Dupilumab improves the molecular signature in skin of patients with moderate-to-severe atopic dermatitis

Jennifer D. Hamilton, PhD,[a]* Mayte Suárez-Fariñas, PhD,[b]* Nikhil Dhingra, BS,[b] Irma Cardinale, MSc,[b] Xuan Li, PhD,[b] Ana Kostic, PhD,[a] Jeffrey E. Ming, MD, PhD,[c] Allen R. Radin, MD,[a] James G. Krueger, MD,[b] Neil Graham, MD,[a] George D. Yancopoulos, MD, PhD,[a] Gianluca Pirozzi, MD, PhD,[c] and Emma Guttman-Yassky, MD, PhD[b,d] *Tarrytown and New York, NY, and Bridgewater, NJ*

Background: Severe atopic dermatitis (AD) has a high unmet need for effective and safe therapeutics. In early-phase trials, dupilumab, a fully human mAb targeting IL-4 receptor α, markedly improved disease activity, but the effect of IL-4/IL-13 blockade on AD at the molecular level has not been characterized.
Objectives: We sought to evaluate dupilumab modulation of the AD molecular signature.
Methods: We performed transcriptomic analyses of pretreatment and posttreatment skin biopsy specimens from patients with moderate-to-severe AD treated weekly with 150 or 300 mg of dupilumab or placebo.
Results: Exacerbation of the AD transcriptome was observed in placebo-treated patients. Dupilumab improved the AD signature in a dose-dependent manner. Expression of genes upregulated in AD lesions decreased in patients treated with dupilumab by 26% (95% CI, 21% to 32%) and 65% (95% CI, 60% to 71%) for treatment with 150 and 300 mg, respectively. Genes downregulated in AD lesions increased by 21% (95% CI, 16% to 27%) and 32% (95% CI, 26% to 37%) with dupilumab (150 and 300 mg, respectively). The molecular changes paralleled improvements in clinical scores. A dupilumab treatment signature of 821 probes (>2-fold change, $P < .05$) significantly modulated in the 300-mg dupilumab group at week 4 compared with baseline was identified in this sample set. Significant ($P < .05$) decreases in mRNA expression of genes related to hyperplasia (*K16* and *MKI67*), T cells, and dendritic cells (*CD1b* and *CD1c*) and potent inhibition of T_H2-associated chemokines (*CCL17*, *CCL18*, *CCL22*, and *CCL26*) were noted without significant modulation of T_H1-associated genes (*IFNG*).
Conclusions: This is the first report showing rapid improvement of the AD molecular signature with targeted anti–IL-4 receptor α therapy. These data suggest that IL-4 and IL-13 drive a complex, T_H2-centered inflammatory axis in patients with AD. (J Allergy Clin Immunol 2014;134:1293-300.)

Key words: *Atopic dermatitis, dupilumab, IL-4 receptor α inhibition, transcriptome, gene expression, skin, T_H2 axis*

From [a]Regeneron Pharmaceuticals, Tarrytown; [b]the Laboratory for Investigative Dermatology, Rockefeller University, New York; [c]Sanofi, Bridgewater; and [d]the Department of Dermatology, Icahn School of Medicine at Mount Sinai, New York.
*These authors contributed equally to this work.
Supported by Regeneron Pharmaceuticals and Sanofi (NCT01259323, NCT01385657).
Disclosure of potential conflict of interest: J. D. Hamilton and A. R. Radin are employed by Regeneron, have patents through Regeneron, and receive stock/stock options as part of their compensation. M. Suárez-Fariñas has received research support from and has consultant arrangements with Regeneron. I. Cardinale and X. Li have received research support from Regeneron. A. Kostic and N. Graham are employed by Regeneron and receive stock/stock options as part of their compensation. J. E. Ming and G. Pirozzi are employed by Sanofi and receive stock/stock options as part of their compensation. J. G. Krueger has received grants paid to the institution and personal fees from Novartis, Pfizer, Janssen, Lilly, Merck, Kadmon, Dermira, Boehringer, BMS, and Paraxel during the conduct of the study; grants paid to the institution from Amgen, Innovaderm and Kyowa; and personal fees from Serono, Biogen Idec, Delenex, AbbVie, Sanofi, Baxter, Xenoport, and Kineta.G. D. Yancopoulos is a board member for Regeneron, is employed as Chief Science Officer for Regeneron, and receives stock/stock options as part of his compensation. E. Guttman-Yassky has received research support from Regeneron, BMS, Janssen, Celgene, and Leo Pharma; has board memberships with Regeneron, Sanofi, Celgene, GlaxoSmithKline/Stiefel, MedImmune, and Leo Pharma; and has consultant arrangements with Regeneron, Celgene, BMS, Dermima, MedImmune, GlaxoSmithKline/Stiefel, and Galderma. The rest of the authors declare that they have no relevant conflicts of interest.
Received for publication August 29, 2014; revised October 13, 2014; accepted for publication October 14, 2014.
Corresponding author: Emma Guttman-Yassky, MD, PhD, Department of Dermatology and the Laboratory for Inflammatory Skin Diseases, Icahn School of Medicine at Mount Sinai Medical Center, 5 East 98th St, New York, NY 10029. E-mail: Emma.Guttman@mountsinai.org.
0091-6749

http://dx.doi.org/10.1016/j.jaci.2014.10.013

Atopic dermatitis (AD), the most common inflammatory skin disorder, has a prevalence of 10% in adults (in the United States) and up to 25% among children (worldwide); approximately 20% of patients have moderate-to-severe disease.[1] Although AD imposes a substantial burden on patients and the health care system,[2-5] the physical and psychological effects are often underestimated. These effects might be reflected by a marked increase in suicidal ideation in patients with AD.[6-8]

Despite the increasing worldwide incidence of AD, treatments are limited, with only 3 approved (depending on country) systemic therapeutic options for patients with severe disease who are inadequate responders to topical agents: oral corticosteroids, oral cyclosporin A (CsA), and UVA1/narrow-band UVB (NB-UVB) phototherapy.[9] These therapies are not uniformly effective, and their use is limited by toxicity (corticosteroids and CsA) or inconvenience (NB-UVB). Thus there is an unmet need for a safe and effective systemic treatment for this subset of patients.

It is thought that AD is fundamentally a disease of barrier dysfunction. However, active AD lesions are always accompanied by underlying immune activation.[10,11] Skin lesions have been best characterized in chronic AD skin and defined as lesions persisting for more than 3 days. Features usually present in chronic lesions include increased infiltration by T cells, dendritic cells (DCs), and eosinophils; increased production of cytokines and chemokines; and reactive epidermal hyperplasia, in which

Abbreviations used
AD: Atopic dermatitis
CsA: Cyclosporin A
DC: Dendritic cell
EASI: Eczema Area and Severity Index
IL-4Rα: IL-4 receptor α
LS: Lesional skin biopsy specimen
NL: Nonlesional skin biopsy specimen
NB-UVB: Narrow-band UVB
qRT-PCR: Quantitative RT-PCR

epidermal differentiation products (ie, filaggrin and loricrin) are highly suppressed.[12-14] Although AD has been classified as a T_H2-dominated disease, other T-cell subsets (T_H22, T_H17, and T_H1 cells) might also contribute to pathogenesis.[9-11,15]

On the basis of the hypothesis that IL-4 and IL-13 are key drivers of clinical disease and that IL-4 receptor α (IL-4Rα) is a requisite receptor for signaling from both cytokines, we tested whether blocking IL-4Rα could modify molecular mechanisms of AD pathogenesis in the skin. Dupilumab, a fully human mAb to IL-4Rα that inhibits both IL-4 and IL-13 signaling, is being tested as a potential therapy for AD, asthma, and nasal polyps. We recently reported positive results in early-phase trials in both patients with AD[16] and a T_H2-enriched subpopulation of asthmatic patients.[17] In this report we relate the efficacy observed in phase 1 studies to molecular changes in the skin.[18] This is the first study to evaluate the relationship between the molecular effects of a targeted T_H2 antagonist and AD pathomechanisms.

METHODS

Study subjects and skin samples

Pretreatment and posttreatment lesional skin biopsy specimens (LSs) and nonlesional skin biopsy specimens (NLs; ≥1 cm from any active lesion) were obtained from 18 adult patients with moderate-to-severe chronic AD (Table I) who participated in 2 phase 1 studies and provided additional consent for the biopsy specimens. Both studies were multicenter, randomized, double-blind, placebo-controlled trials of weekly subcutaneous injections of 150 or 300 mg of dupilumab or placebo for 4 weeks (baseline and weeks 1-3) under institutional review board–approved protocols (NCT01259323 and NCT01385657).[16] Biopsy specimens were collected 1 week after the last dose (protocol-defined end of treatment and primary end point).

Patients were allowed to use emollients, with no additional therapy during treatment. Baseline and week 4 LSs and NLs were obtained from the patients treated with placebo and dupilumab (150 or 300 mg). Disease severity was evaluated by using the Eczema Area and Severity Index (EASI) score[19] at baseline and week 4 (Table I).

RNA analyses

Expression profiling was performed to evaluate the effects of IL-4Rα blockade on LSs and NLs from patients with AD. RNA was extracted, followed by quantitative RT-PCR (qRT-PCR) and Affymetrix Human U133Plus 2.0 arrays (Affymetrix, Santa Clara, Calif) analyses, as previously described.[18] qRT-PCR was used to assess the expression of key AD-related genes and microarray findings (primers and probes are listed in Table E1 in this article's Online Repository at www.jacionline.org).

Statistical analysis

Quality control of microarray chips was carried out with standard QC metrics and R package microarray Quality Control. Images were scrutinized for spatial artifacts by using Harshlight.[20] Expression measures were obtained by using the GCRMA algorithm.[21] A batch effect was observed in the original data, corresponding to the date of hybridization, and linear models in the R *limma* framework were used to adjust the expression values to eliminate this effect. Additionally, the batch-specific coefficients of the linear model were estimated sequentially because the variable "tissue" was unbalanced in the third batch, which contained only NLs. The first 2 batches, containing both LSs and NLs, were used to estimate batch 1 and batch 2 coefficients, whereas only nonlesional pretreatment samples were used to estimate the batch 3 coefficient.

Probe sets with at least 3 samples, expression values of greater than 3, and SDs of greater than 0.1 were kept for further analysis. Expression values were modeled by using mixed-effect models, with time and treatment as fixed factors, and a random effect for each patient. This approach intrinsically models within-patient correlation similar to a paired *t* test and estimates the main effects even with missing values. Fold changes for the comparisons of interest were estimated, and hypothesis testing was conducted with contrasts under the general framework for linear models in the R *limma* package. *P* values from the moderated (paired) *t* tests were adjusted for multiple hypotheses by using the Benjamini-Hochberg procedure. Hierarchic clustering was performed with Euclidean distance and a Mcquitty agglomeration scheme.[20,21]

To evaluate the treatment effect on the published AD transcriptome (differentially expressed genes between lesional and nonlesional skin), we used a previously applied strategy.[22] The improvement in the AD transcriptome of lesional skin was defined as treatment-associated changes in gene expression toward a nonlesional molecular phenotype. A "worsening" or exacerbation was defined as gene expression changes further distinguishing LSs from NLs. The qRT-PCR analysis used log-transformed expression values, as previously reported.[22] Pearson rank correlations were used to evaluate the association of all variables measured by using qRT-PCR with treatment responses. For more information, see the Methods section in this article's Online Repository at www.jacionline.org.

RESULTS

As recently reported, EASI scores improved significantly in adults treated with dupilumab compared with scores in those receiving placebo.[16] The mean percentage change in EASI scores in the biopsy substudy (n = 18) was consistent with that of the parent studies (n = 67; Fig 1, *A*, and Table I). The pretreatment serum IgE level was greater than 150 kU/L in 14 of 18 substudy patients, and the majority of patients had positive Phadiatop test results, indicating sensitization to at least 1 allergen (Table I). EASI-50 data for each treatment arm are also listed in Table I and show that EASI-50, which represents at least 50% improvement in EASI score relative to baseline, was achieved by all but 1 patient in the substudy treated with 300 mg of dupilumab versus none in the placebo group.

Improvement of the AD transcriptome

After 4 weeks of treatment, significant dose-dependent changes from baseline in the previously defined AD transcriptome (differentially expressed genes between lesional and nonlesional AD skin) were detected in LSs by using microarrays in the dupilumab group compared with the placebo arm of the study.[22] Dose-dependent changes of −26% (SEM, 2.17%) and −65% (SEM, 3.45%) in upregulated genes were observed in the patients treated with 150 and 300 mg of dupilumab, respectively, with accompanying changes of +32% (SEM, 2.44%) and +21% (SEM, 1.69%) in downregulated genes (Fig 1, *B*, and see Fig E1 and Table E2 in this article's Online Repository at www.jacionline.org). In placebo-treated patients a worsening of the AD signature was observed by +12% (SEM, 2.46%; 95% CI, 7% to 17%) in upregulated and by −31% (SEM, 3.66%; 95% CI, 25% to 36%) in downregulated genes. These molecular