATION VISIT

1. Record any Adverse Experiences.

2. Concomitant medications review.
3. Perform and record vital signs.
4. Perform a complete physical examination.
5. Perform EASI and IGA.
6. Perform pruritus numerical rating.
7. Perform urine pregnancy test (if applicable).
8. Obtain photographs.

For healthy controls:
1) Clinical consent will be obtained prior to surgery by the surgeon or their research coordinator for surgical tissue that will be resected for clinical purposes and will otherwise be discarded.
2) The surgeon will notify our technician the day before samples are collected
3) On the day of surgery, redundant skin will be obtained in the operating room.
4) The samples will be de-identified at the time of harvest.
5) The surgeon will call our technician (after the samples have been harvested and de-identified) to let them know to come to the operating room suite to pick up the samples.
6) The samples will be transported by our technician from outside the operating room to the lab in a biohazard-labeled container.
7) In the lab, the samples will be given a unique identifier that is unknown to the surgical team. Thus, there will be no way for the surgical team to identify which sample came from which patient and there is no way for the laboratory team to know any patient information about the samples. This is in accordance with an agreement stating that the individual providing the specimen will never provide the recipient research team with identifiable information.

11 ADVERSE EXPERIENCE REPORTING AND DOCUMENTATION

11.1 Adverse Events

Definition of an AE: Any untoward medical occurrence in a subject administered a pharmaceutical product that does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the investigational medicinal product.

Investigational Medicinal Product (IMP) includes the drug under evaluation and the comparator drug(s) if specified as part of the research objective, given at any time during the study. Medical conditions/diseases present before starting the drug of interest are only considered adverse events if they worsen after starting the drug of interest.

The occurrence of adverse events will be sought by non-directive questioning of the patient at each visit during the study. Adverse events also may be detected when they are volunteered by the patient during or between visits or through physical examination, laboratory test, or other assessments. All adverse events will be recorded in the study database including the following information:

1. the severity grade (mild, moderate, severe)
2. its relationship to the drug(s) of interest (suspected/not suspected)
3. its duration (start and end dates or if continuing at final exam)
4. whether it constitutes a serious adverse event (SAE)

AE Severity

The National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0 should be used to assess and grade AE severity, including laboratory abnormalities judged to be clinically significant. The modified criteria can be found in the study manual. If the experience is not covered in the modified criteria, the guidelines shown in below should be used to grade severity. It should be pointed out that the term "severe" is a measure of intensity and that a severe AE is not necessarily serious.

Table 1. AE Severity Grading

Severity (Toxicity Grade)	Description
Mild (1)	Transient or mild discomfort; no limitation in activity; no medical intervention or therapy required. The subject may be aware of the sign or symptom but tolerates it reasonably well.
Moderate (2)	Mild to moderate limitation in activity, no or minimal medical intervention/therapy required.
Severe (3)	Marked limitation in activity, medical intervention/therapy required, hospitalizations possible.
Life-threatening (4)	The subject is at risk of death due to the adverse experience as it occurred. This does not refer to an experience that hypothetically might have caused death if it were more severe.

Table 2. AE Relationship to Study Drug

Relationship to Drug	Comment
Definitely	Previously known toxicity of agent; or an event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is not explained by any other reasonable hypothesis.
Probably	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is unlikely to be explained by the known characteristics of the subject's clinical state or by other interventions.

Possibly	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to that suspected drug; but that could readily have been produced by a number of other factors.
Unrelated	An event that can be determined with certainty to have no relationship to the study drug.

11.2 Serious Adverse Experiences (SAE)

A SAE is any untoward medical occurrence that at any dose:

- results in death,
- is life-threatening,
- requires inpatient hospitalization or prolongation of existing hospitalization,
- results in persistent or significant disability/incapacity,
- is a congenital anomaly/birth defect,
- is otherwise a significant medical event.

This includes any SAEs likely to arise from the trial indication or progression of underlying/concomitant illness(es) (e.g. progression of cancer in oncology trials), unless specified in the protocol as study specific exemptions.

Any SAE, irrespective of causality, occurring after the subject has provided informed consent and until four weeks after the subject has stopped study participation must be reported unless otherwise stated in the protocol. SAEs occurring after four weeks from ending study participation should only be reported if considered by the Investigator attributable to the exposure to the investigational drug(s) during the trial period. This includes the period in which the study protocol interferes with the standard medical treatment given to a subject, even if study treatment has not yet started (e.g. withdrawal of previous treatment during washout period, change in treatment to a fixed dose of concomitant medication).

11.3 Serious Adverse Experience Reporting

Study sites will document all SAEs that occur (whether or not related to study drug) per UCSF IRB Guidelines. The collection period for all SAEs will begin after informed consent is obtained and end after procedures for the final study visit have been completed.

SAEs occurring after four weeks from ending study participation should only be reported if considered by the Investigator attributable to the exposure to the investigational drug(s) during the trial period. This includes the period in which the study protocol interferes with the standard medical treatment given to a subject, even if study treatment has not yet started (e.g. withdrawal of previous treatment during washout period, change in treatment to a fixed dose of concomitant medication).

In accordance with the standard operating procedures and policies of the local Institutional Review Board (IRB)/Independent Ethics Committee (IEC), the site investigator will report SAEs to the IRB/IEC.

Timelines: All serious adverse events (SAEs) must be reported by the sites to Sponsor within 24 hours of occurrence of the SAE. The timelines for investigator initiated trials reporting to Regeneron Pharmaceuticals and Sanofi-Genzyme will be done as per Third Party Study/Investigator Initiated Trial Agreement.

Follow-up reports: SAEs will be followed until resolution or until it is judged to be permanent, and an assessment will be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the drug of interest, the interventions required to treat it, and the outcome.

The Sponsor shall support Regeneron and Sanofi-Genzyme in the following-up of all SAEs so that complete information is available to maintain patient safety and also as part of any commitments by Regeneron and Sanofi-Genzyme to any Health authority OR specific Health authority follow-up requests for the product under investigation.

Pregnancies: Any occurrences of a pregnancy in a patient (or a patients partner) during study participation will be collected. All pregnancies will be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications.

12 DISCONTINUATION AND REPLACEMENT OF SUBJECTS

12.1 Early Discontinuation of Study Drug

A subject may be discontinued from study treatment at any time if the subject, the investigator, or the Sponsor feels that it is not in the subject's best interest to continue. The following is a list of possible reasons for study treatment discontinuation:

- Subject withdrawal of consent (or assent)
- Subject is not compliant with study procedures
- Adverse event that in the opinion of the investigator would be in the best interest of the subject to discontinue study treatment
- Protocol violation requiring discontinuation of study treatment
- Lost to follow-up
- Sponsor request for early termination of study
- Positive pregnancy test (females)

If a subject is withdrawn from treatment due to an adverse event, the subject will be followed and treated by the Investigator until the abnormal parameter or symptom has resolved or stabilized.

All subjects who discontinue study treatment should come in for an early discontinuation visit as soon as possible and then should be encouraged to complete all remaining scheduled visits and procedures.

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in

the subject's source documents Refer to Section 10.13 for early termination procedures.

12.2 Withdrawal of Subjects from the Study

A subject may be withdrawn from the study at any time if the subject, the investigator, or the Sponsor feels that it is not in the subject's best interest to continue.

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents. As noted above, subjects who discontinue study treatment early (i.e., they withdraw prior to Visit 12) should have an early discontinuation visit. Refer to Section 10.13 for early termination procedures. Subjects who withdraw after Visit 1 but prior to Visit 10 should be encouraged to come in for a final visit (and the procedures to be followed would include those for their next scheduled visit). Subjects who sign the informed consent form (ICF) and who are discontinued or withdraw from the study before study product administration will be defined as screen failures. No data will be collected in the CRFs for screen failure subjects.

13 PROTOCOL VIOLATIONS

A protocol violation occurs when the subject, investigator, or Sponsor fails to adhere to significant protocol requirements affecting the inclusion, exclusion, subject safety and primary endpoint criteria. Protocol violations for this study include, but are not limited to, the following:

- Failure to meet inclusion/exclusion criteria.
- Use of a prohibited concomitant medication.
- Non-compliance with study drug regimen.
- Non-compliance with study visit procedures.
- Failure to comply with Good Clinical Practice (GCP) guidelines will also result in a protocol violation. The PI will determine if a protocol violation will result in withdrawal of a subject.

When a protocol violation occurs, it will be discussed with the investigator and a Protocol Violation Form detailing the violation will be generated. This form will be signed by a Sponsor representative and the Investigator. A copy of the form will be filed in the site's regulatory binder and in the Sponsor's files.

14 STATISTICAL METHODS AND CONSIDERATIONS

Prior to the analysis of the final study data, a detailed Statistical Analysis Plan (SAP) will be written describing all analyses that will be performed. The SAP will contain any modifications to the analysis plan described below.

14.1 Analysis of Primary Endpoint

CyTOF: One sample paired t-test comparing percentage change in lesional and non-lesional IL-4 positive, CD4+ T effector cells at weeks 2, 4, 12 compared to baseline.

14.2 Analysis Secondary Endpoints

RNA-seq: Identification of differentially expressed genes at weeks 2, 4, 12 compared to pre-treatment baseline in dupilumab-treated patients within each immune population studied using the statistical program Cuffdiff (paired analysis).

We will also explore whether early molecular changes are predictive of the clinical response to dupilumab at 52 weeks (reduction of EASI and improvement in pruritus score), which could lead to the identification of novel biomarkers.

14.3 Safety Data

Safety and tolerability data will be summarized for the treatment group, which is the 15 subjects who receive treatment with dupilumab.

Adverse event rates will be coded by body system and MedDra classification term. Adverse events will be tabulated and will include the number of patients for whom the event occurred, the rate of occurrence, and the severity and relationship to study drug.

14.4 Sample Size

15 subjects will receive dupilumab for a treatment period of 52 weeks (i.e. last injection on week 50). All subjects will undergo skin biopsies for molecular profiling.

Sample size justification

CyTOF: We estimate the minimum effect size that would be clinically relevant would be a reduction in lesional IL-4 positive, CD4+ T lymphocytes of 10%. The standard deviation of the change in lesional IL-4 positive, CD4+ T lymphocytes in the patient population is estimated at 10%. Therefore, our standardized effect size is 1.00. For the statistical analysis, we will set alpha at 0.05, beta at 0.20 (power = 0.8). The resulting sample size estimate for a one sample paired t test is 9 patients. Our proposal uses a total n=15.

RNA-seq: To determine statistical power to identify differentially expressed genes as measured by RNA-seq, we used the Scotty (Busby et al.) power calculator which uses a t-statistic and incorporates the variance attributable to read depth. Performing RNA-seq on 15 samples before and after treatment to a read depth of 50 million per sample as proposed here, we can detect >97% of transcripts displaying 2X fold change and >80% of transcripts displaying 1.5X fold change with $p<0.01$ (Figure 4).

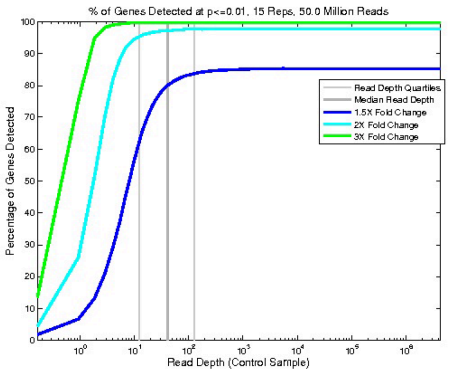

Fig 4. Power to detect differentially expressed genes using RNA-Seq.

15 DATA COLLECTION, RETENTION AND MONITORING

15.1 Data Collection Instruments

The Investigator will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each subject. Study personnel at each site will enter data from source documents corresponding to a subject's visit into the protocol-specific electronic or paper Case Report Form (eCRF) when the information corresponding to that visit is available. Subjects will not be identified by name in the study database or on any study documents, but will be identified by a site number, subject number and initials.

If a correction is required for an eCRF, the time and date stamps track the person entering or updating eCRF data and creates an electronic audit trail. If a correction is made on a paper CRF, the study staff member will line through the incorrect data, write in the correct data and initial and date the change. Changes made to an eCRF should also be made to on a paper CRF, and vice versa, if possible.

The Investigator is responsible for all information collected on subjects enrolled in this study. All data collected during the course of this study must be reviewed and verified for completeness and accuracy by the Investigator. A copy of the CRF will remain at the Investigator's site at the completion of the study.

15.2 Data Management Procedures

The data will be entered into a validated database. The Data Management group will be responsible for data processing, in accordance with procedural documentation. Database lock will occur once quality assurance procedures have been completed.

All procedures for the handling and analysis of data will be conducted using good computing practices meeting FDA guidelines for the handling and analysis of data for clinical trials.

15.3 Data Quality Control and Reporting

After data have been entered into the study database, a system of computerized data validation checks will be implemented and applied to the database on a regular basis. On eCRFs, queries are entered, tracked, and resolved through the EDC system directly. On

paper, query reports (Data Clarification Requests) pertaining to data omissions and discrepancies will be forwarded to the Investigators and study monitors for resolution. The study database will be updated in accordance with the resolved queries. All changes to the study database will be documented.

15.4 Archival of Data

The database is safeguarded against unauthorized access by established security procedures; appropriate backup copies of the database and related software files will be maintained. Databases are backed up by the database administrator in conjunction with any updates or changes to the database.

At critical junctures of the protocol (e.g., production of interim reports and final reports), data for analysis is locked and cleaned per established procedures.

15.5 Availability and Retention of Investigational Records

The Investigator must make study data accessible to the monitor, other authorized representatives of the Sponsor (or designee), IRB/IEC, and Regulatory Agency (e.g., FDA) inspectors upon request. A file for each subject must be maintained that includes the signed Informed Consent, HIPAA Authorization and Assent Form and copies of all source documentation related to that subject. The Investigator must ensure the reliability and availability of source documents from which the information on the CRF was derived.

All study documents (patient files, signed informed consent forms, copies of CRFs, Study File Notebook, etc.) must be kept secured for a period of two years following marketing of the investigational product or for two years after centers have been notified that the IND has been discontinued. There may be other circumstances for which the Sponsor is required to maintain study records and, therefore, the Sponsor should be contacted prior to removing study records for any reason.

15.6 Subject Confidentiality

In order to maintain subject confidentiality, only a site number, subject number and subject initials will identify all study subjects on CRFs and other documentation submitted to the Sponsor. Additional subject confidentiality issues (if applicable) are covered in the Clinical Study Agreement.

16 ADMINISTRATIVE, ETHICAL, REGULATORY CONSIDERATIONS

The study will be conducted according to the Declaration of Helsinki, Protection of Human Volunteers (21 CFR 50), Institutional Review Boards (21 CFR 56), and Obligations of Clinical Investigators (21 CFR 312).

To maintain confidentiality, all laboratory specimens, evaluation forms, reports and other records will be identified by a coded number and initials only. All study records will be kept in a locked file cabinet and code sheets linking a patient's name to a patient identification number will be stored separately in another locked file cabinet. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the FDA. The Investigator must also comply with all

applicable privacy regulations (e.g., Health Insurance Portability and Accountability Act of 1996, EU Data Protection Directive 95/46/EC).

16.1 Protocol Amendments

Any amendment to the protocol will be written by the PI or a sub-investigator with the PI's approval. Protocol amendments cannot be implemented without prior written IRB/IEC approval except as necessary to eliminate immediate safety hazards to patients. A protocol amendment intended to eliminate an apparent immediate hazard to patients may be implemented immediately, provided the IRBs are notified within five working days.

16.2 Institutional Review Boards and Independent Ethics Committees

The protocol and consent form will be reviewed and approved by the IRB/IEC of each participating center prior to study initiation. Serious adverse experiences regardless of causality will be reported to the IRB/IEC in accordance with the standard operating procedures and policies of the IRB/IEC, and the Investigator will keep the IRB/IEC informed as to the progress of the study. The Investigator will obtain assurance of IRB/IEC compliance with regulations.

Any documents that the IRB/IEC may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure, consent forms, information concerning patient recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB/IEC. The IRB/IECs written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated. The IRB/IECs unconditional approval statement will be transmitted by the Investigator to the designee prior to the shipment of study supplies to the site. This approval must refer to the study by exact protocol title and number and should identify the documents reviewed and the date of review.

Protocol and/or informed consent modifications or changes may not be initiated without prior written IRB/IEC approval except when necessary to eliminate immediate hazards to the patients or when the change(s) involves only logistical or administrative aspects of the study. Such modifications will be submitted to the IRB/IEC and written verification that the modification was submitted and subsequently approved should be obtained.

The IRB/IEC must be informed of revisions to other documents originally submitted for review; serious and/or unexpected adverse experiences occurring during the study in accordance with the standard operating procedures and policies of the IRB; new information that may affect adversely the safety of the patients of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

16.3 Informed Consent Form

Informed consent will be obtained in accordance with the Declaration of Helsinki, ICH GCP, US Code of Federal Regulations for Protection of Human Subjects (21 CFR 50.25[a,b], CFR 50.27, and CFR Part 56, Subpart A), the Health Insurance Portability and Accountability Act (HIPAA, if applicable), and local regulations.

The Investigator will prepare the informed consent form, assent and HIPAA authorization and provide the documents to the Sponsor or designee for approval prior to submission to the IRB/IEC. The consent form generated by the Investigator must be acceptable to the Sponsor and be approved by the IRB/IEC. The written consent document will embody the elements of informed consent as described in the International Conference on Harmonization and will also comply with local regulations. The Investigator will send an IRB/IEC-approved copy of the Informed Consent Form to the Sponsor (or designee) for the study file.

A properly executed, written, informed consent will be obtained from each subject prior to entering the subject into the trial. Information should be given in both oral and written form and subjects (or their legal representatives) must be given ample opportunity to inquire about details of the study. If appropriate and required by the local IRB/IEC, assent from the subject will also be obtained. If a subject is unable to sign the informed consent form (ICF) and the HIPAA authorization, a legal representative may sign for the subject. A copy of the signed consent form (and assent) will be given to the subject or legal representative of the subject and the original will be maintained with the subject's records.

16.4 Publications

The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual written agreement among the study Sponsor and participating institutions. The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

16.5 Investigator Responsibilities

By signing the Agreement of Investigator form, the Investigator agrees to:

1. Conduct the study in accordance with the protocol and only make changes after notifying the Sponsor (or designee), except when to protect the safety, rights or welfare of subjects.

11 Personally conduct or supervise the study (or investigation).

12 Ensure that the requirements relating to obtaining informed consent and IRB review and approval meet federal guidelines, as stated in § 21 CFR, parts 50 and 56.

13 Report to the Sponsor or designee any AEs that occur in the course of the study, in accordance with §21 CFR 312.64.

14 Ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.

15 Maintain adequate and accurate records in accordance with §21 CFR 312.62 and to make those records available for inspection with the Sponsor (or designee).

16 Ensure that an IRB that complies with the requirements of §21 CFR part 56 will be responsible for initial and continuing review and approval of the clinical study.

17 Promptly report to the IRB and the Sponsor (or designee) all changes in the research activity and all unanticipated problems involving risks to subjects or others (to include amendments and IND safety reports).

18 Seek IRB approval before any changes are made in the research study, except when necessary to eliminate hazards to the patients/subjects.

19 Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements listed in § 21 CFR part 312.

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APPENDIX 1. EVALUATION SCHEDULE

Visit number	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	
Time of Visit	**Screening**	**Patch testing**	**wk 0**	**wk 2**	**wk 4**	**wk 8**	**wk 12**	**wk 16**	**wk 24**	**Patch testing**	**wk 36**	**wk 44**	**wk 52**	**Early termination**
Inclusion/Exclusion criteria	X													
Informed consent	X													
Review medical history	X													
Physical examination	X		X				X		X				X	X
Vital Signs	X		X	X	X	X	X	X	X		X	X	X	X
EASI and IGA	X		X	X	X	X	X	X	X		X	X	X	X
Pruritus Numerical Rating			X	X	X	X	X	X	X		X	X	X	X
Laboratory assessments*	X													
Urine Pregnancy	X		X		X	X	X	X	X		X	X	X	X
Adverse events			X	X	X	X	X	X	X		X	X	X	X
Review con meds	X		X	X	X	X	X	X	X		X	X	X	X
Biopsy Procedures			X	X	X		X							
Skin swabs and stool sample			X	X	X		X						X	
Photography			X	X	X		X						X	X
Dispense study medication			X	X	X	X	X	X	X		X	X		
Patch testing		X								X				

*CBCPD, BMP, BUN, Cr, AST/ALT/Alk Phos